Impact of inactivity and exercise on the vasculature in humans

THE ROLE OF BLOOD FLOW AND SHEAR STRESS ON ARTERIAL ADAPTATIONS IN HEALTHY MALES

Gurpreet Kaur Birk

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the Degree of Doctor of Philosophy

October 2011

UNIVERSITY HAVE REQUESTED THE FOLLOWING FIGURES BE REDACTED

FIG 2.1 P7

FIG 2.3 P12

FIG 2.7 P18

FIG 2.8 P39

FIG 3.2 P57

FIG 3.3 P58

FIG 9.1 P158

FIG 9.2 P162

Abstract

Exercise training is known to increase endothelial function and provoke arterial remodelling both locally and systemically. This thesis was designed to further examine these relationships by investigating the acute response to different exercise intensities, with and without shear rate modification. Shear rate modification was also used to examine the impact of systemic exercise training on brachial haemodynamics. Finally, the effect of inactivity on vascular function and arterial remodelling were studied using novel models of inactivity.

The aim of Study 1 was to examine the effect of shear stress on upper limb brachial artery dilation during acute cycle exercise of different intensities. The impact of three randomised bouts of 30 mins leg cycling (50, 70 and 85% HR_{max}) on brachial artery blood flow, shear rate (SR) and brachial diameter, was measured bilaterally and simultaneously. SR was further manipulated in one arm via forearm heating (40±1°C) in a water bath (+°C) throughout the exercise bouts. Exercise induced stepwise increases in SR in the unheated arm (~°C) ($P<0.05$). In the +°C arm, SR was significantly greater than in the ~°C limb. Brachial artery diameter increased post-exercise in ~°C by 3% (50%HR_{max}; $P>0.05$ *vs.* baseline), 7% (70%HR_{max}; $P<0.05$) and 9% (85%HR_{max}; $P<0.05$). In the +°C arm, post-exercise brachial diameter increased at all exercise intensities ($P<0.05$) and was significantly greater ($P<0.05$) than in the ~°C limb at 50% (12%), 70%HR_{max} (14%) and 85%HR_{max} (15%). In conclusion, increases in shear rate during incremental lower limb exercise are associated with increases in brachial artery diameter. This response is exaggerated with larger SR induced by localised heating, indicating that leg exercise has systemic effects on arterial diameter and that SR is an important stimulus to vasodilation during exercise in humans.

The second study examined brachial artery flow mediated dilation (FMD) (using high resolution echo-Doppler) pre, and post (0, 1, 2, 24hr) 3 bouts of acute 30 min exercise at different (50, 70 and 85% HR_{max}) intensities. Shear rate area-under-the-curve (from cuff deflation to peak dilatation; SR_{AUC}) was calculated as the eliciting stimulus for FMD. Both baseline diameter and SR_{AUC} were elevated by exercise. With covariate-control of these variables, the change in brachial artery FMD was negligible after exercise (~5 minutes post exercise) at 50% HR_{max} (6.3±2.6 *vs.* 5.9±2.5%; 95%CI for difference: -0.59 to 1.34%) whilst a larger changes in FMD were noted after the exercise bouts at 70% (6.1±1.8 *vs.* 4.7±1.9%; 95%CI for difference: 0.08 to 2.58%) and at 85% HR_{max} (6.6±1.6 *vs.* 3.6±2.2%; 95%CI: 0.41 to 5.42%). A further 2-way ANOVA revealed there were no changes in FMD at any other time-point post exercise (1, 2, 24hrs) and FMD normalised by 1hr post. These data indicate, for the first time, a 'dose-response' relationship between exercise intensity and the reduction in FMD, even when exercise-mediated changes in shear and baseline diameter are accounted for.

The purpose of Study 3 was to examine the contribution of shear stress to changes in vascular function in the non-exercising upper limbs in response to lower limb (systemic) cycling exercise training. Subjects participated in an 8-week cycle training study undertaken at 80% HR_{max}, with unilateral cuff inflation around the forearm

during each exercise bout. FMD, partly NO-mediated endothelial function (i.e. ischaemic handgrip exercise (iEX)), and endothelium-independent dilation to a NO donor (i.e. glyceryl trinitrate (GTN)) were measured at 2, 4 and 8 weeks. Cycle training increased FMD in the non-cuffed limb at week 2 after which, responses returned towards baseline levels (5.8±4.1, 8.6±3.8, 7.4±3.5, 6.0±2.3 at 0, 2, 4 and 8 weeks, respectively; ANOVA: P=0.04). No changes in FMD were observed in the cuffed arm. In addition, no changes were evident in response to iEX or GTN in either the cuffed or non-cuffed arms (P>0.05) across the 8 week intervention period. These data suggest that lower limb cycle training induces a transient increase in upper limb vascular function in healthy young humans which is, at least partly, mediated via shear stress.

Exercise training is associated with rapid changes in endothelial function, which occur within days of starting training. Whilst long-term physical inactivity has a strong effect on vascular structure, little is known about the immediate impact of inactivity on vascular function. Therefore, Study 4 measured changes in vascular function before, during (day 4) and after 8 days of unilateral forearm inactivity induced by wearing a sling on the non-dominant arm. Maximal handgrip strength of the inactive forearm decreased after 8 days, confirming physical deconditioning. There were no significant changes in brachial artery baseline diameter, FMD, iEX or GTN across the 8 days in either arm (P>0.05). A significant decrease in peak blood flow was found in the intervention arm (2-way interaction: P=0.03) that is suggestive of remodelling of forearm resistance vessels. However, measures of (largely and partly) NO-mediated endothelial conduit artery function were not altered across an 8 day period of inactivity.

Whilst increases in mean arterial shear stress are known to induce improvements in arterial function and remodelling in humans, animal data have demonstrated that retrograde shear is associated with pro-atherogenic effects. However, relatively little is known regarding the effect of retrograde shear rate on vascular function in humans *in vivo*. In order to provoke retrograde shear, subjects wore a compression sleeve on one forearm for 8 days. Measurements were taken before and during acute (1hr) exposure to a compression sleeve on baseline day 0. Measurements were taken after 4 and 8 days exposure to the compression sleeve. There were no significant changes in mean or antegrade shear rate during exposure to the compression sleeve. However, the compression sleeve resulted in an immediate increase in retrograde shear rate in 6 subjects (P<0.05, intervention-group), but remained near resting levels in the other 6 subjects (P>0.05, control-group)i.e. subjects in whom the compression sleeve did not increase retrograde shear were the control group. The intervention group demonstrated a significant decrease in FMD after 1 h compression sleeve (P<0.05), but not in the control group (P>0.05). After 8-days using the compression sleeve, no significant changes in FMD, iEX, or GTN-response in the intervention and control group (all P>0.05) were observed. In conclusion, short-term increases in retrograde shear rate decrease FMD, but not chronically.

Data in this thesis provide evidence for the role of blood flow and shear stress, as a result of exercise and inactivity, and its immediate effects upon the vasculature.

Acknowledgements

I still remember the first meeting I ever had with Danny and Dick - and coming out feeling rather overwhelmed by their enthusiasm and passion for research! (Tim warned me!) I didn't realise at the time but this coffee meeting was to be the start of an exciting and challenging journey, otherwise known as the trials and tribulations of a PhD. The completion of which would not have been possible without my supervisors- Ellen Dawson, Tim Cable, Danny Green and Dick Thijssen. It has been an incredible experience to work with you all.

Ellen, simply, thank you. It has been great working with you for the past 3 years. You have taught me a lot, most notably, the correct use of grammar and 'proper' English (aka Yorkshire English ☺). Thank you for always being there for me, through the difficult times and the good! Your door was always open and I will be forever grateful.

Tim, this journey wouldn't have been possible without your support and guidance. Three years ago, you opened this door for me, providing me with an amazing opportunity. Your encouragement from day one has allowed my confidence to grow and I will never forget everything I have learnt from you. Even when you had a million things on your to do list, you always found time to squeeze in a 5 minute catch up, albeit, in the ship. I will always be thankful, for everything.

Danny, although you have been on the other side of the world you have played a huge role in my PhD journey. Your knowledge and passion for research (and wine) is truly admirable and your level of ambition has inspired me to always aim a little higher.

Dick, your direction over the last 3 years has been invaluable, I have always been able to rely on your support, wealth of knowledge and ever-present enthusiasm. Your determination to succeed has kept me motivated throughout my journey and for that I owe you a lot of thanks.

My post grad family, near and far, all of whom have witnessed this journey first hand and have kept me sane, I couldn't have done it without you. Thank you all for sharing this experience with me, I have made some lifelong friends and have so many special memories (and photos)....Procrastination, tea trains, surprise birthday's, CAKE, snake pit posse, MORE CAKE, THE worm! Mojito in one, raving in a cathedral?! To name but a few!!

Thank you to my fellow scanners who helped through the long days of data collection (Toni Tinken, Ellen Dawson, Nik Hopkins & Nic Rowley). I would also like to thank the participants for volunteering their time (and their arm!).

Last but definitely not least, to my wonderful family, I dedicate this thesis to you - Mum, Dad and my 3 beautiful sisters. Mum and Dad, for as long as I can remember you have encouraged me to always achieve my best. Your endless love and support over the years has been invaluable and I couldn't have come this far without you being behind me and always believing in me. Thank you from the bottom of my heart.....(not forgetting, I will forever be indebted to the bank of Mum and Dad!!)

In loving memory of both my grandfathers G. S. Bains and J. S. Birk.

Candidates Declaration

I declare that the work contained in this thesis is entirely my own. Various individuals (mentioned in the acknowledgments) assisted in the collection of data for each study in this thesis.

Published articles completed by the candidate during PhD tenure

Birk GK, Dawson EA, Atkinson CL, Thijssen DH, Cable NT & Green DJ (2012). Brachial artery adaptation to lower limb exercise training: Role of shear stress. *Journal of Applied Physiology*, in press.

Rowley NJ, Dawson EA, **Birk GK**, Cable NT, George K, Whyte G, Thijssen DH & Green DJ. (2011). Exercise and arterial adaptation in humans: uncoupling localized and systemic effects. *Journal of Applied Physiology,* 110**,** 1190-1195.

Oral Communications

European College of Sport Science (ECSS), Liverpool, UK 2011; 8-weeks of cycle exercise training on brachial artery function in humans: the role of arterial shear stress. **Birk GK,** Thijssen DHJ, Dawson, EA, Cable NT and Green DJ.

European College of Sport Science (ECSS), Turkey, June 2010; The effects of 8-days unilateral forearm bracing on brachial artery function in humans. **Birk GK**, Thijssen, DHJ, Dawson EA, Cable NT and Green DJ.

British Association of Sport and Exercise Science, annual student conference (BASES), Hull, UK 2009; The effects of 8-days unilateral forearm bracing on brachial artery function in humans. **Birk GK**, Thijssen DHJ, Dawson EA, Cable NT and Green, DJ.

Poster communications

Liverpool John Moores Institute of Health Research Annual Conference, Liverpool, May 2010; The effects of 8-days unilateral forearm bracing on brachial artery function in humans. **Birk GK**, Thijssen DHJ, Dawson EA, Cable NT and Green DJ.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ACh	Acetylcholine
.avi	Audio video interleave
Ca^{2+}	Calcium
CAD	Coronary artery disease
cGMP	Cyclic guanosine monophosphate
CHD	Coronary heart disease
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DICOM	Digital imaging and communications in medicine
D/U	Doppler ultrasound
EDHF	Endothelial-dependent hyperpolarising factor
EDRF	Endothelial-dependent relaxing factor
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FMD	Flow mediated dilation relative change from baseline
FMDmm	Flow mediated dilation absolute change from baseline
GC	Guanylyl cyclase
GCX	Glycocalyx
GEE	Generalised estimation equation
GLM	General linear model
GTN	Glyceryl trinitrate
HR	Heart rate
HR_{max}	Maximal heart rate
HIT	High intensity training
IMT	Intima media thickness
IP^3	Inositol triphosphate
L-NMMA	NG-monomethyl-L-agrinine
MAP	Mean arterial pressure
mRNA	Messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide

O_2	Oxygen
PVD	Peripheral vascular disease
PAD	Peripheral artery disease
RHBF	Reactive hyperaemic blood flow
RH	Reactive hyperaemia
ROI	Region of interest
SAC	Stretch activated channels
SGC	Soluble guanylate cyclase
SS	Shear stress
SR	Shear rate
SNP	Sodium nitro prusside
SBP	Systolic blood pressure
$VCAM^{-1}$	Vascular cell adhesion molecule
VOP	Venous occlusion plethysmography
+VE	Antegrade blood flow/shear rate
-VE	Retrograde blood flow/shear rate
+°C	Heated forearm water-bath
~°C	Unheated/No waterbath

Chapter 1 | *General Introduction*

1.1. Physical activity, inactivity and cardiovascular risk

Physical inactivity is an independent risk factor for the progression of atherosclerosis and subsequently cardiovascular disease (CVD) (Blair *et al.*, 1995). Endothelial dysfunction is also one of the earliest markers of vascular abnormalities observed in cardiovascular disease and ageing. It has been suggested that augmentation of vasoconstrictor pathways contributes to the endothelial dysfunction observed after physical inactivity (Green *et al.*, 1997). However, it is well recognised that moderate and high intensity exercise training is associated with considerable reductions in the incidence of cardiovascular events (Green *et al.*, 2004; Mora *et al.*, 2007). More specifically, exercise training is known to increase fitness levels, improve endothelial function, exercise capacity, and prevent the progression of atherosclerosis and cardiovascular diseases (CVD) in patients with CVD, and to an extent, asymptomatic individuals (Green *et al.*, 2003; Green *et al.*, 2004; Green *et al.*, 2011). Nevertheless, reductions in traditional risk factors do not appear to fully explain the reduction in cardiovascular morbidity and mortality following exercise training. Collectively, data from exercise training studies from both animal (Jasperse & Laughlin, 2006) and humans (Green *et al.*, 2004; Tinken *et al.*, 2008) suggest that functional and remodelling adaptations of the vasculature in response to exercise training alter with training duration, intensity and the vessel beds involved. Exercise exerts direct effects on the vasculature by the impact of repetitive pulsatile increases in shear stress on the vascular endothelium, which respond by transducing functional and remodelling vascular adaptations which decrease atherosclerotic risk (Laughlin *et al.*, 2008). Consequently, exercise-dependent improvements in endothelial function and remodelling represent a

conditioning effect to the vasculature, which provides a credible explanation for the cardio-protective benefits of exercise, independent of the impact of exercise on traditional CV risk factors (Green *et al.*, 2004; Thijssen *et al.*, 2010).

There is evidence that the link between physical inactivity and cardiovascular risk is explained by changes in endothelial function (Green *et al.*, 2004; Mora *et al.*, 2007; Thijssen *et al.*, 2010). It is likely that the beneficial cardiovascular adaptations are caused by changes in blood flow patterns or shear stress through arteries (Thijssen *et al.*, 2009a). Nevertheless, despite the evidence that physical inactivity is seen as an independent cardiovascular risk factor, there are few physiological studies that have assessed the direct mechanisms that could potentially cause adaptations in response to exercise and physical inactivity conduit artery function and remodelling in humans. It is clear there are strong links between vascular function, remodelling and cardiovascular events, however, the mechanisms responsible for NO bioactivity and the role that other vasodilator substances play in vascular adaptations still remain unclear.

1.2. Aims

Therefore the aims of the studies within this thesis are:

1. Examine exercise intensity dependent and independent (through the use of forearm heating) changes in shear rate at different exercise intensities on brachial artery diameter before, during and immediately post exercise. This will be achieved through study 1 (Chapter 4).
2. Determine the effects of shear rate during different exercise intensities on brachial artery flow-mediated dilation (FMD) responses before and

immediately post cycle exercise. This will be achieved through study 2 (Chapter 5).

3. Examine the role of shear rate upon endothelium dependent (largely and partly) NO mediated and endothelium independent vasodilation of the brachial artery, following 8-weeks lower limb exercise training, with shear rate attenuated in one forearm during the exercise training. This will be achieved through study 3 (Chapter 6).

4. Examine the impact of localised forearm inactivity on brachial artery endothelium dependent (largely and partly) NO mediated and endothelium independent vasodilation. This will be achieved through study 4 (Chapter 7).

5. Determine acute and prolonged changes in retrograde shear rate, and the role of shear rate on endothelium dependent (largely and partly) NO-mediated and independent vasodilation of the brachial artery through the use of compression around one forearm. This will be achieved through study 5 (Chapter 8).

Chapter 2 | *Review of the Literature*

2.1. Background

It is now well accepted that a sedentary lifestyle is a risk factor for cardiovascular disease (CVD). In 2008, CVDs were responsible for more than 17.3 million deaths in the world (WHO, 2011). In particular coronary artery disease (CAD) is one of the most common types of heart disease and is responsible for a high percent of mortality among men and women. Atherosclerosis is the most frequent underlying cause of CAD and peripheral vascular disease (PVD) also referred to as peripheral arterial disease (PAD). Atherosclerosis is a chronic, progressive disease in which plaques (consisting of deposits of cholesterol and other lipids, calcium, and large inflammatory cells called macrophages) accumulate in the walls of the arteries. The plaque formation stimulates the cells of the artery wall to produce substances that accumulate in the inner layer, producing a build-up of atheroma within the cells, and ultimately causing the inner layer of the artery wall to thicken. This in turn reduces the diameter of the artery causing a decrease in blood flow and oxygen delivery eventually leading to arterial occlusions, resulting in ischaemia, usually of the arteries in the lower limbs.

In order to reduce the risk of developing a CVD it is recommended that healthy lifestyle options are chosen, including increasing physical activity, weight management and improving diet. Ominously, it has been stated that nearly 40% of the adult population subject themselves to <10 minutes of continuous physical activity per week, (Blair & Brodney, 1999), and the prevalence of physical inactivity is ever increasing. These lifestyle changes are of special interest because physical inactivity has been proposed as an independent risk factor for atherosclerosis, CVD

and diabetes (Green *et al.*, 2004; Mora *et al.*, 2007; Thijssen *et al.*, 2010). Reductions in both primary (without existing CVD) (Paffenbarger *et al.*, 1986; Sesso *et al.*, 2000; Myers *et al.*, 2002) and secondary (preventing the development of already established CVD) vascular events (Oldridge *et al.*, 1988; Jolliffe *et al.*, 2001) are associated with regular physical exercise. It has been suggested that the most physically active individuals have approximately half the risk of developing CAD compared with sedentary individuals (Thompson, 2003). Additionally, individuals with higher cardiopulmonary fitness demonstrate lower CVD rates compared with individuals that are less fit (Thompson, 2003), with the relative risk of being unfit exceeding that associated with smoking, elevated systolic blood pressure, hypercholesterolemia and obesity (Blair *et al.*, 1996).

These data indicate that exercise training and maintenance of physical fitness influence the occurrence and development of cardiovascular diseases in humans. However, exercise-induced reductions in traditional risk factors, such as obesity, diabetes and high blood pressure only account for 30-40% of the reduction in total CVD risk, and therefore approximately 65% of the training-related reductions in CVD remain unexplained (Figure 2.1).

Figure 2.1 Percentage reductions in CVD events associated with physical activity that is explained by risk factors (adapted from Mora *et al.*, 2007)

One current hypothesis is that exercise training may provide a direct stimulus for adaptations upon the vasculature, which are likely to play a beneficial role in the modification of cardiovascular risk (Green *et al.*, 2004; Green, 2005a; Green *et al.*, 2008b; Thijssen *et al.*, 2010). The potential mechanisms for this will be discussed in more detail within this chapter.

2.2. The Vascular Endothelium

An artery is made up of 3 separate layers; the intima, media and adventitia (Figure 2.2). More specifically, the endothelium is the inner most layer of an artery, and is important due to its vasoactive and anti-atherogenic properties. The endothelium itself consists of a single monolayer of cells that line the interior surface of all blood vessels. Endothelial cells are located at the interface between the blood and the vascular wall, and were surprisingly once considered a passive layer of static cells. It is now well established that the endothelium serves as a dynamically active modulator of vasomotor tone and atherogenic development

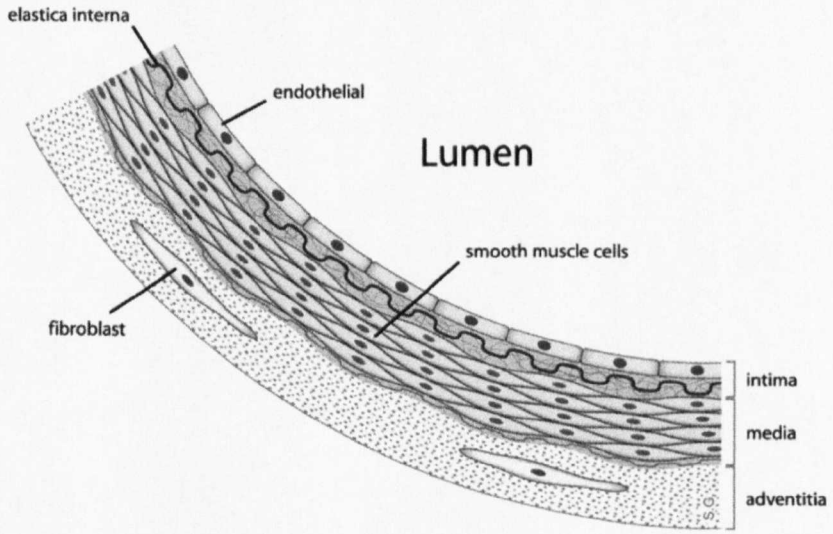

Figure 2.2 Anatomy overview of a human artery.

through the secretion of a number of paracine substances that play a key role in regulating vascular tone, cell growth, platelet and leukocyte inbibition, vasoregulation and vasoprotection (Moncada *et al.*, 1991).

2.2.1. The Identification of endothelium derived relaxing factor (EDRF)

In the 1980's, Furchgott and Zawadski first demonstrated that the endothelium was essential for vasodilation. Results from their study which, apparently occurred by chance, revealed that when acetylcholine (ACh) was applied to rabbit aorta strips, some with endothelium abolished and some with endothelium intact, vasodilation only occurred when the endothelium was present (Furchgott & Zawadzki, 1980). This study led to the (at the time misguided) observation that ACh stimulated the endothelium to release an agent that either relaxed or contracted the vascular smooth muscle, depending on whether the endothelium was healthy or damaged. The contradictory response outlined here was, however due to the fact that in removing the aortic sampling the arteries had been denuded of endothelium. In time it was discovered, that this effect was mediated by a labile factor known as endothelium derived relaxing factor or EDRF that required an intact endothelium, with EDRF later suggested being the inorganic molecule, nitric oxide (NO), (Ignarro *et al.*, 1987). This hypothesis was confirmed with a study that demonstrated vascular endothelial cells synthesise NO and that the biological effects of EDRF could be reproduced by exogenous NO (Ignarro *et al.*, 1987; Palmer *et al.*, 1987). It is now well established that endothelium NO is synthesised by nitric oxide synthase located in the endothelium (eNOS) by the regulation of intracellular calcium concentration (Mulsch *et al.*, 1989; Schmidt *et al.*, 1989).

2.3. Nitric Oxide: Physiological stimulation from the endothelium

The endothelium responds to both physical, and pharmacological stimuli which result in the release of a variety of vasoactive substances such as NO, prostacyclin, endothelins, endothelial cell growth factor, interleukins, adhesion molecules, and fibrinolytic factors (Laughlin *et al.*, 2008). More specifically, given its role in processes associated with the development of atherosclerosis, the endothelial production of nitric oxide has received much research interest. Nitric oxide has a number of roles, dependent on interactions with target cells, and is able to diffuse freely across cell membranes. The biological effects of NO occur by activation of soluble guanylate cyclase (SGC) and subsequent elevations in cyclic guanisine monophospate (cGMP) (Ignarro, 1990; Furchgott & Jothianandan, 1991), which promotes a cascade of events leading to smooth muscle dilatation (see below).

It was not until the late 1980's, that Vallance *et al.* (1989) examined the role of NO in healthy volunteers, by using a specific inhibitor of the synthesis of endothelium-derived NO, N^Gmonomethyl-L-arginine (L-NMMA) which was infused into the brachial arteries. They reported a 50% fall in basal blood flow and attenuated dilator response to infused acetylcholine. This was the first study to discover the importance of NO as an essential factor released from the endothelium allowing for tonic vasomotor control and vasodilation in humans. It was later recognised that, arterial remodelling is shear stress and NO-dependent (Tuttle *et al.*, 2001) and acts in a manner that homeostatically regulates wall shear stress (Tronc *et al.*, 1996). These data provided direct evidence that the dilator action of endothelium derived NO contributes to the control of basal and stimulated regional blood flow in

humans, and therefore suggested that an impairment of production of NO might account for the abnormalities in vascular reactivity that characterise a wide variety of disease states.

2.3.1. Mechanisms that mediate vasodilator function

Endothelial shear stress is the central stress created by the frictional flow of blood on the endothelial surface of the arterial wall, usually expressed in units of dyne/cm^2 (Slager *et al.*, 2005). The nature of flow through a vessel is dependent on the velocity of flow and any obstructions. Flow is usually characterised as either laminar or turbulent (Slager *et al.*, 2005). Laminar flow refers to a smooth undisturbed flow, it can sometimes be known as disturbed laminar flow, characterised by areas of reversed flow (Chatzizisis *et al.*, 2007). On the other hand, turbulent flow is dependent on the velocity and varies continuously over time. The pulsatile nature of arterial blood flow provokes constant changes in direction and magnitude of flow (Chatzizisis *et al.*, 2007). Usually, in straight arterial segments, shear stress is pulsatile and unidirectional with a magnitude that varies within a range of 15 to 70 dyne/cm^2 over the cardiac cycle (Slager *et al.*, 2005). In contrast, in regions where disturbed laminar flow occurs, pulsatile flow generates low or oscillatory shear stress, that is usually evident in the inner areas of curvatures (Slager *et al.*, 2005).

The importance of the vascular endothelium in mediating the vasomotor responses to increases in flow in conduit arteries were examined by Pohl *et al.* (1986), in dogs by investigating the diameter changes of femoral arteries, in response to increases in flow before and after endothelial damage. Their results demonstrated that removal of the endothelial cells abolished dilation and concluded that endothelial cells are obligatory for flow-mediated dilation.

Figure 2.3 (A) Mechanical effects of hemodynamic forces on the vascular wall (Laughin *et al.*, 2008). (B) Hemodynamic forces that act on blood vessels (Hahn and Schwartz, 2009).

The endothelium is normally subjected to mechanical deformation resulting from shear stress and from strain associated with stretch of the vessel wall. These stimuli are detected by a mechano-sensor allowing the influx of extracellular Ca^{2+} and Na^{+} ions into the endothelial cell, through the opening of ion gates (Olsen *et al.*, 1988). This initiates a variety of signalling systems responsible for triggering the functional responses. This results in increases in intracellular Ca^{2+} ion concentration which activates endothelial nitric oxide synthase (eNOS), resulting in the production of NO from the semi-essential amino acid L-arginine (Cooke & Dzau, 1997) (Figure 2.4). Once formed, NO diffuses across the cell membrane and stimulates an increase in

guanylyl cyclase (GC) concentration, resulting in an increase of cGMP levels. cGMP is derived from guanosine triphosphate (GTP) via the catalysis of NO on soluble guanylate cyclase. It acts as a secondary messenger and its likely mechanism of action is activation of intracellular protein kinases in response to the binding of membrane-impermeable peptide hormones to the external cell surface (Vanhoutte *et al.*, 1986). Additionally, cGMP also inhibits the influx of extracellular Ca2+, decreases inositol triphosphate (IP^3) levels and increased vasorelaxation (Vanhoutte *et al.*, 1986). Once cGMP is produced it has the potential to provide protective effects against the development of atherosclerosis, as a result of a reduction in the influx of calcium through L-type calcium channels and by stimulating a cGMP sensitive phosphodiesterase which concludes with a reduction in concentrations of cGMP.

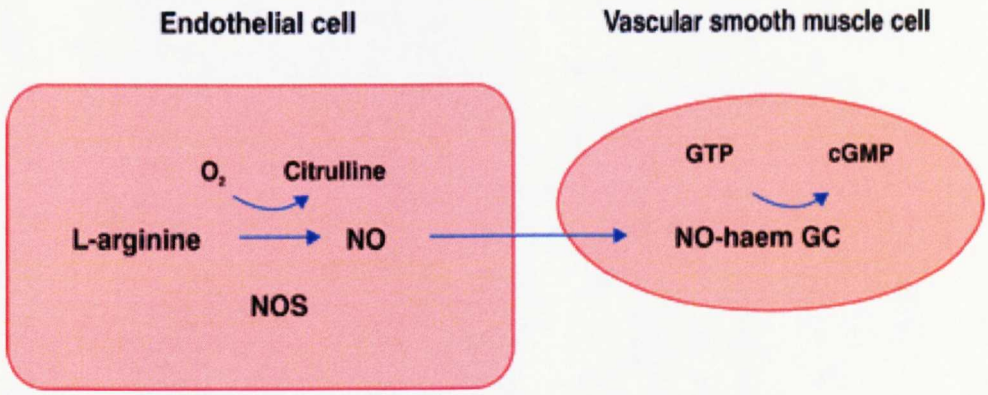

Figure 2.4 The L-arginine NO pathway

A further potential mechanism, critical to the production of NO is the phosphorylation of the amino acid Ser1177 within the eNOS by serine/threonine kinase Akt (protein kinase b) (Dimmeler & Zeiher, 2003; Hambrecht *et al.*, 2003). Shear stress-induced Akt is responsible for the prolonged, calcium-independent

activation of eNOS and acts in addition to the short-term calcium-mediated stimulation of eNOS, leading to NO release and relaxation of the vascular smooth muscle cells. Therefore, the shear stress–induced stimulation of NO is thought to be a potent vasodilator in vivo and may also be related to improved endothelial NO-mediated vasodilator function of arterial conductance vessels prone to atherosceloritic lesion development (Hambrecht *et al.*, 2003).

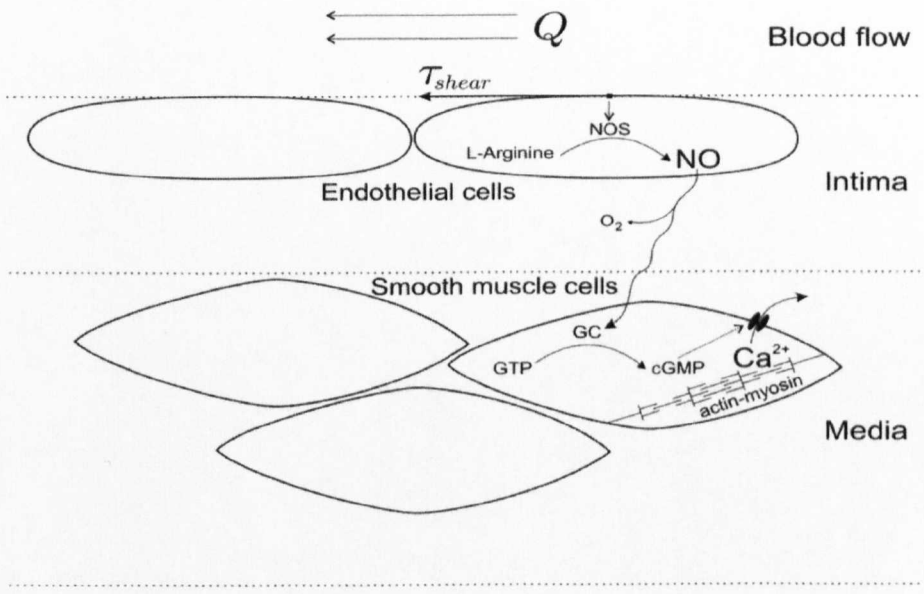

Figure 2.5 A schematic of EDRF-NO shear stress mediated process

An additional mechanism could be related to the endothelial glycocalyx (GCX), which exists as a network of membrane-bound proteoglycans and glycoproteins that line the surface of the lumen. The endothelial GCX was first visualised by Luft over 40yrs ago (Luft, 1966). Although, relatively little is known about this layer, it's importance in vascular biology has recently been acknowledged (Vogel *et al.*, 2000; Nieuwdorp *et al.*, 2005) as playing an essential role in detecting shear stress from increases in blood flow, allowing for the transduction of force to mechanotransduction (biochemical responses (Tarbell & Pahakis, 2006; Tarbell &

Ebong, 2008). These forces are known to induce secretions of several vasoactive substances possibly via internal calcium mobilisation including NO (Furchgott *et al.*, 1985) (Figure 2.5). Although there is evidence indicating that increases in blood flow and shear stress induce the secretion of vasoactive substance from the endothelium, which aid in maintaining the health and tone of the vessel, it is unclear as to whether acute bouts of exercise or exercise training of the lower limb provides a potent stimulus for increases in blood flow and shear stress in the inactive upper limbs (i.e. the brachial artery) to provide beneficial adaptations to endothelial function.

2.3.2. The anti-atherogenic effects of NO

As mentioned at the beginning of this chapter, atherosclerotic vascular disease is a leading cause of morbidity and mortality within the UK, and the elucidation of the processes underlying it is of great potential benefit. Endothelial dysfunction is a precursor to atherosclerosis resulting from reduced NO bioavailability which causes detrimental effects on the health and function of the endothelium. NO plays an essential role in maintaining vascular health and tone and it has many cardio-protective roles some of which include regulation of blood pressure and vascular tone, inhibition of platelet aggregation, leukocyte adhesion, and prevention of smooth muscle cell (SMC) proliferation. Consequently, reduced bioavailability of NO is thought to be one of the central factors common to cardiovascular disease (Laughlin *et al.*, 2008). Decreased endothelial NO production, as a result of endothelial dysfunction, occurs in the early phases of atherosclerosis. NO appears to reduce atherogenesis by inhibiting leukocyte and platelet activation and by

inhibiting smooth muscle cell proliferation (Laughlin *et al.*, 2008). Studies examining subjects at risk for atherosclerosis have demonstrated arterial endothelial dysfunction (Adams & Nash, 1996), and have demonstrated reduced vasodilator responses after pharmacological or physiological stimulation of endothelial NO.

The pathophysiology of atherogenesis is complex, but endothelial dysfunction, caused by cholesterol deposition, represents an important marker of the early phases of atherosclerosis (Anderson *et al.*, 1995). Endothelial dysfunction usually comprises of a combination of reduced endothelium mediated dilation, increased expression of adhesion molecules and inflammatory genes of endothelial cells, and increased oxidative stress, with adaptations in these types of cells referred to as pro-atherogenic (Laughlin *et al.*, 2008). Current evidence is suggestive that endothelial dysfunction is present throughout the atherosclerotic process, and the above 'pro-atherogenic' factors are known to play a key role in atherosclerotic development (Figure 2.6) (Furchgott & Vanhoutte, 1989; Luscher, 1990; Vita, 2002).

An enhanced uptake of low density lipoproteins (LDL) and succeeding oxidation causes an increase in endothelial cell expression of cellular adhesion molecules that recruit circulating monocytes to the intima (Geng & Libby, 2002). The monocytes are then transformed into the activated tissue macrophages and foam cells, which promote the development of the atherosclerotic plaque (Figure 2.6.) (Geng & Libby, 2002). The release of growth factors leading to SMC proliferation, together with accumulation of lipids, causes a progressive narrowing of the vessel lumen, limiting blood flow (Geng & Libby, 2002). However, evidence from studies in animals suggests that NO serves as an anti-atherogenic molecule, and more specifically, it

was reported that exercise appeared to improve endothelial function through the regulation eNOS pathway (Laughlin, 1995; Laughlin *et al.*, 2003). In patients with atherosclerosis, endothelial NO production is usually deficient. In a study by Gokce *et al.* (2002) upper and lower extremity endothelial function was measured in patients with CAD. In response to endurance exercise training, they reported improved endothelial function in peripheral conduit arteries of patients (Gokce *et al.*, 2002). In another study in similar patients, the effect of regular physical activity on endothelial function was assessed. It was concluded that the exercise training leads to an improved endothelium-dependent vasodilatory capacity by upregulating eNOS expression (Hambrecht *et al.*, 2003). Taken together, these data suggest that NO may regulate atherogenesis by several mechanisms, including alteration of LDL modification, platelet and leukocyte function, and SMC proliferation.

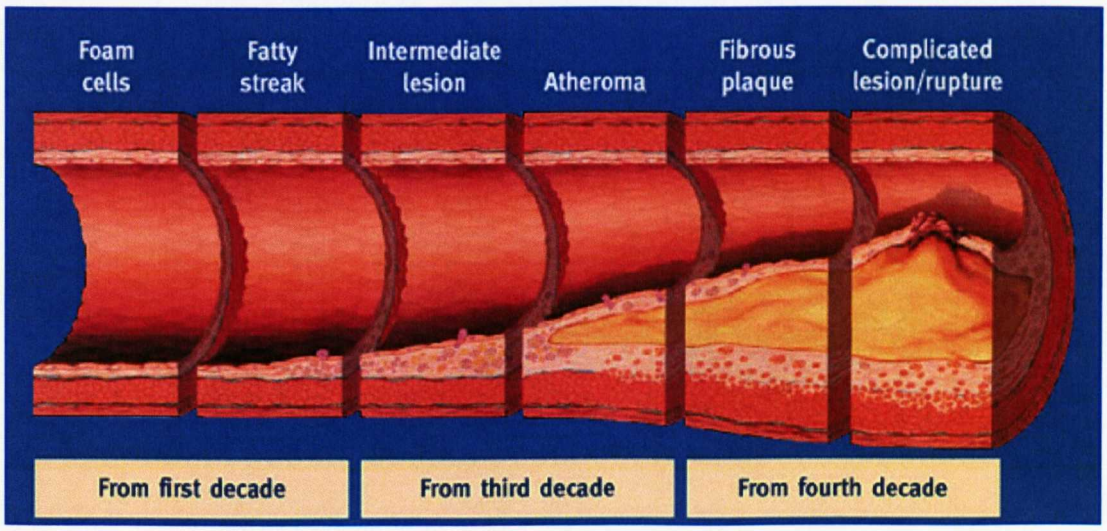

Figure 2.6 Timeline of the stages of atherosclerotic progression within an artery

Raised blood pressure and vasoconstriction are usually associated with the release of proteins called endothelins and the over expression of endothelins are known to

contribute to hypertension and heart disease (Laughlin et al., 2008). In particular, Endothelin-1 (ET-1) is frequently associated with vasoconstriction of an artery and is a key mediator of vascular tone through opposed vasoactive effects (Laughlin *et al.*, 2008). Consequently, dysfunctions of ET-1 signaling are associated with cardiovascular diseases. An additional gene which is equally associated with the progression of cardiovascular disease is the vascular cell adhesion molecule-1 (VCAM-1) which mediates the adhesion of lymphocytes and monocytes to the vascular endothelium. An increase in VCAM-1 levels associated with a decrease in NO bioavailability (Figure 2.7) and therefore an increased risk of developing atherosclerosis, however, there is evidence to suggest exercise training can reverse/reduce this risk (Laughlin *et al.*, 2008), (see section 2.4.).

Figure 2.7 Interactive effects of atherosclerosis and exercise training on markers of endothelial cell phenotype. e NOS, endothelial nitric oxide synthase; ET-1, endothelin-1; NO, nitric oxide; O_2, superoxide; PGI2, prostacyclin; ROS, reactive oxygen species; SOD, superoxide dismutase; VCAM-1, vascular cell adhesion molecule-1(Laughlin et al., 2008).

In summary, due to the anti-atherogenic properties of NO and the stimulus for NO release being increases in blood flow and shear stress, the endothelium has been the target for therapeutic interventions, through both pharmacological and non-

pharmacological (e.g. exercise) options in an attempt to improve endothelial function/dysfunction and NO bioavailability in individuals with cardiovascular disease or risk (Green *et al.*, 2004). In particular it has been demonstrated that exercise training, can improve endothelial dependent function in patients with cardiovascular risk factors, such as high blood pressure, diabetes and obesity (Green *et al.*, 2004).

2.4. Effect of exercise training on the vasculature

Exercise training has been consistently reported as having beneficial effects upon the vasculature in animals (Laughlin *et al.*, 1989; Sessa *et al.*, 1994), patients with cardiovascular risk factors or disease (Green *et al.*, 2004) and to an extent, in healthy subjects (Green *et al.*, 2011). In this section of the chapter, the effects of exercise training on conduit artery function and remodelling in healthy and patient populations, as well as optimal exercise training methods, and potential mechanisms for vascular adaptations in response to exercise training will be discussed in detail.

2.4.1. Exercise training and endothelial function

Exercise training causes an increase in blood flow through the lumen of the vessel resulting in an increase in shear stress that stimulates the release of a number of vasoactive substances, in particular NO. Repeated episodic increases of blood flow and shear stress are thought to be the stimulus responsible for adaptations in the vasculature with short and long -term exercise training.

The majority of studies within the literature that have examined the impact of exercise training in subjects with CVD report an enhanced endothelial function in response to exercise training (Moriguchi *et al.*, 2005; Wisloff *et al.*, 2007). Recent studies indicate that exercise training may improve endothelial function by up-regulating eNOS protein expression and phosphorylation. For example, twelve weeks of endurance exercise training led to an improvement in endothelial function (Edwards *et al.*, 2004). For brevity, studies investigating exercise on endothelial function in healthy and CAD patients are presented in Table 2.1. Comprehensive reviews on this topic can also be found (Green *et al.*, 2004; Mora *et al.*, 2007; Thijssen *et al.*, 2010; Green *et al.*, 2011). Additionally, the impact of 4 weeks of cycling in CAD patients, reported that exercise was associated with significantly higher NO synthase and protein expression as a results of higher shear stress related eNOS phosphorylation, which correlated with in vivo ACh-mediated vasodilator capacity (Hambrecht *et al.*, 2003). These data suggest that enhanced NO bioactivity, *in vivo*, with exercise training improves endothelial function by an up regulation of NO synthase protein expression and by increasing phosphorylation of this particular enzyme. On the other hand, in healthy subjects, an increase in endothelial function has been observed after endurance training (Clarkson *et al.*, 1999; Pullin *et al.*, 2004) but adaptations in the brachial artery were not evident following resistance exercise training (Rakobowchuk *et al.*, 2005; Potter *et al.*, 2008). Similarly, adaptations were not evident in older subjects following cycling exercise training (Moriguchi *et al.*, 2005; Thijssen *et al.*, 2007a). The lack of improvement in brachial artery endothelial function following resistance training and lower limb exercise could possibly be due to an inadequate shear stress

stimulus with resistance exercise or lower limb exercise to enhance eNOS expression and subsequent up-regulation of NO bioavailability.

The studies mentioned above suggest that exercise training induces vascular adaptations in most populations and that there is a strong relationship between adaptations in the exercising muscle bed and associated blood vessel. However, the mechanisms for such adaptations remains unclear, more specifically it is currently not known as to whether an increase in shear stress with lower limb exercise training is responsible for vascular adaptations in the non-exercising upper limbs. Nevertheless, it has been suggested that shear stress is the mechanism responsible for exercise training related adaptations of the vasculature (Tinken *et al.*, 2009; Tinken *et al.*, 2010; Naylor *et al.*, 2011). However, the majority of studies to date, investigating the concept of shear stress-induced adaptations in eNOS expression are based on investigations in animals (Johnson *et al.*, 2001; Woodman *et al.*, 2005), where arterial endothelial cells were shown to exhibit a significant increase in eNOS expression after 6 hours of exposure to shear stress (Davis *et al.*, 2001). Hambrecht *et al.*, (2003), confirmed the exercise training effects on increases in vascular function and eNOS expression in the human vascular system and suggested that the increases in eNOS expression were due to increased levels of shear stress, however, they further go on to state that the exact mechanism by which the endothelial cell sense changes in flow or blood pressure, is still unknown.

2.4.2. Exercise training and vascular remodelling of the conduit vessels

To date, studies examining remodelling adaptations in the conduit arteries report changes in resting arterial diameters as an index of remodelling, following exercise interventions. However, due to vasodilator and vasoconstrictor influences upon resting tone, it has been suggested that baseline diameter is not an optimal index of vascular remodelling following exercise training (Naylor *et al.*, 2005a). Instead, it has been proposed that the peak artery diameter is a more appropriate assessment of conduit structure (Naylor *et al.*, 2005a). Studies examining conduit artery remodelling have found adaptations after 6 weeks of one legged cycle exercise training, in the trained but not untrained femoral artery of healthy subjects (Miyachi *et al.*, 2001). Such adaptations are abolished following detraining, leading to the conclusion that such adaptations are localised rather than systemic. Tinken *et al.* (2008) conducted an 8-week training study and was the first to identify that vascular functional adaptations precede structural adaptations in humans. These authors concluded that, exercise training resulted in a rapid increase in endothelial function between weeks 2 and 4 which subsequently reached near baseline values by week 8. It was reported that the initial modifications in function were replaced by remodelling adaptations between weeks 4 and 8 in healthy humans. Whilst the possibility that late changes occur in the remodelling of upper limb conduit or resistance arteries in response to lower limb cycle exercise cannot be excluded, this seems unlikely as there is limited evidence for systemic effects of exercise on arterial remodelling in humans (Silber *et al.*, 1991; Green *et al.*, 2008a). In fact, comparisons of specific groups of upper or lower limb dominant athletes (Huonker *et al.*, 2003), including the preferred and non-preferred limbs of racquet sportsmen

(Green *et al.*, 1996; Rowley *et al.*, 2011), indicate that changes in artery size predominantly occur in response to local, rather than systemic, stimuli.

Rapid changes in vascular diameter primarily depend on the contractile activation and interaction of actin with myosin in vascular smooth muscle cells (Martinez-Lemus *et al.*, 2009). There are many factors that play a role in the control of vascular smooth muscle cell contraction, more specifically, this includes the calcium-calmodulin dependent phosphorylation of the regulatory myosin cross-bridge cycling, together with, changes in ion flux, membrane potential and intracellular calcium concentration (Hirano *et al.*, 2004). Additionally, rapid vasodilation or vasoconstriction are associated with acute increases or decreases in vascular smooth muscle intracellular calcium (Martinez-Lemus *et al.*, 2009). However, the exact mechanisms for such adaptations in response to systemic exercise remain unclear.

2.4.3. Are vascular adaptations to exercise training local or systemic?

Studies investigating the effects of localised exercise training have primarily found increases in the conduit artery in the exercising muscle bed. In an early study an enhanced endothelial function of the radial artery was reported after 4 weeks of localised hand-grip training and this improvement was NO mediated as indicated by its abolition using an NO blocker (Hornig *et al.*, 1996). Similarly, another study also using hand grip exercise, with the addition of L-arginine supplementation, demonstrated enhanced Ach mediated radial artery diameter change. Additionally, in thebrachial artery an enhanced endothelial function response was observed after

isometric hand grip exercise in patients with primary hypertension (McGowan *et al.*, 2006). Tinken *et al.* (2010) demonstrated improved endothelial function using local handgrip exercise training has been demonstrated to improve endothelial function, that was abolished in the contralateral limb (by cuff inflation on the forearm [60 mmHg] during handgrip), suggesting that shear stress is the principle mechanism for adaptation. In another recent study Wray and colleagues (2011) demonstrated that the relationship between shear stress and brachial artery vasodilation during handgrip exercise was abolished when L-NMMA was infused to block eNOS, data that provides further evidence the link between shear stress and of NO-dependent vasodilation. Taken together these data suggest that local exercise training cause adaptations to endothelial function of the conduit artery in the exercising muscle bed only. Conversely, studies examining whole body exercise e.g. running, walking and cycling illustrate varied results, with some finding increases (Kingwell *et al.*, 1997; Higashi *et al.*, 1999; DeSouza *et al.*, 2000; Goto *et al.*, 2003; Pullin *et al.*, 2004) decreases (Bergholm *et al.*, 1999) or no change (Maiorana *et al.*, 2001; Goto *et al.*, 2003; Moriguchi *et al.*, 2005) in upper limb endothelial function following lower limb exercise training. Additionally, lower limb exercise training studies performed in subjects with CVD usually report an increase in upper limb endothelial function (Maiorana *et al.*, 2001; Goto *et al.*, 2003; Moriguchi *et al.*, 2005; Wisloff *et al.*, 2007). Small muscle group (local) exercise training eliminates the impact of central hemodynamics on vascular adaptation. Conversely, exercise training involving larger muscle groups (whole body/systemic) consists of a local stimulus at the active muscle and a systemic hemodynamic influence due to increases in cardiac output and peripheral changes in sympathetic vasomotor tone supplementing the control

of blood flow (Thijssen *et al.*, 2010). It is apparent that localised and systemic exercise modes signify different influences on endothelial shear stress (Thijssen *et al.*, 2009a; Tinken *et al.*, 2010) and it is likely that the differences in modes of training for small or large muscle mass exercise reflect these differences.

Results of the studies mentioned above remain disparate. This could be due to the participants (asymptomatic) or the various forms of exercise interventions applied. On the other hand, studies examining the impact of exercise training in participants with CV disease or risk factors report beneficial effects of exercise training. A possible explanation for the disparity in the literature may relate to the exercise modality utilised, more specifically the exercise intensity implemented (Goto *et al.*, 2003; Green *et al.*, 2008b). With this in mind, Goto *et al.* (2003) suggests that low intensity exercise may fall below the threshold required for vascular adaptation in healthy participants, they further report increases in upper limb endothelial function in response to moderate intensity only, suggesting moderate-intensity aerobic exercise increases endothelium-dependent vasodilation in humans through the increased production of nitric oxide and that high-intensity exercise likely increases oxidative stress. Furthermore, exercise at higher intensity may be associated with elevated levels of oxidative stress and inflammatory responses after exercise (Bergholm *et al.*, 1999; Goto *et al.*, 2003)and this may diminish any underlying shear and hemodynamic-mediated beneficial adaptations (Laughlin *et al.*, 2008). However, to date the exact mechanisms associated with the large stimulus in the exercising muscle bed and upper limb adaptations following lower limb exercise are less well understood.

A number of studies have examined the impact of leg exercise training on forearm vasodilator capacity and reactive hyperaemic blood flow (RHBF) as an indicator of resistant vessel remodelling, in particular, Silber *et al.* (1991) reported a systemic adaptation in RHBF of the forearm vessels in non-exercising upper limbs of subjects following cycle ergometer training. They also suggested that this effect may be dependent upon the muscle mass involved in the training stimulus, as handgrip training studies demonstrate no change in the contra-lateral limb (Sinoway *et al.*, 1986; Green *et al.*, 1994). However, although this study reported increases in RHBF indicating upper limb resistant vessel remodelling, in healthy subjects, studies that typically examine conduit and resistance vessel remodelling usually report no adaptations in the conduit or resistance arteries of the inactive upper limbs (Dinenno *et al.*, 2001; Huonker *et al.*, 2003). It has been suggested that remodelling of vessels is associated with the vascular smooth muscle. A known factor to impact vascular smooth muscle tone, is the role of blood flow and shear stress for vascular remodelling (Langille & O'Donnell, 1986; Tuttle *et al.*, 2001; Amiri *et al.*, 2004; Bouvet *et al.*, 2007; Loufrani & Henrion, 2008). Blood flow and shear stress mediated release of vasoactive substances (i.e. NO) enable the endothelial cells to play a significant role in causing resistance vessel remodelling (Martinez-Lemus *et al.*, 2009). However, as to whether lower limb exercise can provide a stimulus for an increase in upper limb endothelial function, remains disparate.

2.4.4. Function vs. Remodelling

Studies examining the time-course of vascular adaptations to exercise training in humans have been few. However, there is evidence of time-course adaptation in animals, with 2-4 weeks of training in rats increased endothelial NO synthesis in skeletal muscle arterioles and vasodilator responses to Ach and L-arginine (Sun *et al.*, 1994). Similarly, in another study, the infusion of L-NMMA partly abolished an augmented dilator response in rats (Koller *et al.*, 1995). Additionally, in a study examining the effects of a 4 week exercise training protocol, on aortic tissue of rats, both enhanced vasodilation via Ach infusions, and increased eNOS protein levels were found (Delp *et al.*, 1993; Delp & Laughlin, 1997; de Groot *et al.*, 2005). In conduit vessels, improved endothelium-dependent vasodilation has been observed after as few as 7 days of endurance training in pigs (McAllister & Laughlin, 1997). These findings suggest that increased production of endothelial NO occurs rapidly in response to exercise training, particularly in arteries supplying the exercising muscle beds.

Studies in healthy subjects indicate that exercise-induced increases in brachial artery endothelial function can be seen after 4 weeks training (Pullin *et al.*, 2004) (Clarkson *et al.*, 1999). However, studies that have been performed over a longer duration have not shown consistent improvements in endothelial function. For example, after 16-20 weeks training endothelium-dependent vasodilation was unaltered in pigs (McAllister & Laughlin, 1997) or after 16 weeks of exercise training in rats (Kingwell *et al.*, 1997). Little data exists regarding the time-course of vascular functional and structural adaptation to exercise training in humans. However, as

mentioned previously, Tinken *et al.* (2008) recently assessed the brachial and popliteal artery function and structure in healthy young subjects and indicated that functional adaptations precede changes in artery peak vasodilator capacity (Tinken *et al.*, 2008) supporting the concept that functional adaptations may supersede structural changes and artery remodelling may normalise shear stress. They confirm previous reports in animals that endothelial function rapidly adapts to training and detraining (Pullin *et al.*, 2004; Haram *et al.*, 2006). Enhanced expression of eNOS protein and improved ACh-mediated vasodilation were evident after one week of training in pigs (Johnson *et al.*, 2001), whereas changes were not present after 16 weeks (Johnson & Laughlin, 2000). These data provide evidence that long-term training does not consistently report enhanced vascular function, since such adaptation is masked by remodelling of the vasculature.

To summarise so far, animal studies suggest that short-term exercise training enhances eNOS expression and therefore NO production and bioactivity, producing a short term buffer to the increased shear associated with exercise occurring within the first 4 weeks of exercise interventions. After extended training (4 weeks and beyond), the increased production of NO (and possibly other mediators) induces structural changes in the vessels resulting in an increase in lumen diameter (Brown, 2003). Shear stress may therefore be normalised by changes in the structure of the vessel resulting in endothelial NO activity returning towards baseline levels.

2.5. Mechanisms for vascular adaptations to exercise

As mentioned earlier in this chapter, endothelial dysfunction is characterised by reduced endothelium-dependent dilation and a decrease in pathways that produce

NO, increases in oxidative stress and inflammatory responses (Laughlin *et al.*, 2008). However, as suggested in the above section, exercise or physical activity seems to improve or maintain endothelial function. It is likely that the regulation of this occurs through adaptations in the regulation of eNOS (Hambrecht *et al.*, 2003), increased expression (Hambrecht *et al.*, 2003) and activation of Akt by the phosporylation of eNOS protein (Hambrecht *et al.*, 2003).

Referring back to shear stress as a potential stimulus for vascular adaptations, t has been suggested that the pulsatile nature of shear stress on the cells of the endothelium, are the primary stimulus for vascular adaptations in response to exercise training (Green *et al.*, 2004; Tinken *et al.*, 2009; Thijssen *et al.*, 2010; Tinken *et al.*, 2010). Evidence is increasing regarding shear stress resulting in increased eNOS expression and decreases in ET-1 and decreased VCAM-1 expression in endothelial cells (Laughlin *et al.*, 2008) (Figure 2.7). An early study reported that endothelial cells that were exposed to 24h of shear stress caused increases in eNOS expression (Harrison *et al.*, 1996), with increases observed after as little as 3h exposure to shear stress, this data providing direct evidence that shear stress promotes eNOS expression (Laughlin *et al.*, 2008).

With regards to upper limb adaptations following lower limb exercise, a possible explanation for systemic adaptations in the upper limbs could relate to the impact of lower limb exercise on blood flow and shear stress patterns in the inactive arterial beds. Although there have been arguments for (Green *et al.*, 2008a) and against (Thijssen & Hopman, 2008; Padilla *et al.*, 2011a) a systemic shear stress adaptations in non-exercising limbs, it is still suggested that shear stress is a major

physiological signal for improvements in endothelial function (Pohl *et al.*, 1986; Rubanyi *et al.*, 1986) and remodelling adaptations (Langille & O'Donnell, 1986; Tronc *et al.*, 1996; Tuttle *et al.*, 2001). It is also suggested that the effect, may in part be transduced by NO-mediated effects (Thijssen *et al.*, 2010). Additionally, in a recent study it was suggested that lower limb exercise such as cycling induces a change in the pattern of blood flow in the brachial artery of the inactive limb characterised by increases in antegrade flow during systole as cardiac output increases, as well as large increases in retrograde flow during diastole (Green *et al.*, 2002a), and that these altered patterns of flow may provoke divergent adaptations (Thijssen *et al.*, 2009a).

2.6. Physiological factors affecting blood flow and shear stress

As previously mentioned, one of the most important blood flow-induced mechanical stresses acting on the vessel wall is shear stress. However, there are other physiological factors, such as blood pressure, that influence blood flow and the interaction of these factors during exercise is essential.

The length and diameter of individual vessels, physical characteristics of blood viscosity, flow (laminar *vs.* turbulent) and mechanical forces (shear stress) acting upon the vasculature all impact blood flow through the vessel. More specifically, changes in vessel diameter are most important for regulating blood flow as well as regulating arterial pressure, this enables smaller arteries to adjust blood flow via changes in vessel diameter by releasing substances that either dilate or constrict the vessel (Moncada *et al.*, 1991).

The baroreflex is a homeostatic mechanism for maintaining blood pressure. It provides a negative feedback loop in which an elevated blood pressure inertly causes heart rate to decrease therefore causing blood pressure to decrease, subsequently decreased blood pressure activates the baroreflex, causing heart rate to increase therefore causing an increase in blood pressure. A decrease in mean arterial pressure is detected by the arterial baroreceptors and this initiates a decrease in parasympathetic activity and an increase in sympathetic activity. Furthermore, elevated blood pressure initiates vascular smooth muscle cell contraction, which constricts small resistance arteries to maintain a constant flow of blood in downstream capillaries (Hahn & Schwartz, 2009) resulting in a decrease in blood flow and therefore shear rate through the vessel.

At the onset of exercise, blood flow to the muscle is increased in proportion to the intensity of exercise, however, increased sympathetic nerve activity also increases resulting in constriction of the vessel and therefore reducing blood flow to active muscles in an attempt to maintain arterial blood pressure (Thomas & Segal, 2004). Following on from this, the activation of skeletal muscle fibres, during exercise, results in vasodilation and functional hyperaemia which subsequently causes an increase in the sympathetic activation to the muscles. Therefore causing the vasoconstriction of blood vessels, however this is overcome via the release of vasodilators by a process termed 'functional sympatholysis'. It is suggested that the vasodilating substances associated with contraction promote a 'functional sympatholysis' that limits the ability of the sympathetic nerves to cause vasoconstriction in the active muscles (Remensnyder *et al.*, 1962). Furthermore,

sympathetic nerve activity is essential for vasoconstriction and the maintenance of arterial blood pressure (Thomas & Segal, 2004). However, elevations in sympathetic vascular tone and peripheral vascular tone have been associated with acute impairments in brachial artery endothelial function (Dyson *et al.*, 2006).It is clear that blood flow and shear stress increase with exercise, and that an increase in blood flow and therefore shear stress is beneficial for the endothelium (Furchgott & Zawadzki, 1980; Furchgott & Vanhoutte, 1989), which suggests that the likely mechanism for exercise induced improvements in endothelial function and structure occur due to an up-regulation of NO (Hambrecht *et al.*, 2003). Although, it has previously been reported that lower limb exercise can cause increases in blood flow and shear stress of the brachial artery (Thijssen *et al.*, 2009a), whether lower limb exercise and exercise training can provide a systemic increase in blood flow and shear stress to cause endothelial adaptations in the upper limbs is less well understood.

2.7. Optimal exercise training programs

The disparity in the literature regarding the impact of exercise training amongst different subject populations, could be explained by the different modalities of exercise employed, as it has been suggested that different types, intensities and durations of exercise exert distinctly different physiological and health benefits. For example, recent studies indicate that exercise involving different volumes of muscle mass or exercise intensity may be associated with different shear stress mediated impacts on vascular function (Goto *et al.*, 2003; Green *et al.*, 2004). This could be due to increases in pulse pressure and heart rate generated through repeated bouts

of shear stress during aerobic exercise involving large muscle groups (Green *et al.*, 2004), whereas small muscle group exercise is not associated with such high hemodynamic stimuli. Recent studies also suggest differences in endothelial adaptations to exercise of differing modalities (High Intensity Training) in heart failure and healthy subjects (Wisloff *et al.*, 2007; Tyldum *et al.*, 2009).

Several studies raise the possibility that different modalities or intensities of exercise may impact upon the magnitude of vascular adaptation observed. Bergholm *et al.* (1999) reported that 3 months of high intensity running reduced endothelium-dependent function but not endothelium-independent function (Bergholm *et al.*, 1999). Endothelium-dependent forearm vasodilation improved in a group of healthy subjects at moderate intensity (50% VO_{2max}) (Goto *et al.*, 2003), and in the absence of shear stress. In the same study, when subjects exercised at a higher intensity (70% $VO_{2max,}$) endothelial function did not improve, but in this instance there was evidence for increased oxidative stress. Generally data suggests that exercise below 25% VO_{2max} is insufficient to promote an improvement in endothelial function, whilst moderate intensity exercise enhances NO bioavailability and any improvement in vascular function resulting from high-intensity exercise may be surpassed by excess oxidative stress (Goto *et al.*, 2003), although Wisloff has reported changes in HIT (Wisloff *et al.*, 2007). However, further research needs to be undertaken to further explain the impact of oxidative stress following acute or prolonged exercise upon endothelial function.

2.8. Summary: Exercise training, vascular modifications and CV risk

In summary, exercise or physical fitness is associated with approximately 35% benefit in terms of decreasing the risk of cardiac events. As described above, exercise training improves vascular function in the absence of changes in lipid levels (Lewis *et al.*, 1999), blood pressure (Higashi *et al.*, 1999), glucose tolerance, or BMI (Bell *et al.*, 2007). Significant improvements in conduit endothelial function have been reported, in the absence of changes in plasma lipids, blood pressure, blood glucose, BMI, with no significant correlations between changes in any of these risk factors and the improvements in vascular function (Green *et al.*, 2004). These studies indicate that improvement in risk factors is not an obligatory requirement for improvement in vascular function as a result of exercise training. This raises the possibility that direct effects of exercise on the vasculature, due to repetitive impacts of shear stress, pulse pressure or pulsatility, may therefore contribute to the reduction in CV events associated with exercise training (Green *et al.*, 2008b).

Table 2.1 Summary of exercise training studies of conduit artery function in asymptomatic subjects and patients with CAD undertaking local or systemic exercis training.

Asymptomatic Subjects:				
LOCAL Conduits				
Tinken et al. Hypertension 2010: 55: 312-318	Healthy young males	Handgrip exercise 8 weeks 4 sessions per week - 30-minute of simultaneous handgrip exercise (30-contractions per min) at 30% MVC for 4 weeks, 40% MVC for 2 weeks, and the final 2 weeks at 50% MVC	FMD increased in the brachial artery at weeks 2 and 4	↑
SYSTEMIC Conduits				
Clarkson et al. J Am Coll Cardiol 1999;33:1379.	Healthy army recruits- Brachial D/U	Running 10 weeks. Daily 3 mile run + strength sessions. High volume exercise.	FMD increased from 2.2 to 3.9 %. No change GTN	↑
Thijssen et al, Act Physiol 2007;190:221-228.	FMD & GTN; BA and SFA (5 min occlusion) IMT; CA & SFA	Cycling. 20 min, 3x wk for 8 wks @ 65-85% HR_{max}.	Training increased peak leg BF, arterial compliance and baseline diameter and BF in the SFA. No change in FMD or IMT of SFA. All measures unchanged in BA and CA. **Training effect locally (SFA) but not systemically (BA). No change in FMD.*	↔
Rakobowchuk et al. J Appl Physiol 2005;98:2185-90.	Brachial FMD (4.5 min occlusion)	Resistance training 12 wks, 5 x wk, reaching 90%+ 1RM by wk 12	Increased mean brachial artery diameter at peak and 10 sec post occlusion BF at 6 and 13 wks. No change in FMD. **Localised training effect may also be present with UL weights*	↔

Pullin et al. J Ex Physiol 2004;7:14-22.	Brachial FMD (5 min occlusion) and GTN	Cycling. 30 min, 3 x wk @ 70-80% HR_{max} for 4 weeks followed by 2 weeks detraining.	FMD increased post training but returned to baseline after 2 weeks detraining. No change in response to GTN	↑
Moriguchi et al. Hypertens Res 2005; 28:315-21.	Brachial FMD (4.5min occlusion)	Cycling 60 min, twice wk @ 50% VO_{2max})	No FMD in healthy. No change to GTN.	↔ controls
Sugawara et al. Am J Physiol Heart Circ Physiol 2007; 293:H1466-72.	Femoral BF (US) in response to phentolamine (α-adrenergic blockade) and LNMMA	Aerobic training 30-45min, 4-5 x wk for 12 wks @ 65-75% HR_{max}.	No change in basal limb BF post training. Training increased femoral BF in response to phentolamine (47%). The increase was partially abolished (18%) by LNMMA	 ↑
CAD:				
LOCAL Conduits				
Gokce et al. J Am Coll Cardiol 2002;90:124-7.	Brachial and tibial FMD	Leg Exercise (Cardiac rehab)40 min, 3x weekly 10 weeks	Increased tibial artery response No changes in GTN responses.	↑
SYSTEMIC Conduits				
Gokce et al. J Am Coll Cardiol 2002;90:124-7.	Brachial and tibial D/U	Leg Exercise (Cardiac rehab) 40 min, 3x weekly 10 weeks	No significant change in the brachial artery response (increased, but NSD). No changes in GTN responses.	↔
Walsh et al. J Appl Physiol 2003;95:20-5.	Brachial D/U	Circuit training. 8 weeks 3x wk	FMD improved. No change in GTN	↑

Edwards et al Am J Cardiol 2004;93:617-20.	Brachial FMD	Cycling 15 to 20 min (40-50% HRR) progressing to 40 to 50 min (70-85% HR), 3x wk for 12 wks	FMD improved in trained group	↑
Paul et al. J Cardiopulm Rehabil 2007;27:65-73.	Brachial FMD	ET with various upper and lower body aerobic exercise modalities 3 x wk for 12 wks	NSD brachial diameter, FMD or GTN	↔

Conduits, conduit vessels; D/U, Doppler ultrasound arterial assessment; LNMMA, NG monomethyl-L-arginine; FMD, flow-mediated vasodilation, an endothelium and NO-dependent vasodilator stimulus; GTN, glyceryl trinitrate, an endothelium-independent vasodilator; CAD, coronary artery disease; LOCAL, localised exercise; SFA, superficial femoral artery; CA, carotid artery; IMT, intima-media thickness; RM, rep max; NSD, no significant difference; ET,exercise training; UL, upper limb; MVC, maximal voluntary contraction; BF, blood flow; SYSTEMIC, whole body exercise; ↑, improvement in NO vasodilator function; ↓, deterioration in NO vasodilator function; ↔, no change in NO vasodilator function.

2.9. Effect of inactivity on the vasculature

As discussed in the previous section, the effects of exercise training on endothelial function are not well established with functional adaptations preceding structure, in response to both local and systemic stimuli. However, as mentioned earlier in this chapter, previous data have shown that it is likely that functional adaptations precede structural adaptations, in response to local handgrip exercise training (Figure 2.8B), however, this is not fully identified in vivo in humans using systemic (whole body) exercise. On the other hand, the effects of inactivity and endothelial function are less well understood. It has been hypothesised that vascular adaptations follow a similar pattern to that of exercise but in an opposite manner (Figure 2.8A), however, literature in this area provide inconsistent results. Additionally, the figure below represents hypothesised vascular adaptations to periods of inactivity in relation to the adaptations observed following exercise training in conduit vessels. It is unknown as to whether the figure below is applicable to all exercise training modalities and it is currently uncertain if it would indicate the same pattern of adaptation in resistance vessels following systemic exercise training. To date the exercise related adaptations have only been shown in conduit vessels and in some studies in animal models (Laughlin *et al.*, 1989; Sun *et al.*, 1994; McAllister *et al.*, 1996; McAllister & Laughlin, 1997; Johnson & Laughlin, 2000), and the mechanisms for these adaptations have not been fully investigated.

Figure 2.8 Hypothesised changes in conduit artery function and structure (remodelling) in response to (A) inactivity and (B) exercise training in humans (Thijssen *et al.*, 2010).

This section of the chapter will discuss the disparity amongst the area of inactivity and endothelial function, including possible explanations for the conflicting results, such as the time-course of inactivity, and the various methods employed to inflict localised or whole-body inactivity. To date, the methods used to examine inactivity are varied, ranging from space travel, bed rest, unilateral lower limb immobilisation (ULLS), immobilisation via casting and assessment of subjects with spinal cord injury. However, there have been many limitations identified with these approaches. For example, space flight and bed rest cause changes in plasma volume (Convertino *et al.*, 1986) whilst it is thought that the bed rest models do not completely restrict upper body movement (Bleeker *et al.*, 2005b). Bleeker *et al.* (2004) suggested ULLS is only suitable to study localised deconditioning in one lower limb and state there is an increased risk of deep venous thrombosis. Finally, casting of a limb usually follows a fracture and vascular changes to this model of inactivity may therefore be influenced by the impact of healing and inflammatory processes (Green *et al.*, 1997).

2.9.1. Inactivity and endothelial function

Acute and chronic functional adaptations to inactivity illustrate conflicting results within the literature. For instance, after 5 days of bed rest, no change in brachial artery FMD was observed (Hamburg *et al.*, 2007), however, in another study an increase in brachial artery FMD was reported following 7 days of bed rest (Bonnin *et al.*, 2001). These differences could have occurred due to the upper limbs not been fully restricted during the bed rest studies resulting in the upper limbs remaining active with shear stress levels increased. Furthermore, another potential explanation for the disparity in the results in this area could be due to the different methods used to examine endothelial function, such as the different approaches to the detection of peak arterial diameter (Hamburg *et al.*, 2007). More specifically, it is suggested that specific guidelines should be adhered to in an attempt to standardise the criterion for reliable and valid FMD measurements (Corretti *et al.*, 2002; Harris *et al.*, 2010; Thijssen *et al.*, 2011a). Additionally, it has recently been highlighted that there are many physiological and technical issues that could impact the validity, reproducibility, and interpretation of the assessment of endothelial function (Hijmering *et al.*, 2002; Betik *et al.*, 2004; Pyke *et al.*, 2004; Pyke & Tschakovsky, 2005; Harris & Padilla, 2007; Pyke & Tschakovsky, 2007; Black *et al.*, 2008; Padilla *et al.*, 2008; Thijssen *et al.*, 2008d; Atkinson *et al.*, 2009; Parker *et al.*, 2009; Kizhakekuttu *et al.*, 2010).

In studies examining functional adaptations in response to chronic inactivity periods, results also remain disparate. In patients with traditional cardiovascular risk factors an impaired endothelium-dependent NO-mediated function, following assessment of endothelial function, has usually been demonstrated. In addition, endothelial function of the femoral artery following bed rest (25 and 52 days) (Bleeker *et al.*, 2005b), ULLS (28days) (Bleeker *et al.*, 2005a) and in patients with acute (de Groot *et al.*, 2006b) and chronic (de Groot *et al.*, 2004; de Groot *et al.*, 2005) SCI demonstrates enhanced endothelial function. However, when endothelial function of the superficial femoral artery in SCI subjects is normalised for prevailing shear the endothelial functional response was, in fact, found to remain preserved (de Groot *et al.*, 2004) or only a small increase observed compared with able-bodied controls (de Groot *et al.*, 2004), a finding that again reinforces the association between shear and the NO dilator system. Alternatively, the preserved endothelial function response in deconditioned vessels could be due to an up-regulation of smooth muscle cell sensitivity to NO, initiated by a response to decreases in shear stress and a resultant decrease in eNOS regulation in deconditioned vessels. For example, 28 days of limb suspension (Bleeker *et al.*, 2005a) and after 52 days of bed rest (Bleeker *et al.*, 2005b) resulted in an increased NO smooth muscle sensitivity in response to NO donors.

2.9.2. Inactivity and conduit and resistance vessel remodelling

In a study comparing the femoral artery of chronic SCI individuals and able-bodied subjects, it was demonstrated that baseline shear rate levels almost doubled in the femoral artery of chronic SCI individuals when compared with able-bodied subjects

(de Groot *et al.*, 2004). Additionally, Thijssen *et al.* (2008b) demonstrated that conduit artery diameter decreases markedly in response to physical inactivity, which coincides with a significant increase in conduit artery shear rate. However, Olive *et al.* (2004) examined chronic SCI individuals and able-bodied controls and reported that the significantly smaller femoral vessel diameter in SCI that was no longer evident when diameter was expressed per unit muscle mass. In addition, de Groot *et al.* (2006a) also found that when changes in vascular characteristics and limb volume during the first 6 weeks after a spinal cord injury were examined, femoral artery size decreases substantially, with a concurrent decrease in limb volume was also evident. However, when femoral artery diameter was corrected for limb volume no differences were evident over the 6 week time period and values in SCI were comparable with non SCI individuals (de Groot *et al.*, 2006a). These findings suggest vascular structural adaptations are associated with a decrease in muscle atrophy as a consequence of inactivity.

However, it should be noted that baseline diameter reflects functional influences on vascular tone, as well as artery size, and therefore the use of baseline diameter as an index of structure is potentially corrupted by factors that regulate vascular function (Thijssen *et al.*, 2010). In a study by Naylor *et al.* (Naylor *et al.*, 2005b) the importance of measuring maximal conduit artery diameter was emphasised as a marker of structural changes. Near maximal diameter can usually be demonstrated through the sub-lingual administration of glyceryl trinitrate (GTN) and can therefore reflect the limits of arterial structure and size (Naylor *et al.*, 2005b). In accordance with this, there have been studies indicating that inactivity is associated with

arterial structural remodelling and decreased conduit artery dimensions. These include, a 9% reduction of maximal femoral artery diameter after 4 weeks of limb suspension (Bleeker *et al.*, 2005a), a decrease of 16% after 25 and 52 days of bed rest (Bleeker *et al.*, 2005b) and by 35% in chronic SCI individuals (de Groot *et al.*, 2004).

Recent studies examining the acute and chronic effects of vascular deconditioning suggest that endothelium-dependent and -independent vasodilation is preserved in peripheral conduit arteries (Bleeker *et al.*, 2005a; Bleeker *et al.*, 2005b). A possible explanation for the preserved conduit artery endothelial function after physical inactivity may relate to changes in conduit artery dimensions (Bleeker *et al.*, 2005a; Bleeker *et al.*, 2005b), through changes in shear rate. As a result of the changes in shear rate, deconditioning induces an inward remodelling of conduit arteries that preserves or normalises the vascular function (Bleeker *et al.*, 2005a; Bleeker *et al.*, 2005b).

Peak reactive hyperaemia can be used as an assessment of peak blood flow and as a marker for forearm resistance vessel remodelling in humans (Folkow *et al.*, 1958; Conway, 1963; Sinoway *et al.*, 1986; Silber & Sinoway, 1990; Silber *et al.*, 1991). This method uses an assessment of the reactive hyperaemic blood flow response to 3 minutes of ischaemic handgrip exercise after 5 minutes of ischaemia (see Section 3.3 General Methods, Chapter 3) and it has been suggested that reactive hyperemia in human forearms is caused largely by mechanisms other than NO (Tagawa *et al.*, 1994). Furthermore, it has been suggested that the vascular smooth muscle plays a role in remodelling of vessels and there are many factors that are known to impact

vascular smooth muscle tone. In particular there is evidence for the important role of blood flow and shear stress for vascular remodelling (Langille & O'Donnell, 1986; Tuttle *et al.*, 2001; Amiri *et al.*, 2004; Bouvet *et al.*, 2007; Loufrani & Henrion, 2008). Blood flow and shear stress mediated release of vasoactive substances (i.e. NO) enable the endothelial cells to play a significant role in causing resistance vessel remodelling (Martinez-Lemus *et al.*, 2009).

Studies investigating the effect of inactivity on vascular adaptations usually report a decrease in peak reactive hyperaemia blood flow, which is suggestive of forearm vessel remodelling (Takeshita & Mark, 1980; Sinoway *et al.*, 1986). For instance, Bleeker *et al.* (2005b) report a 28% decrease in peak reactive hyperaemia after 52 days of bed rest. Similarly, after 14 days of bed rest a 31% decrease in peak reactive hyperaemia was observed in the forearm (Shoemaker *et al.*, 1998). In the 1990's, Silber and Sinoway conducted a study examining reactive hyperaemia after 29 days of cast immobilisation of the wrist. As with the other studies mentioned above reactive hyperaemia decreased in the casted arm but did not change in the control arm (Silber & Sinoway, 1990), further supporting the notion that reactive hyperaemia is decreased in response to deconditioning. It has been reported that reactive hyperaemia is 40–60% lower in the legs of SCI compared with controls (de Groot *et al.*, 2004). Taken together, these data provide evidence for rapid remodelling adaptations by reductions in peak reactive hyperaemia after periods of inactivity/deconditioning (Thijssen *et al.*, 2010).

2.9.3. Are vascular adaptations to inactivity local or systemic?

In studies examining whole body inactivity using experimental models such as space flight and bed rest, it has been demonstrated that vascular adaptations are evident throughout the body (Bonnin *et al.*, 2001; Bleeker *et al.*, 2005b). However, inactivity is also associated with changes in systemic cardiovascular parameters (Hamburg *et al.*, 2007). On the other hand, localised inactivity employed by ULLS and cast immobilisation associated with vascular adaptations are only specific to the region of inactivity (Sugawara *et al.*, 2004; Bleeker *et al.*, 2005a; de Groot *et al.*, 2005), do not provoke systemic changes. In studies examining SCI individuals, it has been demonstrated that in the inactive paralysed legs, vascular adaptations are still observed (de Groot *et al.*, 2006b; Sabatier *et al.*, 2006), while upper extremity vascular function and structure are similar to able-bodied controls (Hopman *et al.*, 2002; de Groot *et al.*, 2004; de Groot *et al.*, 2005). These data suggest that vascular adaptations are a result of localised physical inactivity only.

2.9.4. Function vs. Remodelling

Prolonged inactivity is associated with a dose-dependent structural remodelling of arterial diameter (reviews (de Groot *et al.*, 2006a; Thijssen *et al.*, 2010; Thijssen *et al.*, 2011b)). Adaptations in peak blood flow, a marker for resistance vessel remodelling have been observed in a number of different studies, providing further evidence for remodelling adaptations that precede functional adaptations in response to inactivity (Folkow *et al.*, 1958; Conway, 1963; Sinoway *et al.*, 1986; Silber & Sinoway, 1990; Silber *et al.*, 1991). However, the NO-mediated endothelial function shows no change or even an increase after prolonged inactivity (Bonnin *et*

al., 2001; de Groot *et al.*, 2004; Bleeker *et al.*, 2005a; Bleeker *et al.*, 2005b). Referring back to Figure 2.7, it still remains unclear as to whether functional adaptations in response to periods of inactivity precede vascular remodelling, as data in the literature are disparate. In the study mentioned earlier by de Groot et al. (de Groot *et al.*, 2006b), conduit artery characteristics and limb volume were examined in SCI patients and findings reported a rapid decrease in femoral artery diameter which approached vessel size of chronic SCI subjects after only 3 weeks post injury. In another study, rapid adaptations to inactivity were also established in conduit artery size (Sugawara *et al.*, 2004; Bleeker *et al.*, 2005a; Bleeker *et al.*, 2005b). Taken together these data indicate that physical inactivity provides a strong stimulus for rapid structural remodelling of human conduit arteries, which is suggestive that remodelling adaptations precede function.

2.10. Mechanisms for vascular adaptations to inactivity

The benefits of exercise training on the NO pathway are well established (Green *et al.*, 2004; Green *et al.*, 2011), however, the mechanisms for deconditioning effects upon the vasculature are less well known. Studies examining the effects of inactivity on the NO-pathway in conduit and resistance vessels found no alterations to the NO-pathway (Thijssen *et al.*, 2010). There is a possibility that functional vascular adaptations during physical inactivity may be driven through a pathway, other than the NO-pathway. For example, the powerful vasoconstrictors ET-1 and angiotensin-II are demonstrated to contribute to the increased vascular tone after chronic physical inactivity (Thijssen *et al.*, 2007b; Groothuis *et al.*, 2010). Moreover, ET-1

and angiotensin-II are demonstrated to have pro-atherogenic effects (Laughlin *et al.*, 2008).

Changes in blood flow patterns, in particular, increases in retrograde flow and shear rate, have been shown to produce opposing effects to endothelial gene expression (Guo *et al.*, 2007). For example, it is reported that oscillatory shear rate and increases in retrograde shear rate result in increased expression of ET-1 (Ziegler *et al.*, 1998) and an increase in adhesion of molecules, such as VCAM-1 (Chappell *et al.*, 1998; Himburg *et al.*, 2007), consequently resulting in decreased eNOS expression. Taken together, by assessing and manipulating changes in blood flow and shear rate during localised inactivity, this could go some way to explaining the deconditioning effects of localised inactivity on the vasculature.

2.11. Summary: Inactivity, vascular modifications and cardiovascular risk

To summarise, the impact of physical inactivity on traditional risk factors may not adequately explain the increase in cardiovascular mortality associated with deconditioning, as mentioned earlier in this chapter. Therefore, it is possible that inactivity may demonstrate a direct effect upon the vasculature, which could lead to partially explaining cardiovascular risk associated with inactive or sedentary lifestyles. Despite the conflicting data mentioned above, including the various models of inactivity that have been adopted, it remains likely that changes in conduit and resistance vessel structure and function may help to explain the increased cardiovascular mortality and morbidity associated with deconditioning. This interesting hypothesis will be examined, in this thesis (Chapters 7 and 8).

2.12. Summary and Hypotheses

In summary, physical inactivity is an important and independent risk factor for atherosclerosis and cardiovascular diseases. However, little is known about the direct impact of activity and inactivity on vascular function. Exercise training is a potent stimulus to improvement in vascular function and is consequently associated with decreased cardiovascular risk. Animal studies indicate that episodic increases in shear stress are an important stimulus for vascular adaptation. Furthermore, exercise in humans are not only associated with changes in arterial shear stress, but also with large changes in blood pressure which is another potential haemodynamic stimulus for vascular adaptations.

Below are the aims and hypotheses for each study that will make up the chapters in this thesis:

Study 1. The purpose of study 1 is to determine exercise intensity dependent and independent (heating of one forearm using a heated water-bath) increases in shear rate at different exercise intensities on brachial artery diameter before, during and immediately post exercise. This objective will be achieved through study 1 (Chapter 4). *Hypothesis.* Brachial artery dilation is dependent upon the magnitude of shear stress manipulation during cycle exercise.

Study 2. To determine the effects of shear rate during different exercise intensities on brachial artery flow-mediated dilation (FMD) responses before and immediately post cycle exercise. This objective will be achieved through study 2 (Chapter 5).

Hypothesis. The magnitude of decrease in FMD is dependent on the intensity of cycle exercise and shear-stress.

Study 3. To examine the role of shear rate upon endothelium dependent (largely and partly) NO mediated and endothelium independent vasodilation of the brachial artery, following 8-weeks lower limb exercise training, with shear rate attenuated in one forearm during each exercise training session. This objective will be achieved through study 3 (Chapter 6). *Hypothesis.* Cycle exercise training leads to improvements in endothelial function in the brachial artery of the non-exercising upper limb and that such adaptation would be shear stress mediated.

Study 4. Bilateral assessments of the brachial artery will be examined following an acute period of localised inactivity in one forearm. This objective will be achieved through study 4 (Chapter 7). *Hypothesis.* Forearm inactivity leads to an acute impairment of endothelial function, without the presence of a change in vascular structure.

Study 5. Acute and prolonged changes in retrograde shear rate, and the role of retrograde shear rate on endothelium dependent (largely and partly) NO-mediated and independent vasodilation of the brachial artery will be explored following the acute and prolonged use of compression around one forearm. This objective will be achieved through study 5 (Chapter 8). *Hypothesis.* Retrograde shear rate, induced by the compression sleeve, leads to an acute impairment of endothelial function, which is persistent when the compression sleeve is worn for 8 days.

Chapter 3 | *General Methods*

The measurements and protocols undertaken are applied across the majority of studies in this thesis. Therefore, the general methods chapter describes information regarding participants, techniques of measurements and the measures used to assess vascular function. The specific experimental designs and protocols employed for each study can be found within the respective method section in each chapter.

3.1. Inclusion criteria

All participants were provided with relevant information regarding each study, both verbally and through the use of a participant information sheet. Before participating in any experimental measures, all participants were required to sign an informed consent. All participants included in this study were healthy young men; none reported having been diagnosed with cardiovascular diseases, diabetes, insulin resistance or cardiovascular risk factors such as hypercholesterolemia or hypertension. Subjects who smoked or were on medication of any type were excluded. All subjects were recreationally active, typically engaging in low (e.g. walking) and moderate intensity (e.g. jogging) aerobic activities (2-3days/wk); none were elite/competitive athletes. All participants were instructed to refrain from any exercise and the consumption of alcohol for 24h, caffeine for 12h and food overnight prior to morning testing sessions or 4-6h prior to daytime sessions. All study procedures were approved by the Liverpool John Moores Ethics Committee and adhered to the Declaration of Helsinki.

3.2. Experimental Procedures

All assessments were performed in a dedicated and temperature controlled (21-23°C) laboratory, which allowed testing to be undertaken in a relaxed and isolated environment to reduce any stress to the participant.

3.2.1. Heart rate and blood pressure

Initially all participants were asked to rest in a supine position for a period of at least 20-minutes to facilitate baseline assessment of heart rate and blood flow. Heart rate and mean arterial pressure were determined by an automated sphygmomanometer (Dinamap; GE Pro 300V2, Tampa FL).

3.3. Assessment and relevance of arterial function and structure in humans

Arterial function can be measured invasively or non-invasively. Generally it has been observed that individuals who are less fit, older, or have chronic diseases, have lower vascular reactivity than fitter, younger and healthier individuals (Green *et al.*, 1994).

Venous occlusion plethysmography was one of the first methods used to study human vascular physiology *in vivo*. The technique is typically combined with intra-arterial drug administration, usually into the forearm vascular bed to assess changes in forearm blood flow. This was an ideal method to assess the local effect of vasoactive drugs on peripheral resistance vessels without invoking systemic effects. These vasoactive substances include those highlighted in a study by Vallance (1989) which utilised intrabrachial infusion of N^G-monomemethyl-L-arginine (L-NMMA), which is a nitric oxide blocker, to examine basal NO bioactivity in resting muscles

and the contribution of NO to exercise. Similarly, dose-response curves to intra-arterial infusion of endothelial-dependent NO vasodilators such as ACh, which is endothelial-dependent and NO mediated (Newby *et al.*, 1997) have been used to assay changes in the stimulated release of NO induced by exercise training. Typically, ACh leads to a measurable vasodilation in healthy subjects but vasoconstriction in states of endothelial dysfunction or denudation (Raitakari & Celermajer, 2000). Sodium nitroprusside (SNP), a nitric oxide donor, infusion is commonly undertaken to assess endothelium-independent NO vasodilator function. Evaluating the responses to endothelium-dependent and -independent NO vasodilators and NO blockade provides information about the contribution of blood flow control of different components of the NO-dilator system (Benjamin *et al.*, 1995).

Plethysmography has been demonstrated to be a reliable assessment of relative changes induced by pharmacological agents to a resting muscle bed (Joyner *et al.*, 2001). However, this method does entail several limitations including poor temporal resolution and high sensitivity to movement, which could affect measurements of blood flow during and following exercise bouts (Green *et al.*, 2004). Nevertheless, current information in the literature surrounding exercise hyperemia, and the mechanisms responsible for this, have been derived from studies utilising plethysmography (Joyner *et al.*, 2001).

3.3.1. Terason T3000 (Burlington, MA) high-resolution duplex ultrasound machine

The most commonly used non-invasive technique utilises high-resolution duplex ultrasound. Ultrasound can be used to evaluate artery diameter and blood flow to determine functional and structural changes of single conduit arteries. Ultrasound assessment provides absolute blood flow down a single artery, improves the temporal resolution of measurement during exercise (Green *et al.*, 2002c) and it is suggested that this method is less likely to be affected by movement artefacts when compared with the method of plethysmography (Green *et al.*, 2004).

Across all the studies in this thesis two Terason T3000 machines were employed to simultaneously assess artery diameter and blood flow. A 12-MHz multi-frequency linear array probe was attached to each of the high resolution ultrasound machines (T3000; Terason, Burlington, MA). When an optimal image was obtained, the probe was held stable throughout the entire assessment. The ultrasound parameters were set to optimise the longitudinal, B-mode images of the lumen-arterial wall interface. Continuous Doppler velocity was acquired using the lowest possible isonation angle, this was always <60°. Images were captured using Camtasia screen recording software and were saved as .avi files which were then transferred to external hard drives.

3.3.2. Assessment of brachial artery flow mediated vasodilator (FMD) responses to ischaemia

This technique emerged from early work by Anderson and Mark (1989) who reported a brachial arterial vasodilation in response to hyperaemia following forearm cuff occlusion. This was later followed by work by Celemajer and colleagues

(1992) who introduced the assessment of flow-mediated dilation (FMD). This technique is now the most common non-invasive method used to determine conduit artery endothelial function. To briefly describe the technique, a region of tissue distal to the artery being imaged is made ischaemic through cuff inflation. When the cuff is released the reactive hyperaemia results in an increase in blood flow and shear rate down the targeted artery. In response to the increased shear stress, the artery releases vasoactive substances and dilates. When measuring FMD with ultrasound, the image quality of both artery diameter and Doppler velocity is essential. Additionally, the velocity trace is modulated by the isonation angle (Logason *et al.*, 2001), therefore it is important to ensure this is always set to less than 60°. Furthermore, the calculation of FMD using reliable analysis software is equally relevant. It has been suggested that it is important to utilise an appropriate means of analysis that uses edge-detection and wall tracking, as this markedly improves the sensitivity of the technique and is less observer dependent (Woodman *et al.*, 2001; Green *et al.*, 2002c).

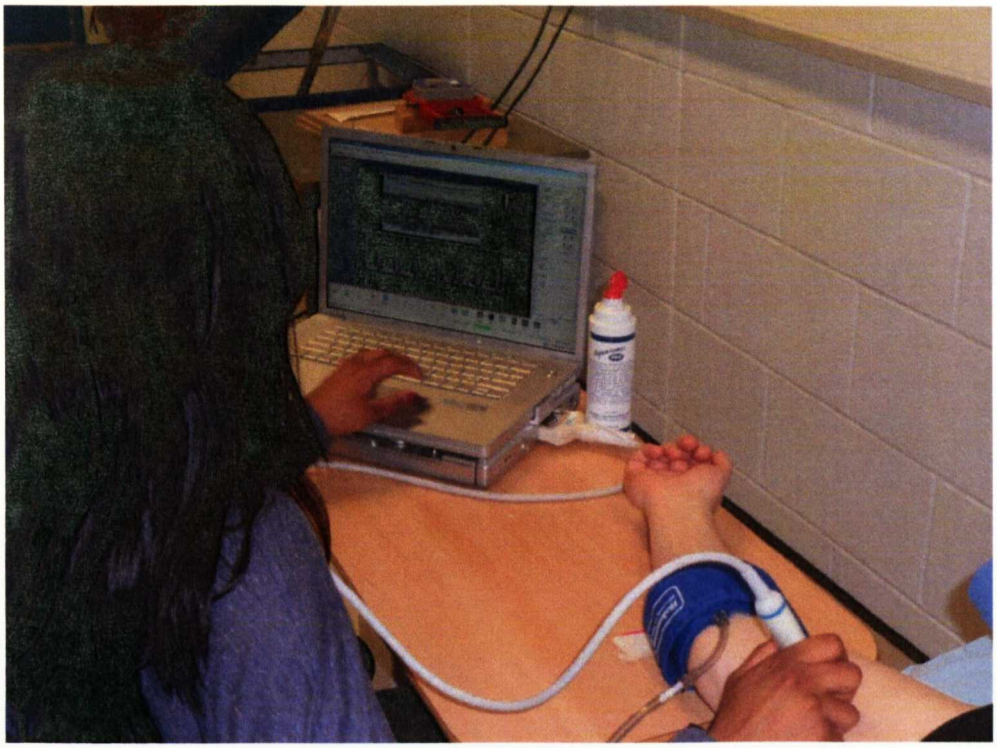

Figure 3.1 Terason T3000 (Burlington, MA) high-resolution duplex ultrasound machine used to assess vascular function during all studies.

For the FMD to be performed correctly the occluding cuff has to be placed distal to the scanned artery. This was highlighted by Doshi *et al.* (2001) (Figure 3.2) who demonstrated that dilation following upper arm occlusion is greater than that observed after wrist occlusion, despite a similar peak flow stimulus. L-NMMA infusion revealed that FMD following wrist occlusion is mediated largely by NO, while dilation following upper arm occlusion is only partially mediated by NO (Doshi *et al.*, 2001).

Figure 3.2 FMD following wrist and upper arm cuff occlusion during control and L-NMMA infusion (Doshi *et al.*, 2001).

The duration of cuff inflation and the impact of this on NO bioactivity is also essential when assessing endothelium-dependent function, as it was revealed that 5 minute periods of ischaemia resulted in a vasodilation that was abolished by the infusion of L-NMMA. However, this was not the case following 15 minutes of ischaemia, suggesting sustained cuff occlusion elicits a response largely dependent on other vasodilator factors, not only NO (Figure 3.3) (Mullen *et al.*, 2001).

Figure 3.3 Fifteen minutes of hand ischemia resulted in a more prolonged episode of reactive hyperaemia (A) and a significantly increased FMD of the radial artery (B) compared with 5 minutes of ischaemia (Mullen *et al.*, 2001).

Therefore, for the technique to be a valid index of conduit artery endothelium-dependent NO function the cuff must be placed distal to the imaged region with a period of 5 minutes occlusion (Doshi *et al.*, 2001; Mullen *et al.*, 2001; Kooijman *et al.*, 2008).

Data in the literature have highlighted the importance of determining the shear rate as the most important stimulus for FMD. Pyke *et al.* (2004) undertook a series of experiments and determined that the shear rate area under the curve (SR_{AUC}), that is the shear rate stimulus from the point that the cuff is released until the maximal dilation, was important for determining the FMD and subsequently it was suggested that the FMD should be normalised for the eliciting SR_{AUC} stimulus (Pyke & Tschakovsky, 2007). However, more recently, it was recommended that normalisation of the data is not required as the relationship between SR_{AUC} and

FMD is often non-linear or varied between samples (Atkinson *et al.*, 2009). As such, the SR_{AUC} is reported in this thesis but the FMD is not normalised for it.

In summary, the above information reinforces the importance of following specific protocols to assess FMD to elicit responses which are primarily mediated by NO. Any deviation from this protocol increases the contribution of vasoactive substances other than NO, such as EDHF, adenosine or prostaglandins or a combination of these (Green *et al.*, 2004).

For all assessments of FMD within this thesis, both arms were extended and positioned at an angle of ~80° from the torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned on the forearm of the imaged arm immediately distal to the olecranon process to provide a stimulus to forearm ischaemia (Corretti *et al.*, 2002). An image of the brachial artery was obtained in the distal one third of the upper arm. A baseline recording of 1-minute was taken before the forearm cuff was inflated (>200 mmHg) for 5-minutes, recordings were not obtained during cuff inflation. Diameter and flow recordings were resumed 30 seconds prior to cuff deflation and continued for 3-minutes thereafter.

3.3.3. Assessment of brachial artery responses to ischaemic exercise (iEX)

To determine endothelial-dependent (partly) NO vasodilation a 1-minute baseline recording of brachial artery diameter, flow and shear rate was taken. For this particular assessment, in contrast with the FMD assessment, the occluding cuff was positioned above the imaged part of the brachial artery, i.e. proximally on the upper arm, and was inflated to >200 mmHg for 5-minutes. During the middle 3-minutes of

cuff inflation, ischaemic handgrip exercise (3kg weight) was performed to the beat of a metronome (30-contractions/min); with no recording during this time. Brachial artery diameter and flow recordings resumed 30 seconds prior to cuff deflation and continued for 3 to 5 minutes thereafter. This protocol results in near maximal dilation of the brachial artery in humans (Naylor *et al.*, 2005b). In addition to the assessment mentioned above, it is suggested that changes in the peak reactive hyperaemic blood flow response (RHBF) initiated from the ischaemic handgrip exercise is associated with remodelling of resistance vessels. Resistance vessels contribute to systemic levels of vascular resistance and blood pressure as well as a downstream supply of blood flow and there is evidence to suggest that adaptations to the peak reactive RHBF provides a prognostic marker for predicting future cardiovascular diseases (Anderson *et al.*, 2011; Lind *et al.*, 2011). Peak RHBF assessment is traditionally used to assess resistance vessel remodelling in humans (Takeshita & Mark, 1980; Sinoway *et al.*, 1986). The peak RHBF response to this stimulus in humans provides a valid and accepted index of resistance artery size or remodelling (Naylor *et al.*, 2005b) which has been used extensively the past 2 decades in human investigations (Sinoway *et al.*, 1986; Sinoway *et al.*, 1987).

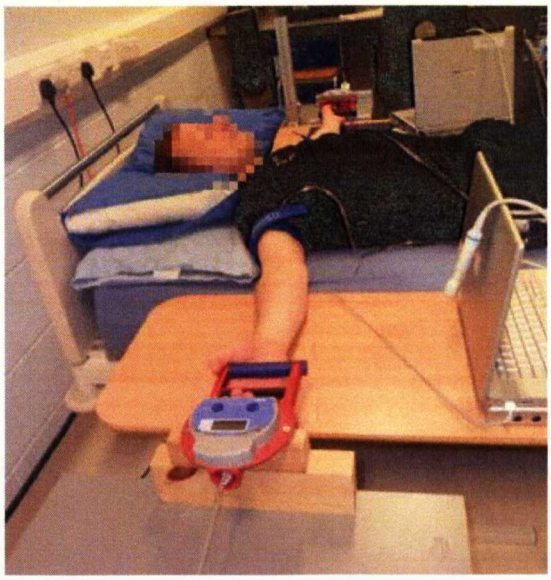

Figure 3.4 Ischaemic hand grip exercise using a handgrip dynamometer and ischaemia via cuff inflation to induce a maximal dilator response.

3.3.4. Assessment of brachial artery endothelium-independent vasodilation to glyceryl trinitrate (GTN)

To assess brachial artery NO-independent vasodilation an additional 1-minute baseline recording of brachial artery diameter was obtained, this was followed by glyceryl trinitrate (GTN) administration sublingually (single spray under the tongue; GTN, 400ug). Measurements of the artery diameter and blood flow were recorded for a further 10 minutes after administration.

GTN is a pharmacological stimulus for NO-independent vasodilation (Moncada *et al.*, 1991) as it is a NO donor. When administered sublingually it results in a dose-dependent increase in the level of cGMP (cyclic guanosine monophosphate) in smooth muscle (Rees *et al.*, 1990). It is now widely accepted that smooth muscle relaxation is associated with the activation cGMP which induces protein phosphorylation. As such GTN provides an endothelial-independent source of NO

with which it challenges the integrity of the smooth muscle and thereby provides an insight of smooth muscle sensitivity and subsequent vasodilation.

3.4. Artery diameter and blood flow analysis

Baseline diameter, blood flow, and shear rate were calculated at ~30Hz for all analysis. To enable this, a custom-designed edge detection wall tracking software was used to perform post-test analysis of brachial artery function and artery diameters were used. The initial phase of image analysis involved the identification of regions of interest (ROI; Figure 3.5) on the first frame of every individual study. These ROI's allowed automated calibration for diameters on the B-mode image and velocities on the Doppler strip.

A ROI was then drawn around the optimal area of the B-mode image and within this ROI a pixel-density algorithm automatically identified the angle-corrected near and far-wall e-lines for every pixel column within the ROI. The algorithm begins by dividing the ROI into an upper half, containing the near wall lumen-intima interface, and a lower half containing the far wall interfaces. The near-wall intimal edge is identified by a Rake routine that scans from the bottom to the top of the upper half of the ROI. The position of the edge is established determining the point where the pixel intensity changes most rapidly. Therefore, typical B-mode ROI's contained approximately 200-300 diameter measures per frame, the average of which was calculated and stored. This process occurred at 30 frames per second.

A final ROI was drawn around the Doppler waveform and automatically detected the peak of the waveform. The mean diameter measures derived from within the B-

mode ROI (above) were then synchronised with the velocity measure derived from the Doppler ROI at 30 Hz. Ultimately, from this synchronised diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) and shear rate (4 times velocity divided by diameter) were calculated at 30 Hz. The shear rate patterns (antegrade and retrograde) were assessed by calculating the area under the curve for all of the antegrade blood flow and shear recordings (Thijssen *et al.*, 2009b).

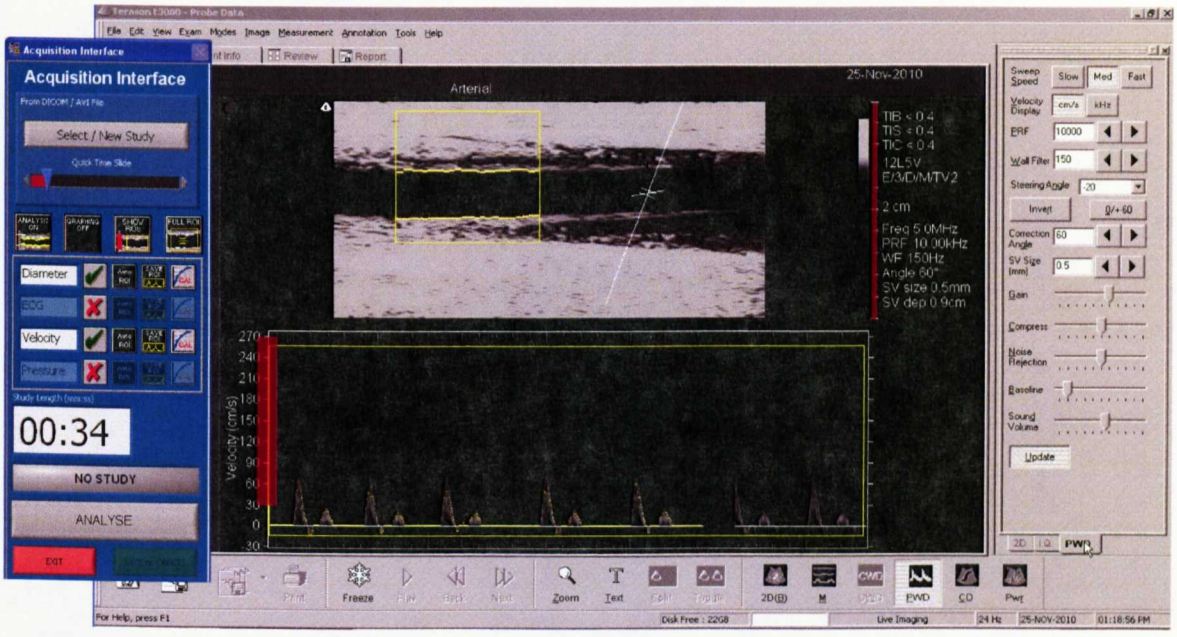

Figure 3.5 2D B-Mode Ultrasound image recording of the brachial artery diameter and velocity in the analysis software with regions of interest (ROI) highlighted in yellow.

It has been previously shown that reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, reduces observer error significantly, and possesses an intra-observer CV of 6.7% (Woodman *et al.*, 2001). Furthermore, this method of blood flow assessment is closely correlated with actual flow through a "phantom" arterial flow system (Green *et al.*, 2002c) .

3.5. Data analysis

Baseline diameter, flow and shear rate were calculated as the mean of data acquired across the 1 minute preceding the cuff inflation period. Peak diameter following cuff deflation was automatically detected according to an algorithm that identified the maximum bracket of data subsequent to performance of a moving window smoothing function. This smoothing routine calculates the median value from 100 consecutive samples, before the window shifts to the next bracket of data, which shares 20% overlap with the preceding bracket. The maximum value of all the calculated median values is then automatically detected and chosen to represent the peak of the post deflation artery diameter curve. FMD, iEX and GTN were calculated as the absolute (mm) and relative (%) rise of the peak diameter from the preceding baseline diameter.

The time to peak diameter (in seconds) was calculated from the point of cuff deflation or administration of GTN to the maximum diameter. Calculations of the FMD (Figure 3.6), iEX (Figure 3.7) and GTN (Figure 3.8) time to peak were therefore observer-independent and based on standardised algorithms, applied to data which had undergone automated edge-detection and wall tracking.

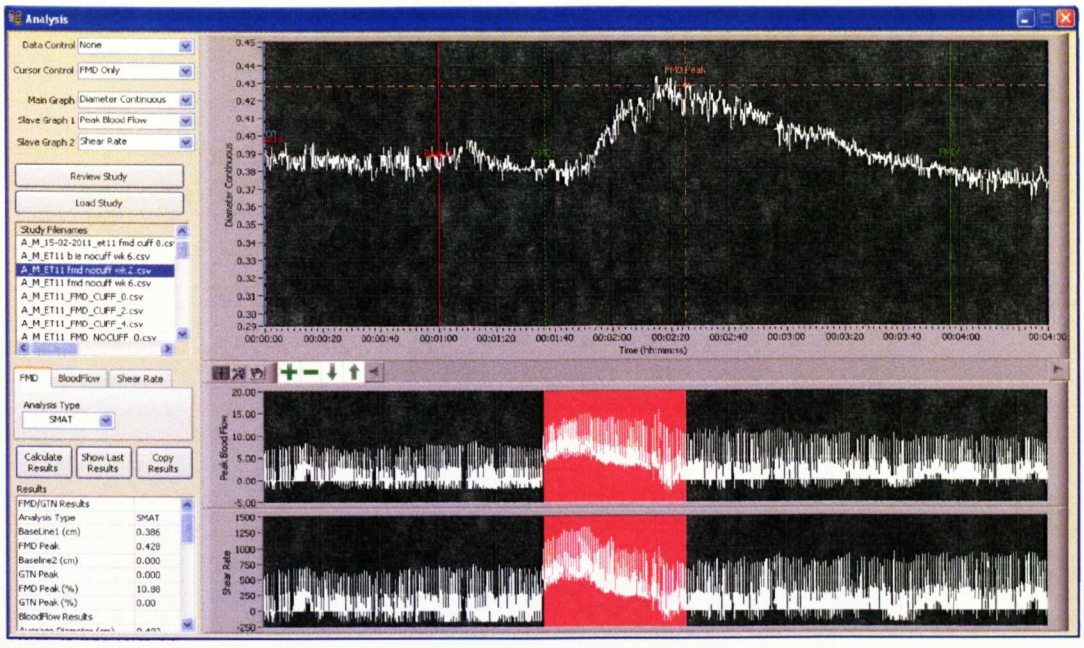

Figure 3.6 Continuous brachial artery diameter and velocity recordings represented in the analysis software during calculation of the FMD. Red lines represent selection of baseline; green placed from cuff release to after peak dilation; orange lines automatically detect peak and calculate the percent change from baseline to peak dilation; area highlighted in red refers to SR_{AUC} calculation.

In accordance with recent findings (Pyke & Tschakovsky, 2005) shear rate stimulus was calculated for endothelium-dependant FMD. The post-deflation shear rate data, derived from simultaneously acquired velocity and diameter measures at 30 Hz, was used to determine the area under the shear rate curve (SR_{AUC}). This is calculated for data up to the point of maximal post-deflation diameter (FMD) for each individual using the trapezoid rule. Peak blood flow ($ml.min^{-1}$) in response to ischaemic exercise stimuli, was calculated from diameter (cross-sectional area) and velocity data, which was recorded as the highest area under the blood flow curve data across a 30 second period following each stimulus, this epoch being selected on the basis of results of previous studies utilising the use of plethysmography.

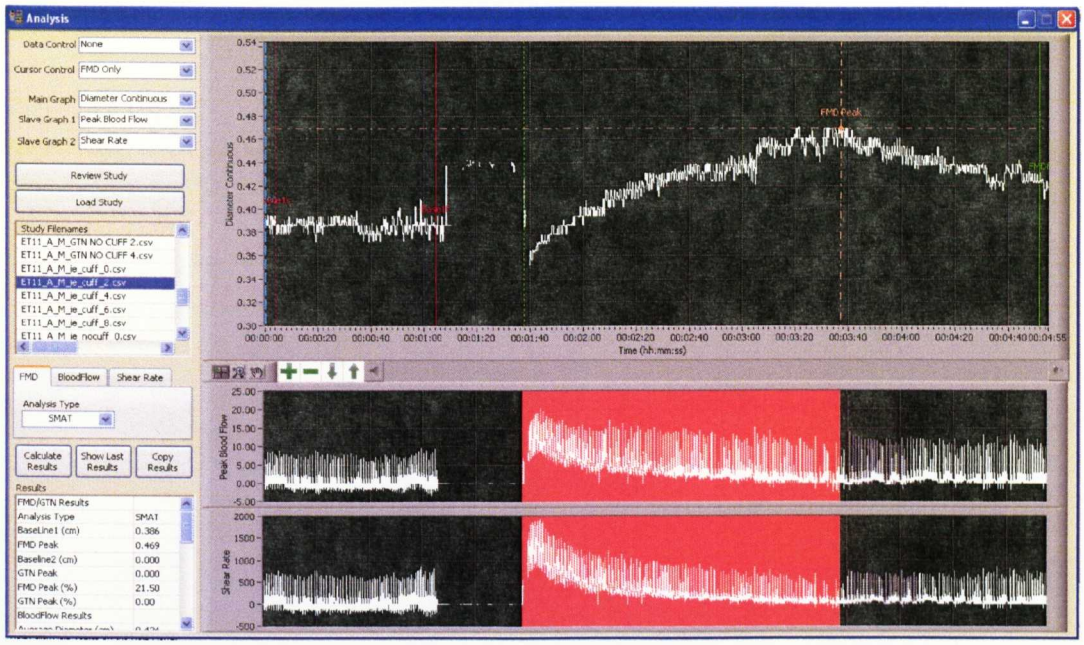

Figure 3.7 Continuous brachial artery diameter and velocity recordings represented in the analysis software during calculation of the iEX. Red lines represent selection of baseline; green placed from cuff release to after peak dilation; orange lines automatically detect peak and calculate the percent change from baseline to peak dilation.

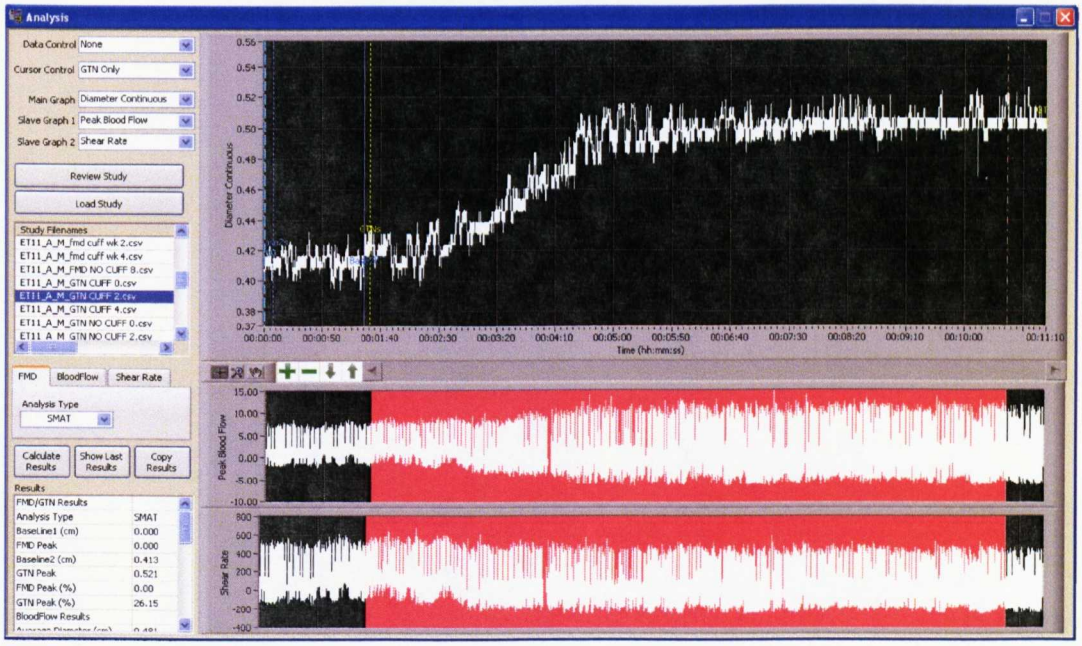

Figure 3.8 Continuous brachial artery diameter and velocity recordings represented in the analysis software during calculation of the GTN. Blue lines represent selection of baseline; yellow lines placed from GTN administration to end point; orange lines automatically detect peak and calculate the percent change from baseline to peak dilation.

3.6. Statistical analysis

Once the data collected for this thesis had been analysed and placed into a spreadsheet, statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, Illinois) software. This sample size was similar to those recruited for previous studies on the effects of exercise intensity, and it was estimated that a 2% change in FMD would be detected with 10 participants assuming that the standard deviation of this change is 2% with statistical power of 80%. All data were reported as mean ± SD unless stated otherwise, statistical significance was assumed at $P<0.05$. Specific analyses are highlighted in each experimental chapter.

Aims and Outcomes of Chapter 4

Chapter 4

Aim: Examine the systemic effect of shear rate during exercise on post exercise brachial diameter.

Chapter 4

Outcome: Dose-dependent increase in shear rate = Dose-dependent increase in brachial artery diameter.

Chapter 4 | *Systemic effect of shear rate during exercise on post-exercise conduit artery diameter in humans.*

4.1. Introduction

Exercise reduces primary (Paffenbarger *et al.*, 1986; Sesso *et al.*, 2000; Myers *et al.*, 2002) and secondary cardiovascular events (Oldridge *et al.*, 1988; Jolliffe *et al.*, 2001), an effect that cannot be fully explained by reductions in risk factors (Mora *et al.*, 2007). It has therefore been suggested that exercise may have a direct anti-atherogenic effect on the vasculature (Green *et al.*, 2008b; Joyner & Green, 2009). It is well established that exercise is associated with increases in blood flow and shear stress across the endothelium. Endothelial cells respond to shear stress by producing localised factors, such as nitric oxide (NO), which is a potent vasodilator and anti-atherogenic agent.

It has been established that lower limb exercise in humans increases blood flow and shear stress in arterial beds distant from the working muscle (Green *et al.*, 2002b; Green *et al.*, 2002c) and that exercise training of the lower limbs improves upper limb arterial function (Green *et al.*, 2008a; Tinken *et al.*, 2008). It has been observed that increases in blood flow and shear stress during leg exercise are associated with production of NO in the brachial artery (Green *et al.*, 2002b; Green *et al.*, 2005), providing a mechanistic explanation for systemic exercise training effects on vascular adaptation. More recently, it has been established that changes in blood flow and shear rate during exercise are obligatory for adaptations in response to exercise training (Tinken *et al.*, 2009; Tinken *et al.*, 2010). The impacts of antegrade and retrograde blood flow and shear rate during exercise on arterial diameter have also been confirmed by others (Simmons *et al.*, 2011).

Despite these previous findings, little is known regarding the systemic impact of shear rate changes during exercise on conduit arterial diameter. The purpose of this study was to examine the effect of increasing shear rate on brachial artery diameter during cycle ergometer exercise undertaken at increasing exercise intensities (50, 70 and 85% HR_{max}) and to better understand the association between systemic increases in shear stress during lower-limb exercise on immediately post exercise diameters of the non-exercising upper limbs. In addition, unilateral forearm heating was utilised to increase shear stress independently of exercise intensity during each exercise bout. The heating intervention provides a stimulus to cause an increase in blood flow and therefore shear stress in one forearm independent of exercise and exercise intensity. The increased rise in temperature to the forearm causes a continuous increase in blood flow and therefore shear stress, resulting in the vessels dilating during the entire exercise bout regardless of the exercise intensity. Therefore, it is hypothesised that upper limb brachial artery dilation would be dependent upon the magnitude of shear stress manipulation during cycle exercise.

4.2. Methods

4.2.1. Subjects

Ten young male subjects (21 ± 2 years) were recruited. Subjects were healthy; none reported having been diagnosed with cardiovascular disease, diabetes, insulin resistance or cardiovascular risk factors, such as hypercholesterolemia or hypertension. Subjects who smoked or were on medication of any type were excluded. All subjects took part in ≤2 hrs of physical activity per week. Informed

consent was gained from all subjects prior to the experimental procedures. The study procedures were approved by the Liverpool John Moores Ethics Committee and adhered to the Declaration of Helsinki.

4.2.2. Experimental design

Brachial artery diameter and velocity was measured in both arms before and immediately after cycling exercise. Exercise was performed for 30 minutes at 50, 70 and 85% of maximal heart rate, in a randomised order. During exercise, one forearm was randomly assigned for submersion in a heated water bath (40°C). Once an arm was selected, this was kept consistent within each subject across the 3 exercise intensities.

4.2.3. Experimental procedures

Subjects were instructed to abstain from strenuous exercise for 24 hrs and from caffeine and alcohol ingestion for 18 hrs before attending the laboratory. They were also asked to fast for 6 hrs prior to each visit. All tests within subjects were performed at the same time of day. First, subjects rested in the supine position for 20 minutes, followed by assessment of heart rate and blood pressure using an automated sphygmomanometer (GE Pro 300V2, Dinamap, Tampa, Fl). This was followed by bilateral assessment of brachial artery diameter and velocity. Subsequently, participants cycled for 30 minutes on an electrically braked ergometer (Monark 874E, Sweden), with resistance adjusted to maintain stable, and individually determined exercise intensity at 50, 70 and 85% HR_{max}.

During each exercise bout, participants submerged one forearm into a heated water bath maintained at 40±1°C (Figure 4.1). The water bath was placed on an adjustable table so that the subject could comfortably place their forearm into the water during the entire cycling intervention. The water bath was maintained at a constant temperature by a thermostatically controlled circulating heater pump (+°C). The unheated arm (~°C) rested comfortably on an adjustable table for assessment of the brachial artery diameter and velocity. Upon cessation of exercise, the arms were removed from the water bath.

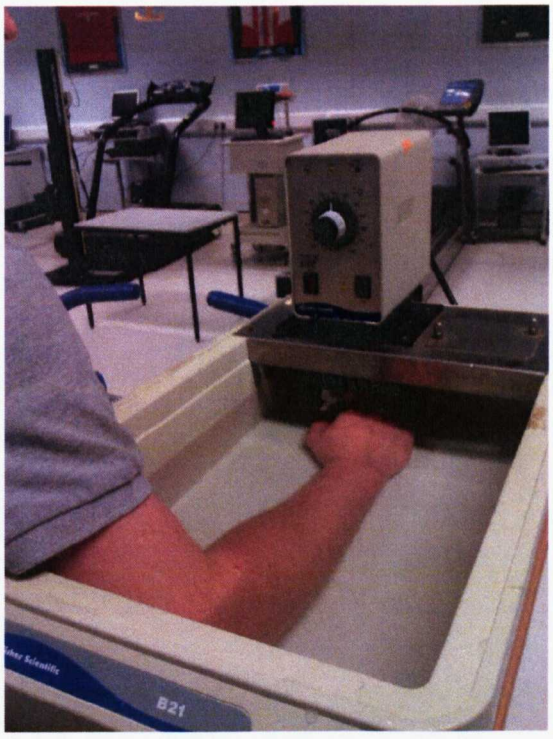

Figure 4.1 Temperature controlled heated waterbath (~40°C)

Duplex ultrasound (T3000; Terason, Burlington, MA) was utilised to simultaneously image brachial artery diameter and velocity in both arms. Settings on both ultrasound machines were identical. Recordings were performed at baseline and during the final 15 minutes of cycling and immediately post-exercise.

4.2.4. Brachial artery diameter, blood flow and shear rate analysis

Analysis of brachial artery diameter, blood flow and shear rates before, during and after the intervention were performed using custom-designed edge-detection and wall-tracking software. This is fully described in Sections 3.4 and 3.5 in the General Methods, Chapter 3.

4.2.5. Statistical analysis

A two-factor ANOVA with repeated measures (pre and post time-points and intensity) was performed for each arm. A further two-factor ANOVA with repeated measures (intensity and arm) was performed separately for the data of shear rates collected during exercise. A one-factor ANOVA was performed for the percent changes in diameter data. Post-hoc analysis *t*-tests were used when significant values were found.

4.3. Results

Participants completed the 30-minute cycle exercise bout at 101±6, 135±4 and 162±4 bpm. There were no significant differences in mean arterial pressure (MAP) pre *vs.* post exercise across the intensities (Table 4.1). There was a significantly higher heart rate after exercise at 70% and 85% (Table 4.1).

Table 4.1 Mean arterial blood pressure (MAP) and heart rate (HR) pre and post exercise at all exercise intensities (50, 70, and 85% of HR_{max}).

	50%		70%		85%	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*
MAP (mmHg)	91±4	91±3	93±8	92±6	90±6	88±6
Heart Rate (bpm)	67±14	65±11	62±13	75±13*	62±11	90±11*

Data are presented as mean ± SD. *Significant from pre-exercise at P<0.05.

4.3.1. Acute effects of cycle exercise on brachial artery shear rate

Shear rate data for 3 subjects, across all intensities, were removed from the analysis due to inadequate brachial artery diameter and velocity recordings during intense exercise (Table 4.2, Figure 4.2). Mean and antegrade shear rate in both arms was the same pre-exercise and rose progressively with increasing exercise intensities (Table 4.2, Figure 4.2). In the ~°C arm, mean and antegrade shear rate during exercise demonstrated a graduated increase with increasing exercise intensity (one-way ANOVA; P<0.05, Figure 4.2). Larger increases in mean and antegrade SR were evident in the contra-lateral arm as a result of heating (significant interaction by two-way ANOVA; P<0.01), and post-hoc analysis revealed a significant difference between arms for mean and antegrade shear rate at 50 and 85% HR_{max} (P<0.05, Table 4.2, Figure 4.2). Retrograde shear rate was significantly different between arms (two-way ANOVA; P=0.02). Post hoc *t*-test revealed this to be lower in the +°C arm compared to ~°C at 70% HR_{max} (P<0.05), but no statistical differences were evident at 50 and 85% HR_{max} (Table 4.2).

Table 4.2 Antegrade and retrograde brachial artery shear rate at all exercise intensities (50, 70, 85% HR_{max}) in the unheated and heated arms. (~°C, unheated; +°C, heated; SR, shear rate). Pre; n=10, 50%; n=9, 70%; n=10, 85%; n=7.

Antegrade SR (s^{-1})	***Pre***	***50%***	***70%***	***85%***	***P-value***
~°C	161±98	235±60	308±66* ‡	328±46*‡	0.01
+°C	168±114	391±154*†	365±94*	468±92*†	<0.01
Retrograde SR (s^{-1})					
~°C	-8±9	-21±25	-16±15	-13±13	0.80
+°C	-12±19	-5±15	-6±9†	-1±2	0.34

Data are presented mean±SD. Post hoc analysis; *Significantly different from pre; † Significantly different from ~°C. ‡ Significantly different from 50%HR_{max} (P<0.05). P-value represents, one-way ANOVA.

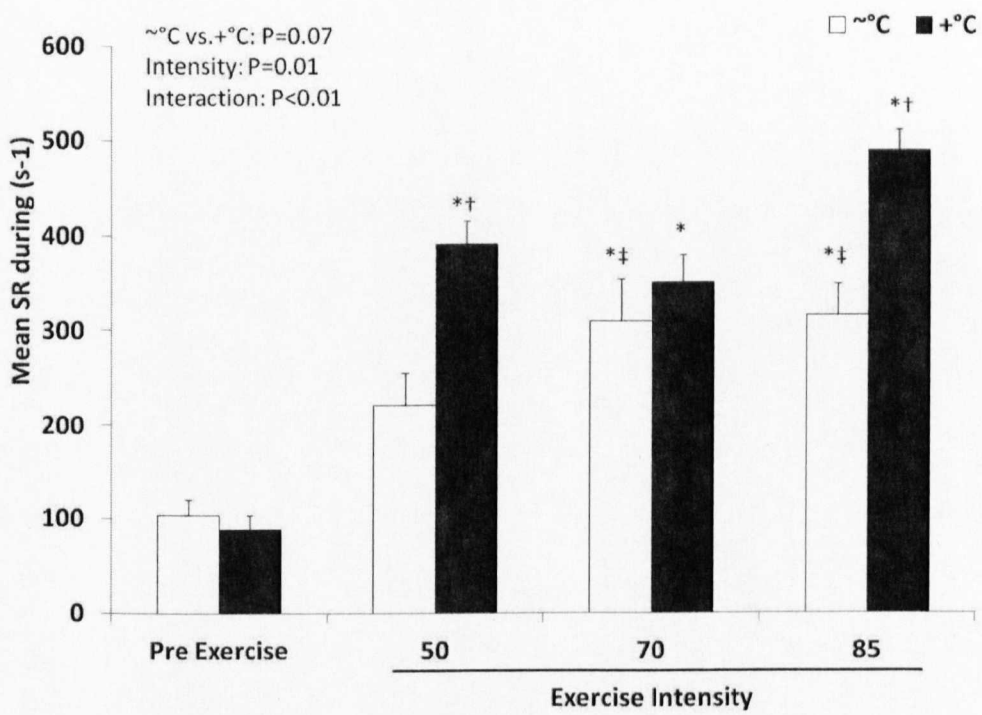

Figure 4.2 Brachial artery mean shear rate (SR) data in the unheated and heated arms during exercise at 50, 70 and 85% (white bars, ~°C, unheated; black bars, +°C, heated). Data are presented as mean ± SE. Post hoc analysis; *Significantly different from pre exercise. †Significantly different from ~°C. ‡Significantly different from 50%HR_{max} (P<0.05).

4.3.2. Brachial artery diameter pre vs. post cycle exercise

Brachial artery diameter demonstrated a significant increase across time in both the +°C and ~°C limbs ($P<0.05$, Figure 4.3, Table 4.3).

Table 4.3. Brachial artery diameter pre and post exercise at all exercise intensities (50, 70, 85% HR_{max}) in the unheated and heated arms. (~°C, unheated; +°C, heated).

		~°C		+°C	
		Pre	***Post***	***Pre***	***Post***
Diameter (mm)	***50%***	4.1±0.4	4.1±1.4	4.2±0.7	4.6±0.5*†
	70%	3.9±0.4	4.2±0.4*	4.0±0.6	4.5±0.4*†
	85%	4.1±0.4	4.4±0.4*	4.1±0.5	4.7±0.4*

Data are presented as mean ± SD. Post hoc analysis; * Significant from pre; † Significant from ~°C at $P<0.05$.

Post-exercise brachial artery diameter was significantly increased compared to pre-exercise at 70 and 85% in the ~°C arm and at all intensities in the +°C arm ($P<0.05$, Table 4.3). Post-exercise brachial artery diameter was significantly larger in the +°C compared to the ~°C arm at 50 and 70% ($P<0.05$, Table 4.3).

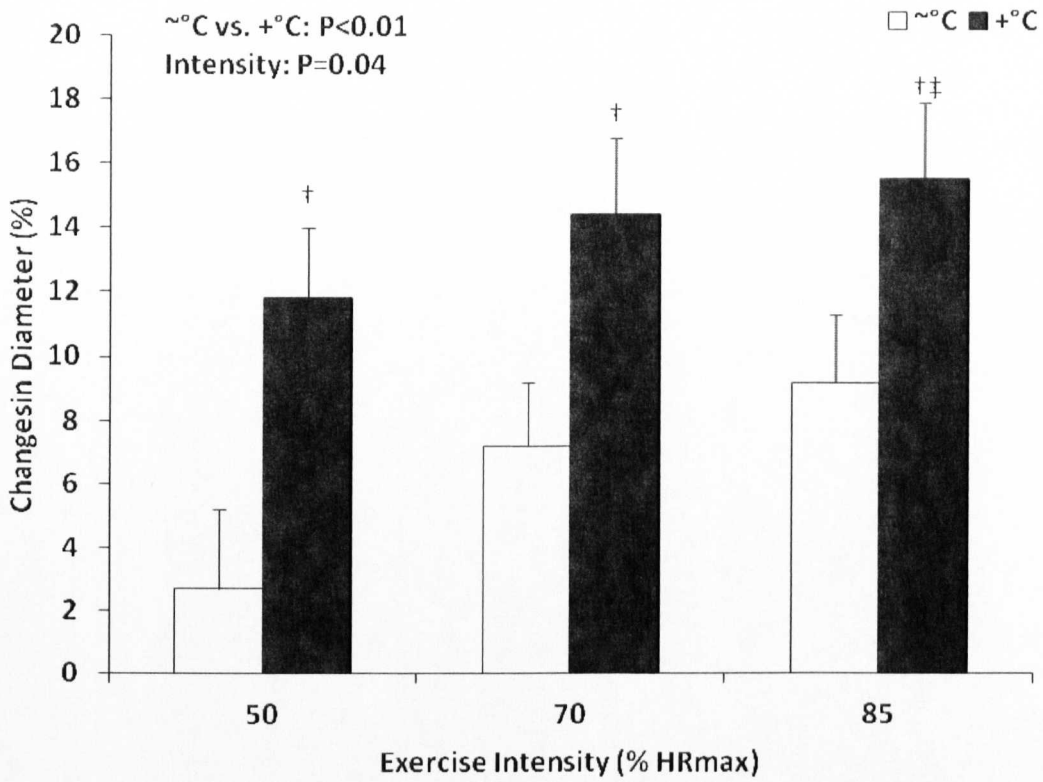

Figure 4.3 Percent change between pre *vs.* post exercise brachial artery diameters in unheated and heated arm (white bars, ~°C, unheated; black bars, +°C, heated) at 50, 70 and 85%HR_{max}. Data are presented as mean ± SE. Post hoc analysis; †Significant difference ~°C *vs.* +°C. ‡Significantly different from 50%HR_{max} ($P<0.05$).

4.4. Discussion

The purpose of this study was to examine the systemic effects of leg cycling exercise on brachial artery shear rate and diameter. Incremental elevations in shear rate were induced by 3 step-wise elevations in exercise intensity, whilst unilateral forearm heating during simultaneous bilateral monitoring at all exercise intensities was utilised to induce additional increments in brachial artery shear rate. The principle finding of the present study is that an 'exercise intensity-dependent' increase in brachial artery shear rate in the unheated arm was observed, which resulted in a concomitant 'exercise intensity-dependent' increase in brachial artery

diameter. This observation suggests an important role of shear rate as a fundamental stimulus for the systemic increase in brachial diameter after leg cycling exercise. To support this notion, heating of the contralateral forearm during each exercise bout induced a significantly higher mean and antegrade shear rate, compared to the unheated arm. This, in turn, resulted in a larger post-exercise brachial diameter in the heated arm. This indicates that cycling exercise leads to a systemic exercise-intensity dependent increase in brachial artery diameter, which is primarily explained by increases in brachial artery shear rate during exercise.

Exercise is a well-known stimulus to induce systemic changes in conduit artery diameter and shear rate, with the change in shear rate being dependent on the type of exercise (Green *et al.*, 2002c; Green *et al.*, 2005; Thijssen *et al.*, 2009a) and the duration of exercise performance (Simmons *et al.*, 2011) and the present findings are in agreement with these previous studies. Data from this study extend previous observations by demonstrating changes in brachial artery diameter in response to different shear rate stimuli. The importance of shear stress as an endothelial stimulus to long term arterial adaptation in function and structure has previously been established in both animals (Langille & O'Donnell, 1986; Laughlin *et al.*, 2008) and humans (Green *et al.*, 2004; Tinken *et al.*, 2008; Thijssen *et al.*, 2010; Tinken *et al.*, 2010). However, the effects of acute exercise on immediate changes in diameter have not been well described. Interestingly, in line with a recent study (Simmons *et al.*, 2011), in the present study an increase in artery diameter after exercise was also observed. More importantly, this demonstrates a strong relationship between the magnitude of shear rate during exercise and magnitude of the post-exercise

artery diameter. Therefore, data from the present study provides support for a central role of shear stress in acute adaptations of conduit arterial diameter to exercise in humans.

Shear stress stimulates the endothelium to release several vasodilators, in particular nitric oxide (Pohl *et al.*, 1986; Laughlin *et al.*, 2008). It has therefore been suggested that shear stress mediated NO production is a key stimulus for conduit artery vasodilation with exercise (Green *et al.*, 2004; Tinken *et al.*, 2009; Tinken *et al.*, 2010; Simmons *et al.*, 2011) and that a functioning, healthy endothelium is necessary for this shear-stress mediated vasodilation to occur (Langille & O'Donnell, 1986). However, exercise is a complex stimulus, resulting in several systemic changes which may influence brachial artery vasodilation (Green *et al.*, 2004; Laughlin *et al.*, 2008). For example, recent data provide evidence that changes in arterial wall thickness occur as a result of systemic, rather than localised and shear-induced adaptation (Rowley *et al.*, 2011). In order to focus on, and specifically manipulate, shear stress during exercise, forearm heating was utilised to locally and unilaterally elevate shear rate during simultaneous bilateral assessments. Excess shear elevation in the heating limb occurs independently of the effect of exercise and any systemic or reflex impact on the vasculature is effectively eliminated by the simultaneous measurements taken in both arms. Heating resulted in an additional increase in shear rate across all intensities and associated additional increase in brachial artery diameter. This observation further supports a central role of shear stress as a principal stimulus for systemic vasodilation in conduit arteries supplying a non-exercising vascular bed during exercise.

In a recent study it was suggested that lower limb exercise such as cycling induces a change in the pattern of blood flow in the brachial artery of the inactive limb characterised by increases in antegrade flow during systole as cardiac output increases, as well as increases in retrograde flow during diastole (Green *et al.*, 2002a). Simmons *et al.* (2011) report an increase in brachial artery retrograde SR at the onset of leg cycling subsequently returns towards baseline values due to thermoregulatory cutaneous vasodilation during prolonged exercise, suggesting that the increases in diameter post exercise are more likely associated with increases in antegrade shear rate rather than retrograde. Additionally, retrograde shear rate is hypothesised as a strong stimulus for endothelial dysfunction in humans (Thijssen *et al.*, 2009b).

These data help to clarify the underlying stimulus for vascular adaptations to exercise training. It is likely that the increases in shear stress and vasodilation with acute exercise are the triggers for long-term vascular adaptations with exercise training. More specifically the exercise-induced increases in flow and shear stress up-regulate NO-mediated function (Gielen *et al.*, 2010). It has previously been demonstrated that eNOS mRNA levels can be increased very soon (2-4 hours) after *in vitro* increases in blood flow (Woodman *et al.*, 1999) and eNOS protein expression can increase after a week of exercise training (Wang *et al.*, 1993). In further support of this, recent studies in humans (Tinken *et al.*, 2009; Tinken *et al.*, 2010) have demonstrated that acute and chronic adaptations to exercise are only evident when there is an increase in shear rate (Tinken *et al.*, 2010; Simmons *et al.*, 2011).

Observations from the present study raise the question why such marked elevations in blood flow and shear rate are present in a vessel supplying a non-exercising vascular bed. As special care was taken to ensure that the subjects did not perform any hand gripping exercise, forearm exercise cannot explain this increase. A contribution from increased arterial blood pressure is likely, but may not fully explain the changes observed in the present study. Recently, an important role for skin vasodilation, secondary to thermoregulatory demand, was demonstrated (Simmons *et al.*, 2011). Increased cutaneous blood flow to the forearm and consequent increase in brachial artery shear rate may contribute to the shear mediated brachial dilation observed in the present study, as supported by the heating data. In addition, a recent study involving repeated forearm heating resulted in chronic changes in upper limb arterial function and structure (Green *et al.*, 2010; Naylor *et al.*, 2011).

During exercise the activation of skeletal muscle fibres by somatic nerves results in vasodilation and functional hyperaemia (Thomas & Segal, 2004). In addition, sympathetic nerve activity is essential for vasoconstriction and the maintenance of arterial blood pressure. Therefore, the interaction between somatic and sympathetic pathways is essential for blood flow control to skeletal muscle during exercise (Thomas & Segal, 2004). The notion that systemic changes in blood flow and shear rate occur in non-exercising regions has been discussed extensively (Green, 2005b; Thijssen & Hopman, 2008; Padilla *et al.*, 2011a). However, it has been reported that haemodynamic stimuli play an important role in endothelial and vascular smooth muscle cell adaptations to exercise training (Laughlin *et al.*, 2008).

Nevertheless, the impacts that exercise-induced changes in hemodynamic profile, pulse pressure, transmural pressure have on systemic adaptations of both endothelial and smooth muscle cells still remain unclear (Newcomer & Padilla, 2011), therefore, subsequent studies to determine this would be of interest.

Limitations. The present study took advantage of a within subject approach (i.e. different exercise intensities causing different shear responses) and bilateral measurements (i.e. heated and unheated arm to induce different shear responses) to isolate the role of shear rate in brachial dilation during leg exercise. Additionally, it may have been beneficial to assess forearm heating as a control independent of exercise, however, it has previously been reported that heating of the forearm independent of exercise is a potent stimulus to replicate increases in shear stress, observed during exercise, to induce vascular improvements (Naylor *et al.*, 2011). A potential limitation of the present study is that healthy male volunteers were recruited. These findings cannot be extrapolated to subjects with cardiovascular disease, particularly that affecting cardiac output.

4.5. Conclusion

In conclusion, progressive lower limb cycling exercise causes an increase in brachial artery shear rate which was associated with a dose-dependent increase in brachial artery diameter. When unilateral localised forearm heating was combined with cycling exercise, an additional increase in brachial artery blood flow and shear rate was observed, and brachial artery dilation was enhanced. These data indicate that cycle exercise has systemic effects on arterial dilation, with an increase in shear rate being an important stimulus to artery vasodilation during exercise in humans.

Aims and Outcomes of Chapter 5

Chapter 4 ***Aim:*** Examine the systemic effect of shear rate during exercise on post exercise brachial diameter.	*Chapter 4* ***Outcome:*** Dose-dependent increase in shear rate = Dose-dependent increase in brachial artery diameter.
Chapter 5 ***Aim:*** Address the role of shear rate on endothelial function following acute leg cycling exercise.	*Chapter 5* ***Outcome:*** Impairment in FMD post exercise is exercise intensity-dependent. FMD is only decreased immediately post exercise.

Chapter 5 | *Effect of exercise intensity on brachial artery flow mediated dilation in healthy humans.*

5.1. Introduction

Exercise reduces primary (Blair & Morris, 2009) and secondary cardiovascular events (Taylor *et al.*, 2004), effects which cannot be fully explained by reduction in traditional risk factors (Maiorana *et al.*, 2003; Mora *et al.*, 2007). It has been suggested that exercise has a direct anti-atherogenic impact on the vasculature (Green *et al.*, 2008b; Joyner & Green, 2009), which may be mediated through shear-stress induced improvement in endothelial function (Tuttle *et al.*, 2001; Green *et al.*, 2004). Whilst the effects of exercise training on the vasculature are well documented, less is known about acute changes in vascular function in response to exercise which may provide the foundation for these adaptations.

Previous studies which have examined the acute effect of exercise on endothelial function have reported conflicting results (Padilla *et al.*, 2006; Dawson *et al.*, 2008; Harris *et al.*, 2008; Tinken *et al.*, 2009; Jones *et al.*, 2010; Varady *et al.*, 2010; Zhu *et al.*, 2010; Johnson *et al.*, 2011). In a series of experiments, it was found that the magnitude and pattern of shear rate modulate endothelial function (Thijssen *et al.*, 2009b; Tinken *et al.*, 2009; Tinken *et al.*, 2010), and that shear rate patterns during exercise depend on the mode of the exercise performed (Green *et al.*, 2005; Thijssen *et al.*, 2009a). Exercise intensity may also modulate the nature of training-induced vascular adaptation (Goto *et al.*, 2003). A significant proportion of the disparity in previous studies which examined the acute effect of exercise on endothelial function may therefore relate to differences in exercise intensity, although this hypothesis has not been directly addressed.

The purpose of this study was to examine brachial artery endothelial function before and immediately following acute cycling exercise at 3 different intensities (50, 70 and 85% maximal heart rate). Therefore, it is hypothesised that the magnitude of decrease in FMD would depend upon exercise intensity.

5.2. Methods

5.2.1. Subjects

Ten young male subjects (21 ± 2 years) were recruited. Subjects were healthy; none reported having been diagnosed with cardiovascular disease, diabetes, insulin resistance or cardiovascular risk factors, such as hypercholesterolemia or hypertension. Subjects who smoked or were on medication of any type were excluded. All subjects took part in ≤2 hrs of physical activity per week. Informed consent was gained from all subjects prior to the experimental procedures. The study procedures were approved by the Liverpool John Moores Ethics Committee and adhered to the Declaration of Helsinki.

5.2.2. Experimental design

Brachial artery diameter and endothelial function (FMD) was measured in one arm (randomised between subjects, but once selected all measurements were always performed in the same arm) before and after (0, 1, 2, 24h) after upright cycling exercise. At least 48 h was taken between subsequent exercise bouts, whilst all tests were performed within 14 days to minimize variability of the primary outcome variables. Exercise was performed for 30 minutes at 50, 70 and 85% of maximal heart rate (in a randomised order).

5.2.3. Experimental procedures

Subjects were instructed to abstain from strenuous exercise for 24 hrs and from caffeine and alcohol ingestion for 18 hrs before attending the laboratory. They were also asked to fast for 6 hrs prior to each visit. Subjects rested in the supine position for 20 minutes, followed by assessment of heart rate and blood pressure using an automated sphygmomanometer (GE Pro 300V2, Dinamap, Tampa, FL). This was followed by assessment of brachial artery diameter and velocity. Subsequently, participants cycled for 30 minutes on an electrically braked ergometer (Monark 874E, Sweden), with resistance adjusted to maintain a stable and individually determined exercise intensity at 50, 70 and 85% HR_{max} (Tanaka *et al.*, 2001). During the exercise bout both arms were rested comfortably on an adjustable table. Duplex ultrasound (T3000; Terason, Burlington, MA) was utilised to simultaneously image brachial artery diameter and velocity in one arm. Recordings were performed at baseline and during the final 15 minutes of cycling and immediately post-exercise. Brachial artery FMD was assessed, before and after exercise (0, 1, 2 and 24h), a full description of the FMD procedure and analysis can be found in Section 3.3.2 in the General Methods, Chapter 3).

5.2.4. Brachial artery diameter, blood flow and shear rate analysis

Analysis of brachial artery diameter, blood flow and shear rates before, during and after the intervention were performed using custom-designed edge-detection and wall-tracking software. This is fully described in Sections 3.4 and 3.5 in the General Methods, Chapter 3.

5.2.5. Statistical analysis

The primary outcome of this study was FMD therefore it was important to control for the influence of known moderators of FMD (shear rate and baseline diameter), since it was likely that these moderators would also be influenced by exercise. Therefore, the effects of exercise intensity and time on the change in logarithmically transformed diameter using a Generalised Estimating Equation which incorporated baseline diameter and shear rate as covariates was analysed. The resulting mean differences between exercise intensities and pre/post were back-transformed to the original units of FMD (%). Two-factor general linear models (GLM) with repeated measures were employed to analyse the effect magnitudes of time and intensity on all other study outcomes. Post-hoc analysis was performed using the least significance difference (LSD) multiple comparison method. All data are reported as mean ± SD unless stated otherwise. Exact P-values and 95% confidence intervals for the effect magnitudes are also cited. An additional two-factor ANOVA with repeated measures was used to analyse heart rate and SR during exercise, mean arterial pressure (MAP) before and after exercise across each exercise intensity and for FMD characteristics at baseline, at all time-points and shear rate during exercise. Post-hoc analysis *t*-tests were performed where significant values were found. The relationship between the percent changes in baseline diameter and mean shear rate were assessed using pearson's R correlations.

5.3. Results

Participants completed the 30-min cycle exercise bouts at 101±6, 135±4 and 162±4 bpm, which corresponded closely with 50, 70 and 85% of HR_{max} (193±1). The

changes in mean arterial pressure (MAP) after exercise were small in clinical terms (≤ 2 mmHg) and not statistically significant across the 3 different exercise intensities (Table 5.1). The magnitude of exercise-mediated tachycardia increased as exercise intensity became higher (Table 5.1).

Table 5.1 Mean arterial blood pressure (MAP) and heart rate (HR) pre and post exercise at all exercise intensities (50, 70, and 85% of HR_{max}).

	50%		**70%**		**85%**	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*
MAP (mmHg)	91±4	91±3	93±8	92±6	90±6	88±6
Heart Rate (beats/min)	67±14	65±11	62±13	75±13*	62±11	90±11*

Data are presented as mean ± SD. *Statistically significant difference from pre-exercise at P<0.05.

5.3.1. Acute effects of cycle exercise on brachial artery shear rate

Shear rate data for 3 subjects, across all intensities, were removed from the analysis due to inadequate brachial artery diameter and velocity recordings during intense exercise (Table 5.2). Mean and antegrade shear rate during exercise were higher compared to baseline data (Figure 5.1 and Table 5.2, respectively). Moreover, mean and antegrade shear rate demonstrated a dose-dependent increase with exercise intensity (two factor GLM; P<0.001, Figure 5.1, Table 5.2).

Table 5.2 Antegrade and retrograde brachial artery shear rate at baseline and during cycling exercise (during final 15 minutes cycling) at all exercise intensities (50, 70, 85% HR_{max}).

	Pre	*50%*	*70%*	*85%*	*P-value*
Antegrade shear rate (s^{-1})	109±42	243±66*	322±63*†	324±40*†	<0.001
Retrograde shear rate (s^{-1})	-6±4	-18±24	-15±14	-12±10	0.47

P-values refer to two factor GLM. Data are presented mean ± SD. Post-hoc significantly different from *pre (P<0.05) or †50%HR_{max} (P<0.05). Data in the table are based on n=7 due to difficulty tracking velocity measures at higher exercise intensities.

5.3.2. Acute effects of cycle exercise on brachial artery endothelial function

Exercise intensity. Exercise mediated an increase in baseline diameter, the magnitude of which depended on exercise intensity. The pre-to-post exercise changes were 4.1±0.4 to 4.1±0.3 mm (P=0.36), 3.9±0.4 to 4.2±0.4 mm (P=0.01) and 4.1±0.4 to 4.4±0.4 mm (P<0.01) for the 50%, 70% and 85% HR_{max} intensities. Exercise also induced a dose-dependent increase in SR_{AUC} immediately after exercise at 50, 70 and 85%HR_{max} (21.8±13.2 to 26.9±8.4 $s^{-1}x10^3$, 20.9±6.0 to 25.4±8.1 $s^{-1}x10^3$ and 19.6±7.0 to 37.7±12.8 s^{-1} $x10^3$, respectively, two factor GLM, interaction; P=0.04). However, there was a poor correlation between the percent change in baseline diameter (Pre vs. Post exercise) and mean SR (R^2=0.13; P>0.05).

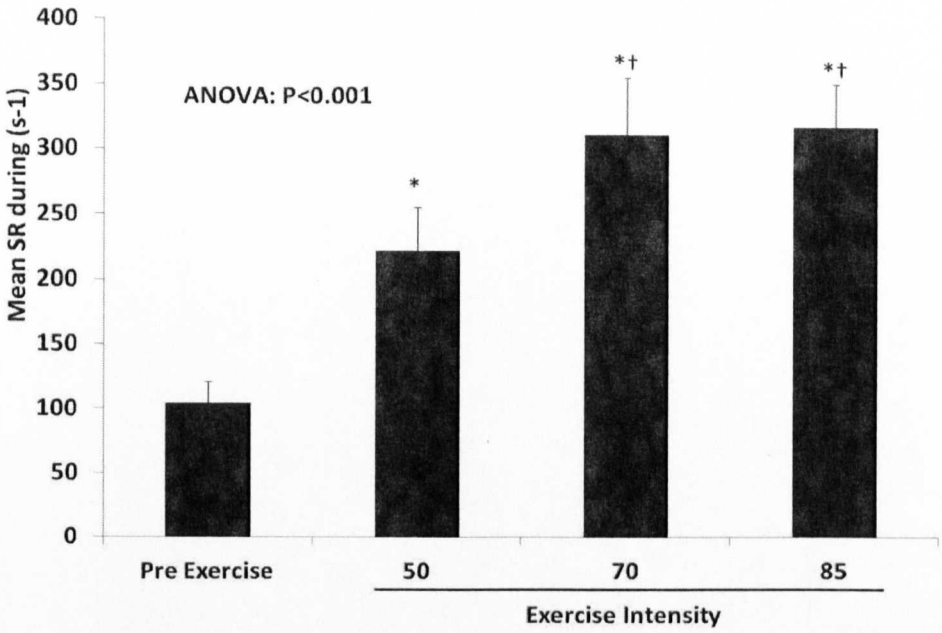

Figure 5.1 Brachial artery mean shear rate at rest and during a 30-min leg cycling exercise bout at 50, 70 and 85% of the individual maximal heart rate in healthy subjects (n=7). Error bars represent SE. Significantly different at $P<0.05$ from *pre-exercise, † significantly different from 50%HR_{max} ($P<0.05$).

No significant difference was found from pre-exercise to 1hr and subsequent values. Consequently, the immediate post-exercise changes were interrogated further. As diameter and SR can influence FMD further analysis was undertaken to correct for these covariates. Therefore, due to the substantial exercise-mediated changes in baseline diameter and SR_{AUC}, a GEE was performed for FMD with baseline diameter and SR_{AUC} entered as covariates. This analysis indicated that the change in FMD after exercise at 50%HR_{max} was negligible in practical terms, whilst larger decreases were apparent after 70%HR_{max} exercise and especially after 85%HR_{max} (Figure 5.2).

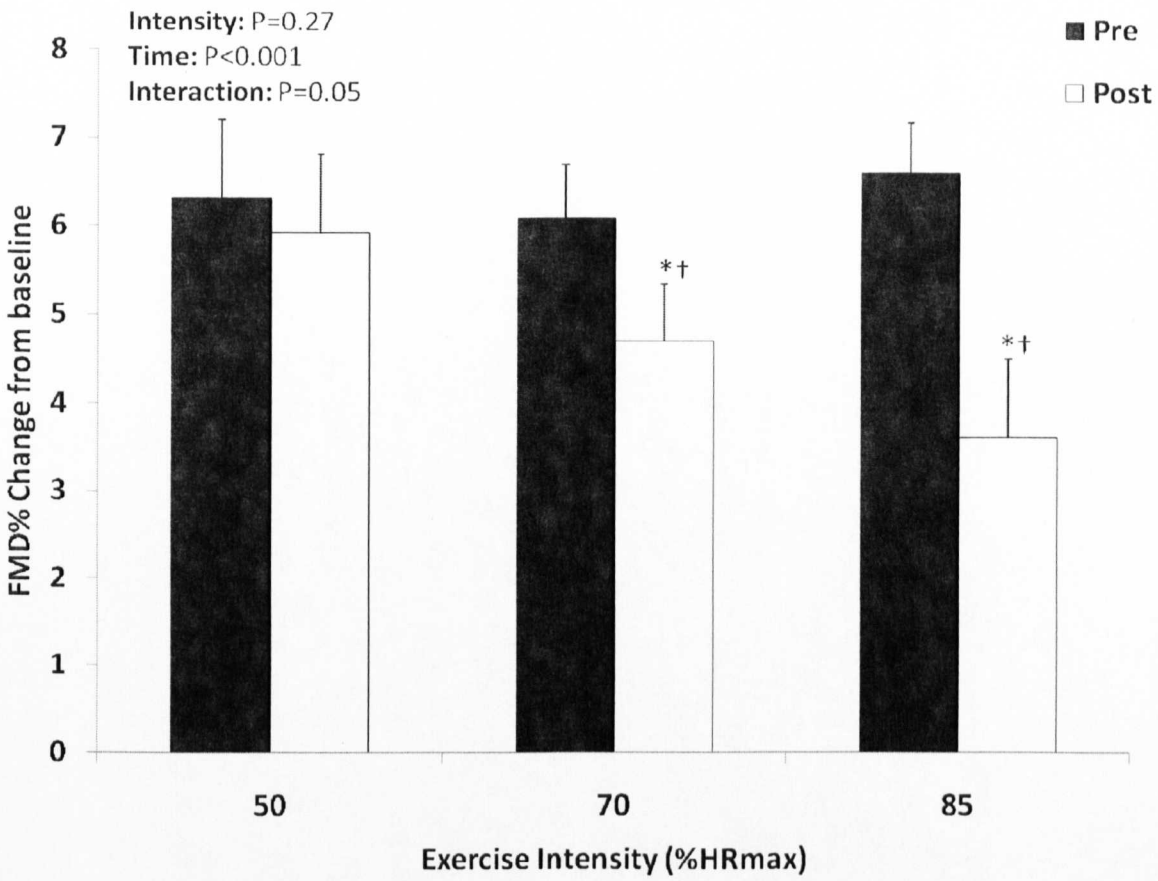

Figure 5.2 Brachial artery flow-mediated dilation (FMD, presented as a relative change from baseline diameter) before and immediately after a 30-minute leg cycling exercise bout performed at 50, 70 and 85% of the individual maximal heart rate in healthy subjects (n=10). Data are presented as mean ± SE. Data from the GEE are presented. *Post hoc statistically significantly different from pre-exercise, † statistically significantly different from 50%HR_{max} (P<0.05).

Time course. Although significant differences in baseline and peak diameter were found between pre-exercise and 1 and 2h after exercise at 70 and 85%HR_{max} (Table 5.3), it was observed that the immediate post-exercise decrease in FMD after 70 and 85%HR_{max} was normalised within 1 h after exercise (Table 5.3).

Table 5.3 Brachial artery characteristics before ('baseline') and after (1h, 2h and 24h) a 30-minute leg cycling exercise bout at 50, 70 and 85% of maximal heart rate in healthy subjects (n=10).

	Baseline	Post Exercise				P value
		0h	1h	2h	24h	
50%						
Baseline diameter (mm)	4.1±0.4	4.1±0.3	4.1±0.3	4.1±0.3	4.0±0.4	0.31
Peak diameter (mm)	4.3±0.5	4.4±0.4	4.4±0.4	4.4±0.4	4.3±0.4	0.68
FMD (%)	6.4±2.9	5.9±2.0	6.9±1.9	6.2±2.4	7.0±1.9	0.58
SR_{AUC} (s^{-1} $x10^3$)	21.8±13.2	26.9±8.4	19.8±9.9†	18.9±9.4†	28.1±11.5	0.06
70%						
Baseline diameter (mm)	3.9±0.4	4.2±0.4	4.1±0.4*	4.1±0.4*†	4.0±0.4†	<0.01
Peak diameter (mm)	4.2±0.5	4.4±0.4	4.3±0.4*	4.3±0.3*	4.2±0.4	0.02
FMD (%)	6.7±2.1	4.8±2.1	5.9±2.7	6.4±2.7	6.6±2.5†	0.03
SR_{AUC} (s^{-1} $x10^3$)	20.9±6.0	25.4±8.1	22.5±8.4	21.1±5.9	23.6±7.7	0.35
85%						
Baseline diameter (mm)	4.1±0.4	4.4±0.4	4.1±0.4†	4.2±0.4*†	4.0±0.4†	<0.01
Peak diameter (mm)	4.3±0.4	4.6±0.4	4.4±0.4†	4.5±0.4*	4.3±0.4†	<0.01
FMD (%)	6.1±1.2	3.4±1.2	6.8±2.7†	5.6±2.1†	6.2±2.5†	<0.01
SR_{AUC} (s^{-1} $x10^3$)	19.6±7.0	37.8±12.8	26.5±9.2†	22.6±11.3†	20.6±11.3†	<0.01

Data are presented mean ± SD. P-value represents one-way repeated measures ANOVA.*Post hoc significantly different from baseline. †post hoc significantly different from immediately post

5.4. Discussion

The purpose of the present study was to determine the magnitude of effect of 30 minutes leg cycling exercise at 3 exercise intensities (50, 70 and 85%) on brachial artery endothelium-dependent vasodilation immediately after cycle exercise. The principle finding from the present study was that FMD decreased to a greater degree immediately post exercise at higher exercise intensities compared with lower intensities. This decrease in FMD was accompanied by, but not fully explained by, increased baseline diameter and shear rate immediately post exercise. Taken together, data from the present study suggest that exercise intensity impacts upon the magnitude of FMD reduction in a 'dose-dependent' manner.

Exercise intensity plays an important role in modulating adaptations in vascular function with exercise training (Goto *et al.*, 2003; Green *et al.*, 2004). In a carefully performed study, Goto and colleagues (2003) introduced the notion that vascular adaptations may be dependent upon repetitive increases in shear stress, but that such adaptations may be offset to some degree at higher exercise intensities by effects of oxidative stress. More specifically, it was suggested that high intensity exercise may impair endothelium-dependent vasodilation due to an increase in reactive oxygen species, resulting in a reduction in NO bioavailability (Davies *et al.*, 1982; Bergholm *et al.*, 1999). Broadly speaking, the present study provides some support for this notion, as exercise at higher intensity was associated with larger acute impacts upon endothelial function. It is important to note that a decrease in endothelial function as a result of an acute bout of exercise may not necessarily be associated with down-regulation as an adaptive response. As highlighted by Padilla

et al. (Padilla *et al.*, 2011a), there are many examples in integrative human physiology of up-regulation to stimuli which challenge pathways acutely, a notion encapsulated in the concept of hormesis.

It is important to note that the largest decrease in FMD in the present study was also accompanied by the largest increase in immediately post-exercise baseline diameter. The observation of an increase in brachial artery diameter in response to lower limb exercise confirms the findings of a recent study which reported systemic, post-exercise increases in resting diameter (Padilla *et al.*, 2011b). This change in baseline diameter raises an important methodological issue regarding the calculation of FMD, as baseline diameter is used in this calculation. While expressing the change in diameter as a percentage of resting diameter is relatively simple, this expression does not fully control for the influence of baseline diameter *per se*, since it is well documented that baseline diameter is inversely related to FMD (Thijssen *et al.*, 2008a; Thijssen *et al.*, 2008c). Additionally, it has already been suggested that differences in baseline diameter, within or between subjects, should be accounted for with more appropriate allometric models involving covariate control of baseline diameter (Thijssen *et al.*, 2008a; Thijssen *et al.*, 2008c). Therefore, a novel analysis method was used to determine whether exercise-induced changes in baseline diameter immediately post exercise accounted for the observed effects on FMD. The dose-dependent decrease in FMD was found with this covariate-control for both baseline diameter and shear rate. Additionally, previously-published data indicate that up to 60% of the inter- and intra-subject variability in FMD is, in fact, explained by inter- and intra-subject differences in baseline diameter (Thijssen *et*

al., 2008a). This specific statistical analysis for pre and immediately post values only was used as there was a particular interest in the immediately post decrease in FMD. Values beyond this time point were analysed without correcting for baseline diameter as by 1 hour post exercise, values related to FMD had already normalised to near resting levels.

Shear stress stimulates the endothelium to release several vasodilators, in particular nitric oxide (Langille & O'Donnell, 1986; Pohl *et al.*, 1986). It has therefore been suggested that shear stress mediated NO production is a key stimulus for conduit artery vasodilation during exercise (Green *et al.*, 2004; Tinken *et al.*, 2009; Tinken *et al.*, 2010; Simmons *et al.*, 2011). However, an exercise intensity-dependent decrease in FMD was observed (Figure 5.2) and a relatively small influence of shear stress (compared with baseline diameter) on FMD. It is, nevertheless, conceivable that large and/or sustained increases in shear may be associated with attenuated endothelial function. For example, NO biosynthesis is dependent upon the continual availability of L-arginine (Jin & Loscalzo, 2010) and several co-factors. Whilst arginine stores do not usually limit NO production in healthy humans, precedent does exist whereby increased availability of this substrate enhances endothelial function, particularly where underlying NOS function is augmented in the face of enhanced NO degradation by oxidant species (Ohara *et al.*, 1993). It is therefore possible that higher levels of shear during intense exercise reduce the biosynthesis of NO and that increases in oxidative stress at higher exercise intensities amplify this (Goto *et al.*, 2003).

It was hypothesised that some of the disparate findings in the literature may relate to the timing of the post-exercise FMD and the time-course of change in endothelial function after acute exercise (Rognmo *et al.*, 2008; Tyldum *et al.*, 2009; Zhu *et al.*, 2010). The finding that FMD measured immediately post exercise was lower than at rest, but normalised within 1h, is in general agreement with most previous studies that examined the FMD immediately post-exercise (Thijssen *et al.*, 2006; Rundell *et al.*, 2007; Dawson *et al.*, 2008; Varady *et al.*, 2010). Other studies which used different timing of the post-exercise FMD measurements, have reported increases (Silvestro *et al.*, 2002; Harvey *et al.*, 2005; Padilla *et al.*, 2006; Thijssen *et al.*, 2006; Tinken *et al.*, 2009; Tyldum *et al.*, 2009). Taken together, these observations emphasise the importance of considering the time-course of post exercise measurements.

Limitations. A potential limitation of this study is that healthy male volunteers were recruited and these findings can therefore not be extrapolated to subjects with cardiovascular disease, women or older subjects. Additionally, indices of oxidative stress were not measured in the present study, but previous research has demonstrated that exercise at higher intensities, consistent with those observed in the present experiment, are associated with production and circulation of reactive oxidant species (Davies *et al.*, 1982; Ji, 1999; Cooper *et al.*, 2002). Studies have also attempted to overcome the production and circulation of reactant oxidant species using antioxidant strategies, with some suggesting that these approaches may modulate NO function (Raitakari *et al.*, 2000; Cooper *et al.*, 2002; Silvestro *et al.*, 2002; Eskurza *et al.*, 2004).

5.5. Conclusion

In conclusion, the present study demonstrates an 'exercise intensity-dependent' effect of leg exercise on brachial artery endothelial function. An immediate and substantial reduction in vasodilator function was observed at the higher exercise intensity, which may relate to the increased production of free radicals or increased oxidative stress, but does not relate to exercise-mediated changes in baseline diameter nor shear stress. This decrease in FMD may form the basis for adaptive vascular response to episodic exercise stimuli.

Aims and Outcomes of Chapter 6

Chapter 4 ***Aim:*** Examine the systemic effect of shear rate during exercise on post exercise brachial diameter.	*Chapter 4* ***Outcome:*** Dose-dependent increase in shear rate = Dose-dependent increase in brachial artery diameter.
Chapter 5 ***Aim:*** Address the role of shear rate on endothelial function following acute leg cycling exercise.	*Chapter 5* ***Outcome:*** Impairment in FMD post exercise is exercise intensity-dependent. FMD is only decreased immediately post exercise.
Chapter 6 ***Aim:*** Examine the role of shear rate in systemic adaptations in the brachial artery in response to leg cycle exercise training across 8-weeks.	*Chapter 6* ***Outcome:*** Rapid and transient increase in endothelial function No adaptation when shear is attenuated.

Chapter 6 | *Role of shear rate in systemic vascular adaptations in the non-active upper limbs during cycling in humans.*

Based on the published article in the Journal of Applied Physiology (2012) entitled, *Brachial artery adaptation to lower limb exercise training: role of shear stress.*

6.1. Introduction

The beneficial effects of exercise training may partly be mediated through direct effects of exercise on the vasculature (Green *et al.*, 2008b; Joyner & Green, 2009). It has been reported that exercise training leads to localised improvements in vascular function and structure in the active regions (Tinken *et al.*, 2010), which may be mediated through repetitive increases in shear stress (Tinken *et al.*, 2009; Tinken *et al.*, 2010; Naylor *et al.*, 2011). Exercise training also results in systemic vascular adaptations beyond the active regions (Linke *et al.*, 2001; Maiorana *et al.*, 2003; Green, 2005b). However, to date, relatively little is known about the hemodynamic stimuli responsible for systemic effects of large muscle group exercise training on the vasculature.

One potential explanation for systemic vascular adaptations to exercise training relates to shear stress. It is has been demonstrated that shear increases in the inactive upper limbs in a 'dose-dependent' manner during leg cycling (Green *et al.*, 2005; Thijssen *et al.*, 2009a; Carter *et al.*, 2011) and others have recently confirmed these observations (Green *et al.*, 2002b; Padilla *et al.*, 2011a; Padilla *et al.*, 2011b). Although the average increase in shear rate (SR) in the upper limbs is smaller than that observed in the active lower limbs (Thijssen *et al.*, 2009a), or in response to handgrip exercise (Green *et al.*, 2005), this can mask substantial changes in the pattern of flow and shear which may contribute to vascular adaptations in the non-active regions as a result of training (Tinken *et al.*, 2009). Therefore, the purpose of this study was to examine upper limb brachial artery endothelium-dependent and -independent dilation before, during and after 8 weeks of lower limb cycling training

in healthy humans. To specifically address the role of shear, subjects exercised with a cuff around one forearm to unilaterally manipulate the exercise-induced increases in this variable (Tinken *et al.*, 2009; Green *et al.*, 2010; Tinken *et al.*, 2010; Naylor *et al.*, 2011). Therefore, it is hypothesised that cycle exercise training would lead to improvements in endothelial function in the non-active upper limb and that such adaptation would be shear stress mediated.

6.2. Methods

6.2.1. Subjects

Eleven healthy men (22±2 years) were recruited to examine the effects of 8-weeks cycle exercise on brachial artery endothelial function in the non-cuffed and cuffed arms. Subjects were healthy; none reported having been diagnosed with cardiovascular diseases, diabetes, insulin resistance or cardiovascular risk factors, such as hypercholesterolemia or hypertension. Subjects who smoked or were on medication of any type were excluded. All subjects were recreationally active (1-5 hours of physical activity per week). Informed consent was gained from all subjects prior to the experimental procedures. The study procedures were approved by the Liverpool John Moores Ethics Committee and adhered to the Declaration of Helsinki.

6.2.2. Experimental design

Effect of upright cycle ergometer exercise on brachial artery shear stress: Impact of forearm cuff inflation. Five additional healthy male subjects (24±2 years), distinct

from those studied above, were recruited to undertake 30 mins of cycle exercise (Monark 874E, Sweden) at 80%HR_{max}. Throughout this exercise bout, a pneumatic cuff was placed around one forearm immediately below the cubital crease and inflated to 60mmHg (Figure 6.1A). The purpose of the forearm cuff is to reduce the exercise-induced increase in blood flow and shear rate in the upstream brachial artery compared with the contralateral arm (Tinken *et al.*, 2009; Tinken *et al.*, 2010) . The contralateral arm remained uncuffed during cycle exercise. Brachial artery diameter and velocity values were simultaneously collected immediately prior to exercise (before cuff inflation) and at 25 minutes during exercise with the cuff inflated. Briefly, subjects rested for 20-minutes whilst seated at rest on a stationary bicycle ergometer in a quiet, temperature controlled room. Baseline bilateral brachial artery diameter and velocity were recorded using high resolution duplex ultrasound for at least 1-minute. Subsequently, subjects performed leg cycling exercise for 30-minutes at 80%HR_{max}. Brachial artery blood flow and shear were recorded during the leg cycling exercise intervention in both the cuffed and non-cuffed arms (Figure 6.1B). Previous studies have demonstrated that placement and inflation of a forearm cuff in this manner attenuates upstream brachial artery shear rate (Thijssen *et al.*, 2009b; Tinken *et al.*, 2009; Green *et al.*, 2010; Tinken *et al.*, 2010; Naylor *et al.*, 2011). For analysis of brachial artery diameter, blood flow and shear rate please refer to Sections 3.4 and 3.5 in the General methods, Chapter 3.

Effect of cycle ergometer training on brachial artery adaptation. Exercise training was performed over an 8-week period with subjects visiting the laboratory 3 times per week. Each laboratory session was supervised and consisted of 30-mins of cycle

exercise (80%HR_{max}) performed at the same time of day. During each 30-min training session, a pneumatic blood pressure cuff was placed below the cubital crease on one forearm and inflated to 60 mmHg throughout the exercise period (Figure 6.1B). Cuff inflation to 60mmHg was selected on the basis of previous experimentation (Tinken *et al.*, 2009; Green *et al.*, 2010; Naylor *et al.*, 2011) in which unilateral cuff inflation was effective in modifying blood flow and shear rate during exercise. The arm selected for cuff placement was randomised, but once selected remained consistent for each subject across the 8-week training period.

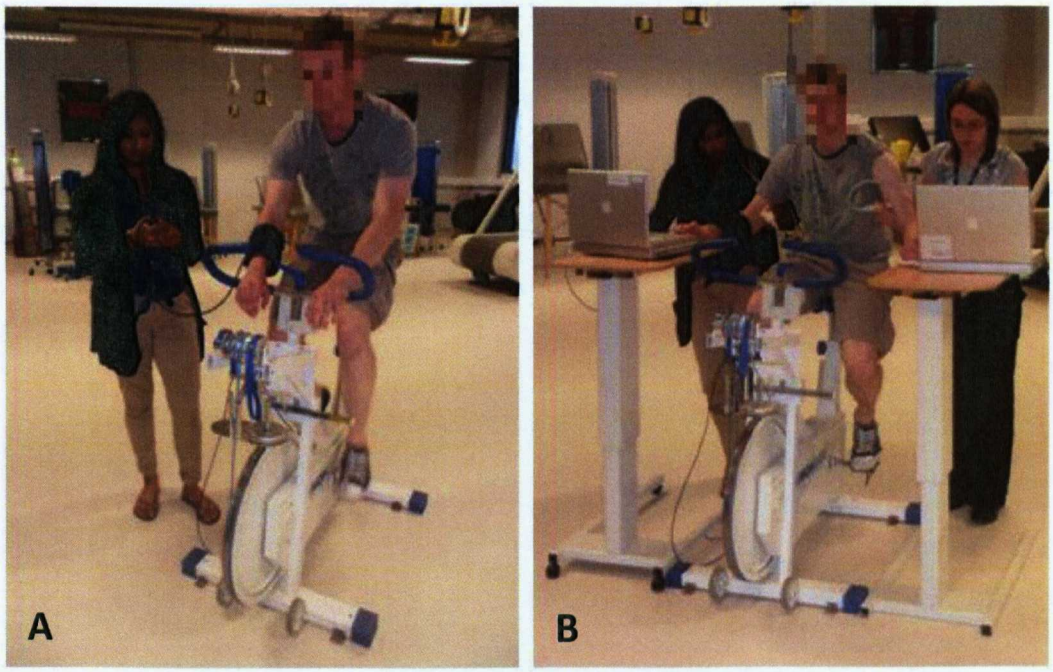

Figure 6.1 (A) Participant during a cycle exercise training session, with one forearm receiving attenuation of shear rate via cuff inflation (B) Measurement of brachial artery diameter, blood flow and shear rate during leg cycling exercise, with one forearm receiving attenuation of shear rate via cuff inflation.

6.2.3. Experimental measures

All studies were conducted in a quiet, temperature controlled environment and each visit for a given subject was performed at the same time of day. Subjects were asked to fast for >4 hours, abstain from alcohol and caffeine for 16 hours, and not to perform any exercise for 24 hours. Study procedures were performed according to recent guidelines (Thijssen *et al.*, 2011a). To examine the role of shear rate in systemic vascular adaptation to 8-weeks of leg cycle training, bilateral baseline FMD, brachial artery diameter response to an ischaemic exercise protocol (iEX) and dilator responses to sub-lingual glyceryl trinitrate administration (400μg GTN) was assessed. These measures were repeated at 2, 4 and 8-weeks (Tinken *et al.*, 2008). For a full description of FMD, iEX and GTN procedures and analysis please refer to Sections 3.3, 3.4 and 3.5 in the General Methods, Chapter 3.

6.2.4. Statistical analysis

Initially, a two-factor ANOVA with repeated measures (with time and cuff placement as the independent factors) was performed for the initial acute blood flow data. Additional, repeated measures ANOVA (with time and cuff placement as independent factors) were used to assess changes in brachial artery vasodilation in response to FMD, iEX and GTN across the 8-week intervention period. Post-hoc analysis *t*-tests were used where significant values were found.

6.3. Results

6.3.1. Acute effects of leg cycling exercise

Mean and antegrade shear rate significantly increased from baseline in both arms ($P<0.05$). Mean and antegrade SR were both significantly larger in the non-cuffed versus cuffed arm during exercise (Figure 6.2; $P<0.05$). In addition brachial artery retrograde shear rate was significantly larger in the cuffed, compared to the non-cuffed arm (Figure 6.2), during exercise.

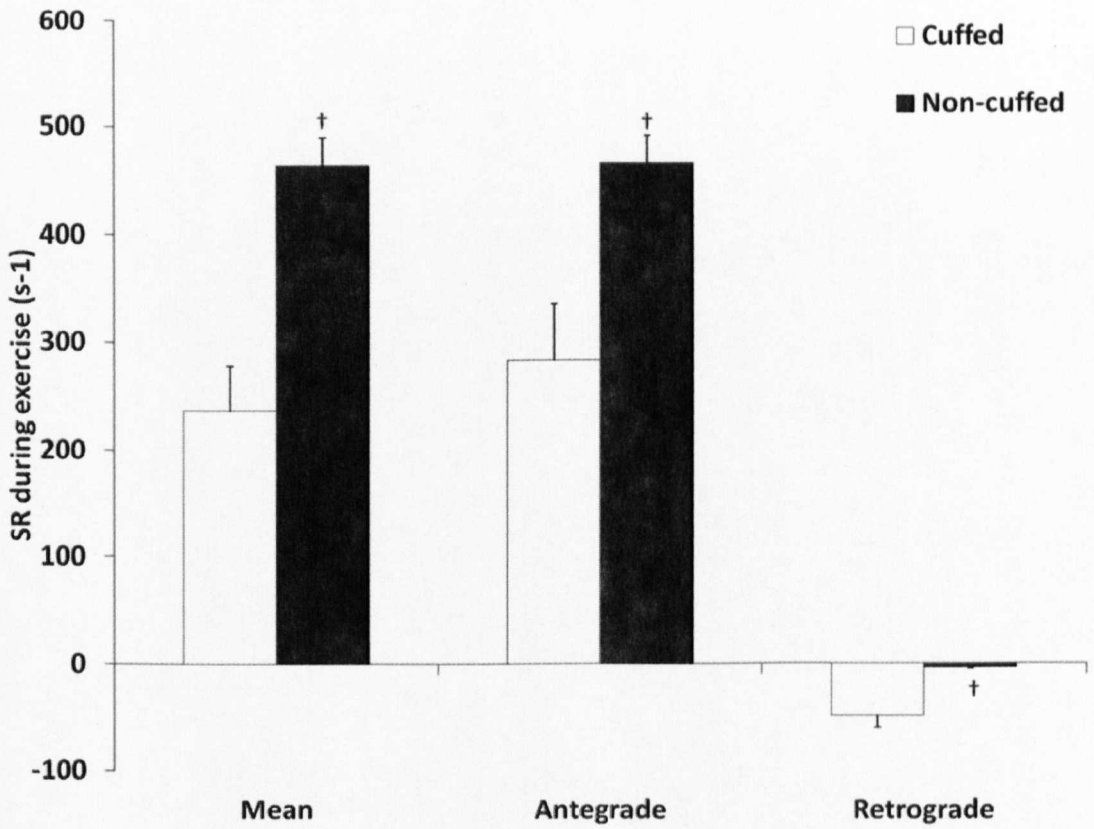

Figure 6.2 Brachial artery mean, antegrade and retrograde shear rate during cycle exercise in the cuffed and non-cuffed arms of healthy, young volunteers (n=5). †Post hoc *t-test* significantly different from cuffed at $P<0.05$.

6.3.2. Chronic effects of eight weeks leg cycling exercise training

Two subjects were unable to complete the study for personal reasons unrelated to the study or its protocols. Across the 8-week exercise training period, there was 91.7% adherence to the training sessions in the remaining 9 subjects. There were no significant changes in mean arterial blood pressure (87±6, 86±7, 87±4, and 85±6, ANOVA: P=0.81) or heart rate (64±3, 61±9, 57±7, and 60±7, ANOVA: P=0.09) between weeks 0, 2, 4 and 8 of leg cycle training. There were no differences in baseline brachial artery characteristics between arms at week 0 (Table 6.1).

Table 6.1 Brachial artery characteristics at 0, 2, 4 and 8 weeks during leg cycle training, in the cuffed and non-cuffed arm.

Variable	Week 0	Week 2	Week 4	Week 8	P-value
Resting diameter (mm)					
Cuffed	3.7±0.6	3.6±0.8	3.7±0.4	3.7±0.4	'time': 0.78
Non-cuffed	3.8±0.4	3.8±0.5	3.7±0.3	3.9±0.4	'time*arm': 0.56
FMD time-to-peak (s)					
Cuffed	44±13	46±16	55±23	43±16	'time': 0.92
Non-cuffed	57±20	49±17	49±14	63±37	'time*arm': 0.27
FMD SR_{AUC} ($s^{-1}x10^3$)					
Cuffed	17.7±7.7	21.6±13.9	19.6±7.7	20.6±9.2	'time': 0.65
Non-cuffed	17.9±9.0	21.2±7.1	23.2±11.3	20.1±9.7	'time*arm': 0.80
iEX (%)					
Cuffed	16.3±5.7	14.8±4.5	14.5±7.0	14.3±4.7	'time': 0.56
Non-cuffed	12.8±4.3	12.9±4.0	14.3±4.7	12.0±5.2	'time*arm': 0.40

Values are means ± SD. FMD, flow-mediated dilatation; iEX, ischaemic handgrip exercise, SR_{AUC}, shear rate area-underneath-curve.

Brachial artery flow mediated vasodilator (FMD) responses to ischaemia. Cycle exercise training induced a significant increase in brachial artery FMD (ANOVA, P=0.04; Figure 6.3A). Post-hoc analysis revealed that brachial artery FMD in the non-cuffed arm increased at week 2 (P<0.05 *vs.* week 0), before returning to near baseline values at weeks 4 and 8 (Figure 6.3A). In the cuffed arm, no changes in FMD were evident at any time point (Figure 6.3A). No change was evident across the 8-week training period in the eliciting shear stress stimulus (SR_{AUC}) to FMD, baseline diameters or time-to-peak dilation (Table 6.1).

Brachial artery responses to ischaemic exercise (iEX). In the non-cuffed and cuffed arms, the brachial artery dilator responses to ischaemic exercise did not significantly differ across the training intervention period (ANOVA, P=0.56; Table 6.1).

Endothelium-independent, NO-mediated dilation (GTN) responses. Leg cycling exercise training induced a significant change in brachial artery GTN by two-way ANOVA (time factor P<0.01) (Figure 6.3B). Post hoc analysis revealed non-significant decreases in GTN responses between weeks 0, 2 and 4, with a significantly increase in GTN in both arms between week 4 and 8. Nonetheless, no significant change in GTN was apparent in either arm when week 8 and week 0 data were directly compared.

FMD/GTN. The ratio of FMD to GTN responses was calculated to reflect changes in endothelial function, were normalised to smooth muscle adaptation. This data reinforced the FMD results, in that the ratio in the non-cuffed was significantly increased at weeks 2 and 4 (ANOVA: P<0.01, Figure 6.3C), relative to week 0, with

week 8 data not significantly different from baseline. No significant changes were apparent in the cuffed limb (Figure 6.3C).

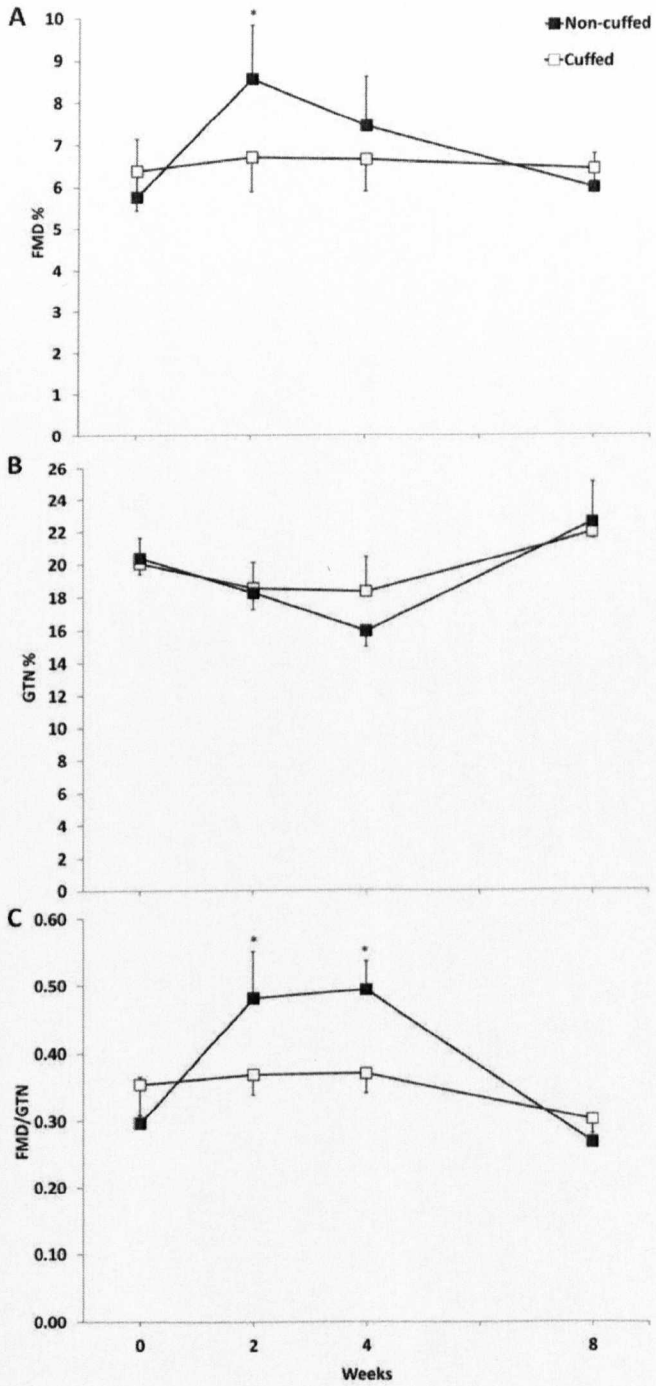

Figure 6.3 (A) Brachial artery endothelium-dependent (FMD), (B) independent (GTN; n=8) and (C) FMD-to-GTN ratio data in the cuffed (white) and non-cuffed (black) arms across an 8 week leg cycle training intervention. Data are presented as mean ± SE. * Post hoc *t-test* significantly different from cuffed at $P<0.05$.

6.4. Discussion

The purpose of the present study was to determine the impact of shear rate on brachial artery endothelium-dependent and -independent function in response to lower limb cycle ergometer training. The principle of the present study was that a rapid increase in endothelium-dependent and largely NO-mediated vasodilator function was apparent in the limb exposed to increased shear stress during leg training, but not in the contralateral cuffed limb in which shear was attenuated. Changes in FMD in the non-cuffed arm were transient, returning to near baseline values at week 8. When FMD responses were normalised for changes in smooth muscle sensitivity, the FMD/GTN ratios also revealed significant increases in artery function in the non-cuffed arm between weeks 0, 2 and 4, with no differences between weeks 0 and 8. These data suggest that lower limb exercise induces vascular functional changes evident in the untrained upper limbs which are attributable, at least in part, to the systemic effects of lower limb exercise on shear stress.

In the present study, cycle exercise training induced endothelial adaptations in brachial artery of the non-cuffed arm only. This is in line with recent studies which examined the impact of shear rate manipulation in response to localised handgrip exercise training (Tinken *et al.*, 2010) and bilateral forearm heating (Green *et al.*, 2010; Naylor *et al.*, 2011) in the presence and absence of cuff inflation. Collectively, these studies suggest that shear stress is a key stimulus responsible for adaptation in conduit artery function in humans. The transient nature of endothelial adaptations, with resolution by 8 weeks, has previously been described and

discussed in detail (Tinken *et al.*, 2008; Naylor *et al.*, 2011). In response to handgrip exercise, the return of FMD to baseline levels was accompanied by increases in iEX mediated dilation, suggesting either that functional changes are superseded by structural remodelling (Green *et al.*, 2011) or that adaptation in endothelial function other than that associated with NO may occur over time. However, in the present study, no changes in GTN or iEX mediated dilation were apparent at 8 weeks. Whilst the possibility that late changes occur in the function or remodelling of upper limb arteries in response to lower limb cycle exercise cannot be excluded, this seems unlikely based on the findings of the current study and there is limited evidence for systemic effects of exercise on arterial remodelling in humans (Silber *et al.*, 1991; Green *et al.*, 2008a). Indeed, comparison of specific groups of upper or lower limb dominant athletes (Huonker *et al.*, 2003), including the preferred and non-preferred limbs of racquet sportsmen (Green *et al.*, 1996; Rowley *et al.*, 2011), indicate that changes in artery size predominantly occur in response to local, rather than systemic, stimuli. This may suggest that the magnitude and/or pattern of shear stress in the brachial artery in response 8 weeks of lower limb exercise is insufficient to induce structural adaptation, despite evidence for initial changes in endothelial function.

The observation that exercise induces shear stress mediated changes in NO signal transduction is not new. In humans, Hambrecht demonstrated up-regulation of eNOS mRNA and protein, including shear stress sensitive phosphorylation components of the enzyme (Hambrecht *et al.*, 2003). Whilst this paper linked exercise and shear stress to eNOS adaptation, it did not directly manipulate or

measure shear stress. Other studies have reported that leg exercise training can induce chronic changes in upper limb resistance and conduit artery function (Maiorana *et al.*, 2003; Gielen & Hambrecht, 2004; Green *et al.*, 2008a). However, the findings in the present study are novel in that they provide the first evidence in humans that exercise of large muscle groups, associated with hemodynamic changes in blood pressure and cardiac output, is also associated with systemic changes in artery function which are mediated by shear stress. The implications of this study are that exercise is capable of inducing systemic benefits in artery function which may be anti-atherogenic.

The mean brachial artery shear stress was characterised in the cuffed and non-cuffed limbs during leg cycle ergometry in the present study, and also the antegrade and retrograde components of shear (and flow). Recent studies indicate that acute bouts of exercise which alter shear are associated with immediate increases in FMD (Tinken *et al.*, 2009). However, the acute FMD response to exercise bouts may be complex, as interventions which increase retrograde shear can be associated with decreases in FMD (Thijssen *et al.*, 2010). The intensity of exercise is relevant in this context as it impacts upon the substrate availability, oxidant effects of exercise which may scavenge NO and direct signal transduction mediated by antegrade/retrograde patterns of shear. In the present study, measures assessed at the end of the 80%HR_{max} exercise bout revealed modest levels of retrograde shear compared to previous cycle studies (Green *et al.*, 2005; Tinken *et al.*, 2009). The explanation for this may be provided by Simmons *et al.*, (2011) who recently demonstrated that retrograde brachial artery shear in response to cycle exercise

decreases with exercise duration, in association with skin vasodilation sub serving thermoregulatory demand (Simmons *et al.*, 2011). Previous cycle studies, which demonstrated a larger retrograde shear component, involved shorter bouts of exercise (Green *et al.*, 2002c; Green *et al.*, 2005) or measures collected at earlier exercise time points at lower intensities of effort.

Limitations. This study involved young healthy male volunteers and the findings from the present study cannot be extrapolated to subjects with cardiovascular disease, or older individuals or women. Vascular function is affected by the sympathetic nervous system, but it seems unlikely that systemic changes in neural activation could example the unilateral impacts of training observed in the present study. Similarly, circulating hormonal impacts of training would, logically, have elicited bilateral changes in artery function, as would also have been the case for direct effects of blood pressure on functional arterial adaptation. Finally, all vasodilator or constrictor pathways were not assessed and it is possible that functional adaptations that superseded the FMD response were missed.

6.5. Conclusion

In conclusion, findings from the present study indicate that systemic adaptation in flow-mediated endothelial function occurs in the non-exercising upper limbs as a result of leg exercise training and that this response is at least partly explained by the impact of cycle exercise bouts on brachial artery shear stress. These findings are the first, to my knowledge, to indicate a systemic effect of exercise training via shear stress dependent mechanisms. Exercise is an intervention which elicits systemic vascular benefit in humans.

Aims and Outcomes of Chapter 7

Chapter 4 ***Aim:*** Examine the systemic effect of shear rate during exercise on post exercise brachial diameter.	*Chapter 4* ***Outcome:*** Dose-dependent increase in shear rate = Dose-dependent increase in brachial artery diameter.
Chapter 5 ***Aim:*** Address the role of shear rate on endothelial function following acute leg cycling exercise.	*Chapter 5* ***Outcome:*** Impairment in FMD post exercise is exercise intensity-dependent. FMD is only decreased immediately post exercise.
Chapter 6 ***Aim:*** Examine the role of shear rate in systemic adaptations in the brachial artery in response to leg cycle exercise training across 8-weeks.	*Chapter 6* ***Outcome:*** Rapid and transient increase in endothelial function No adaptation when shear is attenuated.
Chapter 7 ***Aim:*** examine the effects of acute localised forearm inactivity and associated vascular responses.	*Chapter 7* ***Outcome:*** Significant decrease in peak reactive hyperaemia and forearm girth, but only in the arm that received local forearm inactivity.

Chapter 7 | *Unilateral forearm inactivity on endothelial function in humans.*

7.1. Introduction

Physical inactivity is an independent risk factor for atherosclerosis and cardiovascular diseases (Blair *et al.*, 1989; Manson *et al.*, 1999; Thijssen *et al.*, 2010). Given the low daily energy expenditure, which is characteristic of modern living (Booth *et al.*, 2002), the consequences of physical inactivity are likely to worsen. Recent data suggest that the impact of inactivity cannot be fully explained through modification of traditional risk factors (Thijssen *et al.*, 2010). An alternative explanation for the link between inactivity and cardiovascular events relates to direct effects on vascular remodeling and function (Joyner & Green, 2009).

Rapid improvements in nitric oxide (NO)-mediated endothelial function have been observed during exercise training (Tinken *et al.*, 2008; Tinken *et al.*, 2009). Data derived from animals (Laughlin, 1995) and humans (Tinken *et al.*, 2008) indicate that these rapid increases in vascular function precede structural remodeling during exercise training. Indeed, prolonged exercise training is associated with outward remodeling of artery diameter (Rowley *et al.*, 2011). Prolonged inactivity is also associated with a strong dose-dependent structural inward remodeling of arterial diameter (reviews (de Groot *et al.*, 2006a; Thijssen *et al.*, 2010; Thijssen *et al.*, 2011b)). However, the NO-mediated endothelial function shows no change or even an increase after prolonged inactivity (Bonnin *et al.*, 2001; de Groot *et al.*, 2004; Bleeker *et al.*, 2005a; Bleeker *et al.*, 2005b). It is proposed that changes in NO-mediated endothelial function during physical activity occur *before* structural remodeling. However, little is known about the immediate effects of physical inactivity on vascular function.

The purpose of this study, therefore, was to measure (largely and partly) nitric oxide (NO)-mediated endothelial-dependent dilation and endothelial-independent dilation in humans, before, during (4 days) and after a brief period (8 days) of unilateral forearm inactivity. Since previous models of physical inactivity are associated with practical limitations (i.e. spinal cord injury), non-specificity of local/systemic stimuli (i.e. bed rest), inflammation (i.e. casting after fracture) or health risks (i.e. unilateral lower limbs suspension) (see (Thijssen *et al.*, 2010)), local forearm inactivity was induced using a sling. Therefore, it is hypothesised that forearm inactivity leads to an acute impairment of endothelial function, without the presence of a change in vascular structure.

7.2. Methods

7.2.1. Subjects

Thirteen young male subjects (22±1years) were recruited. Subjects were healthy; none reported having been diagnosed with cardiovascular diseases, diabetes, insulin resistance or cardiovascular risk factors, such as hypercholesterolemia or hypertension. Subjects who smoked or were on medication of any type were excluded. All subjects were recreationally active (2-5 hours of physical activity per week). Informed consent was gained from all subjects prior to the experimental procedures. The study procedures were approved by the Liverpool John Moores Ethics Committee and adhered to the Declaration of Helsinki.

7.2.2. Experimental design

Subjects reported to the laboratory for assessment of brachial artery endothelium-dependent and independent vasodilation on three different occasions; before, during (day 4) and after the 8-day intervention. Local physical inactivity was induced by a sling around the non-dominant forearm (Figure 7.1). Subjects were instructed to wear the sling continuously and were only allowed to remove the sling for sleeping and for personal hygiene purposes (e.g. showering).

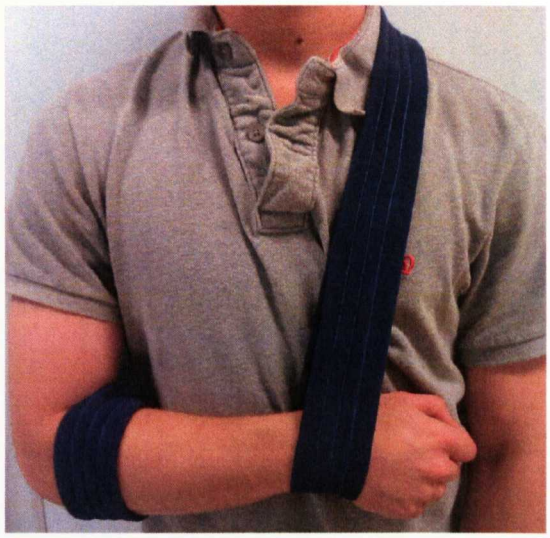

Figure 7.1 Participant wearing "sling" intervention to impose localised inactivity of the forearm.

7.2.3. Experimental procedures

Subjects were instructed to fast for >4 hours, abstain from alcohol and caffeine for 16 hours, and not to perform any exercise for 24 hours (Thijssen *et al.*, 2011a) prior to each laboratory attendance. Upon arrival at the laboratory, body mass (electronic scales, SECA, United Kingdom) and height were measured. Bilateral forearm volume, girth and maximal handgrip strength were also measured. Subjects were then required to rest in a supine position for 20 minutes, followed by assessment of heart rate and blood pressure using an automated sphygmomanometer (GE Pro 300V2, Dinamap, Tampa, Fl).

This was followed by assessment of brachial artery blood flow pattern and brachial artery endothelial-dependent and -independent vasodilation. To examine the impact of localised forearm inactivity on vascular adaptations, bilateral baseline FMD, brachial artery diameter response to an ischaemic exercise protocol (iEX), reactive hyperaemic blood flow (RHBF) and dilator responses to sub-lingual glyceryl trinitrate administration (400μg GTN) were assessed. These measures were repeated at 4 and 8 days of wearing the sling intervention. For a full description of FMD, iEX and GTN procedures and analysis please refer to Sections 3.3, 3.4 and 3.5 in the General Methods, Chapter 3.

7.2.4. Anthropometry

Maximal forearm girths were assessed using a diameter tape (Lufkin, Mexico) at the widest part of the forearm. Forearm volume was determined by the water displacement technique, by immersion of the forearm to the cubital crease. Three measurements of forearm girth and forearm volume were taken on each arm, subsequently calculating the mean of these 3 measures. Maximal voluntary contraction (MVC) of both forearms was assessed using a handgrip dynamometer (Stoelting, Wood Dale, Illinois, USA). Subjects were instructed to perform a 1-second maximal handgrip, followed by 2 minute rest. During this 2 minute rest, subjects performed a MVC using the contra-lateral arm. This procedure was repeated 3 times, and the MVC was calculated as the mean of 3 measurements.

7.2.5. Statistical analysis

A two-factor ANOVA with repeated measures (with time as the independent factor) were used to assess all brachial artery characteristics. A one-factor ANOVA was used for analysis of resting heart rate, MAP, systolic and diastolic BP. Post hoc *t*-tests were used where significant values were found.

7.3. Results

7.3.1. Anthropometric data

Before the sling intervention, there were no significant differences between both arms for forearm girth, volume or strength (Table 7.1). The sling-intervention induced a significant decrease in maximum voluntary contraction in the intervention arm (Post-hoc P=0.03 for Day 0 *versus* Day 8), but not in the control arm (Table 7.1). Analysis of forearm girth revealed a significant interaction-effect across the 8-day period (2-way ANOVA interaction: P=0.05), post hoc analysis revealed a significant decrease in forearm girth between arms at day 8. No change in forearm volume was observed across the 8-day intervention in either arm (Table 7.1).

7.3.2. Brachial artery vascular measurements

Blood flow pattern. No significant changes were observed in baseline mean, antegrade or retrograde blood flow across the 8-day intervention (Table 7.2).

Endothelium dependent, nitric oxide and low-mediated dilation (FMD). The 8-days sling intervention induced no change in brachial artery FMD in either arm (Figure 7.2A). Similarly, when FMD was presented as absolute change from baseline (mm), no effect of the sling-intervention were observed (Table 7.2). No change in resting diameter or shear rate area-under-the-curve across the 8-day intervention was evident in either arm (Table 7.2).

Table 7.1 Characteristics of subjects (n=13) at 0, 4 and 8 days. Forearm characteristics are presented for the intervention (sling) and non-intervention arm (control).

Variable	Day 0	Day 4	Day 8	P-value
Age (years)	22±1			
Height (m)	1.75±0.07			
Weight (kg)	69.7±9.1			
BMI (kg/m^2)	22.7±2.4			
Systolic BP (mmHg)	132±11	131±11	127±9	0.08
Diastolic BP (mmHg)	65±8	65±7	65±7	0.99
Mean BP (mmHg)	88±8	87±7	86±7	0.15
Heart rate (bpm)	52±11	57±7*	55±8	**0.05**
Grip strength (kg)				
Sling	42±8	39±9	38±9	'time' 0.07
Control	44±9	42±8	41±6	'time*arm' 0.85
Forearm volume (ml)				
Sling	1409±202	1364±248	1393±193	'time' 0.23
Control	1434±171	1391±201	1420±174	'time*arm' 0.99
Forearm girth (cm)				
Sling	26.7±1.9	26.6±1.7	26.5±2.0†	'time' 0.72
Control	26.9±1.9	27.1±1.7	27.0±1.9	**'time*arm' 0.05**

P-value refers to a one-way ANOVA or two-way ANOVA (for 'time', 'group' and 'time*group'). †Post-hoc significant from control arm at day 8. Values are means ± SD; BMI, body mass index; BP, blood pressure.

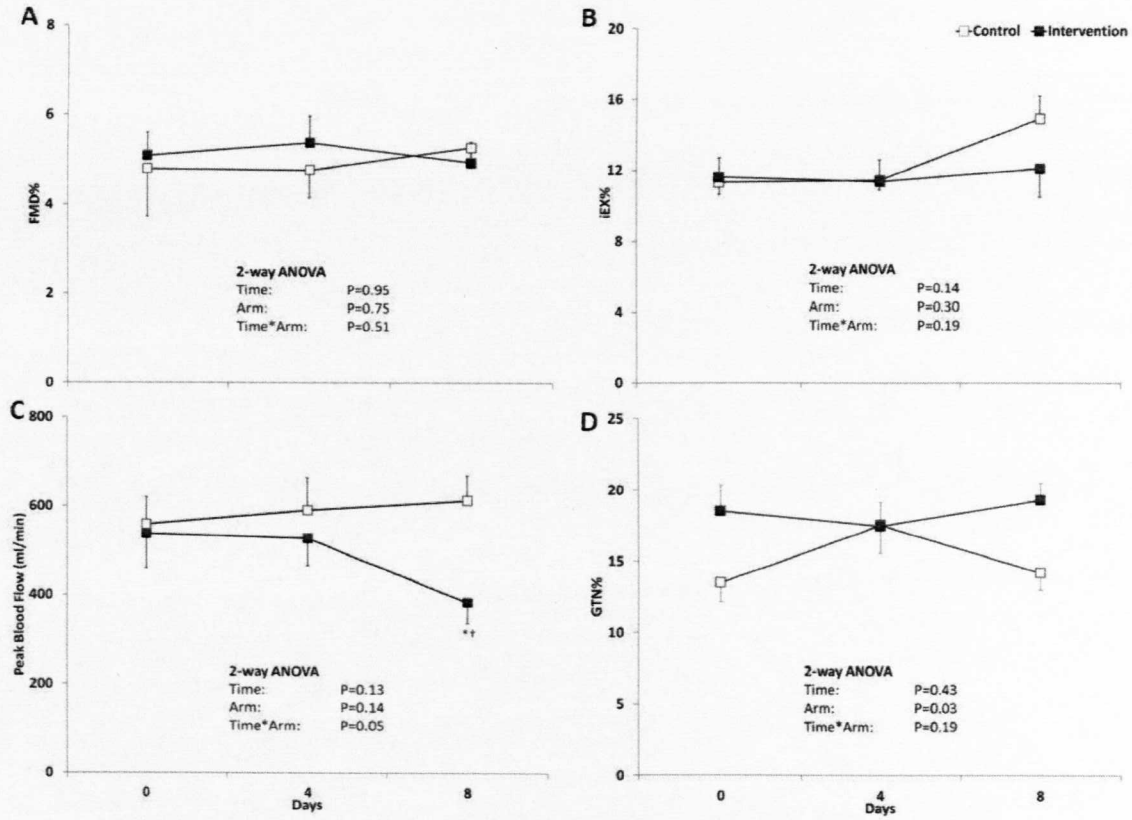

Figure 7.2 Brachial artery nitric oxide-mediated dilation (flow-mediated dilation (FMD), A), partly NO-mediated dilation (ischaemic exercise (iEX), B), peak blood flow (C), and NO-mediated endothelium-independent dilation (glyceryl trinitrate (GTN, D) in the control (solid square) and sling intervention (open square) arms across the 8-day intervention. Due to technical problems 4 subjects were removed for the analysis of iEX peak blood flow (n=9) and 3 subjects were removed from the GTN analysis (n=10). Error bars represent SE.* significantly different from 0 at P<0.05; †significantly different from control arm at day 8.

Table 7.2. Brachial artery characteristics at 0, 4 and 8 days during bracing intervention, in the sling and control arm.

Variable	Day 0	Day 4	Day 8	P-value
Resting diameter (mm)				
Sling	4.0±0.5	3.9±0.5	3.8±0.4	'time': 0.46
Control	4.0±0.6	3.9±0.6	3.9±0.6	'time*arm': 0.46
Mean blood flow (ml/min)				
Sling	83±10	97±20	69±14	'time': 0.41
Control	72±8	67±.11	60±5	'time*arm': 0.67
Antegrade blood flow (ml/min)				
Sling	102±13	118±20	94±13	'time': 0.51
Control	89±8	92±9	79±7	'time*arm'; 0.80
Retrograde blood flow (ml/min)				
Sling	-19±5	-21±5	-26±9	'time': 0.20
Control	-18±4	-26±4	-20±5	'time*arm': 0.16
SR_{AUC} (s^{-1} $x10^3$)				
Sling	16.5±10.2	20.7±11.6	16.1±5.8	'time': 0.50
Control	19.2±7.1	18.4±9.7	17.6±6.4	'time*arm': 0.29
GTN peak diameter (mm)				
Sling	4.7±0.7	4.5±0.4	4.5±0.4	'time': 0.19
Control	4.7±0.7	4.7±0.6	4.7±0.5	'time*arm': 0.78

Values are means ± SD. FMD, flow-mediated dilatation; iEX, ischaemic handgrip exercise, GTN; glyceryl trinitrate; SR_{AUC}, shear rate area-underneath-curve. GTN, n=10, 3 subjects removed due to technical reasons.

Endothelium-dependent responses to ischaemic exercise (iEX). The absolute (mm) and relative (%) change in brachial artery diameter after ischaemic handgrip did not differ significantly across the intervention period in the intervention arm or in the control arm (Table 7.2, Figure 7.2B). Analysis of peak blood flow revealed a significant interaction effect (2-way ANOVA, time*arm; P=0.05). Post-hoc analysis revealed a significant decrease in peak blood flow in the intervention arm at day 8 compared to baseline, but no change in the control arm (Figure 7.2C).

Endothelium independent; glyceryl trinitrate mediated vasodilation (GTN). Brachial artery GTN responses in both the active and inactive arm did not change across the 8-days intervention (Figure 7.2D).

7.4. Discussion

The purpose of the present study was to examine the immediate effect of physical inactivity, induced by wearing a sling, on brachial artery endothelial function in humans. It was observed that wearing the sling induced a trend for a significant decrease in handgrip strength and a significant reduction in peak blood flow, indicating that the intervention successfully induced physical inactivity as well as inward remodelling of forearm resistance vessels (Folkow *et al.*, 1958; Conway, 1963; Sinoway *et al.*, 1986; Silber & Sinoway, 1990; Silber *et al.*, 1991). Nonetheless, it was observed that brachial artery (largely or partly) NO-mediated endothelium-dependent or -independent function was preserved after 4 and 8 days of physical inactivity. These data indicate that physical inactivity leads to a distinct time-course for vascular remodelling between resistance and conduit arteries. Moreover, it was

observed that conduit artery function is preserved during the initial phase of physical inactivity.

Previous models of physical inactivity are associated with limitations to study the immediate impact of physical inactivity. For example, space flight is expensive and impractical (Convertino *et al.*, 1986), unilateral lower limb suspension is associated with an increased risk for venous thrombosis (Green *et al.*, 1997), whilst the frequently used model of strict bed rest is not associated with upper limb inactivity (Bleeker *et al.*, 2005b). To overcome these limitations, in the present study a sling was introduced to induce local forearm physical inactivity. The trend for a significant decrease in handgrip strength and significant decrease forearm girth across 8 days demonstrates the effectiveness of using a sling, i.e. a user-friendly and easy applicable method, to induce forearm inactivity. Moreover, the decreases in forearm peak reactive hyperaemia is in agreement with previous studies that reported a lower peak reactive hyperaemia in the inactive region after bed rest (Shoemaker *et al.*, 1998), cast immobilisation (Silber & Sinoway, 1990), lower limb immobilisation (Bleeker *et al.*, 2005a), and a spinal cord injury (de Groot *et al.*, 2004; de Groot *et al.*, 2006a). As these previous studies adopted a longer time-frame, data from the present study add the novel knowledge that resistance artery remodelling occurs rapidly during physical inactivity.

Previous studies that investigated the effects of prolonged physical inactivity typically reported an increase in conduit artery (NO-mediated) endothelial function (de Groot *et al.*, 2004; Bleeker *et al.*, 2005a; Bleeker *et al.*, 2005c; de Groot *et al.*, 2005; de Groot *et al.*, 2006a; Rakobowchuk *et al.*, 2011). It is important to note that

these studies also demonstrated a strong inward remodelling of the same conduit arteries, possibly interfering with a valid assessment of conduit artery endothelial function (Thijssen *et al.*, 2008a; Thijssen *et al.*, 2008c). Evidence from animals (Laughlin *et al.*, 1989) and humans (Tinken *et al.*, 2009; Tinken *et al.*, 2010) suggest that functional adaptations precede structural adaptations (McAllister *et al.*, 1996; Tinken *et al.*, 2008). It may therefore be possible that physical inactivity initially results in dose-dependent structural remodelling, which are superseded by functional adaptations. Timing of the vascular assessment was therefore crucial in the present study, especially since previous studies found rapid structural changes during models of physical inactivity (Sugawara *et al.*, 2004; Bleeker *et al.*, 2005a; de Groot *et al.*, 2006b). However, in the present study no change in brachial artery baseline diameter was observed, suggesting that the timing of measurements were before significant structural remodelling of the brachial artery. However, there was no significant change in largely or partly NO-mediated endothelial function or in the NO-mediated endothelium-independent dilation was observed in the present study.

One potential explanation for this finding is 8-days are insufficient to induce changes in conduit artery endothelial function. However, it is believed that this is unlikely given the significant remodelling in resistance arteries and decrease in handgrip strength in the present study. Moreover, also other studies have found that changes in conduit artery function can be observed after a relatively short period of physical inactivity (Sugawara *et al.*, 2004) and activity (Laughlin *et al.*, 2003; Tinken *et al.*, 2009). An alternative explanation for the results from the present study relate to the hypothesis that functional adaptations to exercise

training are not simply the opposite of functional adaptations observed during physical inactivity (Thijssen *et al.*, 2010; Rakobowchuk *et al.*, 2011). Whilst the beneficial effects of exercise training on the NO-pathway are well established (Green *et al.*, 2004; Green *et al.*, 2011), most previous studies found that physical inactivity did not alter the NO-pathway in conduit and resistance vessels (Thijssen *et al.*, 2010). Possibly, functional vascular adaptations during physical inactivity may be driven through different ways than the NO-pathway. For example, the powerful vasoconstrictors ET-1 and angiotensin-II are demonstrated to contribute to the increased vascular tone after chronic physical inactivity (Thijssen *et al.*, 2007b; Groothuis *et al.*, 2010). Moreover, ET-1 and angiotensin-II are demonstrated to have pro-atherogenic effects (Newcomer & Padilla, 2011). Future studies should focus on the potential impact of physical inactivity on vasoconstrictor pathways, rather than the NO-pathway (Thijssen *et al.*, 2010; Rakobowchuk *et al.*, 2011), to better understand the impact of physical inactivity on the vasculature. Intra-arterial infusion studies of vasoconstrictors or the low-flow mediated constriction which is believed to reflect vasoconstrictor tone in the radial artery (Gori *et al.*, 2008), may help to unravel these mechanisms.

Furthermore, it has been suggested that the vascular smooth muscle may play a role in remodelling. Many factors are known to impact vascular smooth muscle tone. In particular there is evidence for the important role of blood flow and shear stress for vascular remodelling (Langille & O'Donnell, 1986; Tuttle *et al.*, 2001; Amiri *et al.*, 2004; Bouvet *et al.*, 2007; Loufrani & Henrion, 2008). Blood flow and shear stress mediated release of vasoactive substances (i.e. NO) enable the endothelial

cells to play a significant role in causing resistance vessel remodelling (Martinez-Lemus *et al.*, 2009), more specifically a reduction in blood flow and therefore shear stress with inactivity reduces the shear-stress mediated release in vasoactive substances causing a detrimental effect upon the endothelium (Thijssen *et al.*, 2010). It is suggested that changes in the peak reactive hyperaemic blood flow response (RHBF) initiated from the ischaemic handgrip exercise is associated with remodelling of resistance vessels. Resistance vessels contribute to systemic levels of vascular resistance and blood pressure as well as a downstream supply of blood flow and there is evidence to suggest that adaptations to the peak reactive RHBF provides a prognostic marker for predicting future cardiovascular diseases (Anderson *et al.*, 2011; Lind *et al.*, 2011). The rapid decrease in forearm peak blood flow, i.e. a marker for resistance artery remodelling, did not follow the changes in the conduit artery supplying the forearm resistance vessels. These results suggest that deconditioning of the conduit arteries follow a different time-course compared to that of resistance vessels of the same limb when being exposed to the same stimulus. Such observations are not frequently described when examining vascular adaptations to physical inactivity. However, studies also reported a different time-course of structural adaptations in the conduit and resistance arteries (Bleeker *et al.*, 2005a; Bleeker *et al.*, 2005c; de Groot *et al.*, 2006b). The distinct time-course during exercise and inactivity suggest that the stimuli that induce vascular remodelling vary markedly for conduit and resistance arteries, this hypothesis was first proposed based on data in animals (Laughlin, 1995) and from exercise training in humans (Tinken *et al.*, 2008).

Limitations. Although a decrease in maximal voluntary contraction and peak blood flow in the inactive arm was found, the duration of the sling-intervention may be too short to cause significant changes in the brachial artery endothelial function. Although the sling was convenient and allowed subjects to perform daily activities, this model is highly susceptible for non-compliance. It is recommended future studies to use an accelerometer on the inactive arm as an objective measure to control for arm movement.

7.5. Conclusion

In conclusion, the effects of the forearm inactivity intervention, before, during and after 8-days induced a significant decrease in post-ischaemic limb blood flow. Previous studies have suggested that a reduction in peak blood flow response is a marker of arterial remodelling. Consistent with these findings, data illustrated in the present study indicate a reduction in peak blood flow in response to acute localised inactivity suggestive of remodelling of forearm resistance vessels. However, measures of (largely and partly) NO mediated endothelial conduit artery function were not altered. It is likely that this may be associated with functional adaptations preceding structural adaptations. This could indicate that 8-days of forearm local inactivity has no significant impact on the NO-pathway in the brachial artery. It is possible that non-NO-mediated changes in vascular function may be observed during a period of inactivity.

Aims and Outcomes of Chapter 8

Chapter 4 ***Aim:*** Examine the systemic effect of shear rate during exercise on post exercise brachial diameter.	*Chapter 4* ***Outcome:*** Dose-dependent increase in shear rate = Dose-dependent increase in brachial artery diameter.
Chapter 5 ***Aim:*** Address the role of shear rate on endothelial function following acute leg cycling exercise.	*Chapter 5* ***Outcome:*** Impairment in FMD post exercise is exercise intensity and therefore shear rate-dependent. FMD is only decreased immediately post.
Chapter 6 ***Aim:*** Examine the role of systemic increases in shear rate on brachial artery responses across 8-weeks leg cycle exercise training.	*Chapter 6* ***Outcome:*** Rapid and transient increase in endothelial function. No adaptation when shear is attenuated.
Chapter 7 ***Aim:*** Examine the effects of acute localised forearm inactivity and associated vascular responses.	*Chapter 7* ***Outcome:*** Significant decrease in peak reactive hyperaemia and forearm girth, but only in the arm that received local forearm inactivity.
Chapter 8 ***Aim:*** investigate the role of acute and prolonged retrograde shear rate on vascular responses.	*Chapter 8* ***Outcome:*** FMD is impaired acutely, but only when retrograde shear rate is increased.

Chapter 8 | *Acute and longer- term manipulation of retrograde shear rate on endothelial function in humans.*

8.1. Introduction

Changes in shear stress across the endothelial cell membrane are a key stimulus for adaptation in both vascular function and remodelling of the artery (Langille & O'Donnell, 1986; Pohl *et al.*, 1986; Niebauer & Cooke, 1996; Tuttle *et al.*, 2001; Laughlin *et al.*, 2008). Whilst it is well known that increases in shear stress are beneficial for arterial function, *in vitro* and studies in animals have demonstrated that shear stress patterns characterised by high levels of retrograde shear can be detrimental for vascular health (Harrison *et al.*, 2006; Thijssen *et al.*, 2009b). Such shear pattern lead to expression of pro-atherogenic genes (e.g. increase in ET-1 (Ziegler *et al.*, 1998) and adhesion molecules (Chappell *et al.*, 1998; Himburg *et al.*, 2007) and increase in superoxide (McNally *et al.*, 2003) and NADPH oxidase (De Keulenaer *et al.*, 1998; Hwang *et al.*, 2003), but decreases anti-atherogenic genes (e.g. eNOS expression (De Keulenaer *et al.*, 1998; Hwang *et al.*, 2003)). However, relatively little is known about the impact of acute and short-term changes in retrograde shear stress in humans.

Increases in peripheral vascular tone are believed to contribute to increased upstream retrograde shear (Thijssen *et al.*, 2009c; Padilla *et al.*, 2011a). Previous studies have found that an increased external pressure on the forearm also increases retrograde shear in the upstream brachial artery, without any changes in central arterial pressure or mean shear rate (Tinken *et al.*, 2009; Tinken *et al.*, 2010). Based on these observations, it is hypothesised that a compression sleeve around the forearm may induce a comparable effect on shear rate in the upstream brachial artery, providing a human *in vivo* model to examine the short- and longer-

term effects of manipulation of retrograde shear rate. Therefore, the first aim of this study is to measure endothelial function before and after 1 hour exposure to a compression sleeve in young healthy male subjects *in vivo*. Secondly, endothelium - dependent (i.e. largely and partly nitric oxide (NO) mediated) and -independent dilation was examined before, during (4 days) and after 8 days of wearing the compression sleeve. Therefore, it is hypothesised that an increase in retrograde shear rate, induced by the compression sleeve, will lead to an acute impairment of endothelial function, which is persistent when the compression sleeve is worn for 8 days.

8.2. Methods

8.2.1. Subjects

12 healthy male participants took part in the present study (Table 8.1). Subjects were young and healthy; none reported having been diagnosed with cardiovascular disease, diabetes mellitus, insulin resistance or cardiovascular risk factors (such as hypercholesterolemia or hypertension). Subjects who smoked or were on medication of any type were excluded from participation. Informed consent was gained from all participants prior to the experimental procedures. The study procedures were approved by the Liverpool John Moores University Ethics Committee and adhered to the Declaration of Helsinki.

8.2.2. Experimental design and procedures

Acute compression intervention. On day 0, subjects reported to the laboratory for the assessment of brachial artery function. After baseline testing of the brachial

artery endothelial function on day 0, the acute effect of a 1 h exposure to the compression sleeve on brachial artery blood flow and shear rate patterns was examined. Bilateral brachial artery diameter and velocity was examined every 15 minutes for at least 1 minute (Please see Sections 3.4 and 3.5 in the General Methods, Chapter 3, for a full description of brachial diameter, blood flow and shear rate analysis). Based on the immediate effect of the compression sleeve on brachial artery retrograde shear, subjects were divided into an intervention (i.e. increase in retrograde shear) and control group (i.e. no change in retrograde shear). Immediately after the 1 h intervention, bilateral brachial artery endothelial function was re-examined to assess the immediate impact of the compression sleeve on the endothelial function (Thijssen *et al.*, 2009b) (Please see Section 3.3 in the General methods, Chapter 3, for full description of FMD procedure and analysis). Subsequently, subjects were instructed to wear a compression sleeve around the forearm for 8 days.

Prolonged compression intervention. Subjects reported to the laboratory on three separate occasions over a period of 8 days (day 0, 4 and 8). Subjects were instructed to fast for >4 hours, abstain from alcohol and caffeine for 16 hours, and not to perform any exercise for 24 hours (Thijssen *et al.*, 2011a). Upon arrival at the laboratory, body mass (electronic scales, Seca, England) and height were measured. Subjects then rested in the supine position for 20 minutes, followed by assessment of heart rate and blood pressure using an automated sphygmomanometer (GE Pro 300V2, Dinamap, Tampa, Fl). This was followed by assessment of brachial artery (largely and partly) nitric oxide (NO)-mediated endothelium-dependent and -

independent vasodilation. For a full description of FMD, iEX and GTN procedures and analysis please refer to Sections 3.3, 3.4 and 3.5 in the General Methods, Chapter 3.

8.2.3. Intervention

Subjects were instructed to continuously wear a compression sleeve around the forearm (during daytime and night time). Placement of the compression sleeve around the dominant or non-dominant forearm was randomised between subjects. When selected, the compression sleeve was kept on the same arm throughout the 8-day period. The compression sleeves are developed for (upper limb dominant) athletes to improve performance and provide an equally distributed compression around the forearm (Tube, Herzog Medical, Woudenberg, The Netherlands; Figure 8.1). Information provided by the manufacturers indicates that the compression sleeve provides a pressure of 25-32 mmHg. Subjects were only allowed to remove the compression sleeve for personal hygiene purposes (e.g. showering).

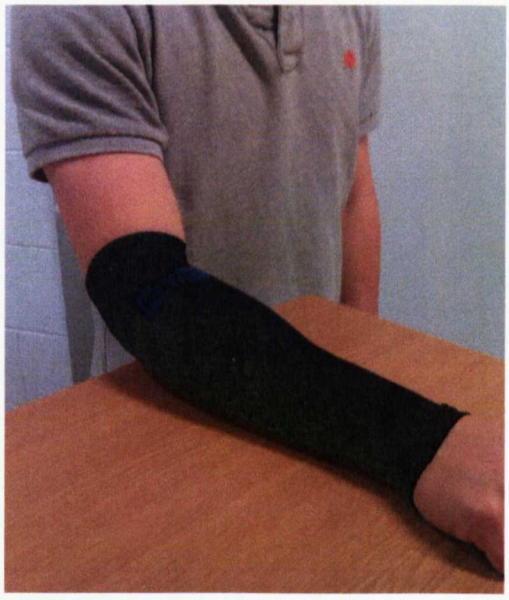

Figure 8.1 Herzog Medical compression sleeve

6.2.4. Statistical analysis

A two-factor ANOVA with repeated measures (time as the independent factor) were used to assess the acute changes in brachial artery blood flow during the acute compression period. An additional two-factor ANOVA repeated measures (with time

as the independent factor) were used to assess the acute changes in brachial artery (largely and partly) nitric oxide (NO)-mediated endothelium-dependent and -independent vasodilation. A one-factor ANOVA was used for analysis of resting heart rate, MAP, systolic and diastolic BP. Post hoc *t*-tests were used where significant values were found. Post-hoc analysis *t*-tests were used where significant values were found. The relationship between the percent changes in BMI and increases in retrograde SR in both the control and the intervention group were assessed using pearson's R correlations.

8.4. Results

8.4.1. Acute effect of compression sleeve on blood flow and endothelial function

Blood flow and shear rate. There were no significant differences between the intervention and control group for mean and antegrade shear rate (P>0.05, Figure 8.2). However, retrograde shear rate was significantly higher in the intervention group compared with the control (P<0.05, Figure 8.2). However, there wasa poor correlation between BMI and increases in retrograde SR, in both the intervention (R^2=0.001) and the control group (R^2=0.21).

Table 8.1 Characteristics of subjects (n=12) before (Day 0), during (Day 4) and after (Day 8) the unilateral compression sleeve intervention.

Variable	Day 0	Day 4	Day 8	P-value
Age (years)	22±3			
Height (m)	1.76±0.07			
Weight (kg)	71.0±10.1			
BMI (kg/m^2)	23.0±3.4			
Systolic BP (mmHg)	131±9	123±17	127±16	0.84
Diastolic BP (mmHg)	67±3	65±4	65±7	0.23
Mean BP (mmHg)	88±4	84±7	86±9	0.99
Heart rate (bpm)	58±14	59±11	54±8	0.73

Values are means ± SD; P-value refers to a one-way ANOVA.BMI, body mass index; BP, blood pressure.

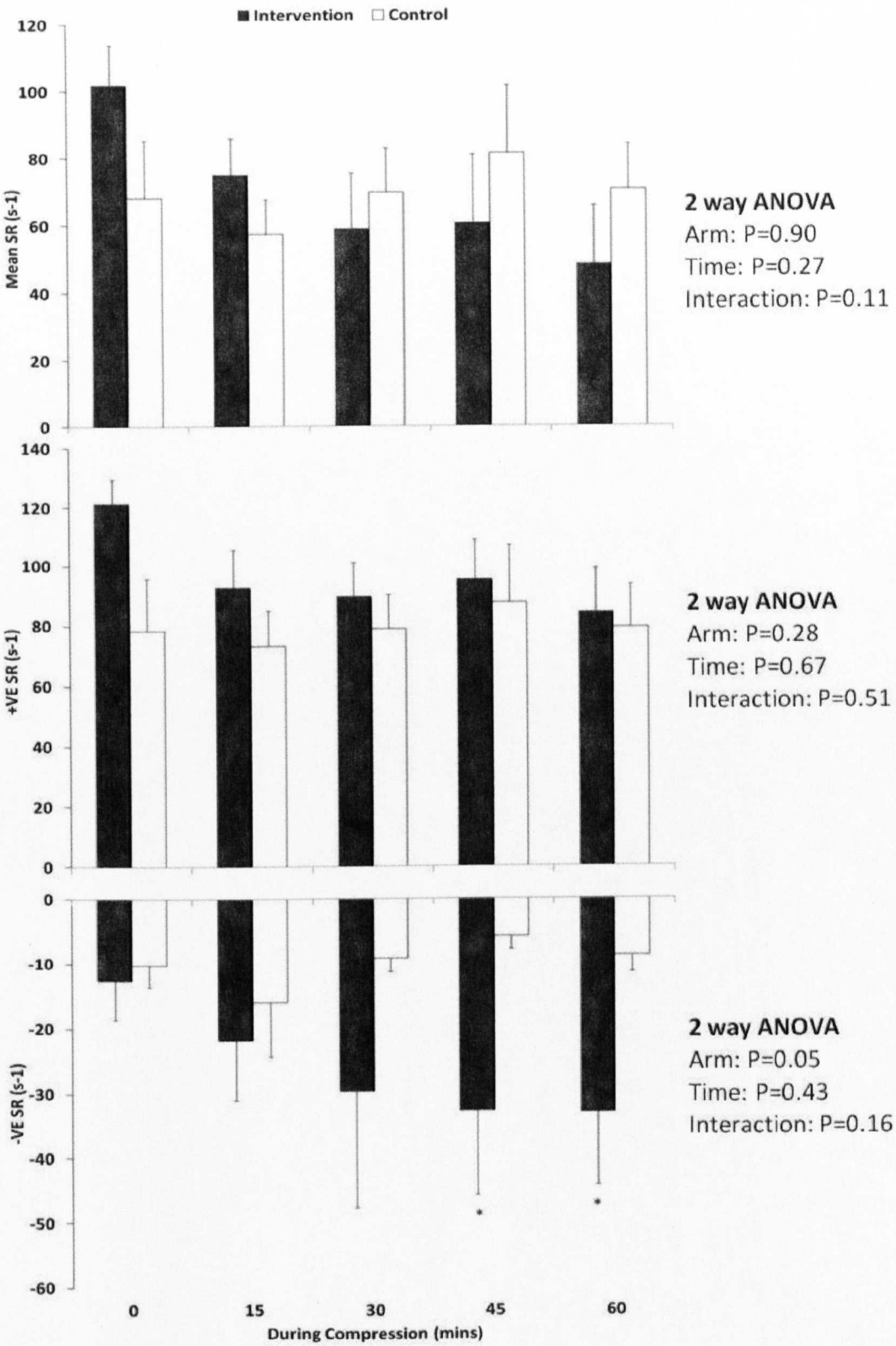

Figure 8.2 Brachial artery mean, antegrade (+ve SR) and retrograde (-ve SR) shear rate at 0, 15, 30, 45 and 60 mins, during compression in the intervention (n=6) and control (n=6). Values are mean ± se. Post hoc analysis; post-hoc significantly different at $P<0.05$ from *control group

Pre and post FMD. Brachial artery FMD was significantly lower immediately after 1 h of the intervention in the intervention group, but not in the control group ($P<0.05$, Figure 8.3 and 8.4). But no changes observed in other FMD parameters (Table 8.2).

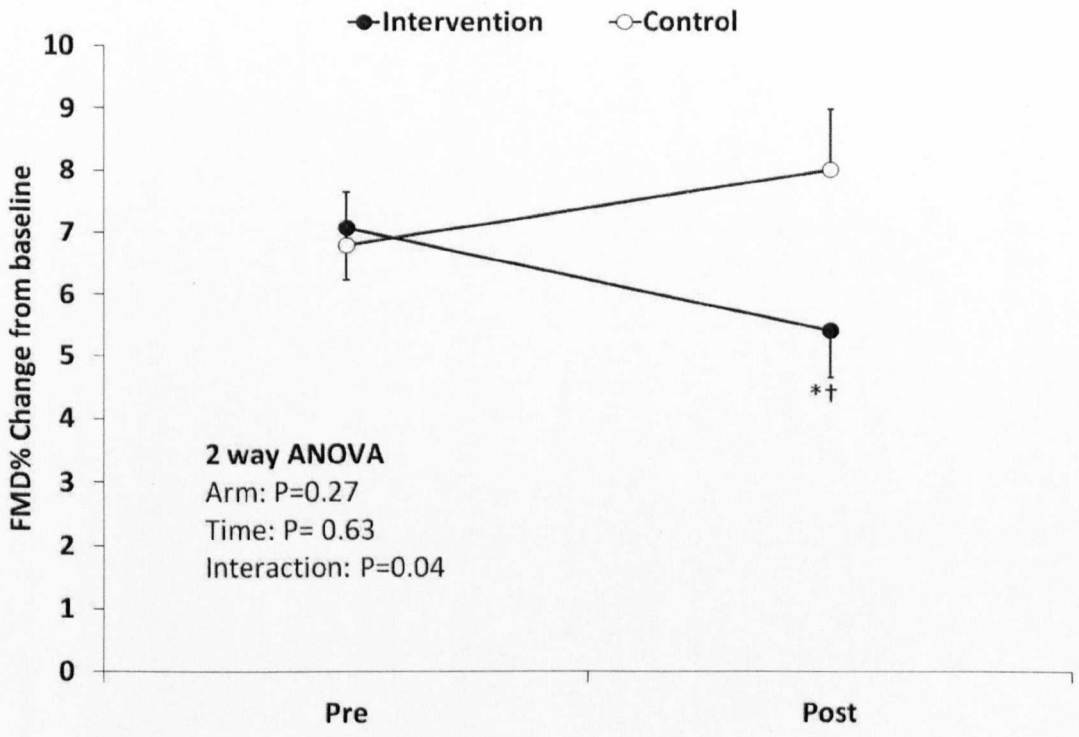

Figure 8.3 Brachial artery flow-mediated dilation (FMD, presented as a relative change from baseline diameter) pre and immediately post an acute compression period of 1hr, in the intervention and non-control. Data are presented as mean ± SE. Data from the 2-way ANOVA are presented. *Post hoc significantly different from pre-compression, † significantly different from the control group ($P<0.05$).

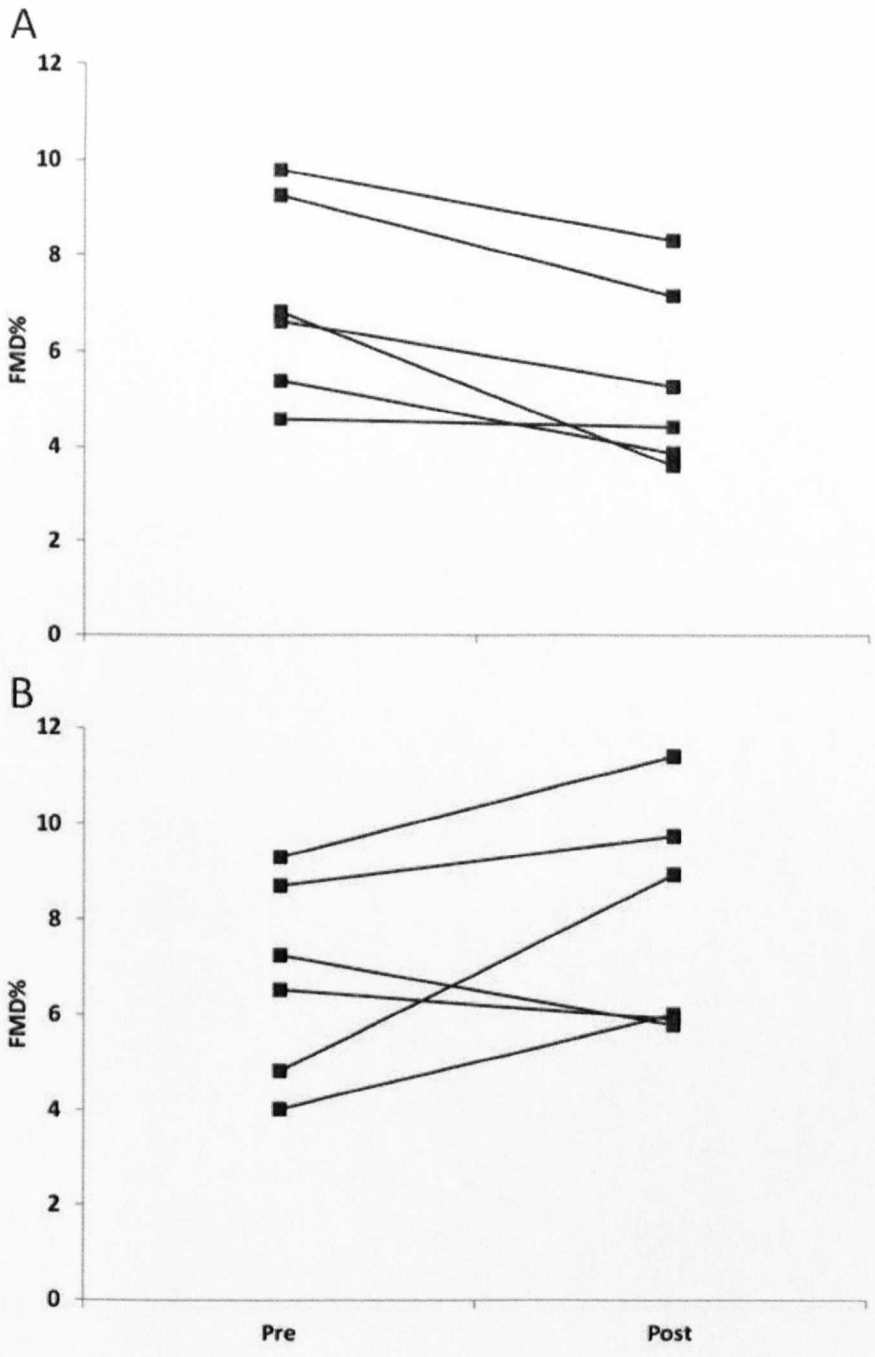

Figure 8.4 Individual data for brachial artery flow-mediated dilation (FMD presented as individual differences from baseline diameter) pre and immediately post an acute compression period of 1hr, in the (A) control and (B) intervention groups.

Table 8.2 Brachial artery FMD parameters before and immediately after 1 h of wearing a compression sleeve in subjects who demonstrated a change in shear pattern ('intervention', n=6) and who demonstrated no change in shear pattern ('control', n=6).

Variable	PRE	POST	P-value
Resting diameter (mm)			
Intervention	3.9±0.3	3.8±0.3	'time': 0.88
Control	4.1±0.5	4.1±0.4	'time*arm' 0.61
Time-to-peak (s)			
Intervention	54±15	48±13	'time': 0.69
Control	44±20	47±25	'time*arm' 0.57
SR_{AUC} (s^{-1} $x10^3$)			
Intervention	21.3±9.1	21.1±6.2	'time': 0.56
Control	14.0±4.4	17.0±7.9	'time*arm' 0.55

Values are means ± SD, FMD, flow-mediated dilatation; SR_{AUC}, shear rate area-under-the-curve.

8.4.2. Longer-term effect of compression sleeve on endothelial function

Before the intervention, no significant differences in brachial artery mean, antegrade and retrograde shear rate between the intervention and control group (Table 8.3, P>0.05) were found. Also, no difference in brachial artery FMD, iEX or GTN were found between both arms.

Table 8.3 Mean, antegrade and retrograde shear rate in the brachial artery at rest 0, 4 and 8 days in the control and intervention arm.

Variable	Day 0	Day 4	Day 8	P-value
Mean SR				
Intervention	107±31	90±47	74±49	'time': 0.56
Control	46±24	83±41	72±42	'time*arm': 0.15
Antegrade SR				
Intervention	123±36	109±37	82±53	'time': 0.34
Control	63±22	88±42	80±36	'time*arm': 0.10
Retrograde SR				
Intervention	-17±10	-19±16	-8±9	'time': 0.12
Control	-18±12	-5±4	-8±8	'time*arm': 0.20

Values are means ± SD. SR; shear rate (s^{-1}).

Across the 8 day intervention, no significant differences were observed in heart rate, mean, systolic and diastolic blood pressure (Table 8.1). Similarly, no significant differences were observed in brachial artery FMD, iEX or GTN during the intervention in the intervention group or control group (P>0.05, Table 8.4).

Table 8.4 Brachial artery FMD, iEX and GTN at 0, 4 and 8 days during compression sleeve intervention in the intervention and control group.

Variable	Day 0	Day 4	Day 8	P-value
Resting diameter (mm)				
Intervention	3.9±0.3	3.7±0.3	4.0±0.6	'time': 0.18
Control	4.1±0.5	3.9±0.5	4.1±0.5	'time*arm': 0.86
FMD (mm)				
Intervention	0.6±0.2	0.6±0.2	0.6±0.2	'time': 0.25
Control	0.6±0.2	0.6±0.2	0.6±0.2	'time*arm': 0.53
FMD (%)				
Intervention	7.1±2.1	6.9±3.0	6.4±3.0	'time': 0.64
Control	6.8±2.1	6.1±2.3	7.9±1.6	'time*arm': 0.53
SR_{AUC} (s^{-1} $x10^3$)				
Intervention	21.1±8.8	17.7±9.4	11.5±6.8	'time': 0.76
Control	14.0±4.4	14.5 ±4.3	20.7±7.2	'time*arm': 0.02
Time to peak (s)				
Intervention	54±15	37±19	35±9	'time': 0.11
Control	44±20	46±15	58±16	'time*arm': 0.07
iEX (%)				
Intervention	14.4±4.0	13.2±3.0	13.7±4.0	'time': 0.16
Control	11.5±4.0	10.6±2.7	9.5±3.4	'time*arm': 0.91
Peak RH				
Intervention	701±248	667±180	802±329	'time': 0.64
Control	562±112	452±102	482±186	'time*arm': 0.37
GTN (%)				
Intervention	17.0±2.9	20.0±5.4	19.2±4.1	'time': 0.49
Control	14.2±5.8	14.0±4.5	15.8±3.8	'time*arm': 0.76

Values are means ± SD, FMD, flow-mediated dilatation; iEX, ischaemic handgrip exercise, GTN; glyceryl trinitrate; SR_{AUC}, shear rate area-underneath-curve; RH, reactive hyperaemia.

8.5. Discussion

The purpose of the present study was to examine the effects of acute and prolonged increases in retrograde shear rate (induced using a forearm compression sleeve) on brachial artery endothelial-dependent and –independent function in humans. The principle finding was that there was an immediate decrease in endothelial function following an acute compression period. Interestingly, this decrease was only evident in those subjects demonstrating a significant increase in retrograde shear rate. However, prolonged use of compression did not induce any changes in endothelial-dependent or –independent function.

In the present study a novel experimental design was utilised to induce increases in retrograde shear rate, and assess endothelial adaptations before and after acute and prolonged exposure of compression. In the present study blood and shear rate was manipulated, independent of exercise. This approach of manipulating blood flow and shear rate to increase retrograde shear rate was evident in 6 subjects, interestingly, this was associated with a significant decrease in FMD immediately post the acute compression exposure. On the other hand, those subjects demonstrating no change in FMD, exhibit no change in retrograde SR. Taken together, the data from the present study demonstrate a decrease in endothelial-dependent (largely) NO dependent function *in vivo*, after short term exposure to increases in retrograde SR.

In addition to this finding, adaptations in endothelial function following the prolonged use of forearm compression were not observed. A possible explanation for this could be that the stimulus was too low, this is consistent with data from

Thijssen *et al.* (Thijssen *et al.*, 2009b) who examined the effects of different pressures of compression on the forearm to induce retrograde shear rate. They report a dose-dependent decrease in FMD, whereby FMD was only reduced in response to the higher compression pressures at 50 and 75 mmHg. Therefore, prolonged modification in artery function in response to elevated retrograde shear may require a more potent stimulus.

Retrograde shear rate is hypothesised as a strong stimulus for endothelial dysfunction in humans (Thijssen *et al.*, 2009b). Increases in blood flow and shear rate with exercise are known to maintain the health and tone of the vessels by initiating the release of various vasoactive and anti-atherogenic substances such as NO (Tronc *et al.*, 1996; Tuttle *et al.*, 2001; Green *et al.*, 2004). However, studies examining elevations in retrograde blood flow and shear rate in animals have demonstrated that increased levels of retrograde shear rate produce opposing effects leading to pro-atherogenic outcomes (Laughlin *et al.*, 2008). There is Increasing evidence indicating that disturbed flow patterns, characterised by the presence of retrograde and oscillatory shear stress, induce a pro-atherogenic endothelial cell phenotype. In particular, retrograde shear rate has been known to initiate the production of ET-1 expression (Ziegler *et al.*, 1998), expression of adhesion molecules (Himburg *et al.*, 2007), and reactive oxygen species (e.g. NADPH oxidase) (De Keulenaer *et al.*, 1998) which are usually associated with increases in pro-atherogenic factors within the vascular wall (Laughlin *et al.*, 2008). Therefore, the short-term increases in retrograde shear rate and the subsequent decrease in

endothelial function, observed in this study, may help to explain the principal cause of atherosclerosis and endothelial dysfunction.

An elevation in peripheral vascular tone are usually associated with characteristics of cardiovascular risk factors, such as obesity (Cardillo *et al.*, 2004) and hypertension (Taddei *et al.*, 1999; Campia *et al.*, 2004; Cardillo *et al.*, 2004), and these conditions are also associated with an impaired endothelial function (Cardillo *et al.*, 2004), therefore it is possible that these increases in retrograde shear rate patterns in the conduit arteries may be a result of increases in vascular tone in the resistance vessel beds (Green *et al.*, 2005). In addition, elevations in sympathetic nervous system tone and peripheral vascular tone are associated with acute impairments in brachial artery endothelial function (Dyson *et al.*, 2006), this could potentially explain the finding in the present study where a decrease in endothelial function was observed. In a recent study, Padilla and colleagues (Padilla *et al.*, 2010), sought to identify the underlying mechanisms of oscillatory shear patterns in conduit arteries, their data suggest that acute elevations in muscle sympathetic nervous activity (MSNA) were associated with an increase in conduit artery retrograde and oscillatory shear. This evidence could suggest that the sympathetic nervous system may play an essential role between MSNA, arterial blood pressure and factors that modulate shear rate patterns, however, future studies would be required to determine this.

Results from the present study suggest that the normalisation of the brachial artery shear pattern across the 8 days of intervention is unlikely to be explained by changes in the brachial artery diameter. Alternatively, it is believed that increases in

retrograde shear rate in conduit arteries may result from an increase in vascular tone in downstream resistance vessels (Green *et al.*, 2005; Padilla *et al.*, 2010). Therefore, changes in forearm resistance artery tone could potentially contribute to a normalisation of the shear pattern. Unfortunately, changes in resistance artery resting tone and/or vascular function was not examined. However, brachial artery peak blood flow, which represents a valid and accepted index of resistance artery size or remodelling (Naylor *et al.*, 2005b) was examined, but no change was found across the 8 day intervention.

Limitations. A potential limitation of this study is that only healthy male volunteers were recruited and these findings cannot be extrapolated to subjects with cardiovascular disease, women or older subjects. Additionally, the stimulus used for compression provides attenuation of between 25-32 mmHg, this pressure may not have been sufficient to induce alterations in endothelial function after prolonged use. A likely explanation relates to between subject differences in the pressure induced by the compression sleeve. Although a correlation between BMI and the intervention or control group was not identified it is still possible that a higher between subject variability regarding the effect of compression on the shear pattern was expected due to the relatively low pressure (25-32 mmHg) (Thijssen *et al.*, 2009b). However, applying higher pressures (i.e. 50-60 mmHg) was not possible as this is associated with subject discomfort when applied for 8 days. Nonetheless, the increase in retrograde shear was associated with a significant decrease in FMD% immediately after 1 h wearing the sleeve, which confirms a previous finding from previous research (Thijssen *et al.*, 2009b). Taken together, these data demonstrate

that 1-h exposure to elevated levels of retrograde shear rate in the brachial artery is associated with a decrease in endothelial function in humans *in vivo*.

8.6. Conclusion

In conclusion, results from the present study provide an insight into the acute and prolonged effects of retrograde shear rate upon endothelial dependent, NO-mediated function, suggesting that acute, but not prolonged, increases in retrograde shear rate cause a decrease in NO-mediated function.

Chapter 9 | *Synthesis*

9.1. Aims and objectives

The principle findings from the studies presented in this thesis will be highlighted and discussed accordingly throughout this chapter. Specifically, the role of shear stress in modulating vascular adaptations in response to acute exercise, exercise training and deconditioning interventions in young, healthy male participants will be explored in this section.

Study 1 (*Chapter 4*) identified the importance of shear rate during acute lower limb exercise for brachial artery vasodilation during exercise at 50, 70 and 85%HR_{max}. This was achieved with a novel intervention of exercise with unilateral forearm heating to manipulate shear in one arm during exercise. Increases in post-exercise brachial artery diameters were evident in a dose-dependent manner in the unheated arm, with largest increases observed at the higher exercise intensities (70 and 85%HR_{max}) only; this was associated with a dose-dependent, exercise-induced increase in shear rate. Unilateral forearm heating resulted in higher levels of shear rate in the heated arm during exercise, independent of the exercise intensity. This resulted in increases in post-exercise artery diameter (compared with pre-exercise) at all intensities, suggesting that shear rate *per se* is a stimulus for vasodilation. Taken together, these data support recent reports that exercise is a known stimulus to induce systemic changes in conduit artery shear rate and subsequent vasodilation (Green *et al.*, 2002c; Green *et al.*, 2005; Thijssen *et al.*, 2009a) (Simmons *et al.*, 2011).

The data reported in Chapter 5 comes from the same experimental model outlined in Chapter 4, but reports that FMD was impaired immediately post-exercise.

Interestingly, as with the data from the previous chapter, the magnitude of decrease in FMD was observed to be dose-dependent, with the magnitude of decrease from pre exercise being greater at intensities of 70 and 85%HR_{max}, but not 50%HR_{max}, an observation concomitant with the largest shear rates. In addition, this study examined endothelial function, before and after exercise (0, 1, 2, 24h) and found that the impairment reported above was transient, since it was evident that FMD return to baseline by 1h post exercise. This observation brings some clarity to the contradictory reports in the literature that FMD is elevated, depressed or unchanged post-exercise, as previous studies have used post-exercise timings which have varied considerably (Padilla *et al.*, 2006; Dawson *et al.*, 2008; Harris *et al.*, 2008; Tinken *et al.*, 2009; Jones *et al.*, 2010; Varady *et al.*, 2010; Zhu *et al.*, 2010; Johnson *et al.*, 2011). Consequently, data from this study confirm the importance of taking multiple measurements post-exercise and that the timing of the immediate post exercise measurements are equally relevant.

The previous chapters assess the importance of acute changes in blood flow and shear rate during acute exercise and with (or without) forearm heating intervention on brachial artery responses both during and following exercise. The third study expanded on the previous work, and examined the effects of prolonged exposure (exercise training) to shear stress on vascular function. Building on previous work (Tinken *et al.*, 2009; Tinken *et al.*, 2010) that examined localised exercise training and associated increases in shear stress, the impact of whole body leg exercise on upper limb adaptations in order to define systemic *vs.* local changes was examined. Lower limb exercise training was performed across 8 weeks, with shear rate

manipulated in one forearm by the inflation of a pneumatic cuff to low pressures. As hypothesised this attenuation in shear rate in the arm that was cuffed resulted in no change in endothelial function across the 8-weeks of exercise. On the other hand, rapid increases in endothelial function were observed in the contralateral arm. These results are in keeping with previous literature which has examined shear stress-mediated adaptations in endothelial function (Tinken *et al.*, 2009; Tinken *et al.*, 2010; Naylor *et al.*, 2011) with localised exercise training, suggesting that shear is the mechanism causing the observed changes in systemic adaptations of the brachial artery.

The main aim of the 4th study was to examine whether inactivity and deconditioning directly effects the vasculature. This study successfully demonstrated the rapid nature of vascular deconditioning via the use of a novel method to impose acute localised deconditioning ("sling") across an 8-day intervention. This method caused a rapid decrease in peak hyperaemic blood flow, indicating resistance vessel remodelling, in the arm with a "sling". This is consistent with previous reports stating physical inactivity results in an inward remodelling of forearm resistance vessels (Folkow *et al.*, 1958; Conway, 1963; Sinoway *et al.*, 1986; Silber & Sinoway, 1990; Silber *et al.*, 1991). In addition to this observation it was observed that wearing the sling induced a trend for a decrease in handgrip strength, providing evidence that the model of inactivity was successful. However, adaptations in endothelial -dependent or -independent vasodilation across the intervention were not observed, suggesting that vascular adaptations to deconditioning do not follow the same mechanisms as those of exercise training (Thijssen *et al.*, 2010).

The final and 5th study (*Chapter 8*) examined the role of acute and prolonged increases in retrograde shear rate on endothelial function, in an attempt to determine the underlying mechanisms of endothelial deconditioning. A compression sleeve to manipulate forearm blood flow and shear rate was used to assess acute and prolonged responses to forearm compression across 8-days. Interestingly, an acute 1hr period of compression resulted in a decrease in endothelial function, but only when retrograde shear was increased. The prolonged use of compression across 8-days illustrated no change in FMD. Taken together, the data demonstrate a decrease in endothelial-dependent (largely) NO mediated function *in vivo*, after short term exposure to increases in retrograde shear rate. Retrograde shear rate is often referred to as a key stimulus for the expression of pro-atherogenic phenotype (Laughlin *et al.*, 2008) and this study provides evidence for this with the acute impairment observed in endothelial function following acute elevations in retrograde shear rate.

The studies presented in this thesis indicate the importance of mean and antegrade shear rate as a potent systemic physiological stimulus for adaptations in the non-exercising upper limbs during acute and prolonged leg cycling exercise. On the other hand, increases in retrograde shear rate are responsible for acute impairments in endothelial function. Finally, there is evidence for rapid structural adaptations to localised inactivity; the mechanisms for this reduction could be related to reductions in blood flow, and thus shear stress, to the localised area of inactivity (Langille & O'Donnell, 1986).

9.2. General Discussion

Shear stress has been referred to as the key stimulus for adaptations of the vasculature (Pohl *et al.*, 1986; Mora *et al.*, 2007; Tinken *et al.*, 2009; Tinken *et al.*, 2010). Studies in animals have provided widespread evidence of the importance of shear stress and blood flow for vascular adaptations (Langille & O'Donnell, 1986) . Extensive data from animals and humans provide evidence for the beneficial effects of exercise training for vascular function and health in different populations (Maiorana *et al.*, 2000; Green *et al.*, 2004; Jasperse & Laughlin, 2006; Mora *et al.*, 2007; Green *et al.*, 2011) and that exercise provides a strong stimulus to cause an increase in blood flow and shear stress (Armstrong *et al.*, 1987). A recent study by Thijssen *et al.* (2009a), demonstrated that different modes of exercise training (large or small muscle groups) are associated with different patterns of blood flow and shear stress, in particular lower limb exercise causes increases in mean, anterograde and retrograde shear rate patterns in the non-exercising upper limbs. Additionally, it has been hypothesised that repetitive increases in shear stress, with exercise training, are the principle stimulus for adaptations of the vasculature by stimulating the endothelium to release several vasodilators, in particular NO (Pohl *et al.*, 1986; Laughlin *et al.*, 2008) which is a potent anti-atherogenic agent (Laughlin *et al.*, 2008). It has therefore been suggested that shear stress mediated NO production is a key stimulus for conduit artery vasodilation with exercise (Green *et al.*, 2004; Tinken *et al.*, 2009; Tinken *et al.*, 2010; Simmons *et al.*, 2011) and that a functioning, healthy endothelium is necessary for shear-stress mediated vasodilation to occur (Langille & O'Donnell, 1986) possibly resulting in altered gene expression.

A likely explanation for exercise training-induced improvements in endothelial function was demonstrated by Hambrecht *et al.* (2003) who provided evidence that *in vitro* and *in vivo* enhancement in endothelial function in response to exercise training was due to a shear stress-dependent increase of endothelium NO synthase (eNOS). Data illustrated in Chapters 4-6 provide an insight into the role of shear stress during and after leg cycling exercise, with and without interventions to either increase or attenuate shear stress and suggests that up-regulation of these signalling systems may occur very rapidly. This proposition warrants further investigation either using direct pharmacological blockers of eNOS or Western blotting techniques to examine eNOS expression.

The results reported in Chapter 4 indicate that exercising at different intensities cause a dose-dependent increase in mean shear rate and subsequent increase in brachial artery diameter after leg cycling exercise, with higher increases in shear rate and diameter only evident at the higher intensities. The role of shear in this process is highlighted by the observation that, when shear rate is increased using a heated water-bath (in one arm), independent of the exercise intensity, increases in brachial artery diameter are observed at all intensities, confirming the importance of shear rate in artery vasodilation. Similarly, a dose-dependent response in FMD was observed in response to an acute bout of exercise (Chapter 5). FMD was significantly lower immediately following exercise at the higher intensities only, normalising and reaching near baseline values by 1hr post-exercise. This study highlights the importance of taking multiple post exercise measurements (i.e. immediately, 1, 2, and 24h post exercise). Interesting, exercise intensity also plays

an important role in modulating adaptations in vascular function with exercise training (Goto *et al.*, 2003; Green *et al.*, 2004). In a carefully performed study, Goto and colleagues (2003) suggested that vascular improvements may be dependent upon repetitive increases in shear stress, but that such adaptations may be offset to some degree as higher exercise intensities create a greater background of oxidative stress, which may ultimately quench NO. More specifically, it was suggested that high intensity exercise may impair endothelium-dependent vasodilation due to an increase in reactive oxygen species, resulting in a reduction in NO bioavailability (Davies *et al.*, 1982; Bergholm *et al.*, 1999). Broadly speaking, the present study provides some support for this notion, as exercise at higher intensity was associated with larger acute impacts upon endothelial function (i.e. a reduction in FMD at the higher intensities). It is important to note that a decrease in endothelial function as a result of an acute bout of exercise may not necessarily be associated with down-regulation as an adaptive response. As highlighted by Padilla *et al.* (2011a), there are many examples in integrative human physiology of up-regulation to stimuli which challenge pathways acutely, a notion encapsulated in the concept of hormesis. More specifically, it is suggested that the stress upon the artery caused by exercise, in particular oxidative stress, could activate mechanisms for the artery's ability to recover from the immediate impairment upon the endothelium post exercise. Therefore repetitive bouts of exercise increase the artery's ability to recover and cope with the increases in oxidative stress caused with exercise, resulting in an up-regulation or expression of eNOS and anti-oxidants. This theory could potentially facilitate in the explanation of exercise training-induced benefits on vascular function.

Results reported in Chapter 4 and 5 go some way in unravelling the disparate data in the literature surrounding acute exercise and endothelial function and highlighting the systemic increases in shear stress during acute leg cycling exercise. Therefore, the next question addressed the role of shear rate, in response to exercise training, on the brachial artery of non-exercising upper limbs. Study 3 (Chapter 6) goes some way in answering this question and providing further novel insight into the role of shear stress as a systemic stimulus during cycling exercise training. When shear rate was attenuated in one forearm during cycling, no training-induced changes in endothelial function were evident. However, in the contralateral limb (exposed to normal exercise-induced increases in shear stress) a rapid increase in FMD was observed (after 2 weeks), a finding that supports the localised training effect of handgrip exercise (Tinken *et al.*, 2008), but extends it by showing shear rate increases endothelial function in the non-active upper limb in response to lower limb (systemic) exercise.

Furthermore, exercise training is usually associated with enhanced endothelial function which is eventually superseded by outwards arterial remodelling (Tinken *et al.*, 2008) and resultant normalisation of endothelial function. These effects are also observed in the vasculature of the inactive as well as active muscle beds (Padilla *et al.*, 2011a), such as arteries supplying skeletal muscles that are not directly involved in the training stimulus. However, consistent with the literature there is only limited data within the literature reporting adaptations in brachial artery remodelling in response to leg cycling exercise (Silber *et al.*, 1991; Green *et al.*, 2008a). Nevertheless, shear stress alone appears to be the most likely mechanism for

functional adaptations (Tinken *et al.*, 2009; Tinken *et al.*, 2010) as these changes were not seen in the arm that had an attenuated shear stress during exercise training. However, the transient nature of improvements observed in the present study may be due to the healthy cohort of subjects recruited to participate in this study and the type and intensity of exercise training conducted for adaptation (Goto *et al.*, 2003) since structural modification may have already been maximised, due to the healthy cohort examined.

Figure 9.1. Hypothesised changes in artery function and structure (remodelling) in response to inactivity (A) and exercise training (B) in humans (Thijssen *et al.*, 2010).

On the other side of the spectrum, it was originally hypothesised that the time-course of vascular adaptations with inactivity *may* follow a similar, albeit downward, pattern to that of exercise training (Thijssen *et al.*, 2010), with functional adaptations preceding structure. However, the literature remains disparate regarding which vascular adaptation (function or remodelling) to inactivity and deconditioning comes first. Referring again to Figure 9.1(B), it is clear that

vascular adaptations in response to exercise training follow a specific time-course, with vascular function preceding vascular remodelling adaptations (Laughlin, 1995; Laughlin *et al.*, 1996; Tinken *et al.*, 2008). Additionally, it is also apparent that these vascular adaptations to exercise training are associated with the increased regulation and expression of NO (Hambrecht *et al.*, 2003), with the principle stimulus for this being increases in shear stress (Tinken *et al.*, 2009; Tinken *et al.*, 2010). On the contrary, it was initially hypothesised that vascular adaptations to inactivity or deconditioning were likely to follow an opposite pattern to those observed with exercise training. This is highlighted from data collected during study 4 (Chapter 7), which illustrates the rapid nature of decreases in forearm resistance remodelling within 8 days of localised inactivity, with no demonstrable change in endothelial function, suggesting that remodelling adaptations precede functional adaptations. Although it has been reported that the reactive hyperaemic response is governed by the contribution of NO and other vasoactive substances (Tagawa *et al.*, 1994), in this particular study, no adaptations in largely or partly NO-mediated modifications were evident in the conduit vessels. It is therefore possible, in contrast to the hypotheses generated earlier and the proposed mechanisms depicted in Figure 9.1(A) that adaptations associated with deconditioning and inactivity follow a different pathway than that of exercise training. It is possible that deconditioning effects with inactivity are associated with pronounced vasoconstriction and an increased expression of ET-1 (Thijssen *et al.*, 2007b; Laughlin *et al.*, 2008; Thijssen *et al.*, 2010), which is known to contribute to increased peripheral resistance in heart failure and hypertension. Moreover, increases in these levels are usually associated with physical inactivity and

cardiovascular disease which are characterised by increased vascular tone (Thijssen *et al.*, 2007b).

Inactivity is associated with increases in retrograde shear rate, which is associated with the expression of pro-atherogenic phenotype (Laughlin *et al.*, 2008; Thijssen *et al.*, 2009b). Therefore, the final study was conducted to determine the acute and prolonged effects of increases in retrograde shear rate upon endothelial function. Retrograde shear rate is usually associated with pro-atherogenic characteristics and acute impairment in endothelial function (Laughlin *et al.*, 2008; Thijssen *et al.*, 2009b). In agreement with this are the results of study 5, which also demonstrated an acute impairment in endothelial function which is only evident when an increase in retrograde shear rate was also observed. Although no adaptations were present over a period of prolonged compression, this is consistent with recent data showing that endothelial function in humans was unchanged following prolonged compression training across 4 weeks (Roseguini *et al.*, 2011). Again this data provides evidence to suggest acute, but potentially not prolonged increases in retrograde shear rate to be unfavourable for endothelial function in humans.

9.3. Limitations and further research

Although the majority of the studies in this thesis took advantage of a within-subject design eliminating any between subject differences, a potential limitation of the studies is that only healthy male volunteers were recruited and these findings cannot be extrapolated to women or subjects with various pathologies or indeed in the healthy aged. It would therefore be intriguing to study such responses in patient groups suffering from risk factors associated with CVD. From a mechanistic point of

view, indices of oxidative stress or interrogate blood flow control pathways with invasive infusion of NO agonists and antagonists (e.g. L-NMMA), or limit oxidative stress with anti-oxidants were not assessed. To have pursued this line would have allowed for a precise quantification of oxidative stress as a function of exercise intensity, and also the exact change in contribution of NO bioavailability to exercise training and models of inactivity. Furthermore, concentration was solely on the NO vasodilator pathways, and therefore it is recommended that future work assay other vasodilator (e.g. bradykinin) and constrictor pathways. Finally, vascular function is affected by the sympathetic nervous system and therefore, future studies should examine the complex interaction between MSNA (muscle sympathetic nerve activity), arterial blood pressure, and other potential modulatory factors of shear rate patterns.

The notion that systemic effects occur in non-exercising regions has been discussed extensively (Green, 2005b; Thijssen & Hopman, 2008; Padilla *et al.*, 2011a). However, it has been reported that haemodynamic stimuli play an important role in endothelial and vascular smooth muscle cell adaptations to exercise training (Laughlin *et al.*, 2008). In particular, endothelial progenitor cells represent immature cells that possess the capacity to contribute to the maintenance of endothelial integrity and function (Laughlin *et al.*, 2008). Nevertheless, the impacts that exercise-induced changes in hemodynamic profile, pulse pressure, transmural pressure and circulating endothelial progenitor cells have on systemic adaptations of both endothelial and smooth muscle cells still remain unclear (Newcomer & Padilla, 2011), therefore, subsequent studies to determine this would be of interest.

9.4. Conclusion

To summarise, increases in blood flow, and therefore shear stress with exercise training are beneficial for the endothelium, and decreases in blood flow and shear stress are associated with rapid decreases in vessel dimensions (Langille & O'Donnell, 1986). Therefore, the decrease in blood flow with inactivity could be associated with an increase in vasoconstrictors (Laughlin *et al.*, 2008) supporting the notion that retrograde shear rate is associated with the expression of pro-atherogenic phenotype. Data from the final study support this, with increases in retrograde shear rate acutely impairing endothelial function. This could provide an explanation for the vascular deconditioning effects observed during a period of inactivity. Finally, data presented in this thesis demonstrate the importance of activity levels on the endothelium. Specifically, the roles of blood flow and associated shear stress during exercise and inactivity, outlined herein, provide a potential insight into the underlying mechanisms for modifications in the vasculature. These data go some way towards closing the 'risk factor gap' between cardiovascular risk and improvements in vascular adaptations highlighting the importance of physical activity (Figure 9.2) for overall cardiovascular health.

Figure 9.2 Percentage reductions in CVD events associated with physical activity that is explained by risk factors (adapted from Mora et al. 2007).

Chapter 10 | *References*

References

Adams DH & Nash GB. (1996). Disturbance of leucocyte circulation and adhesion to the endothelium as factors in circulatory pathology. *Br J Anaesth* **77,** 17-31.

Amiri F, Virdis A, Neves MF, Iglarz M, Seidah NG, Touyz RM, Reudelhuber TL & Schiffrin EL. (2004). Endothelium-restricted overexpression of human endothelin-1 causes vascular remodeling and endothelial dysfunction. *Circulation* **110,** 2233-2240.

Anderson EA & Mark AL. (1989). Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation* **79,** 93-100.

Anderson TJ, Charbonneau F, Title LM, Buithieu J, Rose MS, Conradson H, Hildebrand K, Fung M, Verma S & Lonn EM. (2011). Microvascular function predicts cardiovascular events in primary prevention: long-term results from the Firefighters and Their Endothelium (FATE) study. *Circulation* **123,** 163-169.

Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC & et al. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* **26,** 1235-1241.

Armstrong RB, Delp MD, Goljan EF & Laughlin MH. (1987). Progressive elevations in muscle blood flow during prolonged exercise in swine. *J Appl Physiol* **63,** 285-291.

Atkinson G, Batterham AM, Black MA, Cable NT, Hopkins ND, Dawson EA, Thijssen DH, Jones H, Tinken TM & Green DJ. (2009). Is the ratio of flow-mediated dilation and shear rate a statistically sound approach to normalization in cross-sectional studies on endothelial function? *J Appl Physiol* **107,** 1893-1899.

Bell LM, Watt K, Siafarikas A, Thompson A, Ratnam N, Bulsara M, Finn J, O'Driscoll G, Green DJ, Jones TW & Davis EA. (2007). Exercise alone reduces insulin resistance in obese children independently of changes in body composition. *J Clin Endocrinol Metab* **92,** 4230-4235.

Benjamin N, Calver A, Collier J, Robinson B, Vallance P & Webb D. (1995). Measuring forearm blood flow and interpreting the responses to drugs and mediators. *Hypertension* **25,** 918-923.

Bergholm R, Makimattila S, Valkonen M, Liu ML, Lahdenpera S, Taskinen MR, Sovijarvi A, Malmberg P & Yki-Jarvinen H. (1999). Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilatation in vivo. *Atherosclerosis* **145,** 341-349.

Betik AC, Luckham VB & Hughson RL. (2004). Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions. *Am J Physiol Heart Circ Physiol* **286,** H442-448.

Black MA, Cable NT, Thijssen DH & Green DJ. (2008). Importance of measuring the time course of flow-mediated dilatation in humans. *Hypertension* **51,** 203-210.

Blair SN & Brodney S. (1999). Effects of physical inactivity and obesity on morbidity and mortality: current evidence and research issues. *Med Sci Sports Exerc* **31,** S646-662.

Blair SN, Kampert JB, Kohl HW, 3rd, Barlow CE, Macera CA, Paffenbarger RS, Jr. & Gibbons LW. (1996). Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *Jama* **276,** 205-210.

Blair SN, Kohl HW, 3rd, Barlow CE, Paffenbarger RS, Jr., Gibbons LW & Macera CA. (1995). Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *Jama* **273,** 1093-1098.

Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH & Gibbons LW. (1989). Physical fitness and all-cause mortality. A prospective study of healthy men and women. *Jama* **262,** 2395-2401.

Blair SN & Morris JN. (2009). Healthy hearts--and the universal benefits of being physically active: physical activity and health. *Ann Epidemiol* **19,** 253-256.

Bleeker MW, De Groot PC, Poelkens F, Rongen GA, Smits P & Hopman MT. (2005a). Vascular adaptation to 4 wk of deconditioning by unilateral lower limb suspension. *Am J Physiol Heart Circ Physiol* **288,** H1747-1755.

Bleeker MW, De Groot PC, Rongen GA, Rittweger J, Felsenberg D, Smits P & Hopman MT. (2005b). Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study. *J Appl Physiol* **99,** 1293-1300.

Bleeker MW, Hopman MT, Rongen GA & Smits P. (2004). Unilateral lower limb suspension can cause deep venous thrombosis. *Am J Physiol Regul Integr Comp Physiol* **286,** R1176-1177.

Bleeker MW, Kooijman M, Rongen GA, Hopman MT & Smits P. (2005c). Preserved contribution of nitric oxide to baseline vascular tone in deconditioned human skeletal muscle. *J Physiol* **565,** 685-694.

Bonnin P, Ben Driss A, Benessiano J, Maillet A, Pavy le Traon A & Levy BI. (2001). Enhanced flow-dependent vasodilatation after bed rest, a possible mechanism for orthostatic intolerance in humans. *Eur J Appl Physiol* **85,** 420-426.

Booth FW, Chakravarthy MV & Spangenburg EE. (2002). Exercise and gene expression: physiological regulation of the human genome through physical activity. *J Physiol* **543,** 399-411.

Bouvet C, Belin de Chantemele E, Guihot AL, Vessieres E, Bocquet A, Dumont O, Jardel A, Loufrani L, Moreau P & Henrion D. (2007). Flow-induced remodeling in resistance arteries from obese Zucker rats is associated with endothelial dysfunction. *Hypertension* **50,** 248-254.

Brown MD. (2003). Exercise and coronary vascular remodelling in the healthy heart. *Exp Physiol* **88,** 645-658.

Campia U, Cardillo C & Panza JA. (2004). Ethnic differences in the vasoconstrictor activity of endogenous endothelin-1 in hypertensive patients. *Circulation* **109,** 3191-3195.

Cardillo C, Campia U, Iantorno M & Panza JA. (2004). Enhanced vascular activity of endogenous endothelin-1 in obese hypertensive patients. *Hypertension* **43,** 36-40.

Carter H, Birk GK, Dawson EA, Naylor LH, Cable NT, Thijssen DH & Green DJ. (2011). Effect of shear rate manipulation on conduit artery dilation in humans. ***Submitted***.

Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK & Deanfield JE. (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* **340,** 1111-1115.

Chappell DC, Varner SE, Nerem RM, Medford RM & Alexander RW. (1998). Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. *Circ Res* **82,** 532-539.

Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL & Stone PH. (2007). Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol* **49,** 2379-2393.

Clarkson P, Montgomery HE, Mullen MJ, Donald AE, Powe AJ, Bull T, Jubb M, World M & Deanfield JE. (1999). Exercise training enhances endothelial function in young men. *J Am Coll Cardiol* **33,** 1379-1385.

Convertino VA, Goldwater DJ & Sandler H. (1986). Bedrest-induced peak VO2 reduction associated with age, gender, and aerobic capacity. *Aviat Space Environ Med* **57,** 17-22.

Conway J. (1963). A vascular abnormality in hypertension. A study of blood flow in the forearm. *Circulation* **27,** 520-529.

Cooke JP & Dzau VJ. (1997). Nitric oxide synthase: role in the genesis of vascular disease. *Annu Rev Med* **48,** 489-509.

Cooper CE, Vollaard NB, Choueiri T & Wilson MT. (2002). Exercise, free radicals and oxidative stress. *Biochem Soc Trans* **30,** 280-285.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J & Vogel R. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* **39,** 257-265.

Davies KJ, Quintanilha AT, Brooks GA & Packer L. (1982). Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* **107,** 1198-1205.

Davis ME, Cai H, Drummond GR & Harrison DG. (2001). Shear stress regulates endothelial nitric oxide synthase expression through c-Src by divergent signaling pathways. *Circ Res* **89,** 1073-1080.

Dawson EA, Whyte GP, Black MA, Jones H, Hopkins N, Oxborough D, Gaze D, Shave RE, Wilson M, George KP & Green DJ. (2008). Changes in vascular and cardiac function after prolonged strenuous exercise in humans. *J Appl Physiol* **105,** 1562-1568.

de Groot P, Crozier J, Rakobowchuk M, Hopman M & MacDonald M. (2005). Electrical stimulation alters FMD and arterial compliance in extremely inactive legs. *Med Sci Sports Exerc* **37,** 1356-1364.

de Groot PC, Bleeker MW & Hopman MT. (2006a). Magnitude and time course of arterial vascular adaptations to inactivity in humans. *Exerc Sport Sci Rev* **34,** 65-71.

de Groot PC, Bleeker MW, van Kuppevelt DH, van der Woude LH & Hopman MT. (2006b). Rapid and extensive arterial adaptations after spinal cord injury. *Arch Phys Med Rehabil* **87,** 688-696.

de Groot PC, Poelkens F, Kooijman M & Hopman MT. (2004). Preserved flow-mediated dilation in the inactive legs of spinal cord-injured individuals. *Am J Physiol Heart Circ Physiol* **287,** H374-380.

De Keulenaer GW, Alexander RW, Ushio-Fukai M, Ishizaka N & Griendling KK. (1998). Tumour necrosis factor alpha activates a p22phox-based NADH oxidase in vascular smooth muscle. *Biochem J* **329 (Pt 3),** 653-657.

Delp MD & Laughlin MH. (1997). Time course of enhanced endothelium-mediated dilation in aorta of trained rats. *Med Sci Sports Exerc* **29,** 1454-1461.

Delp MD, McAllister RM & Laughlin MH. (1993). Exercise training alters endothelium-dependent vasoreactivity of rat abdominal aorta. *J Appl Physiol* **75,** 1354-1363.

DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H & Seals DR. (2000). Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* **102,** 1351-1357.

Dimmeler S & Zeiher AM. (2003). Exercise and cardiovascular health: get active to "AKTivate" your endothelial nitric oxide synthase. *Circulation* **107,** 3118-3120.

Dinenno FA, Tanaka H, Monahan KD, Clevenger CM, Eskurza I, DeSouza CA & Seals DR. (2001). Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men. *J Physiol* **534,** 287-295.

Doshi SN, Naka KK, Payne N, Jones CJ, Ashton M, Lewis MJ & Goodfellow J. (2001). Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci (Lond)* **101,** 629-635.

Dyson KS, Shoemaker JK & Hughson RL. (2006). Effect of acute sympathetic nervous system activation on flow-mediated dilation of brachial artery. *Am J Physiol Heart Circ Physiol* **290,** H1446-1453.

Edwards DG, Schofield RS, Lennon SL, Pierce GL, Nichols WW & Braith RW. (2004). Effect of exercise training on endothelial function in men with coronary artery disease. *Am J Cardiol* **93,** 617-620.

Eskurza I, Monahan KD, Robinson JA & Seals DR. (2004). Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. *J Physiol* **556,** 315-324.

Folkow B, Grimby G & Thulesius O. (1958). Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance. *Acta Physiol Scand* **44,** 255-272.

Furchgott RF & Jothianandan D. (1991). Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels* **28,** 52-61.

Furchgott RF, Martin W & Cherry PD. (1985). Blockade of endothelium-dependent vasodilation by hemoglobin: a possible factor in vasospasm associated with hemorrhage. *Adv Prostaglandin Thromboxane Leukot Res* **15,** 499-502.

Furchgott RF & Vanhoutte PM. (1989). Endothelium-derived relaxing and contracting factors. *Faseb J* **3,** 2007-2018.

Furchgott RF & Zawadzki JV. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288,** 373-376.

Geng YJ & Libby P. (2002). Progression of atheroma: a struggle between death and procreation. *Arterioscler Thromb Vasc Biol* **22,** 1370-1380.

Gielen S & Hambrecht R. (2004). The childhood obesity epidemic: impact on endothelial function. *Circulation* **109,** 1911-1913.

Gielen S, Schuler G & Adams V. (2010). Cardiovascular effects of exercise training: molecular mechanisms. *Circulation* **122,** 1221-1238.

Gokce N, Vita JA, Bader DS, Sherman DL, Hunter LM, Holbrook M, O'Malley C, Keaney JF, Jr. & Balady GJ. (2002). Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *Am J Cardiol* **90,** 124-127.

Gori T, Dragoni S, Lisi M, Di Stolfo G, Sonnati S, Fineschi M & Parker JD. (2008). Conduit artery constriction mediated by low flow a novel noninvasive method for the assessment of vascular function. *J Am Coll Cardiol* **51,** 1953-1958.

Goto C, Higashi Y, Kimura M, Noma K, Hara K, Nakagawa K, Kawamura M, Chayama K, Yoshizumi M & Nara I. (2003). Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* **108,** 530-535.

Green D, Cheetham C, Henderson C, Weerasooriya R & O'Driscoll G. (2002a). Effect of cardiac pacing on forearm vascular responses and nitric oxide function. *Am J Physiol Heart Circ Physiol* **283,** H1354-1360.

Green D, Cheetham C, Mavaddat L, Watts K, Best M, Taylor R & O'Driscoll G. (2002b). Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide. *Am J Physiol* **283,** H899-907.

Green D, Cheetham C, Reed C, Dembo L & O'Driscoll G. (2002c). Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *J Appl Physiol* **93,** 361-368.

Green DJ. (2005a). Decreasing cardiovascular risk through exercise training: Understanding the mecahnisms to optimise the benefits. *ACRA Proceedings*.

Green DJ. (2005b). Point-Counterpoint: Flow-mediated dilation does reflect nitric oxide-mediated endothelial function. *J Appl Physiol* **99,** 1233-1234.

Green DJ, Bilsborough W, Naylor LH, Reed C, Wright J, O'Driscoll G & Walsh JH. (2005). Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *J Physiol* **562,** 617-628.

Green DJ, Cable NT, Fox C, Rankin JM & Taylor RR. (1994). Modification of forearm resistance vessels by exercise training in young men. *J Appl Physiol* **77,** 1829-1833.

Green DJ, Carter HH, Fitzsimons MG, Cable NT, Thijssen DH & Naylor LH. (2010). Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. *J Physiol* **588,** 1571-1577.

Green DJ, Fowler DT, O'Driscoll JG, Blanksby BA & Taylor RR. (1996). Endothelium-derived nitric oxide activity in forearm vessels of tennis players. *J Appl Physiol* **81,** 943-948.

Green DJ, Maiorana A, O'Driscoll G & Taylor R. (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol* **561,** 1-25.

Green DJ, Maiorana AJ & Cable NT. (2008a). Exercise training does induce vascular adaptations beyond the active muscle beds. *J Appl Physiol* **105,** 1002-1004.

Green DJ, O'Driscoll G, Joyner MJ & Cable NT. (2008b). Exercise and Cardiovascular Risk Reduction: Time to Update the Rationale for Exercise? *J Appl Physiol* **105,** 766-768.

Green DJ, O'Driscoll JG, Blanksby BA & Taylor RR. (1997). Effect of casting on forearm resistance vessels in young men. *Med Sci Sports Exerc* **29,** 1325-1331.

Green DJ, Spence A, Halliwill JR, Cable NT & Thijssen DH. (2011). Exercise and vascular adaptation in asymptomatic humans. *Exp Physiol* **96,** 57-70.

Green DJ, Walsh JH, Maiorana A, Best MJ, Taylor RR & O'Driscoll JG. (2003). Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *Am J Physiol Heart Circ Physiol* **285,** H2679-2687.

Groothuis JT, Thijssen DH, Rongen GA, Deinum J, Danser AH, Geurts AC, Smits P & Hopman MT. (2010). Angiotensin II contributes to the increased baseline leg vascular resistance in spinal cord-injured individuals. *J Hypertens* **28,** 2094-2101.

Guo D, Chien S & Shyy JY. (2007). Regulation of endothelial cell cycle by laminar versus oscillatory flow: distinct modes of interactions of AMP-activated protein kinase and Akt pathways. *Circ Res* **100,** 564-571.

Hahn C & Schwartz MA. (2009). Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol* **10,** 53-62.

Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW & Schuler G. (2003). Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* **107,** 3152-3158.

Hamburg NM, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, Gokce N, Ruderman NB, Keaney JF, Jr. & Vita JA. (2007). Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. *Arterioscler Thromb Vasc Biol* **27,** 2650-2656.

Haram PM, Adams V, Kemi OJ, Brubakk AO, Hambrecht R, Ellingsen O & Wisloff U. (2006). Time-course of endothelial adaptation following acute and regular exercise. *Eur J Cardiovasc Prev Rehabil* **13,** 585-591.

Harris RA, Nishiyama SK, Wray DW & Richardson RS. (2010). Ultrasound assessment of flow-mediated dilation. *Hypertension* **55,** 1075-1085.

Harris RA & Padilla J. (2007). Proper "normalization" of flow-mediated dilation for shear. *J Appl Physiol* **103,** 1108; author reply 1109.

Harris RA, Padilla J, Hanlon KP, Rink LD & Wallace JP. (2008). The flow-mediated dilation response to acute exercise in overweight active and inactive men. *Obesity (Silver Spring)* **16,** 578-584.

Harrison DG, Sayegh H, Ohara Y, Inoue N & Venema RC. (1996). Regulation of expression of the endothelial cell nitric oxide synthase. *Clin Exp Pharmacol Physiol* **23,** 251-255.

Harrison DG, Widder J, Grumbach I, Chen W, Weber M & Searles C. (2006). Endothelial mechanotransduction, nitric oxide and vascular inflammation. *J Intern Med* **259,** 351-363.

Harvey PJ, Morris BL, Kubo T, Picton PE, Su WS, Notarius CF & Floras JS. (2005). Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. *J Hypertens* **23,** 285-292.

Higashi Y, Sasaki S, Kurisu S, Yoshimizu A, Sasaki N, Matsuura H, Kajiyama G & Oshima T. (1999). Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. *Circulation* **100,** 1194-1202.

Hijmering ML, Stroes ES, Olijhoek J, Hutten BA, Blankestijn PJ & Rabelink TJ. (2002). Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation. *J Am Coll Cardiol* **39,** 683-688.

Himburg HA, Dowd SE & Friedman MH. (2007). Frequency-dependent response of the vascular endothelium to pulsatile shear stress. *Am J Physiol Heart Circ Physiol* **293,** H645-653.

Hirano K, Hirano M & Kanaide H. (2004). Regulation of myosin phosphorylation and myofilament Ca2+ sensitivity in vascular smooth muscle. *J Smooth Muscle Res* **40,** 219-236.

Hopman MT, Groothuis JT, Flendrie M, Gerrits KH & Houtman S. (2002). Increased vascular resistance in paralyzed legs after spinal cord injury is reversible by training. *J Appl Physiol* **93,** 1966-1972.

Hornig B, Maier V & Drexler H. (1996). Physical training improves endothelial function in patients with chronic heart failure. *Circulation* **93,** 210-214.

Huonker M, Schmid A, Schmidt-Trucksass A, Grathwohl D & Keul J. (2003). Size and blood flow of central and peripheral arteries in highly trained able-bodied and disabled athletes. *J Appl Physiol* **95,** 685-691.

Hwang J, Ing MH, Salazar A, Lassegue B, Griendling K, Navab M, Sevanian A & Hsiai TK. (2003). Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation. *Circ Res* **93,** 1225-1232.

Ignarro LJ. (1990). Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu Rev Pharmacol Toxicol* **30,** 535-560.

Ignarro LJ, Byrns RE, Buga GM & Wood KS. (1987). Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ Res* **61,** 866-879.

Jasperse JL & Laughlin MH. (2006). Endothelial function and exercise training: evidence from studies using animal models. *Med Sci Sports Exerc* **38,** 445-454.

Ji LL. (1999). Antioxidants and oxidative stress in exercise. *Proc Soc Exp Biol Med* **222,** 283-292.

Jin RC & Loscalzo J. (2010). Vascular Nitric Oxide: Formation and Function. *J Blood Med* **2010,** 147-162.

Johnson BD, Padilla J & Wallace JP. (2011). The exercise dose affects oxidative stress and brachial artery flow-mediated dilation in trained men. *Eur J Appl Physiol.*

Johnson LR & Laughlin MH. (2000). Chronic exercise training does not alter pulmonary vasorelaxation in normal pigs. *J Appl Physiol* **88,** 2008-2014.

Johnson LR, Rush JW, Turk JR, Price EM & Laughlin MH. (2001). Short-term exercise training increases ACh-induced relaxation and eNOS protein in porcine pulmonary arteries. *J Appl Physiol* **90,** 1102-1110.

Jolliffe JA, Rees K, Taylor RS, Thompson D, Oldridge N & Ebrahim S. (2001). Exercise-based rehabilitation for coronary heart disease. *Cochrane Database Syst Rev,* CD001800.

Jones H, Green DJ, George K & Atkinson G. (2010). Intermittent exercise abolishes the diurnal variation in endothelial-dependent flow-mediated dilation in humans. *Am J Physiol Regul Integr Comp Physiol* **298,** R427-432.

Joyner MJ, Dietz NM & Shepherd JT. (2001). From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *J Appl Physiol* **91,** 2431-2441.

Joyner MJ & Green DJ. (2009). Exercise protects the cardiovascular system: effects beyond traditional risk factors. *J Physiol* **587,** 5551-5558.

Kingwell BA, Arnold PJ, Jennings GL & Dart AM. (1997). Spontaneous running increases aortic compliance in Wistar-Kyoto rats. *Cardiovasc Res* **35,** 132-137.

Kizhakekuttu TJ, Gutterman DD, Phillips SA, Jurva JW, Arthur EI, Das E & Widlansky ME. (2010). Measuring FMD in the brachial artery: how important is QRS gating? *J Appl Physiol* **109,** 959-965.

Koller A, Huang A, Sun D & Kaley G. (1995). Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles. Role of endothelial nitric oxide and prostaglandins. *Circ Res* **76,** 544-550.

Kooijman M, Thijssen DHJ, de Groot PCE, Bleeker MWP, van Kuppevelt HJM, Green DJ, Rongen GA, Smits P & Hopman MTE. (2008). Flow-mediated dilatation in the femoral artery is nitric oxide mediated in humans. *Journal of Physiology (London)* **586,** 1137-1145.

Langille BL & O'Donnell F. (1986). Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science* **231,** 405-407.

Laughlin MH. (1995). Endothelium-mediated control of coronary vascular tone after chronic exercise training. *Med Sci Sports Exerc* **27,** 1135-1144.

Laughlin MH, McAllister RM, Jasperse JL, Crader SE, Williams DA & Huxley VH. (1996). Endothelium-medicated control of the coronary circulation. Exercise training-induced vascular adaptations. *Sports Med* **22,** 228-250.

Laughlin MH, Newcomer SC & Bender SB. (2008). Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol* **104,** 588-600.

Laughlin MH, Overholser KA & Bhatte MJ. (1989). Exercise training increases coronary transport reserve in miniature swine. *J Appl Physiol* **67,** 1140-1149.

Laughlin MH, Rubin LJ, Rush JW, Price EM, Schrage WG & Woodman CR. (2003). Short-term training enhances endothelium-dependent dilation of coronary arteries, not arterioles. *J Appl Physiol* **94,** 234-244.

Lewis TV, Dart AM, Chin-Dusting JP & Kingwell BA. (1999). Exercise training increases basal nitric oxide production from the forearm in hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol* **19,** 2782-2787.

Lind L, Berglund L, Larsson A & Sundstrom J. (2011). Endothelial function in resistance and conduit arteries and 5-year risk of cardiovascular disease. *Circulation* **123,** 1545-1551.

Linke A, Schoene N, Gielen S, Hofer J, Erbs S, Schuler G & Hambrecht R. (2001). Endothelial dysfunction in patients with chronic heart failure: systemic effects of lower-limb exercise training. *J Am Coll Cardiol* **37,** 392-397.

Logason K, Barlin T, Jonsson ML, Bostrom A, Hardemark HG & Karacagil S. (2001). The importance of Doppler angle of insonation on differentiation between 50-69% and 70-99% carotid artery stenosis. *Eur J Vasc Endovasc Surg* **21,** 311-313.

Loufrani L & Henrion D. (2008). Role of the cytoskeleton in flow (shear stress)-induced dilation and remodeling in resistance arteries. *Med Biol Eng Comput* **46,** 451-460.

Luft JH. (1966). Fine structures of capillary and endocapillary layer as revealed by ruthenium red. *Fed Proc* **25,** 1773-1783.

Luscher TF. (1990). Imbalance of endothelium-derived relaxing and contracting factors. A new concept in hypertension? *Am J Hypertens* **3,** 317-330.

Maiorana A, O'Driscoll G, Dembo L, Cheetham C, Goodman C, Taylor R & Green DJ. (2000). Effect of aerobic and resistance exercise training on vascular function in heart failure. *Am J Physiol* **279,** H1999-H2005.

Maiorana A, O'Driscoll G, Dembo L, Goodman C, Taylor R & Green D. (2001). Exercise training, vascular function, and functional capacity in middle-aged subjects. *Med Sci Sports Exerc* **33,** 2022-2028.

Maiorana A, O'Driscoll G, Taylor R & Green D. (2003). Exercise and the nitric oxide vasodilator system. *Sports Med* **33,** 1013-1035.

Manson JE, Hu FB, Rich-Edwards JW, Colditz GA, Stampfer MJ, Willett WC, Speizer FE & Hennekens CH. (1999). A prospective study of walking as compared with vigorous exercise in the prevention of coronary heart disease in women. *N Engl J Med* **341,** 650-658.

Martinez-Lemus LA, Hill MA & Meininger GA. (2009). The plastic nature of the vascular wall: a continuum of remodeling events contributing to control of arteriolar diameter and structure. *Physiology (Bethesda)* **24,** 45-57.

McAllister RM, Kimani JK, Webster JL, Parker JL & Laughlin MH. (1996). Effects of exercise training on responses of peripheral and visceral arteries in swine. *J Appl Physiol* **80,** 216-225.

McAllister RM & Laughlin MH. (1997). Short-term exercise training alters responses of porcine femoral and brachial arteries. *J Appl Physiol* **82,** 1438-1444.

McGowan CL, Levy AS, Millar PJ, Guzman JC, Morillo CA, McCartney N & Macdonald MJ. (2006). Acute vascular responses to isometric handgrip exercise and effects of training in persons medicated for hypertension. *Am J Physiol Heart Circ Physiol* **291,** H1797-1802.

McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H & Harrison DG. (2003). Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol* **285,** H2290-2297.

Miyachi M, Tanaka H, Yamamoto K, Yoshioka A, Takahashi K & Onodera S. (2001). Effects of one-legged endurance training on femoral arterial and venous size in healthy humans. *J Appl Physiol* **90,** 2439-2444.

Moncada S, Palmer RM & Higgs EA. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* **43,** 109-142.

Mora S, Cook N, Buring JE, Ridker PM & Lee IM. (2007). Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation* **116,** 2110-2118.

Moriguchi J, Itoh H, Harada S, Takeda K, Hatta T, Nakata T & Sasaki S. (2005). Low frequency regular exercise improves flow-mediated dilatation of subjects with mild hypertension. *Hypertens Res* **28,** 315-321.

Mullen MJ, Kharbanda RK, Cross J, Donald AE, Taylor M, Vallance P, Deanfield JE & MacAllister RJ. (2001). Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia. *Circ Res* **88,** 145-151.

Mulsch A, Bassenge E & Busse R. (1989). Nitric oxide synthesis in endothelial cytosol: evidence for a calcium-dependent and a calcium-independent mechanism. *Naunyn Schmiedebergs Arch Pharmacol* **340,** 767-770.

Myers J, Prakash M, Froelicher V, Do D, Partington S & Atwood JE. (2002). Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med* **346,** 793-801.

Naylor L, Arnolda L, Deague J, Playford D, O'Driscoll G & Green DJ. (2005a). Reduced diastolic function in elite athletes is augmented with the resumption of exercise training. *J Physiol* **563,** 957-963.

Naylor LH, Carter H, FitzSimons MG, Cable NT, Thijssen DH & Green DJ. (2011). Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. *Am J Physiol* **300,** H664-669.

Naylor LH, Weisbrod CJ, O'Driscoll G & Green DJ. (2005b). Measuring peripheral resistance and conduit arterial structure in humans using Doppler ultrasound. *J Appl Physiol* **98,** 2311-2315.

Newby DE, Boon NA & Webb DJ. (1997). Comparison of forearm vasodilatation to substance P and acetylcholine: contribution of nitric oxide. *Clin Sci (Lond)* **92,** 133-138.

Newcomer SC & Padilla J. (2011). Racket sports as a model of studying vascular adaptations: a comeback after a quarter of a century. *J Appl Physiol* **110,** 1156-1157.

Niebauer J & Cooke JP. (1996). Cardiovascular effects of exercise: role of endothelial shear stress. *J Am Coll Cardiol* **28,** 1652-1660.

Nieuwdorp M, Meuwese MC, Vink H, Hoekstra JB, Kastelein JJ & Stroes ES. (2005). The endothelial glycocalyx: a potential barrier between health and vascular disease. *Curr Opin Lipidol* **16,** 507-511.

Ohara Y, Peterson TE & Harrison DG. (1993). Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* **91,** 2546-2551.

Oldridge NB, Guyatt GH, Fischer ME & Rimm AA. (1988). Cardiac rehabilitation after myocardial infarction. Combined experience of randomized clinical trials. *Jama* **260,** 945-950.

Olive JL, Slade JM, Bickel CS, Dudley GA & McCully KK. (2004). Increasing blood flow before exercise in spinal cord-injured individuals does not alter muscle fatigue. *J Appl Physiol* **96,** 477-482.

Olsen R, Santone K, Melder D, Oakes SG, Abraham R & Powis G. (1988). An increase in intracellular free Ca2+ associated with serum-free growth stimulation of

Swiss 3T3 fibroblasts by epidermal growth factor in the presence of bradykinin. *J Biol Chem* **263,** 18030-18035.

Padilla J, Harris RA, Fly AD, Rink LD & Wallace JP. (2006). The effect of acute exercise on endothelial function following a high-fat meal. *Eur J Appl Physiol* **98,** 256-262.

Padilla J, Johnson BD, Newcomer SC, Wilhite DP, Mickleborough TD, Fly AD, Mather KJ & Wallace JP. (2008). Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus. *Cardiovasc Ultrasound* **6,** 44.

Padilla J, Simmons GH, Bender SB, Arce-Esquivel AA, Whyte JJ & Laughlin MH. (2011a). Vascular effects of exercise: endothelial adaptations beyond active muscle beds. *Physiology (Bethesda)* **26,** 132-145.

Padilla J, Simmons GH, Vianna LC, Davis MJ, Laughlin MH & Fadel PJ. (2011b). Brachial artery vasodilatation during prolonged lower limb exercise: role of shear rate. *Exp Physiol* **96,** 1019-1027.

Padilla J, Young CN, Simmons GH, Deo SH, Newcomer SC, Sullivan JP, Laughlin MH & Fadel PJ. (2010). Increased muscle sympathetic nerve activity acutely alters conduit artery shear rate patterns. *Am J Physiol Heart Circ Physiol* **298,** H1128-1135.

Paffenbarger RS, Jr., Hyde RT, Wing AL & Hsieh CC. (1986). Physical activity, all-cause mortality, and longevity of college alumni. *N Engl J Med* **314,** 605-613.

Palmer RM, Ferrige AG & Moncada S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327,** 524-526.

Parker BA, Trehearn TL & Meendering JR. (2009). Pick your Poiseuille: normalizing the shear stimulus in studies of flow-mediated dilation. *J Appl Physiol* **107,** 1357-1359.

Pohl U, Holtz J, Busse R & Bassenge E. (1986). Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension* **8,** 37-44.

Potter K, Hankey GJ, Green DJ, Eikelboom JW & Arnolda LF. (2008). Homocysteine or renal impairment: which is the real cardiovascular risk factor? *Arterioscler Thromb Vasc Biol* **28,** 1158-1164.

Pullin CH, Bellamy MF, Bailey D, Ashton M, Davies B, Willaims S, Goodfellow J, Wilson JF & Lewis MJ. (2004). Time course of changes in endothelial function following exercise in habitually sedentary men. *J Ex Phys (Online)* **7,** 14-22.

Pyke KE, Dwyer EM & Tschakovsky ME. (2004). Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. *J Appl Physiol* **97,** 499-508.

Pyke KE & Tschakovsky ME. (2005). The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol* **568,** 357-369.

Pyke KE & Tschakovsky ME. (2007). Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation? *J Appl Physiol* **102,** 1510-1519.

Raitakari OT, Adams MR, McCredie RJ, Griffiths KA, Stocker R & Celermajer DS. (2000). Oral vitamin C and endothelial function in smokers: short-term improvement, but no sustained beneficial effect. *J Am Coll Cardiol* **35,** 1616-1621.

Raitakari OT & Celermajer DS. (2000). Testing for endothelial dysfunction. *Ann Med* **32,** 293-304.

Rakobowchuk M, Crozier J, Glover EI, Yasuda N, Phillips SM, Tarnopolsky MA & MacDonald MJ. (2011). Short-term unilateral leg immobilization alters peripheral but not central arterial structure and function in healthy young humans. *Eur J Appl Physiol* **111,** 203-210.

Rakobowchuk M, McGowan CL, de Groot PC, Hartman JW, Phillips SM & MacDonald MJ. (2005). Endothelial function of young healthy males following whole body resistance training. *J Appl Physiol* **98,** 2185-2190.

Rees DD, Palmer RM, Schulz R, Hodson HF & Moncada S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br J Pharmacol* **101,** 746-752.

Remensnyder JP, Mitchell JH & Sarnoff SJ. (1962). Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res* **11,** 370-380.

Rognmo O, Bjornstad TH, Kahrs C, Tjonna AE, Bye A, Haram PM, Stolen T, Slordahl SA & Wisloff U. (2008). Endothelial function in highly endurance-trained men: effects of acute exercise. *J Strength Cond Res* **22,** 535-542.

Roseguini BT, Sheldon R, Stroup A, Bell JW, Maurer D, Crist BD, Laughlin MH & Newcomer SC. (2011). Impact of chronic intermittent external compressions on forearm blood flow capacity in humans. *Eur J Appl Physiol* **111,** 509-519.

Rowley NJ, Dawson EA, Birk GK, Cable NT, George K, Whyte G, Thijssen DH & Green DJ. (2011). Exercise and arterial adaptation in humans: uncoupling localized and systemic effects. *J Appl Physiol* **110,** 1190-1195.

Rubanyi GM, Romero JC & Vanhoutte PM. (1986). Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol* **250,** H1145-1149.

Rundell KW, Hoffman JR, Caviston R, Bulbulian R & Hollenbach AM. (2007). Inhalation of ultrafine and fine particulate matter disrupts systemic vascular function. *Inhal Toxicol* **19,** 133-140.

Sabatier MJ, Stoner L, Mahoney ET, Black C, Elder C, Dudley GA & McCully K. (2006). Electrically stimulated resistance training in SCI individuals increases muscle fatigue resistance but not femoral artery size or blood flow. *Spinal Cord* **44,** 227-233.

Schmidt K, Mayer B & Kukovetz WR. (1989). Effect of calcium on endothelium-derived relaxing factor formation and cGMP levels in endothelial cells. *Eur J Pharmacol* **170,** 157-166.

Sessa WC, Pritchard K, Seyedi N, Wang J & Hintze TH. (1994). Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res* **74,** 349-353.

Sesso HD, Paffenbarger RS, Jr. & Lee IM. (2000). Physical activity and coronary heart disease in men: The Harvard Alumni Health Study. *Circulation* **102,** 975-980.

Shoemaker JK, Hogeman CS, Silber DH, Gray K, Herr M & Sinoway LI. (1998). Head-down-tilt bed rest alters forearm vasodilator and vasoconstrictor responses. *J Appl Physiol* **84,** 1756-1762.

Silber D, McLaughlin D & Sinoway L. (1991). Leg exercise conditioning increases peak forearm blood flow. *J Appl Physiol* **71,** 1568-1573.

Silber DH & Sinoway LI. (1990). Reversible impairment of forearm vasodilation after forearm casting. *J Appl Physiol* **68,** 1945-1949.

Silvestro A, Scopacasa F, Oliva G, de Cristofaro T, Iuliano L & Brevetti G. (2002). Vitamin C prevents endothelial dysfunction induced by acute exercise in patients with intermittent claudication. *Atherosclerosis* **165,** 277-283.

Simmons GH, Padilla J, Young CN, Wong BJ, Lang JA, Davis MJ, Laughlin MH & Fadel PJ. (2011). Increased brachial artery retrograde shear rate at exercise onset is abolished during prolonged cycling: role of thermoregulatory vasodilation. *J Appl Physiol* **110,** 389-397.

Sinoway LI, Musch TI, Minotti JR & Zelis R. (1986). Enhanced maximal metabolic vasodilatation in the dominant forearms of tennis players. *J Appl Physiol* **61,** 673-678.

Sinoway LI, Shenberger J, Wilson J, McLaughlin D, Musch T & Zelis R. (1987). A 30-day forearm work protocol increases maximal forearm blood flow. *J Appl Physiol* **62,** 1063-1067.

Slager CJ, Wentzel JJ, Gijsen FJ, Schuurbiers JC, van der Wal AC, van der Steen AF & Serruys PW. (2005). The role of shear stress in the generation of rupture-prone vulnerable plaques. *Nat Clin Pract Cardiovasc Med* **2,** 401-407.

Sugawara J, Hayashi K, Kaneko F, Yamada H, Kizuka T & Tanaka H. (2004). Reductions in basal limb blood flow and lumen diameter after short-term leg casting. *Med Sci Sports Exerc* **36,** 1689-1694.

Sun D, Huang A, Koller A & Kaley G. (1994). Short-term daily exercise activity enhances endothelial NO synthesis in skeletal muscle arterioles of rats. *J Appl Physiol* **76,** 2241-2247.

Taddei S, Virdis A, Ghiadoni L, Sudano I, Notari M & Salvetti A. (1999). Vasoconstriction to endogenous endothelin-1 is increased in the peripheral circulation of patients with essential hypertension. *Circulation* **100,** 1680-1683.

Tagawa T, Imaizumi T, Endo T, Shiramoto M, Harasawa Y & Takeshita A. (1994). Role of nitric oxide in reactive hyperemia in human forearm vessels. *Circulation* **90,** 2285-2290.

Takeshita A & Mark AL. (1980). Decreased vasodilator capacity of forearm resistance vessels in borderline hypertension. *Hypertension* **2,** 610-616.

Tanaka H, Monahan KD & Seals DR. (2001). Age-predicted maximal heart rate revisited. *J Am Coll Cardiol* **37,** 153-156.

Tarbell JM & Ebong EE. (2008). The endothelial glycocalyx: a mechano-sensor and -transducer. *Sci Signal* **1,** pt8.

Tarbell JM & Pahakis MY. (2006). Mechanotransduction and the glycocalyx. *J Intern Med* **259,** 339-350.

Taylor RS, Brown A, Ebrahim S, Jolliffe J, Noorani H, Rees K, Skidmore B, Stone JA, Thompson DR & Oldridge N. (2004). Exercise-based rehabilitation for patients with coronary heart disease: systematic review and meta-analysis of randomized controlled trials. *Am J Med* **116,** 682-692.

Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME & Green DJ. (2011a). Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol* **300,** H2-12.

Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT & Green DJ. (2008a). Heterogeneity in conduit artery function in humans: impact of arterial size. *Am J Physiol Heart Circ Physiol* **295,** H1927-1934.

Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT & Green DJ. (2009a). Brachial artery blood flow responses to different modalities of lower limb exercise. *Med Sci Sports Exerc* **41,** 1072-1079.

Thijssen DH, Dawson EA, Tinken TM, Cable NT & Green DJ. (2009b). Retrograde flow and shear rate acutely impair endothelial function in humans. *Hypertension* **53,** 986-992.

Thijssen DH, de Groot P, Kooijman M, Smits P & Hopman MT. (2006). Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *Am J Physiol Heart Circ Physiol* **291,** H3122-3129.

Thijssen DH, de Groot PC, Smits P & Hopman MT. (2007a). Vascular adaptations to 8-week cycling training in older men. *Acta Physiol (Oxf)* **190,** 221-228.

Thijssen DH, Ellenkamp R, Kooijman M, Pickkers P, Rongen GA, Hopman MT & Smits P. (2007b). A causal role for endothelin-1 in the vascular adaptation to skeletal muscle deconditioning in spinal cord injury. *Arterioscler Thromb Vasc Biol* **27,** 325-331.

Thijssen DH, Green DJ & Hopman MT. (2011b). Blood Vessel Remodeling and Physical Inactivity in Humans. *J Appl Physiol.*

Thijssen DH, Green DJ, Steendijk S & Hopman MT. (2009c). Sympathetic vasomotor control does not explain the change in femoral artery shear rate pattern during arm-crank exercise. *Am J Physiol Heart Circ Physiol* **296,** H180-185.

Thijssen DH & Hopman MT. (2008). Counterpoint: exercise training does not induce vascular adaptations beyond the active muscle beds. *J Appl Physiol* **105,** 1004-1006; discussion 1006-1007.

Thijssen DH, Kooijman M, de Groot PC, Bleeker MW, Smits P, Green DJ & Hopman MT. (2008b). Endothelium-dependent and -independent vasodilation of the superficial femoral artery in spinal cord-injured subjects. *J Appl Physiol* **104,** 1387-1393.

Thijssen DH, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MT & Green DJ. (2010). Impact of inactivity and exercise on the vasculature in humans. *Eur J Appl Physiol* **108,** 845-875.

Thijssen DH, van Bemmel MM, Bullens LM, Dawson EA, Hopkins ND, Tinken TM, Black MA, Hopman MT, Cable NT & Green DJ. (2008c). The impact of baseline diameter on flow-mediated dilation differs in young and older humans. *Am J Physiol Heart Circ Physiol* **295,** H1594-1598.

Thijssen DHJ, Bullens LM, van Bemmel MM, Dawson EA, Hopkins ND, Tinken TM, Black MA, Hopman MTE, Cable NT & Green DJ. (2008d). Does arterial shear explain the magnitude of flow-mediated dilatation (FMD): A comparison between young and older humans. . *Am J Physiol Heart Circ Physiol,* 00980.02008.

Thomas GD & Segal SS. (2004). Neural control of muscle blood flow during exercise. *J Appl Physiol* **97,** 731-738.

Thompson PD. (2003). Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. *Arterioscler Thromb Vasc Biol* **23,** 1319-1321.

Tinken TM, Thijssen DH, Black MA, Cable NT & Green DJ. (2008). Time course of change in vasodilator function and capacity in response to exercise training in humans. *J Physiol* **586,** 5003-5012.

Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT, Newcomer SC, Laughlin MH, Cable NT & Green DJ. (2009). Impact of shear rate modulation on vascular function in humans. *Hypertension* **54,** 278-285.

Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT & Green DJ. (2010). Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension* **55,** 312-318.

Tronc F, Wassef M, Esposito B, Henrion D, Glagov S & Tedgui A. (1996). Role of NO in flow-induced remodeling of the rabbit common carotid artery. *Arterioscler Thromb Vasc Biol* **16,** 1256-1262.

Tuttle JL, Nachreiner RD, Bhuller AS, Condict KW, Connors BA, Herring BP, Dalsing MC & Unthank JL. (2001). Shear level influences resistance artery remodeling: wall dimensions, cell density, and eNOS expression. *Am J Physiol Heart Circ Physiol* **281,** H1380-1389.

Tyldum GA, Schjerve IE, Tjonna AE, Kirkeby-Garstad I, Stolen TO, Richardson RS & Wisloff U. (2009). Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise. *J Am Coll Cardiol* **53,** 200-206.

Vallance P, Collier J & Moncada S. (1989). Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* **2,** 997-1000.

Vanhoutte PM, Rubanyi GM, Miller VM & Houston DS. (1986). Modulation of vascular smooth muscle contraction by the endothelium. *Annu Rev Physiol* **48,** 307-320.

Varady KA, Bhutani S, Church EC & Phillips SA. (2010). Adipokine responses to acute resistance exercise in trained and untrained men. *Med Sci Sports Exerc* **42,** 456-462.

Vita JA. (2002). Nitric oxide-dependent vasodilation in human subjects. *Methods Enzymol* **359,** 186-200.

Vogel J, Sperandio M, Pries AR, Linderkamp O, Gaehtgens P & Kuschinsky W. (2000). Influence of the endothelial glycocalyx on cerebral blood flow in mice. *J Cereb Blood Flow Metab* **20,** 1571-1578.

Wang J, Wolin MS & Hintze TH. (1993). Chronic exercise enhances endothelium-mediated dilation of epicardial coronary artery in conscious dogs. *Circ Res* **73,** 829-838.

Wisloff U, Stoylen A, Loennechen JP, Bruvold M, Rognmo O, Haram PM, Tjonna AE, Helgerud J, Slordahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen O & Skjaerpe T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* **115,** 3086-3094.

Woodman CR, Muller JM, Rush JW, Laughlin MH & Price EM. (1999). Flow regulation of ecNOS and Cu/Zn SOD mRNA expression in porcine coronary arterioles. *Am J Physiol* **276,** H1058-1063.

Woodman CR, Price EM & Laughlin MH. (2005). Shear stress induces eNOS mRNA expression and improves endothelium-dependent dilation in senescent soleus muscle feed arteries. *J Appl Physiol* **98,** 940-946.

Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA & Green D. (2001). Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* **91,** 929-937.

Wray DW, Witman MA, Ives SJ, McDaniel J, Fjeldstad AS, Trinity JD, Conklin JD, Supiano MA & Richardson RS. (2011). Progressive handgrip exercise: evidence of nitric oxide-dependent vasodilation and blood flow regulation in humans. *Am J Physiol Heart Circ Physiol* **300,** H1101-1107.

Zhu W, Zeng J, Yin J, Zhang F, Wu H, Yan S & Wang S. (2010). Both flow-mediated vasodilation procedures and acute exercise improve endothelial function in obese young men. *Eur J Appl Physiol* **108,** 727-732.

Ziegler T, Bouzourene K, Harrison VJ, Brunner HR & Hayoz D. (1998). Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells. *Arterioscler Thromb Vasc Biol* **18,** 686-692.