Exercise intensity as a mediator of central and peripheral vascular integrity

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others. *Further details of the jointly-authored publications and the contributions of the candidate and the other authors to the work should be included below this statement.*

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Abstract

Vascular homeostasis is a vital element of health. Exercise plays a role in maintaining the integrity of the vascular endothelium via transient increases in endothelial shear stress that accompany exercise-induced hyperaemia. This hyperaemia and stimulus it presents to the vasculature is governed by exercise intensity. Exercise is the primary therapy in cardiac rehabilitation contexts, a population with typically elevated cardiovascular risk factors and compromised vascular integrity. In the UK, cardiac rehabilitation may be ineffective for improving long-term health outcomes. This thesis demonstrates that the exercise intensities achieved by patients in UK cardiac rehabilitation were variable and generally low. The exercise performed had no impact upon indices of peripheral vascular structure or function and little effect upon short-term health outcomes. A service-level intervention was implemented to increase the dose of exercise achieved by patients via an increase in intensity and examine its effects upon the vasculature. In a subsequent cohort, the intervention was unsuccessful at modifying the exercise intensities that were achieved or indices of peripheral vascular structure or function. However, a positive relationship between the intensity achieved at the end of the programme and changes in vascular function was found. Although peripheral vascular integrity is easily studied it is perhaps less relevant to health compared to central vascular integrity. Assessments of the central circulation during exercise have previously relied upon high-risk, invasive techniques. Therefore, to understand the stimulus presented to the central vasculature by exercise at intensities akin to those

achieved in cardiac rehabilitation, a novel technique was applied: using cardiac magnetic resonance imaging during cycling in a healthy cohort. This technique was unable to capture the changes in perfusion of the central circulation that should have occurred with exercise at different intensities but successfully demonstrated reproducible assessments of cardiac dynamics during exercise of different intensities.

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List of Abbreviations

- ACPICR - Association of Chartered Physiotherapists in Cardiac Rehabilitation
- ATP – adenosine triphosphate
- BACPR – British Association for Cardiovascular Prevention and Rehabilitation
- CABG – coronary artery bypass graft
- CAD – coronary artery disease
- cIMT – carotid intima-media thickness
- CMD – coronary microvascular resistance
- CMR – cardiac magnetic resonance
- CPET – cardiopulmonary exercise testing
- CR - Cardiac Rehabilitation
- CRF - Cardiorespiratory Fitness
- CV – cardiovascular
- CVD – cardiovascular disease
- DBP – diastolic blood pressure
- DNA – deoxyribonucleic acid
- ECM – extracellular matrix
- EDV – end diastolic volume
- eNOS – endothelial nitric oxide synthase
- ESV – end systolic volume
- FMD – flow-mediated dilatation
- HDL – high density lipoprotein
- HR – heart rate
- HR_{max} – maximal heart rate
- HRR – heart rate reserve
- ISWT – intermittent shuttle walking test
- LDL – low density lipoprotein
- LV – left ventricle
- MI – myocardial infarction
- MMPs – matrix metalloproteinases
- MRI – magnetic resonance imaging
- MVPA – moderate to vigorous physical activity
- $M\dot{V}O_2$ – myocardial oxygen uptake
- NO – nitric oxide
- PA – physical activity

- PCI – percutaneous coronary intervention
- PO_2 – partial pressure of oxygen in the blood
- ROS – reactive oxygen species
- RPP – rate-pressure product
- RV – right ventricle
- SBP – systolic blood pressure
- SD - standard seviation
- ShMOLLI – shortened modified look locker inversion
- SMC – smooth muscle cell
- UK - United Kingdom
- $\dot{V}O_{2\ max}$ – maximal oxygen uptake
- $\dot{V}O_{2\ peak}$ – peak oxygen uptake

Chapter 1 **Introduction**

As with any multiscale system in biology, the role of the vascular endothelium in the health of a human organism is determined by its place within an integrated physiological system that comprises many levels organisation. From the molecular, cellular, tissue, organ, organ system and organismal scales, the behaviours and characteristics of each system at a given scale are subject to the boundaries of the system immediately superior to it and congruent with the processes that transcend these systems. Interactions across these levels of organisation are co-ordinated in order to execute physiological processes whereby each level of organisation may affect, be affected by or simultaneously affect and be affected by another (Noble, 2012). Examining the properties of any cell, tissue or organ in isolation, whilst essential for understanding physiological machinery, is detached from its true function in the dynamic environment of an organism. Therefore, to avoid a reductionist perspective of the role of the vascular endothelium in health and disease we must consider the context of this organ within the integrated physiological system that is the human body.

The vascular endothelium lines the entirety of the vascular system. It plays a crucial role in an increasingly apparent number of physiological processes including the function, remodelling and patency of the circulatory system, which is vital for the survival of all living tissues. Some of its function at

ascending scales of organisation include: providing a selective molecular barrier to the components of the blood, enabling the sensing of local blood flow, directing vascular proliferation and degeneration, transduction of circulating endocrine signals, regulating of local and global inflammation, mediating cardiovascular homeostasis and a contributor to exercise intolerance. All of these factors and more contribute to the vitality and normal functioning of a human organism.

At an even higher level of organization than the organism itself is the organism in the context of its interactions with its environment. These interactions are shaped by a multitude of factors that determine both the internal and external stimuli that the body is exposed to. One of the most pluripotent internal stimuli that regulates the integrity of the endothelium is physical activity. Arguably, physical activity is the medium through which humans engage with their dynamic external environments and is therefore an inherent feature of normal physiological function. As such, physical activity in all its gradations - from sedentariness to the extreme of intense and prolonged exercise – impacts upon the regulation of many physiological systems which affect and are effected by vascular integrity.

Though endothelial integrity plays a part in various aspects of health, the most notable contribution is its role in the development of cardiovascular disease (CVD); the largest cause of death worldwide (WHO, 2016). CVD is characterised by the progressive formation and development of atherosclerotic plaques often within the coronary arteries. The initiation of

the atherosclerotic cascade and progression towards overt, symptomatic CVD is characterised by vascular endothelial dysfunction (ED).

CVD progresses with age and is exacerbated with deteriorating endothelial function via number of mechanisms, which lead to the eventual manifestation of potentially fatal cardiac events such as a myocardial infarction (MI) - where part of the coronary arterial tree becomes occluded by a thrombus originating from an atherosclerotic plaque. Vascular beds downstream of the occluded region are deprived of blood flow leading to often-irreversible damage to the affected cardiac tissue, subsequent dysfunction of the heart and, if untreated, death. Prior to the era modern medicine, MIs were usually fatal. Now people can survive beyond the incidence of an MI with optimal medical therapy and reactive or prophylactic revascularisation of the occluded region. This creates a patient phenotype characterised by severe systemic CVD with impairments in vascular integrity in the shape of stiffened arteries, endothelial dysfunction and microvascular dysfunction.

Exercise is one therapy that can be applied with the aim of improving vascular integrity with a view to improving health. Exercise, being a natural stressor, provides a stimulus that produces a hormetic response. However, exercise is multifaceted and not all exercise provides an equal stimulus to the vascular endothelium. Furthermore, the stimulus presented by exercise is systemic with local heterogeneities dependent on the metabolic activity of tissue. Similarly, the pathophysiology of CVD acts systemically upon the vasculature with local manifestations in the form of atherosclerotic plaques.

Systemic effects of exercise upon vascular integrity reflect the systemic exercise stimulus and local effects the local stimulus. Some of the most vital vessels susceptible to CVD are those of the coronary circulation. Impaired function of the coronary endothelial function is related to peripheral (systemic) endothelial function and prognostic of cardiac events. Although exercise training can preserve and enhance this function, little is known about how the stimulus presented by exercise effects these vessels in humans – especially when exercise is applied therapeutically in diseased populations. Consequently, it is unclear how and whether current exercise prescriptions provide a sufficient stimulus to enhance the integrity of the coronary vasculature or how to optimise exercise prescriptions for this purpose.

One example of where exercise is employed as stimulus to improve vascular integrity in a population in which vascular integrity is compromised is cardiac rehabilitation (CR). Cardiac rehabilitation is a primarily exercise-based therapy that, in the UK, aims to reduce cardiovascular mortality and hospital re-admissions in individuals with coronary heart diseases, improve functional capacity, quality of life, support an early return to work, promote “long-term healthier living” (BACPR, 2017) and improve endothelial function (ACPICR, 2015). In a broader context, cardiac rehabilitation programmes “are designed to limit the physiological and psychological effects of cardiac illness, reduce the risk for sudden death or re-infarction, control cardiac symptoms, stabilise or reverse the atherosclerotic process, and enhance the psychosocial and vocational status of selected patients” (Balady et al.,

2007). Whilst exercise is the primary therapeutic component of cardiac rehabilitation, comprehensive treatments including education around aimed at modifying lifestyle-related risk factors such as psychological stress, dietary habits and medication adherence (Dalal et al., 2015).

UK cardiac rehabilitation is governed by the British Association for Cardiac Prevention and Rehabilitation (BACPR) and their standards for exercise prescription are derived from the standards of the Association of Chartered Physiotherapists in Cardiac Rehabilitation (ACPICR, 2015) as cardiac rehabilitation originally was led exclusively by physiotherapists. These standards prescribe exercise of 2-3 sessions per week of 20-60 minutes of primarily moderate intensity aerobic exercise of 40-70% of individual heart rate reserve (HRR) or V̇O2max and “low risk or more active individuals should work towards the higher end of these intensity targets: 70% HRR”. The format of this exercise is comprised of “cardiovascular and strength training” with “rhythmic activity of the large muscle groups of the body at a prescribed dosage” using “a continuous or interval training approach” (ACPICR, 2015). Whilst the duration of the programme varies locally the average duration in the UK is 56 ± 4 sessions (BHF, 2015).

1.1. **Thesis aims**

The aims of this thesis are twofold. The first aim is to characterise and examine the effects of a chronic exercise stimulus upon the peripheral

vasculature integrity in a population with CVD where an explicit purpose of exercise is to improve vascular endothelial function. The second aim is to examine the acute effect of exercise intensity upon the central microcirculation including intensities observed in cardiac rehabilitations.

1.2. **The structure of the thesis**

The remainder of the thesis is structured as follows:

- Chapter 2 provides a comprehensive literature review
- Chapter 3 comprises 3 parts:
    - Part 1 presents the methods, findings and a discussion of the findings of the first study in cardiac rehabilitation.
    - Part 2 presents the methods and findings of a subsequent study in cardiac rehabilitation that builds upon the finding in Part 1.
    - Part 3 provides further analysis of the combined data from parts 1 & 2.
- Chapter 4 presents the methods, findings and a discussion of the findings of a study examining the acute stimulus presented by exercise of different intensities in the central vasculature using Cardiac Magnetic Resonance Imaging.
- Chapter 5 provides a discussion of the findings presented in Chapters 3 & 4 and their implications with consideration of the literature discussed in Chapter 2 as well as further literature. The chapter then draws a conclusion and makes suggestions for future research.

Chapter 2 **Literature review**

2.1. **Pathophysiology of cardiovascular disease**

2.1.1. **The vascular endothelium in heath and disease**

2.1.1.1. **The vascular endothelium: function and structure**

The vascular endothelium consists of a unicellular layer, which lines the lumen of the entire human vascular system. This monolayer plays a crucial role as a barrier and gatekeeper to the components of blood to the tissues, as a regulator of local tissue blood flow commensurate with dynamic demands and a transducer of mechanical, hormonal, neurological, immunological, autocrine and paracrine signals. Though several of these functions are key to the discussions found in this thesis, many less relevant aspects of endothelial physiology will be omitted for brevity.

The vascular system is self-maintaining and self-proliferating. With aging and pathology, the ability of the vascular system to perform these functions declines, leading to a progressive degradation of functional and morphological characteristics of endothelial tissue.

Morphologically, a healthy endothelium appears squamous with spindle-shaped cells aligning in the direction of blood flow. In-vivo, the endothelium is in constant contact with flowing viscous blood resulting in frictional force in the form of fluid shear stress being inflicted upon the intraluminal membranes of endothelial cells. Shear stress is an ever present, highly

periodic element of the dynamic local environment of the vascular endothelium. It exists wherever blood flow is found. The mechanotransduction of shear stress via a range of potential cellular apparatuses is vital for local sensing of blood flow and subsequent local blood flow regulation via endothelium-dependent mechanisms. The apparatuses that have been identified as having a role in the sensing of shear stress include: the glycocalyx, caveolae, G-protein-coupled receptors, tyrosine kinase receptors, primary cilia, membranous K^+ ion channels (Johnson et al., 2011), integrins and cadherin (Conway and Schwartz, 2015) at cell to cell junctions. On top of this, shear stress provides a physiological signal that plays a key role in regulating the properties of endothelial cells.

Blood flow - and therefore shear stress - is pulsatile in nature and has been shown to vary considerably in magnitude across the vascular tree (Lipowsky et al., 1978; Kuo et al., 1995). Differential sensitivities to shear stress longitudinally across the vascular tree may be key to the regulation of haemodynamics (De Hert, 2012). As changes in vascular tone at any given point have both upstream and downstream consequences, integration of local and global blood flow control mechanisms is essential. Even more so, substantial increases in shear stress can be induced by increases in cardiac output (CO): the rate at which blood is pumped from the heart. Blood flow is determined primarily by CO which can increase 4-6 fold during maximal exercise from resting levels (Rowell, 1993).

The endothelium is a plastic tissue. In response to physiological levels of shear stress, the developing endothelium becomes aligned to the direction

of blood flow (Figure 1). In contrast, chronic exposure to low shear stress and pathological modification of the structures responsible for the mechanotransduction of shear stress can cause endothelial cells to align entropically (McCue et al., 2006; Noria et al., 2004).

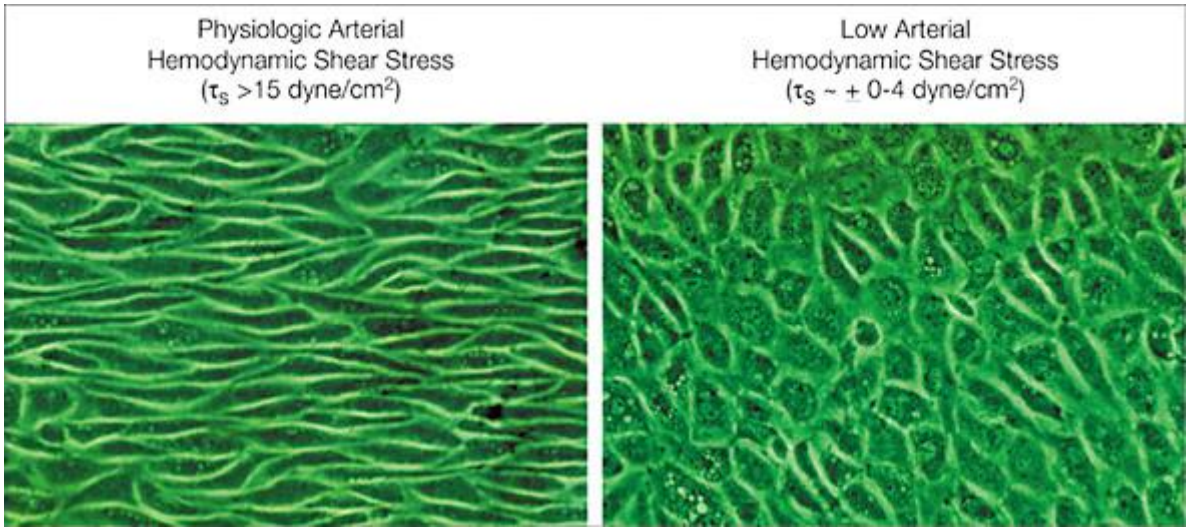

Figure 1: Morphological responses of the vascular endothelium to exposure to physiological vs pathological levels of shear stress (Malek et al., 1999)

2.1.1.2. **Endothelial dysfunction and the atherosclerotic cascade**

Preceding overt pathological modifications to the endothelial morphology, the functional properties of endothelial cells degrade. Endothelial function is a broad, ill-defined term encompassing the capacities of multiple functions of endothelial cells. Conversely, endothelial dysfunction refers to the attenuation of these capacities. Endothelial dysfunction is recognised as an initiating step in the process of atherogenesis and the subsequent atherosclerotic cascade (Figure 2) and is a prominent characteristic of the

majority of cardiovascular diseases (Mozaffarian et al 2016). Endothelial dysfunction develops following sufficient acute and chronic exposure to a range of pathological agents (smoking, hyperlipidaemia, hyperglycaemia, hypercholesterolaemia, hypertension, etc.) which induce an atherogenic endothelial phenotype. This is exacerbated by a lack of exposure to periodic increases in shear stress, which promote a “healthy” endothelial phenotype. In this case, a healthy endothelium would be defined as free from dysfunction and not prone to the acceleration of atherogenesis.

As endothelial function decreases, the endothelium becomes more adhesive, displaying an upregulation of genes that encode vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 (Chatzizisis et al., 2007), and increased permeability of the endothelial membrane (Wedmore and Williams, 1981; Ross, 1999). A consequence of this is an accumulation of lipoproteins and leukocytes around sites that express a pro-atherogenic endothelial phenotype, contributing to the formation of early atherosclerotic lesions (Figure 2).

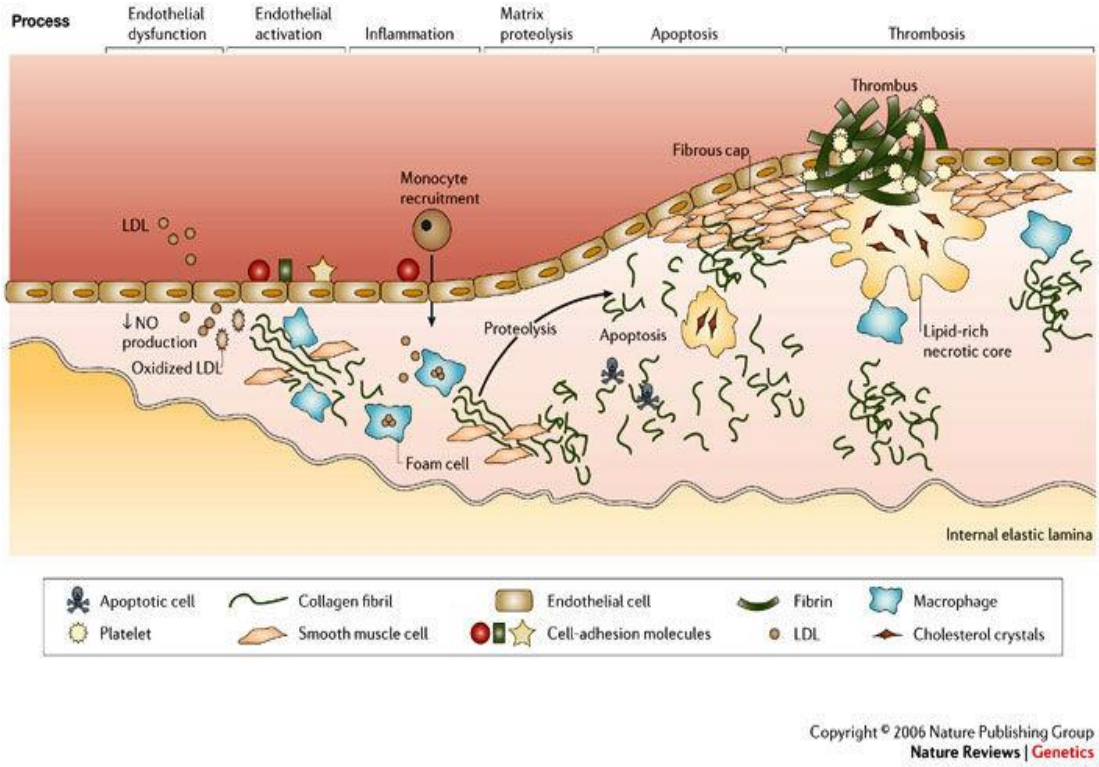

Figure 2: The atherosclerotic cascade. Endothelial dysfunction is associated with a reduction nitric oxide (NO) availability and increased membrane permeability, leading to the migration and oxidation of low-density lipoproteins (LDLs) into the intima of the artery and causing endothelial activation. As a result, the intima extracellular matrix propagates through the accumulation of collagen fibrils and the endothelium upregulates the expression of cell-adhesion molecules. These adhere to circulating monocytes which extravasate into the intima. This inflammatory process results in the scavenging of oxidized LDLs by monocytes, creating foam cells which accumulate with smooth muscles cells, oxidized LDLs and collagen fibrils in the growing extracellular matrix. Eventually, smooth muscles and foam cells senesce leading to apoptosis and the release of cholesterol from LDLs. Cellular debris collects, forming a fibrous cap between the now necrotic intimal pool of lipids and the endothelium. Finally this cap can degrade and rupture leading to the formation of a thrombus in the lumen of the vessels with the agglomeration of fibrin and platelets (Watkins and Farrall, 2006).

The key initiating step of atherogenesis is the accumulation of apolipoprotein B containing lipoproteins in the subendothelial space (Williams, K.J. and Tabas, 1995). Hepatic apolipoprotein B containing lipoproteins are secreted

as very low density lipoproteins which are converted to atherogenic low density lipoproteins (LDLs) via lipoprotein lipase in the circulation. Dyslipidaemia is a major risk factor for the development of CVD that is classified by elevated levels of LDLs and low levels of high density lipoproteins (HDLs). Elevated levels of circulating LDLs can be a major cause of injury to the endothelium particularly when modified through oxidation by reactive oxygen species. In individuals with increased levels of chronic oxidative stress the likelihood of this modification occurring is augmented. HDLs are generally thought to possess anti-atherogenic properties through their actions of removing cholesterol from tissues and their ability to inactivate oxidized phospholipids, however modification of these in disease states which are commonly comorbid with CVD (such as diabetes and metabolic syndrome) can have deleterious effects of these properties of HDL (Kontush and Chapman, 2006). Additionally, LDL adhesion to the endothelium is also promoted through mediators of inflammation such as tumour necrosis factor α and interleukin-1 (Stopeck et al., 1993). There are two means by which LDL then passes through the endothelium into the arterial intima and exacerbating the forming lesion. First, LDL is capable of simply diffusing through endothelial cell junctions to enter the intima. Once inside the intima modified LDL can act upon vascular smooth muscle cells (SMCs) and endothelial cells directly to enhance the inflammatory response by causing further oxidation of lipid membranes, proteins and damaging DNA (Wang, J.C. and Bennett, 2012). Secondly, oxidised LDL is chemotactic for monocytes, which in turn are capable of

binding with oxidised LDL via scavenger receptors and internalising them via endocytosis. This scavenging minimises the impact modified LDLs can have on endothelial cells. Part of the inflammatory response of the endothelium to injury is to promote the migration of monocytes into the intima.

Monocytes differentiate into macrophages once in the intima and act to scavenge LDLs. Once internalised in a macrophage, LDLs can undergo even greater extents of oxidation and cholesterol esters can accumulate leading to the formation of foam cells. Foam cells are a consequence of the protective effect of the inflammatory response as they prevent modified LDLs from oxidising and damaging any surrounding structures, however they contribute to an ever growing lipid pool that forms in the intima.

One final component of early atherogenesis is the hypertrophy and proliferation of vascular SMCs into the intima. This process is mediated by the inflammatory response, which is affected by both the actions of LDL and macrophages interacting with endothelial cells or upon SMCs directly. SMCs are notably responsible for the formation of a fibrous plaque on the outside of this growing atheroma and the elaboration of an abounding extracellular matrix (ECM) (Libby and Theroux, 2005). Components of the ECM - notably proteoglycans – may bind with LDL, increasing the propensity of oxidative modification of LDL. As oxidized LDL is a contributor to the chronic inflammatory process (Berliner et al., 2001), increases in ECM propagation leading to LDL retention will accelerate the process of atherosclerosis.

The accumulation of LDLs, foam cells, SMCs in the intima leads firstly to pathological intima thickening, then through a positive feedback loop of

inflammatory responses the “response to injury” is continued leading to the development of early fibroatheromas. Atherosclerosis is sometimes referred to as a non-resolving inflammatory condition for this reason (Moore and Tabas, 2011). Contrary to previous beliefs, early fibroatheromas are not associated with stenosis of coronary arteries as all remodelling to accommodate the thickening intima and expanding lipid core occurs abluminally (Libby and Theroux, 2005). Non-stenotic plaques may remain stable and asymptomatic for a number of years but can develop into advanced lesions, which can prove particularly dangerous.

As with any reparative process, the high cell turnovers caused by the processes of SMC migration and the proliferation of macrophages into the subendothelial space to quell the damage endured by the endothelium cannot continue indefinitely – leading to replicative senescence. This is exemplified by the abnormally short telomeres found in these cells present in atherosclerotic plaques, indicative of an accelerated cellular ageing process (Wang, J.C. and Bennett, 2012). However, whether this shortening is caused by replicative senescence or oxidative stress induced DNA damage is unclear (Matthews et al., 2006). Shorter telomere lengths in leukocytes are associated with greater risks of CVD (Haycock et al., 2014) and endothelial cell dysfunction induced by telomere shortening may contribute to atherosclerosis (Minamino et al., 2002). When macrophages, foam cells and SMCs become senescent, they accumulate and contribute to the growing plaque. Consequently, a number of these cells undergo apoptosis leading to the conversion of the intimal lipid pool into a necrotic core, which comprises

the debris – a key feature of the late fibroatheroma. Leukocytes, including activated T cells, continue to congregate around the site of the plaque. One of the responses of T cells to inflammation is to promote fibrinolysis of unnecessary connective tissue that is often formed in the early stages of an inflammatory response. In the case of atherosclerosis, this can be hazardous as the ability of SMCs to synthesise new collagen is inhibited. Macrophages in plaques have a tendency to overproduce matrix-metalloproteinases (MMPs) (Moore and Tabas, 2011) which are one of the few groups of enzymes capable of catalysing the breakdown of collagen. Modified LDL can also induce endothelial MMP expression (Huang, Y. et al., 2001). Without the sufficient formation of new collagen by SMCs and with augmented degradation of collagen by MMPs, the ECM starts to break down and the fibrous cap begins to thin and become vulnerable (Libby, 2013).

At this stage, there are two main outcomes for the plaque: rupture or erosion. A plaque rupture involves the formation of luminal thrombus consisting largely of platelets. A thrombus may or may not be occlusive however; a plaque rupture is the most common cause of lethal coronary thrombosis. Notably, plaque rupture is predicted by an increased presence of circulating inflammatory markers (Libby, 2013) and is correlated with an elevated ratio of total:HDL cholesterol (Yahagi et al., 2016). A plaque erosion involves a focal desquamation/denudation of the endothelium of a plaque causing the formation of a relatively smaller luminal thrombus compared with a rupture. Following either event, remodelling of the site of the atherosclerotic lesion occurs. However, erosions tend to show negative

remodelling (luminal narrowing) and ruptures positive remodelling (intimal expansion), suggesting an added stenosis in the event of an erosion. Additionally, there is a greater prevalence of microemboli in the myocardium following erosion (71%) versus ruptures (42%) (Schwartz, R.S. et al., 2009).

Beyond these events, an underlying pathology of episodic rupture and healing may occur resulting from “silent” plaque ruptures or erosions in the epicardial vessels. Whilst acutely insubstantial and non-symptomatic, these events have progressive deleterious effects on the myocardium and additional negative remodelling is likely to result, causing further stenosis (Yahagi et al., 2016) resulting in reduced blood flow in stenotic and occluded vessels leading to further endothelial dysfunction.

2.1.1.3. **Shear stress and the vascular endothelium**

Haemodynamic forces, particularly endothelial shear stress, mediate the phenotype of endothelium, its function and as such its propensity for developing atherosclerosis. Anatomical heterogeneities within and across blood vessels cause variations locally upon magnitudes of endothelial shear stress (Soulis et al 2006) and as a direct consequence phenotypic heterogeneity in the endothelium is observed across the vasculature (Aird, 2007). As such, the susceptibility of regions of the endothelium that are predisposed to lower magnitudes of shear stress to express an atherogenic phenotype are increased, reflecting the increased prevalence for atherosclerotic plaques in specific sites of the vascular tree.

The manifestation of local variations in endothelial phenotype is attributable to local variations in shear stress; however, variations in endothelial health are primary determined by global factors. For example, pathological agents - with the potential to damage the endothelium and contribute to endothelial dysfunction- circulate in the blood and therefore exert global negative effects on the endothelium. In contrast, substantial increases in cardiac output that occur during physical activity cause large increases in blood flow and promote positive effects upon the endothelium that prevent ED. Mitigation of the negative effects of these pathological agents and chronic low shear stress environments plays an important role in vascular homeostasis and maintains the function of the endothelium (Figure 3).

A host of mechanisms have been found to explain the effects of chronic low shear stress on the endothelial phenotype. Low magnitude shear stress is known to attenuate nitric oxide-mediated atheroprotection (Chatzizisis et al., 2007), promote the uptake of LDLs (Sprague et al., 1987) and pro-inflammatory macrophages (Hansson, 2005), increase oxidative stress (Hwang et al., 2003), increase the migration, differentiation and proliferation of SMCs (Chatzizisis et al., 2007), promote the degradation and decrease the rate of synthesis of the ECM in the vessel wall and fibrous cap (Chatzizisis et al., 2011).

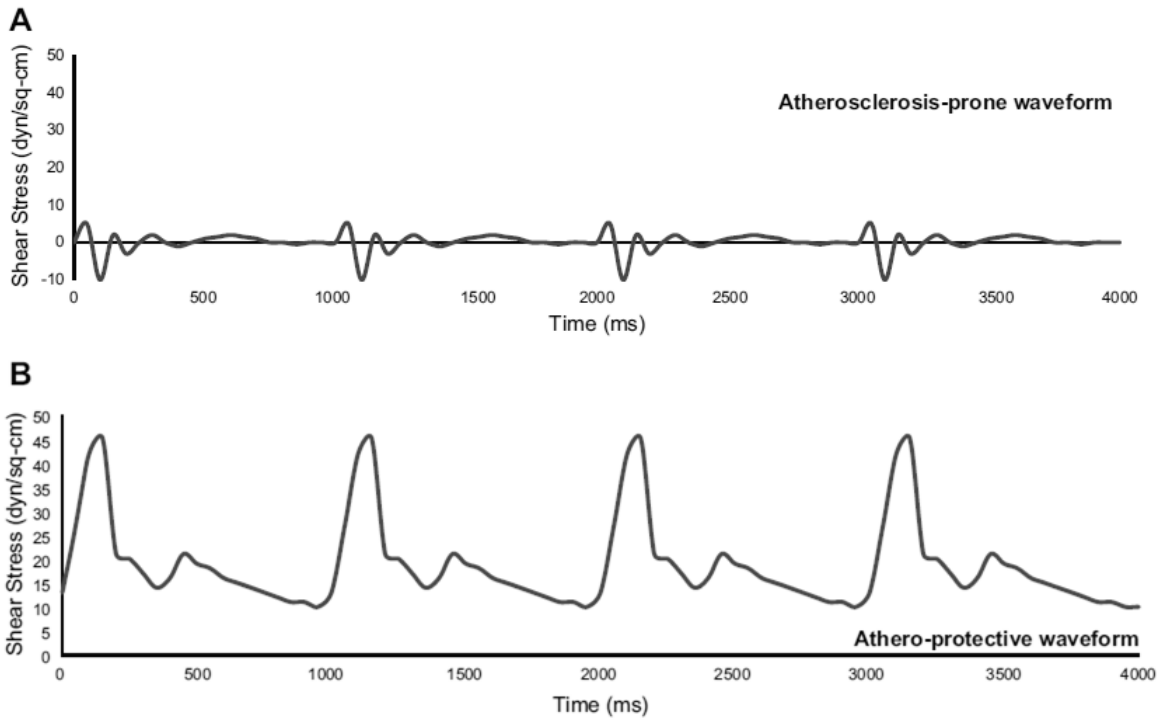

Figure 3: Shear stress profiles for pro-atherogenic (A) and anti-atherogenic (B) waveforms. Adapted from Hopkins, P.N. (2013).

2.1.2. **CVD in the peripheral macrovasculature**

Vascular endothelial function plays a significant role in atherosclerosis and the development of CVD (see section 2.1.1.2). The functional status of the vasculature is often assessed by its ability to regulate vascular tone, however, the regulation of vascular tone can be partitioned into endothelium-dependent and endothelium-independent functions. Both of these functions have been implicated in the development of CVD via independent mechanisms. Endothelium dependent dilatation will be the main focus of the following discussion as it is more modifiable with physical activity and appears to play a greater part in the affecting the cardiovascular risk (Halcox et al., 2002).

Endothelium-dependent dilatation is modulated by the bioavailability of nitric oxide, a vasoactive compound that is produced by endothelial cells via the enzyme endothelial nitric oxide synthase (eNOS). Following the mechanotransduction of a shear stress signal of a sufficient magnitude, eNOS is activated resulting in the generation of NO. In opposition to the generation of NO is the quenching of NO by reactive oxygen species (ROS) which arise from a number of sources (Figure 4). The primary form of ROS in the vascular wall is superoxide, the bulk of which is produced by NADPH oxidase and mitochondria (Madamanchi et al., 2005). ROS, such as superoxide and hydroperoxy radicals, have a high affinity for NO which reduces the bioavailability of NO (Beckman et al., 1990). ROS are also scavenged by endogenous antioxidant systems, which preserve NO bioavailability and protects cellular ultrastructures from oxidative damage. An

imbalance of ROS generating over ROS scavenging effects leads to a state of oxidative stress. Current evidence indicates that increases in oxidative stress account for a significant portion of endothelial dysfunction (Heitzer et al., 2001; Schulz et al., 2011) due to the rapid oxidative inactivation of NO by superoxide producing peroxynitrite. Peroxynitrite oxidizes (6R-) 5, 6, 7, 8-tetrahydrobiopterin (BH_4) which is an essential cofactor for eNOS. If BH_4 becomes depleted as a result of persistent oxidative stress, eNOS becomes uncoupled which causes it to not only stop generating NO but also start generating superoxide thereby aggravating the state of oxidative stress. Oxidative stress mediated reductions in endothelial function predispose the vasculature to atherosclerosis. Positive feedback from oxidative stress leads to oxidation of LDLs which have been shown to activate NADPH oxidase (Heinloth et al., 2000) and another source of superoxide: xanthine oxidase (Stepp et al., 2002). Oxidative stress begets oxidative stress, accelerating atherosclerosis.

Shear stress appears to regulate the vascular redox biology via a number of mechanisms. In particular, increases in eNOS expression following exposure to pulsatile flow and increases in NADPH derived superoxide generation following exposure to oscillatory flow in cell culture models (Hwang et al., 2003) highlight the role which the pattern and direction of shear stress exposure have upon the regulation of oxidative stress. Further evidence exists for the mechanisms of the anti-atherosclerotic effect of exposing endothelial cells to adequately high magnitudes of shear stress through upregulation of endogenous antioxidant and anti-inflammatory systems of

the endothelial cells such as superoxide dismutase (Dimmeler et al., 1999) which is the primary cytosolic enzyme regulating cellular superoxide (Crapo et al., 1992). In short, the mechanisms by which shear stress modulates endothelial function and the susceptibility of the endothelium to atherogenic processes are grounded in the promotion (or lack thereof) of an anti-inflammatory and pro-antioxidant cellular phenotype.

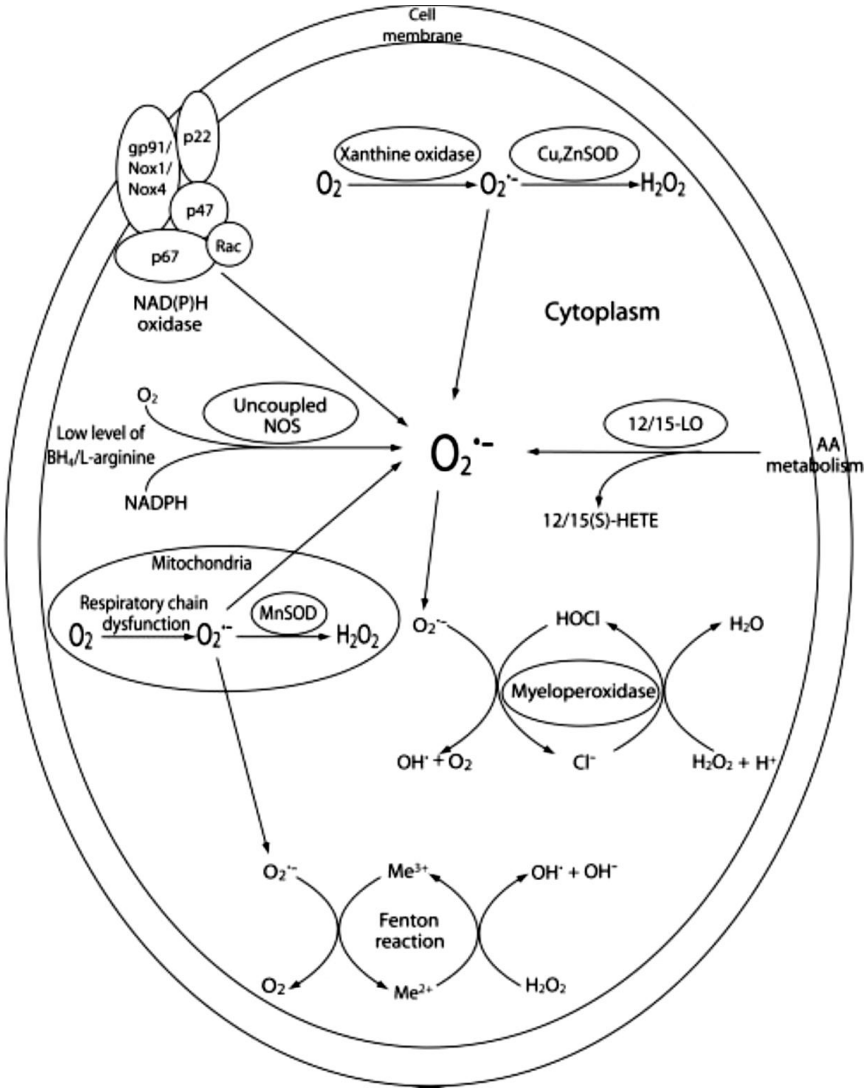

Figure 4: Cellular pathways of reactive oxygen species handling (Madamanchi et al., 2005)

In humans, it is neither practical nor necessary to know the endothelial phenotype that is expressed at all points of an individual's vascular tree to adequately assess the properties of the vascular endothelium. Flow-mediated dilatation (FMD) is a technique that provides a non-invasive, practicable index of systemic endothelial function. Typically performed in the brachial artery, FMD is a measure of the change in diameter of a conduit artery in response to hyperaemia following a 3-5 period of occlusion. This response has been demonstrated to represent endothelium-dependent vasodilatation as it is largely NO-dependent (Kooijman et al., 2008; Thijssen et al., 2011). FMD is an independent risk factor for CVD with every 1% increase in endothelial function being associated with a 12-13% decrease in CV event risk in diseased populations (Ras et al., 2013; Matsuzawa et al., 2015). Similarly, coronary artery endothelial dysfunction is considered to be an independent predictor of coronary artery disease (CAD) progression and cardiovascular event rates (Schächinger et al., 2000). Essentially, vasodilator responses to acetylcholine in the brachial and coronary arteries have been shown by to be highly correlated (Anderson, T.J. et al., 1995b), thus changes in endothelial function in the brachial artery are thought to translate to changes in coronary artery endothelial function. The systemic nature of endothelial dysfunction provides some justification for this assumption though experimental evidence for concurrent improvements in brachial and coronary endothelial function is lacking.

A number of studies, using a range of modalities, have established the positive effect of increasing shear stress upon endothelium-dependent

dilatation through the induction of hyperaemic blood flows. Exposure to repeated passive heating (Imamura et al., 2001; Naylor et al., 2010), lipid-lowering therapy (O'Driscoll et al., 1999), anti-hypertensive therapy (Iwatsubo et al., 1997), antioxidant therapy (Anderson, T.J. et al., 1995a), passive movement (Trinity et al., 2012; Mortensen et al., 2012; Brunt et al., 2016; Broxterman et al., 2017), repeated bouts of brief occlusion (Hodges et al., 2018), oestrogen replacement therapy in post-menopausal women (Gerhard et al., 1998), acute exercise (Harris et al., 2008; Pyke, K. et al., 2008) and exercise training (Green, D.J. et al., 2017) are all modalities that have been shown to increases local blood flow beyond resting levels through mainly NO-mediated mechanisms.

In a similar fashion, the dependence of FMD upon vascular redox biology has been demonstrated by abolishing the attenuation of FMD in individuals with different disease states with established endothelial dysfunction through the use of exogenous antioxidant therapies (Taddei et al., 1998) and anti-inflammatory agents.

Whilst vascular endothelial function can provide useful prognostic information relating to CVD, it becomes a less prognostic biomarker with increasing age. The prevalence and progression of CAD also progresses distinctly with age, therefore in patients with manifest CAD the co-dependency of endothelial function and aging cannot be dismissed. As vascular function declines in older compared to younger populations (Thijssen et al., 2009), the structural properties of the vessels may play an increasingly important role in the vascular health with age.

The time course of adaptations in chronic vascular function is often in the order of weeks (Tinken et al., 2008). Structural adaptations in the vasculature have been shown to follow a similar though delayed time course, presumably due the interrelated haemodynamic stimuli that drive functional and structural adaptations (Laughlin, M Harold. and Roseguini, 2008). Vascular structure in health and disease can vary across several dimensions including: the stiffness of resistance and conduit vessels, the thickness of vessel walls, the diameter of vessels and the profuseness of microvascular networks.

Arterial stiffness is a key structural property of the vasculature, which describes the resistance to deformation of a vessel. With increased arterial stiffness comes an increase in blood pressure. Elevated blood pressure beyond 140 mmHg systolic and/or 90 mmHg diastolic is classified as hypertension; one of the primary risk factors for the development of CAD (Kannel et al., 1987). Approximately 73% of individuals with CAD have hypertension (Wong et al 2007). Hypertension can be both an inciting factor for CAD through endothelial injury (primary hypertension) and a result of CAD via structural remodelling of the arterial walls and loss of endothelial function (secondary hypertension).

Arterial stiffness can be measured indirectly by measuring pulse wave velocity (PWV), β-stiffness index (SI), intima-media thickness (IMT), pulse pressure (PP) and distensibility (Glasser et al., 1997; Nichols, W.W. et al., 2008; O'Rourke et al., 2002). Aortic PWV tends to increase exponentially

with age (Nichols, W.W. et al., 2008) and is typically elevated in CAD patients (Weber et al., 2004). An underlying mechanism for the age-associated stiffening of central and peripheral arteries is believed to be an increased expression of the protein collagen and a loss of elastin in the adventitial layer of arteries (McEniery et al., 2007; Najjar et al., 2005). Collagenous fibres are ~300 fold stiffer than elastin fibres (Sumner, D.S. et al., 1969; Burton, 1954) so greater forces are require to achieve a given degree of deformation. This leads to a reduction in the elasticity of the vessel wall which causes pulse pressure (the difference between systolic and diastolic blood pressure) to increase because vessel diameter does not increase commensurately when accommodating the fluctuations in flow that occur during the cardiac cycle. The presence of stiffened arteries increases the velocity and amplitude at which pulse pressure waves propagate down the arterial tree resulting in an increased systolic blood pressure. Even in normotensive individuals the presence of increased stiffness in the aorta or carotid artery is predictive of an increased risk of the development of hypertension and accelerated progression of blood pressure with age (Mitchell, G.F., 2014). Both common carotid and aortic PWV had a strong positive correlation with carotid IMT and the presence of carotid and aortic plaques (van Popele et al., 2001) suggesting that arterial stiffening may contribute to the progression of atherosclerosis and CVD. In addition, greater arterial stiffness is also implicated in a higher risk of cardiovascular events (Bots et al., 1997), cardiovascular mortality and all-cause mortality (Laurent et al., 2001; Meaume et al., 2001; Najjar et al., 2005). As stiffer

arteries deform less easily their capacity to dilate is reduced. A strong negative association between FMD in the brachial artery and aortic PWV has been observed in a cross-sectional cohort of males over a range of ages (18-81 years) (McEniery et al., 2006) though both remain independent predictors of all-cause and cardiovascular mortality in hypertensive patients (Perticone et al., 2001; Laurent et al., 2001).

With the progression of CAD, vessel walls undergo remodelling and become thicker. Specifically, excessive proliferation of vascular SMCs into the intimal and medial layers of the vessel walls and/or accumulation of lipids in the form of an atheroma leads to the inward, abluminal remodelling of the walls which reduces the resting diameter of the vessel (Ross, 1999) (Figure 5).

Volumetric flow rate along a vessel is determined by Poiseuille's law:

$$Volumetric\ flow\ rate = \frac{\Delta pressure\ x\ \pi\ x\ radius^4}{8\ x\ length\ x\ viscosity}$$

where $\Delta\mathrm{pressure}$ is the proximal to distal pressure gradient at a given length of vessel. To maintain blood flow to downstream tissues in response to a morphologically diminished lumen diameter the blood pressure gradient along a vessel must increase or a vessel must dilate and increase in diameter. Poiseulle's law dictates that small changes in vessel diameter have large effects on flow rates relative to changes in pressure. Blood viscosity does not change acutely with changes in flow but alterations in blood rheology have been related to typical CVD risk factors which lead to greater blood viscosities (Jeong et al., 2010; Lowe et al., 1980). This encroachment of the vessel wall towards the lumen causes the effective

vasodilatory reserve of a vessel to decrease, as some degree of the vessel's vasodilatory capacity will be utilised to maintain the resting blood flow. Simultaneously, blood flow increases are achieved through an increased pressure differential through augmentation of systolic pressure but regulation of mean arterial pressure, which is typically due to an increase in the pressure distally rather than proximally due to vasodilation of the arterioles (Björnberg et al., 1989). A cause and consequence of compensatory increases in blood pressure is an increase in circumferential stress applied to the vessel wall leading to further inward remodelling (Figure 5). Remodelling of the arteries likely occurs to normalise shear stress and circumferential wall stress (Laughlin, M Harold, 1995). In the case of shear stress, NO is known to play a role in remodelling response (Tronc et al., 1996).

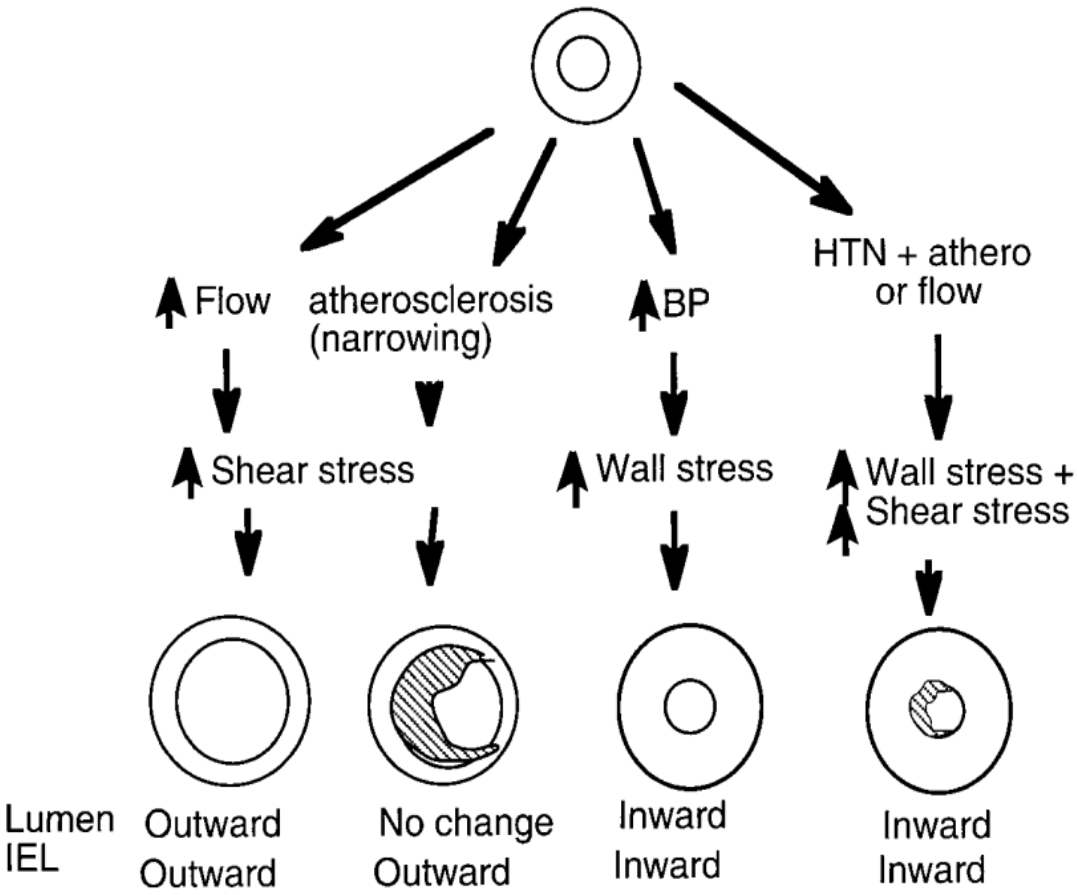

Figure 5: Vascular remodelling in health and disease. Increases in blood flow along an artery cause an increase in shear stress. To normalise this shear stress the internal elastic lamina (IEL) expands abluminanally with concurrent increase in lumen diameter. The opposite effect is seen with chronically low flow. Atherosclerosis cause narrowing of lumen diameter via the encroachment of plaques into the vessel. Compensatory increases in the IEL diameter allow lumen diameter to be maintained. Increases in blood pressure increase the circumferential stress exerted upon the vessel wall causing compensatory vascular smooth muscle cell hyperplasia to normalise wall stress. This hyperplasia leads to a thickening of the IEL and leads to reductions in the size of the lumen of resistance arteries. A combination of these factors leads to further thickening of the IEL and inward remodelling of the lumen. From Berk (2001).

2.1.3. **CVD in the peripheral microvasculature**

The pathogenic actions of many risk factors for CVD act upon the entire vascular tree and not just the major arteries where atherosclerosis tends to present.

It has been suggested that microvascular dysfunction is both a cause and consequence of primary hypertension (Serné et al., 2007), a key CVD risk factor. Hypertension begets hypertension, which increases afterload, placing additional stress on the heart. Despite a differing physiology of the microvasculature compared to larger arteries, microvascular dysfunction is also largely NO-mediated and correlated with CVD risk factors such as hypertension (Serné et al., 2007), hypercholesterolemia (Khan et al., 1999), coronary endothelial dysfunction (Khan et al., 2008) and is intensified with aging (Holowatz et al., 2008). Findings from several studies have shown that lower hyperaemic forearm blood flow, an indicator of microvascular structure and function, is associated with greater cardiovascular disease risk (Huang, A.L. et al., 2007) and central arterial stiffness (Mitchell, G.F. et al., 2005). As with impairments in endothelium-derived NO function in other vessels, increasing exposure to exercise-induced shear stress may provide a stimulus to prevent such dysfunction. Increased CVD risk score is related to a greater microvascular dysfunction of the nail microvascular beds as indicated by attenuated vasodilation and capillary recruitment responses to adenosine and sodium nitroprusside administration (Ijzerman et al., 2003). Systolic blood pressure accounts for 74% of the variance in endothelium-dependent function once other CV risk factors were accounted for,

highlighting the close relation between the microvasculature and blood pressure (Ijzerman et al., 2003). As the microvasculature contains a considerable proportion of the body's vascular endothelium, it is perhaps unsurprising that systemic endothelial dysfunction is related to microvascular function.

Capillaries are found at the distal end of the vascular tree and lack the ability to regulate flow via changes in vessel diameter. Acute changes in blood flow distribution to capillaries is governed by the terminal arterioles and vessels further upstream. As capillary pressure is elevated in hypertensive individuals (Williams et al., 1990), a greater arterial blood pressure is required to maintain perfusion.

To maintain pressure in circumstances of chronic low flow, capillary beds rarefy. Structural rarefaction of the microvasculature is well documented in hypertensives (Greene et al., 1989; Hansen-Smith et al., 1990) and is typically characterised by an anatomical reduction in capillary number. Capillaries are perfused intermittently and alternately. In addition, capillaries periodically become functionally non-perfused (also term functional rarefaction) which is exacerbated by hypertension (Serné et al., 2001) and can be observed during post-occlusive hyperaemia. Whether capillary rarefaction precedes the onset of hypertension as a consequence of the deleterious effects of increases blood pressure on the microvasculature, remains, however, unclear.

Often assessments of the peripheral microcirculation are considered for their relation to the coronary microcirculation. Indeed, patients with anginal chest

pain and normal coronary arteries show evidence of cutaneous capillary rarefaction compared with healthy controls (Antonios et al., 2001). One implication of this is that the pathological processes that create microcirculatory abnormalities – be they structural or functional in nature – are pluripotent and exert their effects on the all of the small vascular beds. Structural rarefaction of the peripheral microvasculature in CAD is also particularly relevant to the symptoms intermittent claudication in peripheral vascular disease which follows a common aetiology as other cardiovascular co-morbidities (Ouriel, 2001). Additionally, the function of the cutaneous circulation, reflecting the general microcirculation, is correlated with coronary endothelial function (Khan et al., 2008)

2.1.4. **CVD in the central macrovasculature**

Atherosclerosis of the coronary arteries (i.e. the central macrovasculature) has historically been the focal element of cardiovascular disease. Presumably, this is due to the critical early pursuits of identifying and treating the causes of myocardial infarction, which are mainly plaque embolizations and ruptures (Ross, 1999; Libby, 2013; Libby and Theroux, 2005). Upon the discovery that occlusive coronary thrombi were the inciting factor for myocardial infarctions (Davies et al., 1976), and their origins from coronary atheromas, interventions to address CAD rapidly advanced in the field of cardiology. Of all CV diseases, CAD has received significant attention as it the best associated with CV mortality. CAD is amongst the most extensively researched diseases in medicine.

The heart is a highly metabolically active organ that requires an ample supply of oxygen and nutrients from the blood to maintain the periodic contractions of the cardiac muscle. These contractions are required to generate the heart's pumping action, which is vital to circulate blood around the body and sustain life. When a region of the myocardium becomes ischaemic, the contractile properties of cardiac muscle cells (cardiomyocytes) are impaired as low capillary oxygen partial pressures restricts rates of oxidative phosphorylation in the mitochondria. This limits the bioenergetic flux of cardiomyocytes leading to weakened contractions in affected cells.

The pumping action of the heart can be represented as a dynamical system. The electrical impulses propagate throughout the heart via finely harmonised patterns. Such synchronicity elegantly produces coordination between the timing of the contractions of the individual contractile units to produce an efficient pumping action. The precision of this phasic harmonisation extends even to produce the superposition of multiple vortices of blood descending the dynamic geometry of the ventricles to exploit the increased contractile force provided by the Frank-Starling mechanism in early systole. When the contractile properties of a region of cardiac muscle are attenuated through ischaemia the efficiency of the pumping action is reduced through a disruption in the synchronicity of the system. Overall cardiac output (CO) is often unaffected by this reduced efficiency as feedback mechanisms induce compensatory increases in contractile force in non-ischaemic regions and heart rate. As the properties of the heart which produce its highly

synchronised action are passive components of the system derived from structural orderedness, the reductions in the efficiency of the pumping action cannot be rectified through autoregulation of the active properties of the system. Consequently, the non-ischaemic regions of cardiac muscle will chronically perform more work and be subject to additional stress over the remainder of the subject's lifespan.

Whilst this paints a picture of the effect that unresolved ischaemia can have on cardiac function at rest, it does not differentiate between the effects of gradations of ischaemia (from mildly flow limiting to total occlusion), the size of the affected region or the emergence of ischaemia ensuing from physiological stress.

Total occlusion of a vessel supplying cardiomyocytes, in the absence of collateral vessels or timely restoration of flow results in cardiomyocyte death, apoptosis and the development of collagenous myocardial scar tissue. Understandably, more severe local reductions in contractile function and more profuse ischaemia and scarring will exacerbate the reductions in cardiac function observed. Furthermore, greater impairments in contractile function following an infarct are associated with worsened long-term outcomes (Yu et al., 2005).

Ischaemia and its manifest symptom angina, are often not present at rest. When the oxygen requirements of the cardiac tissue are low as occurs at rest, blood flow across a stenosis may not be limiting depending on the severity of the stenosis. In accordance with the Bernoulli principle, greater reductions in distal pressure and consequently perfusion occur when oxygen

requirements are increased beyond the autoregulatory capacity of the coronary vasculature.

The pathophysiology of atherosclerosis and remodelling of the coronary arteries with exposure to CVD risk factors draws many parallels with the peripheral vasculature, which is seen in Section 2.1.2. High total blood cholesterol, high blood LDL levels, low HDL levels, elevated plasma triglycerides, hypertension, smoking, diabetes, obesity and physical inactivity have all been identified modifiable risk factors in the development of CAD (Wilson, P.W., 1994). Endothelial dysfunction predisposes arteries to the development of atherosclerosis (Section 2.1.1.2) and the coronary arteries are particularly prone to the development of atherosclerosis (Dock, 1946). It is well-established that coronary endothelial function, the capacity of the coronary arteries to dilate in response to pharmacological or physiological stress is reduced in patients with CV risk factors (Reddy et al., 1994) and with established CAD (Hambrecht et al., 2000; Sixt et al., 2009). Both endothelium-dependent and endothelium-independent functions are depressed in the coronary arteries of this population (Chauhan et al., 1997) and at least endothelium-dependent function is prognostic of future CV event risk (Schächinger et al., 2000; Al Suwaidi et al., 2000). As endothelial dysfunction is considered to be an inciting event in the atherosclerotic cascade (see Section 2.1.1.2), impaired vasodilatory capacity of atherosclerotic coronary arteries would be expected. Extant atherosclerosis of the coronaries is associated with impaired endothelium-dependent vasodilation in response to increases in blood flow (Gordon et al., 1989; Cox

et al., 1989). That coronary endothelial function confers a prognostic effect in patients with established CAD suggests that endothelial integrity relates to cardiovascular event risk in a way that extends beyond merely the development of atherosclerotic plaques. This predictive effect may arise because the coronary artery endothelial function is reflective of the ability of the coronary circulation to regulate myocardial blood flow or as a marker of diffuse CAD across the vascular tree.

The atherogenesis in the coronary arteries is determined by blood flow patterns (Asakura and Karino, 1990) and shows structural remodelling with increases in internal elastic lamina area and increases in wall thickness with the progression of atherosclerosis (Miao et al., 2009; Glagov et al., 1987). Furthermore, the development of atherosclerosis in the coronaries is associated with impaired perfusion to the myocardium downstream (Wang, L. et al., 2006).

2.1.5. **CVD in the central microvasculature**

The central microvasculature, that is the coronary microcirculation, is less well characterised in vivo typically due to technical difficulties in measuring its function and imaging it as opposed to the epicardial vessels of the macrovasculature, which are routinely imaged via angiography in clinical practice. Indeed the labelling of the symptoms of obstructive coronary artery disease fails to acknowledge a microvascular component to the disease. A “plaque-centric” theory of myocardial blood flow limitation as a primary

consequence of epicardial stenoses may not adequately capture the contribution of the whole vascular tree to myocardial ischaemia.

“Coronary microvascular function” was rarely described in research literature before the concepts of pathological coronary microvascular dysfunction (CMD) or microvascular angina became established themselves amongst the rhetoric of the cardiology field. Physiologists, by contrast, have described local myocardial blood flow for hundreds of years. As CAD received more attention in the medical field, particularly with the advent of the angiogram, treating macrovascular obstructions as the major cause of cardiac morbidity and mortality has been of primary concern (Crea et al., 2016).

Much of the mechanistic research in this field has been carried out using disease models in large quadruped mammals due to the technical challenges of assessing the coronary microcirculation in vivo in humans. Only more recently with technological advances have we been able to less invasively and non-invasively measure indices of myocardial perfusion.

Whether CMD can exist independent from CVD risk factors is unknown but current evidence does not support this possibility. Microvascular dysfunction may be systemic in nature and it may not necessarily have risk factors exclusive to its occurrence in the coronary vascular bed. As such, CMD appears to share common aetiological risk factors with peripheral microvascular function such as endothelial function (Sax et al., 1987), hyperglycaemia (Picchi et al., 2010), hypercholesterolaemia (Wyss et al., 2005) and hypertension (Camici and Crea, 2007). In healthy subjects, peripheral endothelial function as assessed by FMD shows a moderate

positive correlation with myocardial perfusion reserve (Stolen et al., 2004), presumably due to the influence of endothelial function albeit in quite different vascular beds (peripheral macrovascular versus central microvascular). CMD is characterised by an impaired ability of the coronary microvasculature to increase perfusion to the myocardium via a reduction in vascular resistance in response to a stressor. This impaired response can be derived from functional limitations dependent upon endothelial function but also structural elements of microvascular resistance. Specifically, the length of the vessels, the total cross-sectional area of the vessels and the density of vessels perfusing the myocardium comprise the structural components of central microvascular resistance.

The pathogenesis of CMD may be rooted in inflammatory processes which concomitantly induce atherogenesis in the large epicardial arteries but causes dysfunction of the smaller vessels (Crea et al., 2016; Michaels et al., 2000). Impairments in coronary microvascular endothelial function, characterised by attenuated increases in coronary flow velocity and coronary vascular resistance in response to acetylcholine infusion, are predictive of CV event risk (Halcox et al., 2002).

One of the major consequences of myocardial ischaemia as a result of myocardial hypoperfusion is angina – chest pain caused by an inadequate oxygen supply to the heart. Whilst it was previously believed that this resulted from upstream (epicardial) flow limitation, it is now thought that CMD contributes to ischemic pain. Additionally, the occurrence of angina is related to impairments in brachial artery FMD which is prognostic of CV

events in this population suggesting there are commonalities in the aetiology of ED and CMD (Neunteufl et al., 2000). Pertinent to this notion is the existence of patients without obstructive CAD who present with angina upon exertion (known as cardiac syndrome X). Emerging evidence, though debated, suggests the pathophysiology of this condition is microvascular in origin (Jones et al., 2012).

A further cause of coronary microvascular impairments with CVD is coronary microembolization, a process whereby microemboli – commonly originating from ruptured, eroded or denuded atherosclerotic plaques – obstruct the vessels of the coronary microcirculation (Heusch et al., 2004). In animal models, microvascular obstruction related hypoperfusion demonstrates a clear short term cause of contractile dysfunction but current models may fail to address the bioactive properties of physiological microemboli (Heusch et al., 2009). The extent of these microvascular impairments – at least following an acute myocardial infarction – is prognostic of CV event risk (Wu, K.C. et al., 1998) and periprocedural myocardial infarction during reperfusion interventions (Wu, Z. et al., 2014). In many cases CMD may be induced iatrogenically through procedures used to treat CAD that ablate atherosclerotic plaques, exposing the coronary microcirculation to microemboli (Crea et al., 2013).

CMD and CVD are highly related and can both contribute to myocardial ischemia though the relative contributions of each may vary. By combining the measurement of coronary flow velocity reserve and fractional flow reserve (the ratio of the pressure distal to a stenosis compared to the

proximal pressure), it is possible to discern whether impairments in coronary vascular function are primarily epicardial (macrovascular) or microvascular in nature (van de Hoef et al., 2014). Discordance between the development of coronary flow limitations in the macrovascular and microvascular beds occurs in 31-37% of intermediate coronary stenosis and is associated with much greater risk of cardiac events when coronary flow limitation is microvascular in origin (i.e. high fractional flow reserve but low coronary flow velocity reserve). This has important implications for targeted treatment of CAD and CMD according to the source of coronary flow impairment. Lastly, the scarcity of evidence for treatments for established CMD highlights the need for further research in this area (Marinescu et al., 2015).

2.2. **Acute effects of exercise upon the vasculature**

Following the onset of exercise, blood flow to working skeletal muscle increases. This occurs due to a an increase in the relative proportion of total cardiac output/blood flow through a redistribution of blood flow away from tissues and organs that are non-essential to transient physical activity and an absolute increase in blood flow through an increased cardiac output. Blood flow to non-exercising tissues also increases (Tanaka et al., 2006) as a consequence of exercise. Increases in blood flow lead to increases in endothelial shear stress which activates signalling pathways within endothelial cells and subsequently vascular smooth muscle cells leading to the release of vasodilators of which the protagonist is NO.

Both endothelium-dependent and endothelium-independent mechanisms exist for exercise-induced changes in vascular function. Mechanistically the role of endothelium-dependent vascular dysfunction has a stronger rationale that associates it with the aetiology of atherosclerosis and long-term outcomes such as CV event risk.

Understanding the mechanisms that influence the acute vascular response to exercise are key to understanding the effects exercise has upon vascular health. Moreover, an understanding of how exercise acts as a stimulus to the vasculature on an acute basis affords a better understanding of how repeated bouts of exercise have a cumulative and chronic effect upon vascular health.

2.2.1. **Acute effects of exercise upon the peripheral macrovasculature**

The acute effects of exercise on the peripheral vasculature are mediated primarily by increases in endothelial shear stress. This has been demonstrated in arteries perfusing active tissues by Tinken et al. (2010). By comparing the effects of 30 minutes of bilateral handgrip exercise on forearm blood flow and FMD with unilateral cuffing to modify blood flow, they found that cuffing one of the arms prevented increases in shear rate (an index of shear stress derived from measuring blood flow and vessel diameter) during exercise compared to the non-cuffed arm. Subsequently, changes in FMD from pre-exercise values were only increased in the non-

cuffed arm, which were attributed to the stimulus of shear stress delivered during the exercise bout.

A similar experiment was attempted by Padilla et al. (2011) to highlight the role of shear stress upon endothelial function in vascular beds of inactive tissue using leg cycling exercise, bilateral blood flow assessments of the brachial arteries and unilateral cuffing of one arm. Unfortunately, practical limitations pertaining to the pattern of brachial artery blood velocity in the non-cuffed arm “complicated the interpretation of the data, thus minimising the usefulness of this technique”. To overcome these methodological limitations, another line of reasoning was used to understand the role of shear stress in the acute stimulus provided by exercise to the peripheral vasculature. Passive heating of the forearms is known to induce increases in shear stress through increases in blood flow that results from decreases in downstream cutaneous microvascular resistance via thermoregulatory processes. Padilla et al. (2011) exploited this to produce a shear stress stimulus to the brachial artery that was comparable to that induced by exercise. Importantly this induced comparable magnitudes of brachial artery vasodilatation during exercise albeit through a means which differs from exercise in blood flow pattern.

Exercise-induced vasodilation is driven by changes in pulse pressure and not heart rate as demonstrated by Green, DJ et al. (2001) who found no change in brachial diameter or the contribution of NO to blood flow regulation using a cardiac pacing stimulus of a similar magnitude to exercise but in the absence of changes in pulse pressure.

Exercise hyperaemia is greater in vascular territories perfusing active skeletal muscle as blood flow increases accordingly withmetabolic demand. The degree of hyperaemia experienced is exercise intensity dependent (Andersen and Saltin, 1985). As such, arteries supplying blood to exercising skeletal muscle experience greater exercise-induced shear stress than arteries supplying less active tissues. Consequently, during exercise peripheral blood vessels supplying active muscle undergo a greater increase in NO-mediated dilation than vessels perfusing inactive or less active tissues.

Inferences about the systemic effects of exercise are made by comparing the effects of exercise on the vasculature of active to non-active vascular territories. Within inactive muscle beds, changes in blood flow are also exercise intensity dependent. According to Green, Daniel et al. (2002) brachial artery blood flow shows a biphasic response during leg cycling exercise, initially decreasing at low workloads then increasing with further increments in workload. This finding is believed to be due to a “steal” effect, whereby a suction of blood flow occurs from the inactive limbs towards the working limbs during diastole resulting in increased retrograde (decelerative) flow in the brachial arteries. Importantly, anterograde blood flow in the inactive limbs increased over twofold at the highest exercise intensity measured which highlights the systemic effect the exercise-induced increases in blood flow can have on the vasculature.

The use of FMD to assess the acute effects of exercise on endothelial function has previously been thought to be a useful indicator for chronic

exercise-induced endothelial adaptations to a particular exercise protocol, presumably as the common agent of both phenomena is shear stress. FMD has been shown to increase, decrease, and be unchanged following acute exercise (Dawson et al., 2013). A number of factors resulting from heterogeneities in study design may explain these findings.

One factor contributing to heterogeneous in study outcomes is the exercise dose received by subjects, that is the intensity and duration characteristics of the exercise stimulus. Exercise at higher intensities can have acute negative effects on FMD (Birk, G. et al., 2013; Dawson et al., 2018). The intensity threshold for these effects is unknown but the underlying mechanism has been attributed to excess production of ROS by mitochondria at higher intensities which acts to quench NO, reducing its bioavailability (Bergholm et al., 1999) and therefore reducing FMD. Additionally, longer duration exercise may be more likely to induce acute decreases in FMD (Johnson et al., 2012) by depleting endogenous antioxidant/NO generating capacities.

Another factor is the time between the cessation of exercise and assessment of FMD. Whilst high intensity exercise but not moderate intensity can lead to acute decreases in FMD immediately after exercise (Birk, G. et al., 2013), when FMD is assessed at a longer interval post exercise (1-2 hours) the effects of exercise dose are less clear. Other potential factors include: exercise modality (resistance exercise acutely decreases in FMD due to the effects of blood pressure independent of shear; antioxidant status, cardiorespiratory fitness because of the depletory effects of ROS generation during exercise on NO bioavailability;

inflammation, sympathetic nervous activity, smooth muscle dysfunction and oestrogen bioavailability all may also confound acute FMD responses to exercise (Dawson et al., 2013). Repeated bouts of exercise do not appear to confer additional benefits once a certain, unknown volume of shear stress has been accumulated in the brachial artery (Pyke, K.E. and Jazuli, 2011) and superficial femoral artery (King et al., 2017). Whether arteries perfusing the habitually active vascular beds of primary locomotor muscles respond differently to magnitudes and durations of shear stress is currently unclear. It is easier to increase absolute blood flow to the lower limbs than the upper due to their greater muscle mass, yet in spite of this higher shear stresses are still easier to obtain in the upper limbs due to smaller arterial diameters. Data from Wray et al. (2005) suggest that for a given increase in shear rate a greater vasodilation is observed in the conduit vessels of the upper over the lower limb, however further heterogeneities exist between the vascular reactivity of the deep versus common femoral arteries with a fourfold greater, albeit small, increase in diameter per increment in shear rate. Shear rate is a surrogate measurement for shear stress with the assumption of a constant blood viscosity. The role of the artery size upon the magnitude and timing of the FMD response in healthy individuals has also been explored by (Thijssen et al., 2008) with smaller responses being observed in larger arteries. The implications of this finding regarding interpretations of endothelial function are at present unclear.

Clearly current evidence supports the notion that an acute bout of aerobic exercise can improve endothelial function. From a different angle, acute

exercise may not only improve the function of the peripheral microvasculature but it may also stimulate protective mechanisms that attenuate damage. Seeger et al. (2014) show that a bout of exercise can ameliorate the transient decline in FMD that occurs following prolonged ischaemia but only when the exercise was performed in an interval format but not continuously. A ~40% decrease in FMD was present in both subjects who performed a continuous exercise bout and a control group following an ischaemia-reperfusion injury in healthy, young subjects with unimpaired endothelial function. Interval exercise induced brief periods of ischaemia and included time spent at work rates (i.e. intensities) of twice the magnitude as the continuous bout (100% vs 50% of maximum workload during and incremental which will likely have induced higher blood flow and as such a greater shear stress stimulus. Although these factors may contribute, the mechanisms by which interval exercise induces a preconditioning effect upon the endothelium are not known.

2.2.2. **Acute effects of exercise upon the peripheral microvasculature**

The peripheral microvasculature is extensive and spans the entire body. Most relevant to health and in relation to the effects of exercise are the microvasculature of skeletal muscle and the cutaneous microcirculation.

At the onset of exercise, the oxygen demands of the skeletal muscle tissue increase. This leads to vasodilation of resistance vessels –primarily arterioles- via a network of local metabolic and mechanosensitive factors,

driving consequent increases in conduit artery blood flow. As such, many of the acute responses of the peripheral microvasculature are similar to the peripheral macrovasculature (see Section 2.2.1). The degree of skeletal muscle perfusion is tightly coupled with the metabolic demands of the tissue. Assessing the changes in acute perfusion of the peripheral microvasculature supplying skeletal muscle in response to exercise is a challenging and largely unexplored area. The response of the cutaneous microcirculation to acute exercise is less tightly coupled to the intensity of the exercise but still highly related; increases in cutaneous blood flow during exercise aid thermoregulation by offsetting exercise-induced heat gain. The cutaneous microcirculation has been proposed to provide a non-invasive window into systemic microcirculatory function and dysfunction (Holowatz et al., 2008). A number of studies have demonstrated that acute exercise leads to reductions in cutaneous vascular resistance (Gonzalez-Alonso et al., 1995; Taylor, W.F. et al., 1989; Rendell et al., 1997).

2.2.3. **Acute effects of exercise upon the peripheral vasculature in CVD**

A dearth of research exists describing the differences and relevance of differences in acute vascular responses to exercise in chronic disease populations with established endothelial dysfunction (Dawson et al., 2013). One study in individuals with CAD revealed acute increases in FMD following moderate intensity continuous exercise and high intensity interval exercise with no difference between protocols and no effects on endothelium-independent function (Currie et al., 2012).

Some insights may however be gained from the existing studies in populations with CVD risk factors. In overweight inactive men, an attenuated increase in FMD is observed following acute exercise bout – independent of exercise intensity – compared to active overweight men (Harris et al., 2008). In contrast, Tjonna et al. (2008) show increases in FMD following a single bout of exercise were sustained for up to 72 hours in untrained individuals with metabolic syndrome following aerobic interval exercise but, following continuous moderate intensity exercise, an increase was seen immediately after - but not 24 hours after - acute exercise. This suggests there may be exercise intensity dependent effects on the acute FMD response to exercise. In line with studies describing the acute effects of exercise intensity in healthy populations (see Section 2.2.1), Farsidfar et al. (2008) found acute increases in FMD in patients with CAD following moderate intensity exercise but FMD was unchanged from baseline values following maximal exercise.

2.2.4. **Acute effects of exercise upon the central macrovasculature**

“Exercise is the most important physiological stimulus for increasing myocardial oxygen demands” (Duncker and Bache, 2008).

As with the observed coupling of blood flow to the metabolic rate of skeletal muscle tissue, cardiac muscle tissue, which makes up the majority of the myocardium is the primary metabolically active component of the heart and is the main driver of central blood flow. Oxygen consumption in the heart is principally required for muscular contraction with basal metabolism utilising

~20% of total oxygen consumption (Yaku et al., 1993). The oxygen cost of a single heart beat or myocardial contraction is determined by ventricular work: comprised of ventricular wall tension/strain and myofibrillar shortening; and contractility: the energetic costs of Ca^{2+} handling required for contraction (Araki et al., 2001; Suga, H, 1990). Total myocardial contractile oxygen uptake MVO_2 is a product of ventricular work, contractility and heart rate.

Increases in heart rate are the main factor responsible for exercise-induced increases in MVO_2, representing 50-70% of total increases in oxygen demand (Figure 6).

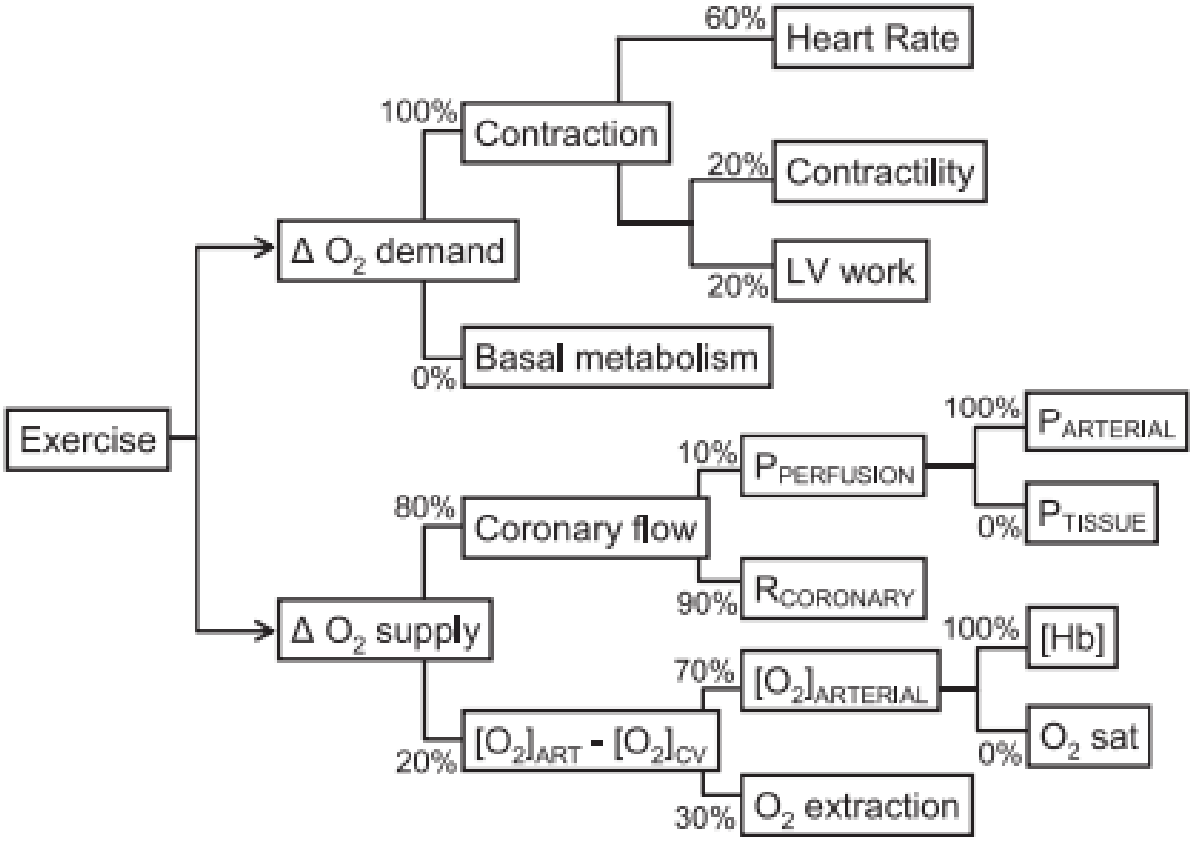

Figure 6: Myocardial oxygen supply-demand dynamics in response to exercise. From Duncker and Bache (2008).

In the healthy heart, increases in coronary blood flow are the major facilitator of increases in MVO_2 during exercise. This change in coronary blood flow is achieved primarily through a reduction in the vascular resistance of the coronary artery. Exercise increases coronary blood flow proportionally to heart rate with 3 to 5-fold increases from the resting level observed during maximal exercise. Animal models have provided extensive evidence that, even during maximal exercise, pharmacologically induced reductions in coronary vascular resistance allow greater coronary artery blood flows to be induced. There appears to be a substantial vasodilator reserve present in healthy coronaries. This surplus blood supply subsequently leads to increases in regional contractile function and $M\dot{V}O_2$ suggesting that coronary flow may play a role in regulating contractile function.

The other component of coronary blood regulation is coronary perfusion pressure. Perfusion pressure in the coronary vasculature is similar to peripheral vascular beds of active skeletal muscle, pressure gradients present along the vascular tree are modulated by changes in aortic pressure (cardiac output), the resistance of distal vascular beds (systemic vascular resistance) and extravascular pressure fluctuation caused by muscular contractions (muscle pumping action). The myocardium is periodically subjected to both intramural and extramural compressive forces due to the normal fluctuations in the cardiac cycle and during ventilation. These extravascular forces overcome lesser intravascular pressures resulting in impedance of flow in the coronary microvasculature. In the normal coronary circulation this effect is not believed to be limiting during exercise as there

still exists a vasodilatory reserve, however, under pathological conditions it may have a greater physiological significance.

Extravascular compression is most prominent during systole due to the generation of large ventricular pressures. Accordingly, myocardial perfusion is much greater during diastole and increasing the time spent in diastole increases myocardial perfusion.

Whilst the systolic temporal component of the cardiac cycle is shorter than the diastolic period at rest, with increasing heart rates (as when exercising) diastolic time decreases at a greater rate than systolic time. Therefore, the ratio of the time spent in systole and diastole increases with increasing heart rate.

Exercise brings about increases in coronary perfusion pressure (aortic sinus pressure) through increased stroke volume and contractile force which coincides with and increased transmural (extravascular) pressure generated by the same means. The net effect is that perfusion pressure increases by only 20-30% during exercise. In spite of this, coronary blood flow can increase 3-5 fold via reductions in coronary vascular resistance. These reductions in vascular resistance are derived from increases in the vessel diameter in the coronary arteries and downstream resistance vessels, particular within terminal arterioles. Dynamic vessel diameter modification across the vascular trees represents the active component of total coronary resistance, which is regulated in response to acute exercise. As in the peripheral vasculature, the passive element of coronary vascular resistance is derived from the profuseness of the vascular territory.

Increases in arteriovenous oxygen difference (i.e. oxygen extraction) constitute the other main mechanism by which increased myocardial oxygen demands are met. Arterial oxygen content has been shown to increase during exercise through increases in the haematocrit proportion of the blood as a result of plasma extravasation of fluid from the capillaries (von Restorff et al., 1977).

A number of studies have demonstrated that during heavy but not light intensity exercise (Duncker and Bache, 2008) myocardial oxygen extraction increases. Myocardial oxygen extraction at rest is relatively high (70-80%) even compared to active skeletal muscle tissue (~50%). As such, there is little leeway for increases in oxygen extraction to contribute to increases in $M\dot{V}O_2$ though it does occur in humans (Heiss et al., 1976). A potential explanation for the occurrence of this phenomenon only during high intensity exercise may be due to a rightward shift in the haemoglobin oxygen dissociation curve induced by a decrease in blood pH as a consequence of an increased dependence on anaerobic glycolysis for ATP production to maintain contractile function.

A number of mechanisms regulate coronary vascular resistance. Coronary blood flow is highly responsive to metabolic signals that allow rapid adjustment to dynamic myocardial metabolic demands. ATP is progressively released by erythrocytes when the local partial pressure of oxygen (PO_2)falls as occurs during exercise. Increases in plasma ATP following a drop in PO_2 during exercise have been suggested to mediate vasodilation of the coronary vessels during exercise (Duncker et al., 1995a). ATP produces

vasodilation via its action on endothelial purinergic receptors leading to the production of NO, which may contribute to vasodilation and consequent increases in blood flow.

A role for histamine-mediated regulation of vascular tone is well established mechanistically and has been explored during exercise in the peripheral vasculature (McCord and Halliwill, 2006). Despite evidence of the presence of histidine receptors in the coronary circulation (Miller and Bove, 1988), there has been little investigation of the role of histidine or histamine regarding the regulation of coronary vascular tone.

NO represents another possible contributor to exercise hyperaemia with established mechanisms of action. NO production can be upregulated through transduction of shear stress signals by endothelial cell as discussed previously. Another source of NO is circulating erythrocytes which will release NO in response to increases in PO_2 gradients (Allen et al., 2009). Existing data from canine and swine models, however, show that NO-blockade in isolation during exercise does not affect and may even increase coronary blood flow. A similar case exists for the role of prostanoids. Other endothelium-derived hyperpolarising factors have not been studied for their effects on coronary vasodilation in humans. The role for NO and prostaglandins may lie in their ability to negate the vasoconstrictor action of endothelin during exercise yet data is scarce and lacking in humans.

Autonomic control plays a small part in the modulation of coronary blood flow. Adrenergic activation of α and β control mechanism exerts conflicting

effects on vascular tone, however the net effect during exercise is a β-adrenergic feed-forward signal towards vasodilatation. This has been suggested to be responsible for up to 25% of exercise hyperaemia (Duncker and Bache, 2008).

Though the effects of blocking multiple individual potential regulators of myocardial blood flow have yielded little in the way of defining a critical locus of vascular control. One reason for this could be that it is the cumulative synergistic action of a combination of mechanisms in a linearly additive manner that regulates coronary vascular tone (Tune et al., 2004). On a more tangible level, coronary endothelial function, the capacity of the coronary arteries to dilate in response to pharmacological or physiological stress is reduced in patients with CV risk factors (Reddy et al., 1994) and with established CAD (Hambrecht et al., 2000; Sixt et al., 2009).

2.2.5. **Acute effects of exercise upon the central microvasculature**

Perfusion of the myocardium increases linearly with increases in exercise intensity in healthy human subjects (Laaksonen et al., 2007). A unique element of microvascular perfusion in cardiac muscle tissue is the heterogeneity of the transmural distribution of blood flow. Broadly, there are distinctions between the patterns of blood flow within the subepicardial and subendocardial layers of the ventricular wall at rest and during exercise. Similar to coronary artery function, transmural flow distribution is not believed to present physiological limitations to cardiac function in healthy

humans. Ventricular contraction induced systolic compression disproportionately affects the subendocardial vessels due to their proximity to the ventricular cavity. Not only is subendocardial flow impeded during systole, but it may also flow in a retrograde manner towards the subepicardium. Anterograde subendocardial flow therefore occurs principally during diastole. As heart rate increases during exercise, the diastolic interval of the cardiac cycle decreases disproportionately reducing time available for subendocardial perfusion. To ameliorate this effect and maintain a necessary transmural pressure gradient, vascular resistance is lowest in the subendocardial vascular territories. Furthermore, the subendocardial vessels tend to be longer and more profuse than subepicardial vessels, which contributes to a vascular waterfall effect. Blood amassed in the epicardial and subepicardial vessels - where perfusion pressure exceeds extravascular pressure - can promptly cascade into the low resistance subendocardial vascular beds when extravascular pressures are relieved during diastole. By these mechanisms, mean transmural perfusion is maintained during exercise. A vasodilatory reserve in the healthy subendocardium may exist even at maximal exercise as various experiments have exhibited increased subendocardial flows using exogenous vasodilators during maximal exercise (Rouleau et al., 1979).

2.2.6. **Acute effects of exercise upon the central vasculature in CVD**

To this researcher's knowledge, studies examining the effects of acute exercise on coronary blood flow and myocardial perfusion in humans with CAD are lacking.

One study used maximal exercise as a diagnostic tool for detecting coronary stenoses in patients with angiographically determined CAD using thallium myocardial perfusion imaging (Josephson et al., 1982), however dipyridamole induced vasodilation proved a more sensitive tool. A stated concern was that many potential CAD patients could not perform maximal exercise, which may explain why historically pharmalogical stressors have been used over the physiological stress of exercise as a diagnostic tool in clinical populations.

Despite having reduced coronary endothelial function (see Section 2.1.4), the finding that patients with severe coronary stenosis produce substantially more NO during exercise than healthy control subjects has been attributed to exercise-induced myocardial ischaemia (Node et al., 1998). This finding implies that it may not be a lack of NO bioavailability *per se* that causes impairments in coronary artery endothelial function, rather that there are insufficiencies in local NO-dependent vasodilatory pathways.

In animal models of CAD, exercise-induced increases in coronary and myocardial blood flow are generally attenuated compared to healthy controls. In swine with severe familial hypercholesterolemia and diffuse coronary atherosclerosis, myocardial oxygen extraction was impaired versus

control swine by myocardial blood flow and transmural blood flow distributions during fixed speed treadmill running were not different (Bender et al., 2016). This increase in oxygen extraction likely occurred as oxygen delivery was inadequate to match oxygen demands. This may have occurred as the CAD swine had impaired coronary artery endothelial function as assessed by vasodilatory responses to sodium nitroprusside infusion.

In a canine model of coronary occlusion, treadmill running based exercise-associated increases in myocardial blood flow were impaired depending upon the degree of constriction and exercise intensity, with the greatest impairments seen with more severe occlusion and more intense exercise (Bache, R. and Schwartz, 1983). The transmural distribution of myocardial blood flow was also markedly affected by these two factors resulting in severe exercise-induced subendocardial hypoperfusion.

2.3. **Chronic effects of exercise upon the vasculature**

In all but severely limited individuals, chronic exercise training provides the most potent stimulus for improvements in cardiorespiratory fitness. A low cardiorespiratory fitness is known to have stronger associations with an increased risk of cardiovascular morbidity and mortality in healthy individuals (Myers et al., 2002) and those with CVD (Kavanagh, T. et al., 2002; Kavanagh, Terence et al., 2003) compared to traditional risk factors. The reasons for this strong association may exist because cardiorespiratory

fitness provides an integrative measure of the function of numerous bodily systems, particularly the cardiovascular system, which contribute to mortality/health. Another reason may be that - to an extent - an individual's cardiorespiratory fitness reflects the chronic accumulation of health promoting stimuli from acute bouts of physical activity (see Section 2.2) and as a consequence reflects the lifelong effect of exercise upon the vasculature (Seals et al., 2008).

2.3.1. **Chronic effects of exercise upon the peripheral macrovasculature**

Vascular adaptations to exercise are primarily dependent on exercise-induced increases in shear stress (Tinken et al., 2010). This has been demonstrated through experiments, which diminished elevations in blood flow to one of two forearms performing handgrip training through the inflation of a blood-pressure cuff. As a result of the prevention of exercise-induced increases in shear stress during training, the cuffed limb did not exhibit the functional and structural vascular adaptations seen in the non-cuffed (contralateral) limb. Furthermore, a study by Birk, G.K. et al. (2012) adds credence to the hypothesis of the focal role of shear stress upon chronic exercise-induced vasodilation using a similar arm cuffing protocol but instead using leg cycling exercise training. 8 weeks of leg cycling exercise induced changes in FMD in the non-cuffed but not cuffed arm that was attributable - in part - to the greater shear stress experienced in the non-cuffed arm during exercise. In the absence of exercise, models using local

and systemic passive heating to induce increases in shear stress using the same cuffing protocol to deprive a limb of shear stress have shown a similar dependence upon shear stress as a mediator of chronic vascular adaptations (Carter et al., 2014; Naylor et al., 2010). While these studies showed increases in FMD and brachial artery diameter, there was no effect observed upon the peak post-occlusive brachial artery blood blow which is thought to give an index of the structural properties of the resistance arteries (Green, D.J. et al., 2017). The remodelling of the resistance arteries may well require a more localised shear stress stimulus than conduit vessels, which may only be induced by increasing the metabolic demands of the local tissues such as skeletal muscle.

Goto et al. (2003) examined the effect of 12 weeks of cycling at different intensities (mild - 25%, moderate - 50% & high - 75% of $\dot{V}O_{2max}$) upon endothelium-dependent vasodilatation and markers of oxidative stress. They showed that moderate intensity but not mild or high intensity cycling augmented endothelial function as assessed by changes in forearm blood flow following acetylcholine administration. Additionally, only the moderate intensity group saw a decrease in markers of oxidative stress. Though the high intensity group would have been exposed to a greater shear stress stimulus, a follow-up experiment demonstrated that the high but not mild or moderate intensity exercise led to an acute increase in markers of oxidative stress (Goto et al., 2007). As discussed in section 2.2.1, exercise intensity modulates ROS balance and NO bioavailability. NO plays a role in shear stress induced remodelling of arteries (Tronc et al., 1996), therefore higher

intensity exercise may not elicit comparable vascular adaptations to moderate intensity exercise because of a lack of NO.

Longitudinal changes in cardiorespiratory fitness and physical activity are related to changes in arterial structure according to data from Ferreira, I. et al. (2003). They found that longitudinal increases in cardiorespiratory fitness were strongly associated with increases in the diameters of arteries perfusing muscular vascular territories and less stiff arteries but not changes in the thickness of the intimal layer of the carotid artery.

Aging is one process that leads to decrements in peripheral vascular endothelial function and arterial stiffening over time (Seals et al., 2011). These maladaptations are not seen in active older individuals compared to inactive individuals (Tanaka et al., 1998; DeSouza et al., 2000) suggesting that regular lifelong physical activity can provide healthful effects that offset maladaptations of the vascular system.

2.3.2. **Chronic effects of exercise upon the peripheral microvasculature**

The effects of exercise training in the peripheral microcirculation have not been extensively researched and the means by which exercise may effectively modify these vessels are unclear. One well-established phenomenon is that increases in the density of capillaries in skeletal muscle following exercise training occur as verified through the use of muscle biopsies (Andersen and Henriksson, 1977).

Though unable to isolate the muscular vascular beds, non-invasive techniques assessing increases in limb blood flow (commonly the forearm) provide insight into the endothelium-dependent and endothelium-independent function of the general microvasculature in response to exercise training. Cross-sectional data suggest cutaneous microvascular function tends to decline with age, but this decline can be ameliorated by through chronic exercise training. Tew et al. (2010) showed that older athletic individuals showed similar hyperaemic microvascular responses to young individuals that were superior to those of an older sedentary cohort. In addition, peak hyperaemic responses to local heating and post-occlusion were moderately correlated with cardiorespiratory fitness across all older individuals. Moreover, exercise training responses and improvements in cardiorespiratory fitness (CRF) have both been demonstrated to prevent age-associated declines in endothelium-derived NO function in cutaneous microvessels (Black et al., 2008).

2.3.3. **Chronic effects of exercise upon the peripheral vasculature in CVD**

Regular physical activity partially attenuates the negative effects that CVD risk factors such as hypertension and diabetes have upon vascular health (Nyberg et al., 2015). Specifically, increased NO bioavailability and decreased ROS generation in vascular territories perfusing skeletal muscle enhance vascular function.

Cardiac rehabilitation is one setting where individuals with overt CAD perform exercise training. A review by Taylor, R.S. et al. (2006) revealed that ~15% of improvements in CV mortality following cardiac rehabilitation could be explained by reductions in systolic blood pressure, which is in part determined by vascular endothelial function (Bleakley et al., 2015).

The SAINTEX-CAD trial compared the effects of 12 weeks of aerobic interval exercise or aerobic continuous exercise upon endothelial function and CRF and found improvements in FMD in both groups but a superior improvement in the continuous group (Conraads, V. M. et al., 2015). Interval training constituted exercise at high exercise intensities (85-90% $\dot{V}O_{2peak}$) and continuous training more moderate intensities (60-70% $\dot{V}O_{2peak}$). Therefore, this finding is in line with the previous acute studies in healthy and CVD populations exhibiting greater endothelial function responses (see Sections 2.2.2 & 2.2.3) and adaptations following moderate intensity exercise (see Section 2.3.1). On top of this, absolute changes in FMD were correlated with increases in CRF with training, which may suggest that improving endothelial function contributes to improvements in CRF because CRF is a measure of the integrative function of multiple physiological systems. Presumably this is due to enhancement of the perfusion of active skeletal muscle which improves oxygen utilisation (Andersen and Saltin, 1985). Endothelial dysfunction mediated limitations to CRF may also confer some of the high prognostic power of measures of CRF for this reason (Kavanagh, Terence et al., 2003; Kavanagh, T. et al., 2002).

Cornelissen, Véronique A et al. (2014) showed similar improvements in FMD and also found a positive correlation between changes in FMD and CRF following 12 weeks of cardiac rehabilitation. Several other studies in cardiac rehabilitation cohorts have demonstrated improvements in FMD with exercise training (Moholdt et al., 2012; Luk et al., 2012; Vona et al., 2009).

2.3.4. **Chronic effects of exercise upon the central macrovasculature**

The most comprehensive review of the responses of the coronary circulation to exercise training to date by Laughlin, M Harold et al. (2011) highlights the considerable reliance upon data animal models that underpins the current understanding of the human coronary circulatory responses to exercise training.

Frequent augmentations of myocardial blood flow through regular exercise training provides stimulus for functional and structural adaptations to the coronary circulation. Coronary vascular adaptations are in part reactionary to changes in cardiac muscle tissue following exercise training. For example, Windecker et al. (2002) describe concurrent increases in left ventricular mass and the cross-sectional area of the left anterior descending coronary artery which likely occurs to enable adequate perfusion per unit mass of the newly hypertrophied myocardium. Furthermore, coronary artery flow-mediated dilatation function was positively correlated with $\dot{V}O_{2peak}$ and coronary flow velocity reserve increased in a manner that was positively correlated with maximal exercise workload, emphasising again the

importance of vascular function to the highly prognostic measure that is CRF.

Comparing athletes with physiologically hypertrophied hearts with pathologically hypertrophied hearts in hypertensives and sedentary individuals, Kozakova et al. (2000) show that exercise-trained individuals have an augmented coronary vasodilation in response to dipyridamole. Whether this effect may be caused by an increase in NO bioavailability is unclear. An increase in eNOS content of the coronary arteries, as was seen in exercise-trained canines (Sessa et al., 1994) may result in increased NO bioavailability. Conversely, in exercise-trained swine eNOS protein content is shown not to increase in the coronary arteries but was increased in all other precapillary vessels comparted to sedentary swine (Laughlin, M. et al., 2001).

Little is known about the effects of exercise intensities, volumes and frequencies on coronary vascular adaptations to exercise in healthy individuals. Substantially more research has been conducted in disease individuals and disease models which is likely attributable to the lack of a limiting effect of coronary perfusion upon CRF or normal function in healthy individuals.

2.3.5. **Chronic effects of exercise upon the central microvasculature**

In one of the few studies to invasively assess the coronary circulation in exercising humans, Heiss et al. (1976) compared the acute effects of

matched-intensity (65% of $\dot{V}O_2$ reserve) exercise on myocardial blood flow in trained and untrained healthy subjects. Trained individuals had lower myocardial blood flows at rest, which may be attributable to lower resting heart rates as indices of myocardial work, and $M\dot{V}O_2$ per stroke were not different from the untrained individuals.

Additionally, during exercise, trained individuals showed a much smaller increase in myocardial blood flow (~2-fold vs ~3-fold) from rest to exercise that paralleled a smaller coronary flow reserve in trained individuals. A similar fractional utilisation of coronary flow reserve was observed during exercise, however $M\dot{V}O_2$ per stroke decreased in trained and increased in untrained individuals (-14% vs + 37%) versus resting values. The reasons for this observation are unclear but may relate to increased cardiac efficiency as a result of increased in preload and greater reductions in peripheral resistance during exercise.

Studies in animals of the effects of aerobic exercise training generally support an increase in myocardial flow reserve but human data does not corroborate this finding. Kalliokoski et al. (2002) found no difference in resting perfusion or perfusion reserve in endurance trained subjects using positron emission tomography imaging. They did, however, show an inverse relationship between $\dot{V}O_{2max}$ and myocardial perfusion reserve. Along a similar line, Laaksonen et al. (2007) reported that during exercise at a given relative exercise intensity endurance trained individuals revealed similar myocardial perfusion per gram of tissue compared to untrained individuals.

Equally, whilst myocardial perfusion resistance decreased from rest to exercise, it was not different between the two groups.

A subsequent study by Kalliokoski et al. (2003) showed that myocardial perfusion resistance at rest and during exercise was in fact elevated in highly trained individuals, which contributed to an increase mean transit time of the blood and thus increased myocardial oxygen extraction but no subsequent change in myocardial efficiency .

2.3.6. **Chronic effects of exercise upon the central vasculature in CAD**

Peripheral artery endothelial function is correlated with coronary artery endothelial function in individuals with CVD (Takase et al., 1998). This relationship is attributed to the systemic nature of the pathophysiology of endothelial dysfunction and atherosclerosis. Changes in peripheral arterial function, especially since the adoption of the FMD technique, are less invasive to assess than measures of coronary artery endothelial function and therefore have been used as a more practical index to reflect the progression of CVD and CV event risk. Thus, changes in peripheral artery function with exercise training (see Section 2.3.1) infer changes in coronary artery function. Furthermore, this application may justify the claims that vascular function may account for up to 40% of CVD risk (Green, D.J. et al., 2004).

Endothelial dysfunction is a precursor to the development of atherosclerosis (see Section 2.1.1.2) and manifests in the central vasculature in individuals

exposed to CV risk factors (see Section 2.2.4). Just as exercise training is an effective stimulus to induce beneficial functional and structural adaptations in the peripheral vasculature, coronary vascular beds are responsive to exercise training.

Coronary endothelial function in inpatients with CAD, as measured by diameter and blood flow responses to acetylcholine and adenosine infusions, are improved by 4 weeks of daily intermittent exercise training of ~2 hours in duration (Hambrecht et al., 2000). This study demonstrated a 29% increase in coronary blood flow reserve in the exercise training group with no changes observed in the control group. This finding provides a good justification for the therapeutic use of exercise training in populations with or at risk for CAD. One caveat is the huge time commitment associated with this format of high frequency, high volume exercise training that limits the generalisability of this finding. Several studies have shown that exercise training leads to an increase in NO bioavailability in the coronary arterioles (Laughlin, M. et al., 2001; Rush et al., 2003), which may explain in part the mechanism of improved coronary endothelial function.

In patients with type 2 diabetes mellitus (a condition associated with impaired peripheral artery endothelial function) and CAD, a four week daily exercise program with a total of six months of multi-factorial intervention showed improvements in coronary endothelial function at six months but not four weeks (Sixt et al., 2009). Whether the pathology of diabetes or the lower volume exercise intervention explain the lack of effect of four weeks of

exercise training in this study versus that of Hambrecht et al. (2000) is unclear.

Schuler et al. (1992) demonstrated that in CAD patients the adoption of a regimen of intensive physical exercise and a low-fat diet over 12 months led to decreases in exercise-induced myocardial ischaemic indices, as determined via myocardial perfusion scintigraphy. These improvements in perfusion were concomitant with increases in maximal exercise work rate and estimates of myocardial oxygen consumption (rate-pressure product).

From a more structural standpoint exercise may potentially exert beneficial effects upon coronary atheroma morphology. Hambrecht et al. (1993) examined the effects of leisure time physical activity upon coronary plaque progression and found that luminal encroachment of coronary plaques only occurred in individuals who expended less than ~1400 kcal/week. Conversely, individuals who expended greater than 2200 kcal/week experienced regression of coronary plaques, thereby highlighting that the effects of physical activity upon coronary atheroma morphology may be dose-dependent and that exercise of a sufficient dose may alter atheroma morphology. A study by Schuler et al. (1992) showed that a 12-month combined exercise and diet intervention did not reduce minimal coronary lesion diameter, suggestive of negligible plaque volume regression, rather it did show a reduced rate of progression compared to a control group. The ETICA trial found that following a coronary angioplasty or stenting exercise training three days weekly for six months reduced atherosclerotic plaque

progression but not restenosis rates versus non-exercising control subjects (Belardinelli, R. et al., 2001).

In contrast to these studies, Madssen et al. (2014) showed that coronary plaque characteristics, as assessed by intravascular ultrasound, were unaffected by 12 weeks of exercise training using either high intensity interval training or moderate intensity continuous training approaches. Despite this, a small reduction in the necrotic core volume of atheromas located distal but not proximal to an implanted stent was observed with exercise training. Whether exercise can effectively reduce atherosclerotic burden is equivocal. Nevertheless, the finding that it can reduce the progression of atherosclerosis is an encouraging one that may explain in part the role of exercise in reducing CV event risk.

Studies examining the effects of exercise training in isolation on coronary atheroma morphology in humans are unlikely, as patients will almost definitely be receiving standard medical care, such as statin therapy, alongside exercise, which will concurrently alter plaque burden and composition. The physiological effects of exercise in CAD should be considered to act amongst a milieu of other treatment effects. Nevertheless, the interactions of such treatment effects are incompletely understood.

2.3.7. **Chronic effects of exercise in coronary microvascular dysfunction**

In patients with stable angina, when comparing the effects of percutaneous coronary intervention (PCI) with 12 months of daily exercise training, Hambrecht et al. (2004) observed a greater number of ischaemic events in the PCI group than the exercise training group despite a much smaller observed stenotic burden (77.9% vs 11.8% at baseline respectively). Both groups improved their work rate at the ischaemic threshold and myocardial perfusion, but only the exercise training group saw increases in cardiorespiratory fitness or exercise tolerance. That the exercise training group saw improvements in myocardial perfusion in the absence of changes in stenosis diameter and of a comparable magnitude to the far less stenosed PCI group suggests that exercise training improved myocardial perfusion via adaptations that were microvascular in nature.

One of the other outcomes of the ETICA trial was a greater increase in the number of reversible myocardial defects found using thallium myocardial scintigraphy with exercise training and that the thallium uptake score index only increased in the training group (Belardinelli, R. et al., 2001). Improvements in thallium uptake were strongly correlated with improvements in coronary risk profile.

A combination of 6-weeks of exercise training and a low-fat diet reduced resting myocardial blood flow and myocardial flow reserve concurrent with increases in exercise capacity in older patients with CVD risk factors (Czernin et al., 1995). The minimal coronary vascular resistance achieved

with dipyridamole administration also decreased with exercise training, which correlated with improvements in exercise capacity. Whether this change reflects an improvement in endothelium-independent dilatation of the epicardial vessels of coronary microvasculature during exercise is unclear. Interestingly, these short-term training effects contrast with the long term training effects observed by Heiss et al. (1976), which show an inverse trend. This is because their study relativized measures to left ventricular mass whilst the study of Heiss et al. (1976) did not; a factor that explains the discrepancies in findings of changes in myocardial perfusion reserve.

Exercise training has not been demonstrated to reduce angina pain symptoms in patients with cardiac syndrome X but may reduce the onset time and/or exercise intensity at which this pain is experienced (Asbury et al., 2008; Eriksson et al., 2000). Whether this ischaemic pain reduction is a reflection of decreased exercise-induced hypoperfusion or not remains to be elucidated.

The EXCITE trial examined the effects that 4-weeks of either moderate (60% of ischaemic threshold work rate) or high intensity (intervals from 70 to 95% of ischaemic threshold work rate) four times daily for 30 minutes, 5 times per week would have on coronary collateral growth in symptomatic CAD patients (Möbius-Winkler et al., 2016). Compared to usual care, both exercise groups saw improvements in collateral flow index and ischaemic threshold - changes which were associated with one another. Only in the moderate intensity group were reductions in angina found following training. In

addition, improvements in diastolic function were observed but only in the high intensity training group.

There is a current debate in the literature as to the precise mechanisms by which myocardial perfusion increases in response to exercise training. Increases in endothelial function in the coronary arteriolar beds with exercise training may allow greater reductions in coronary vascular resistance and thereby increase myocardial blood flow (Thengchaisri et al., 2007). Equally, arteriogenesis of the main coronary resistance vessels in response to exercise training is well documented in stenotic animal models (Duncker and Bache, 2008) and shows an increase in arteriolar cross-sectional area following early angiogenic adaptations. Previous work that has induced angiogenesis via an exogenous recombinant human vascular endothelial growth factor infusion demonstrated successful increases in myocardial perfusion via increased collateralization at rest but not during pharmacological stress (Hendel et al., 2000). This result has been attributed to the lack of influence of collateral vessels on coronary flow reserve in non-collateral dependent (patent) vascular territories. Exercise training-induced collateral development to collateral dependent regions in disease models has also been demonstrated (Roth et al., 1990), which is associated with improvements in regional contractile function. Little is known about the exercise dose and intensities required to achieve these adaptations.

A combination of flow (shear stress) induced and hypoxic milieu interact to stimulate remodelling of the coronary microcirculation. This is achieved via a combination of upregulation of vascular endothelial growth factor and

hypoxia-inducible factor 1 alpha (HIF-1a) signalling to induce arteriogenesis leading to an increased total cross-sectional area of resistance vessels as documented in swine by Bache, R.J. et al. (1981) and by inducing angiogenesis. These adaptations in theory provide a mechanism for improvements in myocardial perfusion reserve though many other mechanisms, singly or in combination, may contribute.

2.4. **Summary**

The pathophysiological role of low endothelial shear stress and an absence of periodic increases endothelial shear stress in the acceleration of atherosclerosis and CVD risk is well established. Exercise, when delivered in adequate intensities, plays a role in vascular homeostasis and reducing CVD risk, by providing a hyperaemic stimulus to the vascular endothelium, a signal that translates to the promotion of a healthy endothelial phenotype. The stimulus that exercise provides is heterogeneous throughout the vasculature though pathological processes of CVD act systemically.

Assessments of the integrity of the peripheral vasculature confer information about systemic CVD risk, and the effect of exercise upon these in humans gives insight into how elements of exercise prescription such as intensity could be optimised to maximise the positive effects of exercise upon vascular integrity.

Whilst the central vasculature is arguably more pertinent to CV events, the effects of exercise and the determinants of the stimulus that exercise provides to the central vasculature in humans is less well studied relative to the peripheral vasculature. Of further importance is developing an understanding the effect of exercise in remedying endothelial dysfunction as seen in individuals with CVD or a high prevalence of CVD risk factors. Much of the research examining the effects of exercise upon vascular integrity is limited to healthy subjects, while the effects on the central vasculature have been limited to animal models. The overarching concept that exercise can benefit vascular endothelial function through the effect transient increase in blood flow is supported throughout this literature review, yet evidence that this is realised in applied contexts with diseased populations is lacking. Additionally, knowledge of how to optimise exercise prescription to improve central vascular integrity especially in diseased populations is scarce.

The acute stimulus to the peripheral and central vasculature provided by exercise is shear stress dependent and therefore blood flow dependent. A key determinant of exercise hyperaemia is exercise intensity. Understanding the factors that influence the acute stimulus provided by exercise is important to understand the effects of chronic exposure to acute exercise stimuli, particularly as the chronic effects of exercise are more pertinent to health.

Chapter 3 **Characterisation of physiological responses to cardiac rehabilitation in a UK community-based cohort**

This chapter consists of three parts.

3.1. **Part 1**

3.1.1. **Introduction**

Endothelial dysfunction is a key component of the atherosclerotic cascade and by extension CVD risk. Exercise has been shown to reduce CVD risk, and is attributed to improvements in endothelial function (Green, D.J. et al., 2008). Endothelial function reflects, in part, the health of the endothelial tissue as unimpaired endothelial function confers anti-thrombotic and anti-inflammatory properties and resists local hypoperfusion and ischaemia through the release of local vasodilators. It is also possible that other factors explain the association between endothelial function and CVD risk as this relationship may be confounded by exercise-induced reductions in CVD risk factors such as blood pressure and blood glucose level (Cornelissen, Veronique A and Smart, 2013; Holloszy et al., 1986). As such, exercise can play a role in the reduction of CVD risk due to its pluripotent effects.

One exercise-based therapy, in which the secondary prevention of CVD is a major goal, is cardiac rehabilitation (CR). Exercise is a fundamental component of the rehabilitation process (Jenni et al., 2012) and it has been demonstrated that no additional benefits on all-cause mortality or

cardiovascular mortality are gained by performing CR programmes that are comprehensive versus primarily exercise-based . The latter include lifestyle interventions (O'Connor et al., 1989) and patient education (which in itself does not modify mortality or CV event rate (Anderson, L. et al., 2017)). Though it is clear that exercise can be effective for reducing multiple elements of CVD risk, in the most recent meta-analysis and systematic review of the topic, CR participation was not associated with reduced all-cause mortality and only had very modest effects on cardiovascular mortality when compared to non- participation (Anderson, L. et al., 2016). The largest randomised-control trial (RCT) included in this meta-analysis was from a UK cohort and failed to show a reduction in mortality, CV event rate or rehospitalisation rates with CR participation (West et al., 2012). These outcomes are at odds with the literature that shows that exercise, being the primary therapeutic component of CR, reduces CVD risk and modifies mortality risks through improvements in other physiological markers such as cardiorespiratory fitness (Swift et al., 2013). Consequently, questions have been raised over whether CR is delivered effectively as a therapy for improving long term outcomes.

The overall lack of benefits seen in the RCTs of CR included in the meta-analysis of (Anderson, L. et al., 2016) have been attributed to a number of factors. First, in the era of modern CR, there may be less health gains to be accrued from CR due to better medications and medical management (Anderson, L. et al., 2016). Second, previous data that showed CR was a beneficial treatment had been influenced by better uptake and adherence to

medications (Giannuzzi et al., 2008). Thirdly, the CR programme employed by (West et al., 2012) provided an "underdosage" of exercise (Conraads, Viviane M et al., 2012).

There is some plausibility behind the first suggestion. Survival from cardiac events has improved tremendously with medical advances over the past few decades, which could have influenced the phenotype of patients who enter CR. Much older and frailer patients who previously would not have survived a cardiac event are now partaking in CR and a much greater proportion of women are engaging in CR (Roger et al., 2010; Dunlay et al., 2014; Doherty et al., 2018; Pashkow, 1993). Additionally, many CVD risk factors are altered through a cocktail of medications including statins, beta-blockers, anti-hypertensives and antiplatelet agents. It has been suggested that as a consequence of optimal medical therapy there may be little to gain from further reductions in risk factors such as blood pressure or thrombotic risk profiles via exercise training (Anderson, L. et al., 2016). Furthermore, older individuals may be more resistant to exercise training adaptations as they often have other co-morbidities such as having stiffer arteries (Mitchell, G.F., 2008; Najjar et al., 2005) which may depreciate the role of the endothelial vasodilator function, and having a lesser adaptive responses to exercise (Cuthbertson et al., 2005; Seals et al., 2008). The second suggestion, that benefits of CR have been derived in the past from its effects on medication adherence, is again plausible and has been observed in a modern cohort (Shah et al., 2009) but the magnitude of the effect is hard to discern as historical evidence of medication adherence is lacking. The third suggestion

by Conraads, Viviane M et al. (2012), that exercise in some CR programmes may suffer from an "underdosage" is also tenable but this notion has been incompletely explored.

As with most treatments, the effects of exercise upon many health indices are dose dependent, that is, the greater the dose, the greater the effect (to a point). The key here is that low doses of exercise will have little to no effect, hence an underdosage of exercise could explain the lack of benefits accrued by patients attending CR. Several elements comprise exercise dose: frequency, intensity, duration and modality.

Sandercock, G. et al. (2013) have found that in the UK the dose of CR is lower, due to having on average half the total number of exercise sessions, compared to programmes in Europe and North America (18 vs 36). Furthermore, programmes with more sessions were associated with greater changes in CRF. The total number of CR sessions only explains part of the dose achieved by patients in CR. One element which is inconsistently reported in the literature is the intensity of exercise prescribed: very little data exists to verify that the prescribed intensity of exercise is actually achieved (Powell et al., 2018). As a consequence, it is challenging to make inferences about the effects of exercise across different CR programmes on physiological indices of health. Exercise intensity is the only element of exercise dose that can be manipulated without the need for additional resource. Thus, exercise intensity is particularly crucial to the delivery of an effective exercise dose in resource limited healthcare models. To further our understanding of the contributions of exercise to patients’ health outcomes

following CR, it is necessary to develop a more complete picture of the characteristics of the exercise dose received by patients.

Based upon the discussed literature, I hypothesise that patients undergoing UK CR may experience an underdosage of exercise and physical activity which will not sufficiently impact upon indices of vascular integrity or cardiovascular risk to confer long-term health benefits.

The present study sought to characterise the exercise characteristics undertaken in a cohort of UK patients undergoing community-based phase III CR and to examine the impact of the programme on changes in habitual physical activity and vascular integrity.

3.1.2. **Methods**

Participants were recruited following referral to phase III CR within a local Community Healthcare NHS Trust subsequent to myocardial infarction (MI) or elective revascularisation (percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG)). Participants were excluded in the presence of diagnosed arrthymias, heart failure, valvular disease or orthopaedic limitations and recruited from 3 separate centres prior to the onset of their CR programme. Written informed consent was gained by a member of the research team from participants prior to participation. Information pertaining to medical condition and medication use was provided by the nursing team. The study was approved by the local NHS ethics

committee (REC reference: 15/WA/0404; IRAS project ID: 188338) and complied with the Declaration of Helsinki.

3.1.2.1. **Experimental protocol**

Participants undertook an incremental shuttle walk test (ISWT) and assessments of blood pressure and weight at a baseline assessment clinic prior to the start of the CR programme. Participants then underwent a 6 week programme of twice weekly exercise sessions and one weekly lifestyle education session. Immediately prior to the 2nd exercise session ultrasound assessments of carotid arterial stiffness and vascular endothelial function were measured. A tri-axial accelerometer was then given to the participant to wear for a 7-day period to record habitual physical activity (PA). During 3 subsequent exercise sessions each participant wore a heart rate (HR) monitor to record heart rate (HR) continuously. These measures of in-exercise characteristics were collected in three separate exercise sessions: session 2 (START), 6/7 (MID) and 11 (END). Assessment of PA was repeated during the final week of CR and measures of carotid arterial stiffness and endothelial function on the 11th exercise session. Participants attended a follow-up assessment clinic on completion of the programme where a second ISWT took place as well as measures of systolic blood pressure (SBP), diastole blood pressure (DBP) and body weight. Participants continued with usual medication use throughout and changes in medication usage and dosage during the study period were recorded.

3.1.2.2. **Incremental shuttle walk test**

The ISWT is a field test of exercise capacity commonly used in clinical populations (Singh et al., 1992). It is a graded exercise test whereby participants walk around two shuttles spaced 10m apart at a gradually increasing pace. A standardised audio track is used to signal when a participant should walk from one shuttle to another using a series of beeps. The test consists of 12 possible levels of increasing speed each with a duration of 1 minute. The first stage requires the participant to walk 30 m (3 shuttles) with the interval between beeps decreasing each level such that an additional 10 m must be walked to complete the subsequent level. A change in level and an increase in speed is signalled via a series of beeps. A HR monitor was worn throughout the test (Polar FT1 pulse, Polar Electro, Finland) and HR recorded at the end of each stage. The test was terminated when the participant failed to reach a shuttle before a beep on two consecutive occasions. The test was conducted by an exercise instructor who worked within the CR service. The total distance walked during the test was recorded.

3.1.2.3. **Physical activity**

Habitual physical activity was objectively measured using tri-axial accelerometers (ActiGraph GTX3+, Actigraph LLC, Florida, USA) worn on the hip using elastic belts. Participants were instructed to wear the

accelerometer for 7 consecutive days, including during CR sessions, and complete an adjunct wear time log collected by a member of the research team. Monitors sampled acceleration at a rate of 30 Hz with data collected in 10 second epochs. Data reduction was performed to produce 60 second sample epochs which were used for subsequent analysis. Wear time analysis was performed using ActiLife software (Actigraph LLC, Florida, USA) with a valid wear period defined as >4 days of >10 hours of wear. Periods of >60 minutes of consecutive zero readings were considered as non-wear. Time spent at different intensities were allocated into banding using cut points for light (<1800 counts per minute (cpm)), moderate (1800-3799 cpm) and vigorous (≥3800 cpm) activity selected based on those validated in a post-CR population by Prince et al. (2015). Moderate to Vigorous physical activity (MVPA) bouts were defined as continuous periods of > 10 minutes with greater than 1800 cpm. Sedentary bouts were defined as periods of valid wear time exceeding 60 minutes with <150 cpm. Sedentary breaks were defined as interruptions in periods of sedentary time with activity of >150 cpm.

3.1.2.4. **Vascular integrity**

Assessments of vascular integrity were performed in a subsample of participants. Vascular integrity comprised carotid artery compliance, carotid intima-media thickness (cIMT), brachial and carotid artery diameters and endothelial function. All assessments of vascular integrity were performed on concurrent days and by a single researcher (this author).

3.1.2.5. **Subject Preparation**

Participants were instructed to arrive early to their session following a > 4 hour fast and having abstained from alcohol ingestion, smoking, caffeine ingestion, exercise and vasodilator medication usage (e.g. sub-lingual glyceryl trinitrate) for >12 hours. Though this period of fasting is not in line with recommendations (Thijssen et al., 2011), there is sparse evidence for the effects of shorter fasting periods upon assessments of vascular function, and high adherence to longer fasting periods may be unachievable in clinical populations with impaired glucose regulation (such as those attending CR). The use of other prescription medications was not prohibited. Participants lay supine for >10 min before measures were taken and were instructed to remain still throughout all vascular imaging.

3.1.2.6. **Carotid artery assessments**

Measures of cIMT and arterial compliance were measured in the right common carotid artery by ultrasound (Vivid I, GE Vingmed Ultrasound, Horten, Norway) with a 10MHz probe (9L, GE Vingmed Ultrasound, Horten, Norway). Images were recorded for a minimum duration of 20 seconds. Two recordings were taken ~2 cm distal to the carotid bulb and using longitudinal views. cIMT and compliance were calculated using automated edge-detection software (Vascular Research Tools 6, Medical Imaging

Applications-LLC, Iowa, USA). cIMT was derived from the average IMT of the near and far carotid wall imaged in anterior and posterior facing planes.

Compliance was calculated as:

$$C = (Dmax - Dmin) / \Delta P$$

where D_{max} and D_{min} are the mean maxima and minima of carotid artery diameter oscillations with pulse over a 30-second measurement period and ΔP is the difference between systolic and diastolic blood pressure.

3.1.2.7. **Brachial artery assessment**

Following the carotid examination, flow-mediated dilatation (FMD) was measured and analysed using duplex ultrasound equipped with a 10 MHz linear array ultrasound transducer probe (9L, Vivid I, GE Vingmed Ultrasound, Horten, Norway) with adherence to the guidelines described by Thijssen et al. (2011). All scans were performed by a single researcher (this author) who had a previously determined intra-rater reliability for the FMD technique, expressed as a within-subject coefficient of variation of 10.8%; where an acceptable value is considered to be <20%. Participants remained in a supine position with their right arm abducted to a ~60-80˚ angle, their wrists supinated and arms supported with inflatable cushions to remain at an equal elevation to the heart. The ultrasound probe was placed on the upper arm proximal to a blood pressure cuff placed on the proximal aspect of the forearm to minimise blood flow by inflating the cuff to > 50 mmHg above systolic pressure. The cuff was attached to sphygmomanometer with a

trigger valve to deflate the cuff as quickly as achievable without the use of specialist equipment. Baseline measures of brachial artery diameter and blood flow were taken before to occlude forearm blood flow for a period of 5 min, as this period produces a predominantly NO-dependent FMD (Kooijman et al., 2008). Images were recorded continuously at 15 frames per second from 30 seconds prior to the release of the cuff and thereafter for a 3 min period, using Duplex (combined linear B-mode and Doppler) ultrasound to record blood flow using an angle of insonation of ≤60˚, as this angle is considered to give a near optimal trade-off between the error rate of Doppler ultrasound velocity estimates and linear B-mode imaging quality (Logason et al., 2001). In all FMD procedures the insonation angle was kept constant at 60˚ and manual steering of the ultrasound beam angle was used to obtain the most accurate data achievable. To ensure the site of scanning was reproduced when repeating measures, the distance from the distal aspect of the ultrasound probe to the ipsilateral medial epicondyle was measured with a tape measure. Also, when repeating ultrasound measurements, the previously recorded image for a participant was displayed to aid alignment of the probe and reproduce a comparable image. Brachial artery diameter and blood flow were analysed using automated edge-detection software (Vascular Research Tools 6, Medical Imaging Applications-LLC, Iowa, USA). The data were processed through the application of quality control within algorithms and, where needed, manually adjustment of the detected edges. Exported data was smoothed through the application of a 1 second (15-point) moving average to the diameter data and a 15 second moving

average to the blood flow data to reduce extraneous noise. FMD was defined as the percentage change in brachial artery diameter from baseline to peak dilatation. The time to peak diameter and peak hyperaemic blood flow were also recorded.

Mean blood flow velocity was calculated using the following equation:

$$Mean\ blood\ flow\ velocity\ (m \cdot s^{-1}) = \frac{f_d c}{2 f_t cos\theta}$$

where f_d represents the Doppler frequency c the average velocity of sound in tissue (1540 m·s^{-1}), f_t the transmitted frequency and θ the angle of insonation.

Shear rates were estimated using measures of blood flow obtained using intensity-weighted mean velocities outputted from the software. Shear rate was calculated for 60 and 90 seconds post-occlusion as:

$$Shear\ rate\ (s^{-1}) = \frac{8\ \times VTI}{D}$$

where VTI is the Velocity Time Integral of Doppler flow and D is brachial artery diameter. This equation was adopted and is recommended when the Doppler sample gate encompasses the entirety of the lumen (Harris et al., 2010). Shear rates are considered synonymous with shear stress for ease of interpretation. Blood flow is assumed to be laminar as only straight sections of the brachial artery were imaged and blood is assumed to follow the properties of a Newtonian fluid. It is assumed that blood viscosity, the other factor that influences fluid shear stress, remains constant between assessments of FMD.

The velocity time integral (VTI) represents mean blood flow velocity was calculated using the trapezium rule for velocity each frame:

$$VTI\ (cm) = \frac{VTI_1 + VTI_2}{2} \times time$$

3.1.2.8. **Exercise training**

Each CR session consisted of a warm-up, ~24 minutes of circuit training style exercise (1-2 min per station) and a standard cool down. Sessions adhered to Association of Chartered Physiotherapists in Cardiac Rehabilitation (ACPICR) guidelines, using a prescribed intensity of 40-70% of heart rate reserve (HRR) and a rating of perceived exertion of 2-4 on the Borg CR10 scale (ACPICR, 2015). Patients' individual heart rate prescriptions were presented to them on clipboards prior to each session. During the three sessions where heart rate was recorded, HR monitors were worn throughout (Polar RS800CX, Polar Electro, Finland) and data outputted in 5 second epochs. Heart rate monitors (Polar FT1 pulse, Polar Electro, Finland) were also used as part of usual care. Resting blood pressures and resting heart rate were taken manually by the nursing team via manual sphygmomanometers before and after each session as per usual practice.

Percentage heart rate reserve (HRR) was calculated as:

$$HRR\ = \frac{HR\ -\ RHR}{HRmax\ -\ RHR}$$

$$HR\ max\ =\ 205.8 - (0.685\ x\ age)$$

where HRmax is maximum predicted HR (calculated using the Inbar formula (Inbar et al., 1994)) and RHR is resting HR. For patients using β-blockers an additional 20 bpm were subtracted from maximal HR in accordance with British Association for Cardiovascular Prevention and Rehabilitation (BACPR) guidelines (ACPICR, 2015). Where changes in β-blockers usage were recorded HRR calculations were adjusted accordingly.

The exercise circuits at each centre consisted of a very similar assortment of exercises. The circuit was preceded by a ~ 10-minute warm-up that consisted of walking and standing exercises plus some light static stretching. The circuited was succeeded by a 5-minute, slower tempo version of the warm-up. The circuits were organised into 12 stations at which there were 5 levels of exercise options prescribed. Different levels described different variations of an exercise via gradations of the speed, range of motion or complexity of the movement. Patients were prescribed a level to follow throughout the whole circuit which was progressed or regressed at the exercise instructor's discretion. Two minutes were spent at each exercise station with the exercise instructor signalling when to move on to the next station. Minimal time was to be spent transitioning between stations. Approximately half of the stations were solely "cardiovascular" (aerobic) in nature whilst the remainder of stations were mixtures of 1 minute of "active recovery" (light resistance or gentle aerobic exercise) and 1 minute of "cardiovascular" exercises depending on the level the patient was working at which an interval training approach. Rest periods were atypical and were taken at an individual's liberty (often to drink some water). There were 5

levels of exercises with the majority of patients beginning on level 3 and then progressing at varying rates throughout the programme. Higher exercise levels eliminated “active recovery” exercises in favour of “cardiovascular” exercises with the intention of patients completing a full circuit of “cardiovascular” exercise as the ideal prescription for able patients. Examples of “cardiovascular” exercises include: stationary cycling, rowing machine, brisk walking/jogging, variations of stepping onto and off a platform/step, mini-trampoline bouncing, bouncing a swiss ball whilst briskly walking, side steps, step backs, low kicks, knee raises, leg curls with arm movements.

Examples of “active recovery” exercises include: weighted bicep curls, weighted tricep extensions, weighted front raises, weighted lateral raises, lunges, sit-to-stand, swiss ball squats, wide-stance squats, press-ups with hands on a wall, toe-taps in-front or behind from a standing position, sways, seated knee extensions.

3.1.3. **Statistical analysis**

Data are presented as mean ± SD. All statistical analysis was completed using SPSS (IBM SPSS Statistics for Windows, Version 24.0. IBM Corp Armonk, NY, USA). Data were assessed for normal distribution using Shapiro-Wilk, and subsequently non-normally distributed data were log transformed. Non-parametric analyses were performed on variables that remained non-normally distributed following transformation. A comparison of

heart rate data between the 3 monitored exercise sessions was undertaken as both time spent above a series of HRR thresholds and as mean %HRR achieved in each session and was analysed via Friedman's test, Wilcoxon-signed rank tests and one-way repeated measures ANOVA. Frequency analysis was performed for the number of participants achieving more than 8 minutes, or more than 12 minutes above HRR thresholds in each session. Groupings were identified as cardiac pathology (MI, PCI and CABG) and co-morbidities (hypertension and diabetes). Further groupings were created by splitting mean heart rates per session into tertiles and by whether participants accumulated more than 8 or more than 12 minutes above HRR thresholds. In order to assess whether variables changed from pre to post CR a repeated measures ANOVA was used with time and cardiovascular risk factors (blood pressure, weight and resting heart rate), measures of ISWT performance, parameters of daily PA and measures of vascular integrity as the repeated measure, the inclusion of age and time since cardiac event as covariates, together with pathology and co-morbidity status as fixed factors.

Relative FMD was calculated by entering logarithmically transformed diameter changes (ln Peak diameter - ln baseline diameter) into an ANCOVA model with time as a fixed factor and logarithmically transformed baseline diameter as a covariate. Covariate-adjusted means were used to derive baseline-corrected relative FMD values (as described by Atkinson and Batterham (2013)). To assess whether differences in vascular

adaptations with training differed by pathology (MI, PCI, CABG), these were also included within the ANCOVA model as fixed factors.

Pearson's and Spearman correlations were performed between baseline values and changes following CR in ISWT performance variables, age, blood pressure, habitual physical activity and vascular integrity. Alpha was accepted as ≤ 0.05 unless stated otherwise.

3.1.4. **Results**

Participant characteristics are displayed in Table 1. The proportion of males and diabetics in the sample was similar to that typically seen in UK CR (70% and 23%: NACR, 2014, NACR, 2015).

Table 1: Participant Characteristics

Baseline characteristics (n = 48)	
Sex (% Male)	73
Age (years)	62 ± 12
Height (m)	1.70 ± 0.1
Weight (kg)	82.4 ± 15.5
Systolic Blood Pressure (mmHg)	129 ± 20
Diastolic Blood Pressure (mmHg)	71 ± 10
Diabetic (%)	15
Cardiac event (%)	
AMI	73
CABG	19
PCI	69
MI + PCI	50
Days since cardiac event	72 ± 30
AMI	70 ± 29
PCI	68 ± 29
CABG	100 ± 18
MI + PCI	69 ± 30
Medication use (%)	
ACE inhibitors	85
Antiplatelet agents	95
β-adrenergic antagonists	95
Statins	90
Aspirin	100

3.1.4.1. **In-exercise characteristics**

Table 2 displays the group mean time per monitored session spent above HRR thresholds from 40-80%. The average %HRR achieved during the three monitored exercise sessions was unchanged (START 42 ± 16 %, MID 48 ± 17 % and END 47 ± 17 %; p=0.16), whilst only time spent above 55% HRR differed between the 3 sessions (p=0.02). Time spent above 55% HRR differed between the START (6.7 ± 9.3 min) and END (9.5 ± 10.7 min; p=0.02) but not the START and MID (9.7 ± 10.5 min: p=0.09) or MID and END (p=0.87) of the programme. From the START to the END of the programme, the average increase in time spent above 55% HRR was 3.1 ± 12.7 min (range = - 21 – 24 min), with 22% of participants achieving an increase of at least 5 min. There were no interactions with sex, age or pathology (p>0.05). Frequencies of participants accumulating at least 8 and 12 min above different intensities in each exercise session are displayed in Table 3, whilst the variability in heart rates achieved by participants in the START and MID sessions are shown in Figure 7 & Figure 8.

Table 2: Mean time spent above heart rate reserve thresholds during each measured session

HRR threshold	Time spent above HRR threshold (min) START (n=44)	MID (n=40)	END (n=39)
40%	19.2 ± 15.9	23.1 ± 14.4	23.5 ± 15.0
50%	11.2 ± 13.8	15.1 ± 13.6	15.0 ± 14.9
55%	8.4 ± 12.7	11.4 ± 13.2	12.0 ± 14.2
60%	6.4 ± 11.4	8.8 ± 12.2	9.6 ± 13.2
65%	5.0 ± 10.3	6.6 ± 10.4	7.7 ± 12.0
70%	3.8 ± 8.8	4.5 ± 8.2	5.5 ± 9.9
80%	1.7 ± 5.4	1.8 ± 4.4	2.7 ± 6.5

Table 3: Number of participants accumulating 8 & 12 minutes above each HRR threshold in each measured session

HRR threshold	START n=44		MID n=40		END n=39	
	8 min	12 min	8 min	12 min	8 min	12 min
40%	29	24	31	29	28	27
50%	17	16	24	21	20	17
55%	13	11	15	13	17	14
60%	11	9	11	9	14	11
65%	8	5	10	9	10	8
70%	4	4	8	6	6	5
80%	4	3	4	3	3	3

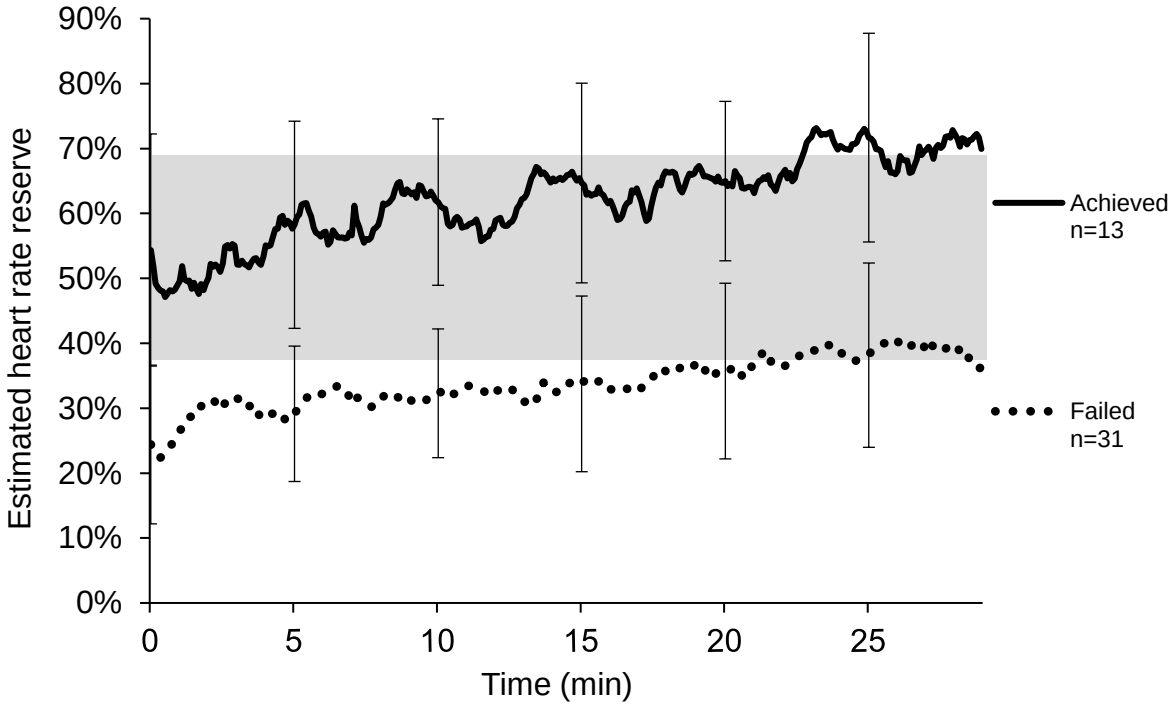

Figure 7: Mean ± SD heart rate traces throughout the START session grouped for those patients who achieved > 8 minutes above 55% HRR and those who failed to achieve this. The shaded area represents the target heart rate zone.

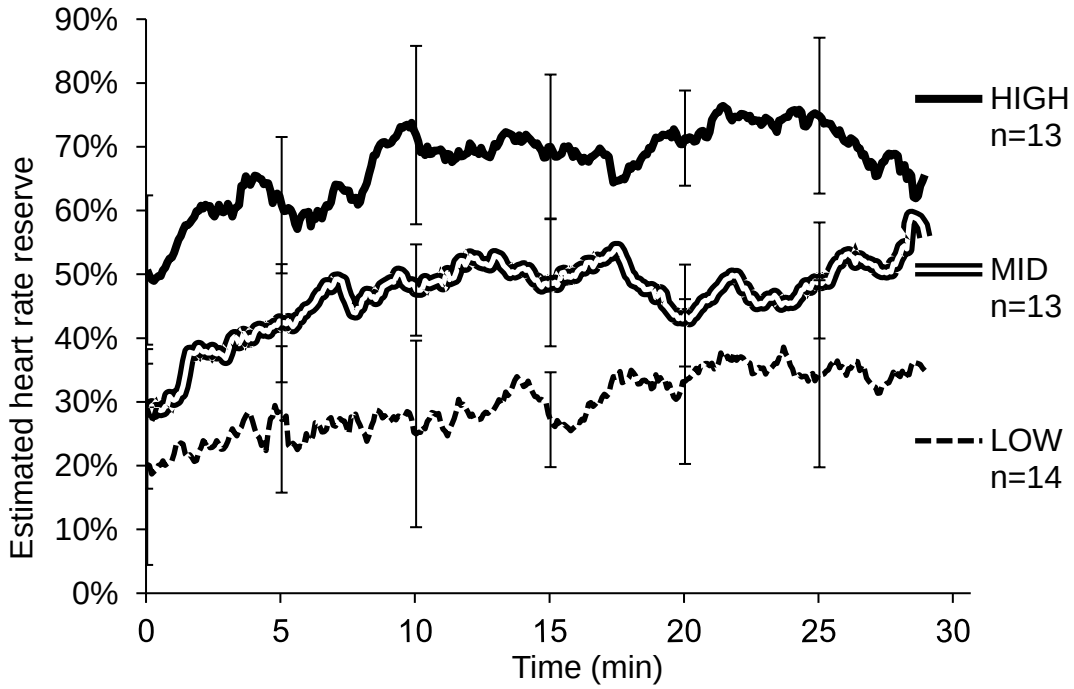

Figure 8: The variability in the %HRR attainment during the MID session. Data are mean ± SD HR divided into tertiles of the mean session HR.

3.1.4.2. **The impact of six weeks of CR**

The impact of six weeks of CR upon performance and physiological measures can be seen in Table 4. No changes were observed in assessments of habitual physical activity or vascular integrity following CR ($p>0.05$); only systolic blood pressure and parameters related to ISWT performance changed post CR. Systolic blood pressure decreased by an average of - 5 ± 13 mmHg ($p = 0.03$), with no interaction of pathology, gender or age ($p > 0.05$). Peak HR and the distance and speed achieved during the ISWT increased following the CR programme ($p < 0.001$). Changes in walk speed and distance were correlated with the increase in peak HR achieved during the test ($\rho = 0.56$; $p < 0.001$ and $\rho = 0.54$; $p < 0.001$ respectively), but not changes in habitual physical activity or heart rates achieved in exercise sessions ($p > 0.05$).

A number of baseline characteristics were correlated with the change in ISWT distance which were: age ($\rho = - 0.51$; $p > 0.001$), SBP ($\rho = - 0.42$; $p > 0.01$), % of daily time spent doing vigorous ($r = 0.41$; $p = 0.01$) or MVPA ($r = 0.40$; $p = 0.017$) and time since cardiac event ($r = - 0.38$; $p = 0.01$). The change in SBP was unrelated to changes in or baseline values of habitual physical activity, vascular integrity, exercise heart rates, body weight, pathology or gender ($p > 0.05$), but was correlated with baseline SBP ($r = - 0.39$; $p = 0.01$).

Table 4: Cardiovascular risk factors, ISWT performance, physical activity, assessments of vascular integrity PRE and POST cardiac rehabilitation.

	n	PRE	POST	*p*-value
Cardiovascular risk factors				
Weight (kg)	47	82.4 ± 15.5	81.8 ± 15.5	0.12
Systolic blood pressure (mmHg)	46	129 ± 20	124 ± 20	0.01*
Diastolic blood pressure (mmHg)	46	71 ± 10	71 ± 9	0.67
Resting heart rate (beats · min^{-1})	46	64 ± 9	64 ± 10	0.49
ISWT performance				
Distance (m)	47	439 ± 116	632 ± 213	< 0.001*
Speed (ms^{-1})	47	1.5 ± 0.2	1.8 ± 0.3	< 0.001*
Peak heart rate (bpm)	43	101 ± 11	116 ± 17	< 0.001*
Daily physical activity				
Steps	29	6337 ± 2943	6539 ± 3866	0.81
Sedentary activity (%)	29	55 ± 10	54 ± 10	0.66
Light activity (%)	29	39 ± 14	37 ± 9	0.59
Moderate activity (%)	29	6.1 ± 2.2	6.5 ± 3.6	0.72
Vigorous activity (%)	29	1.7 ± 1.9	1.9 ± 2.0	0.36
Sedentary activity (min)	29	483 ± 110	460 ± 87	0.16
Light activity (min)	29	326 ± 82	319 ± 94	0.21
Moderate activity (min)	29	53 ± 21	57 ± 36	0.97
Vigorous activity (min)	29	15 ± 17	17 ± 18	0.17
MVPA (mins)	29	68 ± 31	74 ± 46	0.73
MVPA bout number	29	0.8 ± 0.9	0.8 ± 0.9	0.19
Time MVPA bouts (min)	29	28 ± 26	33 ± 35	0.21
Time in Sedentary bouts (min)	29	104 ± 77	100 ± 70	0.62
Vascular integrity				
Brachial arterial diameter (mm)	17	4.13 ± 0.88	4.23 ± 0.84	0.69
Flow-mediated dilatation (mm)	17	0.22 ± 0.21	0.14 ± 0.23	0.96
Flow-mediated dilatation (%)	16	6.8 ± 1.0	4.4 ± 4.7	0.12
Shear stress AUC_{60} (a.u.)	17	3583 ± 1548	3305 ± 1165	0.40
Carotid intima-media thickness (mm)	13	0.74 ± 0.13	0.68 ± 0.17	0.10
Carotid artery compliance (mm·mmHg·10^{-3})	13	9.0 ± 3.9	9.6 ± 3.9	0.51

ISWT: incremental shuttle walk test; MVPA: moderate to vigorous physical activity; AUC_{60}: area under curve for 60 seconds. *denotes a significant difference at $p < 0.05$

3.1.5. **Discussion**

These data demonstrate that the dose of exercise achieved by patients in an UK outpatient phase III cardiac rehabilitation is highly variable and participants predominantly exercised at the lower end of the prescribed 40-70% HRR range. The CR programme did not induce an overall change in prognostic factors such as markers of vascular integrity, body weight or habitual physical activity. Combined these findings allude to the possibility that patients accumulate an exercise dose which is insufficient to drive improvements in health. Considering that many rehabilitation programmes are resource-limited, the only element of exercise dose that can be manipulated without additional expenditure is intensity. These data suggest there is much room for optimisation of the delivery of exercise in UK CR and does not support that the current format of UK CR is fit for purpose.

In UK CR, it is currently recommended that, in the absence of significant co-morbidities such as heart failure or orthopaedic impairments, CR patients should exercise within the exercise intensity range of 40-70% of HRR (ACPICR, 2015). In the present cohort, patients were asked to achieve this HRR target during a circuit training style programme. When given this prescription, the data suggests that the majority of patients spend most of the time exercising around the lower end of this prescribed intensity range and in many cases below the target intensity. Participants in the study generally exercised at intensities of less than 55% of HRR for the majority of the observed sessions (Table 3). Whilst only ~30% of participants accumulated 8 minutes above 40% HRR (~ a third of session duration) in the

START session (Figure 7), 8% of patients failed to accumulate any time above this threshold in the three monitored sessions. Mean HR per session was on average < 55% HRR and did not progress across the programme. Exercise has a dose-response relationship with CRF (Huang, G. et al., 2016) which is the strongest predictor of mortality in patients following CR (Kavanagh, T. et al., 2002; Kavanagh, Terence et al., 2003; Keteyian et al., 2008). In exercise training programmes where the volume of exercise (frequency and duration) performed is fixed (such as the one described in this study), the only method of modifying the dose of exercise received is by varying the intensity of exercise that is performed. Although mean training intensity influences responses of CRF to CR (Savage et al., 2009), the pattern of exercise and amount of time spent at higher intensities can have independent effects upon CRF (O'Donovan et al., 2005; Kemi et al., 2005; Huang, G. et al., 2016; Warburton et al., 2005). In the present study, there was no association with mean exercise intensity or time spent at higher intensities and changes in measures of vascular integrity, habitual physical activity, ISWT distance or speed. This is in part attributable to the small number of our sample that achieved higher training intensities and the low overall volume of exercise achieved relative to non-UK sample populations (Sandercock, G. et al., 2013).

Despite finding clear improvements in ISWT performance, which is a proxy test for exercise capacity, we found no discernible changes in any physiological variables following participation in CR other than systolic blood pressure. The mean walk distance increased by 207 ± 12 m which is well in

excess of the minimally clinically meaningful difference of 70 m reported by Houchen-Wolloff et al. (2015). A familiarisation effect likely partially contributes to this increase as participants did not undergo a practice test (Hakamy et al., 2017), however the strongest correlate of changes in walk test distance was change in peak heart rate during the test. Peak HR achieved during the ISWT increased concomitantly with ISWT distance by 20 ± 13 bpm which by far exceeds the ~ 10 bpm increase in chronotropic response to cardiopulmonary exercise testing following other exercise programmes in CAD patients (Conraads, V. M. et al., 2015; Vanhees et al., 1995). This, together with the substantial increase in mean walk distance and the moderate correlation between changes in peak HR and ISWT distance, implies that the changes in walk distance observed were unlikely to represent simply a physiological adaptation to exercise training. Instead, participants may have been more motivated to exert themselves more in the follow-up test, masking training responses to the CR programme. As such, the validity of the changes in ISWT performance observed as a marker of changes in exercise capacity is questionable. In similar CR programmes in the UK where patients undertook a median of 15 sessions at 40-70% of HRR, no mean change in CRF or peak HR was observed from cardiopulmonary exercise testing but an increase in exercise test duration and peak power were observed (Nichols, S. et al., 2018). As there was an absence of changes in peak HR in the study of Nichols, S. et al. (2018), it could be argued that the changes in ISWT in the present study may not reflect changes in CRF.

Physical activity has a dose-response relationship with CVD risk (Sattelmair et al., 2011).Therefore, modifying habitual physical activity in CR is essential as both a behavioural outcome that will contribute to secondary CVD prevention and as a means of driving health gains. Indices of PA were unchanged by the CR programme in the present study. A recent systematic review and meta-analysis of the effect of CR on physical activity found PA significantly increased with CR participation in only 38/145 trials assessed (Dibben et al., 2018). All but one of the participants in the present study attained the minimum recommended guidelines of 150 minutes of MVPA per week at baseline. Whilst a risk of self-selection bias in the sample choosing to take part in CR generally and in this study specifically is possible, a large proportion (> 90%) of patients attending CR (Pattyn et al., 2016) and the general population (> 80%) achieve this guideline (Lear et al., 2017). As such, greater volumes of PA than the current recommendations are likely to be needed for PA-mediated improvements in health. Furthermore, Ayabe et al. (2004) have previously demonstrated that whilst PA on training days increases during CR, PA levels on days without structured exercise sessions are unchanged. That CR programmes appear ineffective for improving habitual PA highlights the need for targeted PA interventions in CR to provide effective secondary CVD prevention, particularly as large volumes of PA are needed to induce beneficial changes in CVD risk markers (Hambrecht et al., 1993). The adopted cut-points for physical activity intensity thresholds in the present study were derived from data in a CR cohort (Prince et al., 2015) because post-MI (or CR) patients have greater

metabolic costs during walking activity (Buckley et al., 2016). These cut-points were derived from a less heterogeneous population and therefore their application may not have been adequately representative of the true exercise intensities all participants.

A combination of a possibly low exercise dose achieved by most patients - due to both low exercise intensity and volume - and lack of changes in habitual physical activity may explain the inability of this CR programme to induce healthful physiological responses. In this study, other than ISWT parameters, the only CVD risk factor that changed following CR was SBP.

Although SBP was inversely related to FMD at baseline – in agreement with previous literature (Benjamin et al., 2004) - FMD was unchanged following CR. There are several potential explanations behind this. First, despite observing a reduction in SBP with CR, FMD may be more resistant to change following exercise training – in part due to the inherently greater measurement error/variability involved in this technique. Second, the mechanism by which SBP changed with CR may diverge from those that relate SBP and FMD. For example, alterations in autonomic regulation of blood pressure via endothelium-independent mechanisms may not concurrently affect endothelial function and blood pressure. One potential mechanism is an alteration in the sensitivity or the baroreceptor reflex which is known to be depressed in individuals with CAD (Nasr et al., 2005) or hypertension (Bristow et al., 1969) and has been shown to improve following exercise training in these individuals (Laterza et al., 2007). Whilst there exists an inverse relation between systolic blood pressure and endothelial

function, it is unclear whether hypertension may cause (Rossi et al 2007) or be caused by endothelial dysfunction (Shimbo et al 2010). As systolic blood pressure has intra-individual between subject variability of 10-12 mmHg (Kronish et al., 2016; Muntner et al., 2011), it is also possible that with our small sample size a mean reduction of 5 mmHg may be a spurious finding or due to other factors such as better adherence to antihypertensive medication prescription. Another CVD risk factor that was unaffected by the CR programme was body weight though this may be unlikely to change across the course of a 6-week exercise and diet programme and does not typically occur in post-MI CR patients (Lawler et al., 2011), particularly in non-obese individuals and in the absence of changes in physical activity.

A comparatively higher exercise dose study by Cornelissen, Véronique A et al. (2014) demonstrates that a CR programme of 12 weeks of 3 exercise sessions per week performed continuously at a prescribed intensity of 60% HRR - progressing to a median intensity of 80% HRR in the final week- can increase FMD by 3.1% (+37%) and peak oxygen uptake by 3.1 ml/min/kg (+22%). As an increase in FMD of 1% decreases future risk of CV events by 13% (Inaba et al., 2010) and an increase of 1 ml/min/kg associated with a ~15% decrease in all-cause mortality in patients with CAD (Keteyian et al., 2008), the improvements in these biomarkers found by Cornelissen et al. (2014) exemplify the remarkable potential health gains that can be achieved in CR programmes with higher doses of exercise.

Current attempts to answer the question of why patients in the UK specifically may not be receiving benefits from CR have explored the factors

affecting the potency of CR at a programme level (i.e. patient uptake, time since cardiac event, adherence to guidelines and provision of additional services) (Dalal et al., 2015; Sumner, J. et al., 2017). However, a major issue with the evaluation of exercise-based CR is the lack of available data regarding the achieved – rather than simply prescribed – dose of exercise (Powell et al., 2018). The largest randomised control trial in modern CR was performed in the UK using identical exercise prescription guidelines to the present study and failed to show reductions in mortality (West et al., 2012), which was attributed – in part - to an “underdosage” of exercise (Conraads, Viviane M et al., 2012). The data in the present study demonstrates that adherence to an exercise prescription identical to that used in the RAMIT is poor and results in a highly variable but generally low dose of exercise as is clear in Figure 8. Furthermore, this dose does not appear adequate to improve vascular health or habitual physical activity, which are key factors in secondary CVD prevention. Thus, in combination with the low total exercise volume found in UK CR relative to other European and North American cohorts (Sandercock, G. et al., 2013), the data supports the notion that UK CR may not provide a sufficient exercise dose to improve long-term health. Additionally, the lack of fidelity to the exercise prescription in this study calls into question the potency of the – already considerably variable (Anderson, L. et al., 2016) – exercise prescriptions across CR trials. Future randomized control trials concerning the efficacy of cardiac rehabilitation should report the exercise intensities achieved by patients and explore the feasibility of more intense exercise intensity prescriptions and provide more robust

evidence for the impact that total exercise dose achieved in CR has on patients' long term outcomes.

3.1.6. **Conclusion**

The present study has characterised the exercise perform by patients in a community-based CR programme in the UK. The majority of patients spend most of their exercise sessions at exercise intensities of ~40% of HRR and which progresses little throughout the programme. All patients achieved the recommended PA levels of 150 minutes of MVPA per week and PA was unchanged following CR. There was an absence of training effects on measures of endothelial function and arterial stiffness.

3.2. **Part 2**

3.2.1. **Introduction**

Following this study, it was clear that the exercise dose received by some patients was likely to be low. Consequently, there was a considerable opportunity to enhance exercise dose within exercise sessions. Therefore a subsequent study was undertaken with the objective of increasing the intensity of exercise performed as part of usual practice in CR via a service-level intervention and repeating the outcome measures of the previous study to observe whether that simple modification of the CR programme would be sufficient to induce a change in patient outcomes. The strengths of this approach are the generalisability of the study in the context of at least UK CR, a lack of cost for enhancement of the therapy and the opportunity to investigate whether a 6-week CR programme can be effective when better optimised. I hypothesised that the intervention would be effective at increasing the dose of exercise achieved and that this would confer improvements in FMD, SBP, ISWT performance and indices of habitual physical activity.

3.2.2. **Methods**

The methods of data collection for this study were identical to the previous one including the techniques used and experimental protocol. The cohort from the previous study will now be referred to as the “original” cohort and the cohort recruited for the present study as the “improved practice” cohort.

Between the two studies, the results of the first study was presented to CR staff including all exercise instructors, nurses and healthcare assistants who are present during CR sessions. Particular attention was paid to the low exercise intensities being achieved by many patients and the potential importance that exercise intensity has regarding the outcomes of patients following CR. Further discussions with staff regarding how practice may be improved with respect to the exercise intensities achieved by patients highlighted the frequent lack of awareness and attention of staff and patients to heart rate targets during exercise. This may have resulted in patients largely adopting volitional exercise intensities during CR sessions which could have led to inadequate exercise intensities being achieved by many individuals. A proposed (non-evidence based) solution to this inattention was to provide patients with target heart rate zones on a name badge which they wore during CR sessions and make reference to these numbers during exercise sessions. This would give patients and CR staff an immediately available and easily accessible reference to ensure that an adequate exercise intensity was being achieved (if not strived for). Additionally visual cues were also provided at exercise stations to encourage patients to monitor their heart rate and respond by altering their work rate if it was outside of the target range. A training session was provided for CR staff to emphasise how to increase patient exercise intensities with the targets of keeping heart rates above 40% HRR at all times and increasing heart rate to > 55% HRR during exercise stations with a primarily “aerobic” focus (e.g. brisk walking, stationary cycling, rowing machine). These targets were

chosen, firstly, to comply with the BACPR guidelines (ACPICR, 2015) and secondly to be realistic and practicable (as the halfway point of the HRR target zone was immediately calculable from visual cues). Since most patients did not achieve even 8 minutes exercising above 40% HRR in the original cohort, achievement of this target would appear to infer a substantive change in practice.

3.2.3. **Statistical analysis**

All statistical analyses to assess whether HRs were different between exercise sessions and whether variables changed pre to post CR in this "improved practice" cohort were performed in an identical manner to the previous study (see Section 3.1.3) including: cardiovascular risk factors (blood pressure, weight and resting heart rate), measures of ISWT performance, parameters of daily PA and measures of vascular integrity. To assess for differences in mean HRR achieved and time spent above HRR thresholds ranging from 40-80% during the measured sessions, comparisons were made between the original and improved practice cohorts via repeated measures ANOVA with time as the repeated measure (START, MID and END) and study cohort included as a fixed factor.

Comparisons of the effect of the CR programme upon each of the remaining variables between the two cohorts were made with repeated measures ANCOVA with time (PRE-POST) as the repeated measure factor, age and time since event as covariates and the pathology, co-morbidities and study

cohort as fixed factors. Comparisons of the magnitude of change (Δ) of a variable between cohorts were made if a significant study cohort by time interaction was observed. These were carried out as an ANCOVA of the change in said variable PRE-POST with its baseline values included as a covariate and study cohort as a fixed factor.

3.2.4. **Results**

Participant characteristics are displayed in Table 5 below.

Table 5: Participant characteristics at baseline

Baseline characteristics (n = 12)	
Sex (% Male)	83
Age (years)	68 ± 12
Height (m)	1.73 ± 0.13
Weight (kg)	78 ± 14
Systolic Blood Pressure (mmHg)	126 ± 23
Diastolic Blood Pressure (mmHg)	66 ± 7
Diabetic (%)	17
Cardiac event (%)	
AMI	83
CABG	50
PCI	33
MI + PCI	25
Days since cardiac event	
AMI	94 ± 25
PCI	95 ± 45
CABG	106 ± 23
MI + PCI	74 ± 17
Medication use (%)	
ACE inhibitors	83
Antiplatelet agents	92
β-adrenergic antagonists	83
Statins	83
Aspirin	100

Table 6: Time spent exercising above estimated heart rate reserves thresholds from 40-80% in the START, MID and END sessions

HRR threshold	Time spent above HRR threshold (min) START (n=12)	MID (n=9)	END (n=7)
40%	13.9 ± 11.2	21.0 ± 9.4	22.0 ± 7.8*†
50%	8.2 ± 9.1	14.5 ± 11.1	15.4 ± 9.9†
55%	6.2 ± 8.5	11.6 ± 11.1	10.8 ± 9.8*†
60%	4.4 ± 7.7	8.4 ± 10.6	7.0 ± 8.4
65%	3.1 ± 6.9	6.1 ± 10.4	4.3 ± 6.7
70%	2.2 ± 5.4	4.8 ± 8.9	3.0 ± 6.7
80%	1.0 ± 3.2	2.1 ± 4.2	0.6 ± 1.7

Data are mean ± SD. * $p < 0.05$ vs START, † $p < 0.05$ vs MID

Table 7: Number of participants accumulating 8 & 12 minutes above each HRR threshold in each measured session

HRR threshold	START n=12		MID n=9		END n=7	
	8 min	12 min	8 min	12 min	8 min	12 min
40%	8	7	7	7	6	6
50%	6	3	7	6	5	5
55%	3	3	4	3	4	2
60%	2	2	3	3	2	2
65%	2	1	2	2	2	1
70%	1	1	2	2	1	1
80%	1	0	2	0	0	0

Data are mean ± SD.

The mean HRR achieved during the exercise sessions were not different between the START (41 ± 17%), MID (51 ± 16%) and END (50 ± 12%) sessions (p=0.06). Between the START, MID and END session, there were differences in the time spent above individual HRR thresholds of 40% (p=0.015), 50% (p=0.022) and 55% (p=0.022) with generally greater periods above these being achieved as the programme progressed (see Table 6 above). No differences were observed for time spent above higher HRR thresholds (p>0.05). The frequencies of participants accumulating at least 8 or 12 minutes exercising above HRR thresholds of 40-80% are displayed in Table 7 above. During the START and MID sessions less than half of participants achieved at least 8 minutes above 55% of HRR. There was no main effect of cohorts on the mean HRR achieved (see Figure 9) or time spent above any HRR threshold during START, MID or END sessions (all p > 0.05).

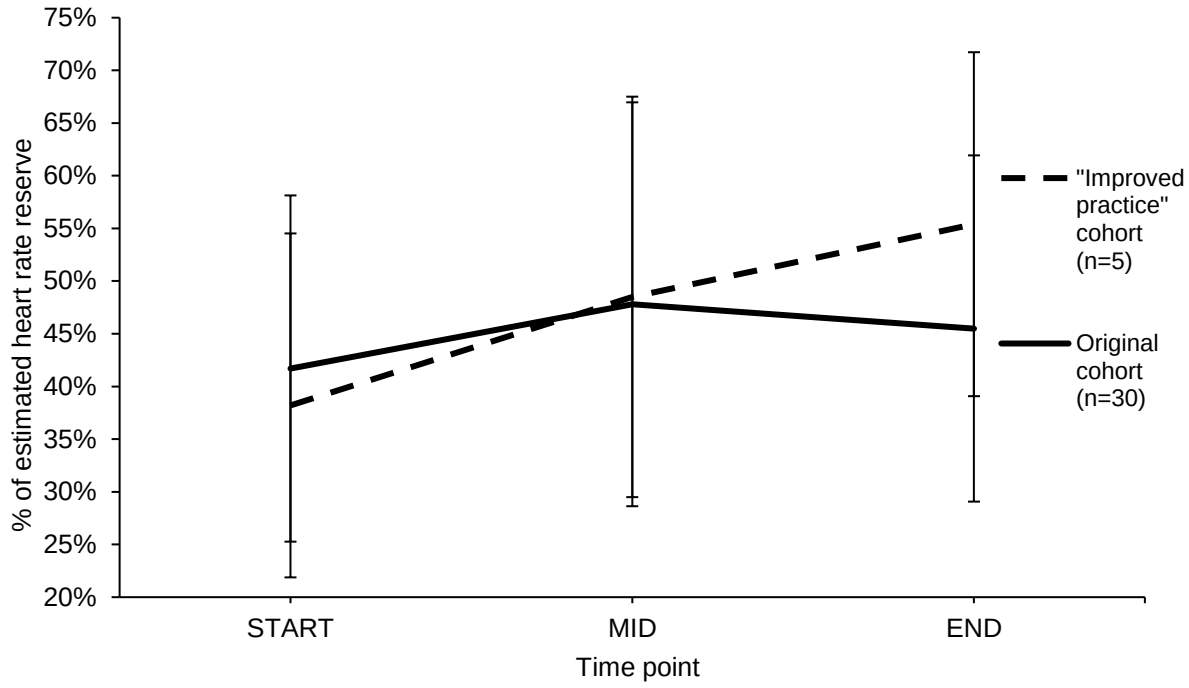

Figure 9: A comparison of estimated heart rate reserve achieved during each measured session. Data are mean ± SD. There was no main effect for time in the heart rates achieved across sessions ($p > 0.05$) and no time by cohort interaction between each cohort ($p > 0.05$).

Table 8: A comparison of PRE-POST cardiac rehabilitation measures of cardiovascular risk factors between the original and improved practice cohort

	Original cohort				"Improved practice" cohort				Pooled cohort	
Cardiovascular risk factors	n	PRE	POST	p-value (time)	n	PRE	POST	p-value (time)	p-value (time pooled)	p-value (time*cohort)
Weight (kg)	47	82.4 ± 15.5	81.8 ± 15.5	0.12	10	76.6 ± 14.2	76.1 ± 14.1	0.56	0.82	0.90
Systolic blood pressure (mmHg)	46	129 ± 20	124 ± 20	0.01*	12	126 ± 23	133 ± 21	0.14	0.29	0.02†
Diastolic blood pressure (mmHg)	46	71 ± 10	71 ± 9	0.67	12	66 ± 7	69 ± 9	0.36	0.98	0.93
Resting heart rate (beats · min^{-1})	46	64 ± 9	64 ± 10	0.49	12	63 ± 10	64 ± 10	0.49	0.41	0.45

Data are mean ± SD. *denotes a significant main effect for time $p < 0.05$. †denotes a significant time by cohort interaction $p < 0.05$

Table 9: A comparison of PRE-POST cardiac rehabilitation measures of incremental shuttle walk test performance between the original and improved practice cohort

	Original cohort				“Improved practice” cohort				
ISWT performance	n	PRE	POST	p-value (time)	n	PRE	POST	p-value (time)	p-value (time pooled)	p-value (time*cohort)
Distance (m)	47	439 ± 116	632 ± 213	< 0.001*	12	501 ± 214	638 ± 243	< 0.001*	< 0.001*	0.97
Speed (ms^{-1})	47	1.5 ± 0.2	1.8 ± 0.3	< 0.001*	12	1.6 ± 0.4	1.9 ± 0.4	< 0.01*	< 0.001*	0.31
Peak heart rate (bpm)	43	101 ± 11	116 ± 17	< 0.001*	10	104 ± 18	117 ± 26	0.64	< 0.001*	0.80

Data are mean ± SD. *denotes a significant main effect for time p < 0.01

Table 10: A comparison of PRE-POST cardiac rehabilitation measures of parameters of daily physical activity between the original and improved practice cohort

Daily physical activity	Original cohort				"Improved practice" cohort					
	n	PRE	POST	p-value (time)	n	PRE	POST	p-value (time)	P value (time-overall)	p-value (time*cohort)
Steps	29	6337 ± 2943	6539 ± 3866	0.81	10	6548 ± 2957	6686 ± 3753	0.84	0.97	0.92
Sedentary activity (%)	29	55 ± 10	54 ± 10	0.66	10	65 ± 7	65 ± 8	0.76	0.52	0.73
Light activity (%)	29	39 ± 14	37 ± 9	0.59	10	24 ± 5	24 ± 5	0.59	0.92	0.63
Moderate activity (%)	29	6.1 ± 2.2	6.5 ± 3.6	0.72	10	7.9 ± 2.7	8.4 ± 3.1	0.27	0.75	0.55
Vigorous activity (%)	29	1.7 ± 1.9	1.9 ± 2.0	0.36	10	3.2 ± 2.3	3.0 ± 2.7	0.79	0.27	0.35
Sedentary activity (min)	29	483 ± 110	460 ± 870	0.16	10	568 ± 80	559 ± 790	0.39	0.93	0.60
Light activity (min)	29	326 ± 826	319 ± 94	0.21	10	209 ± 476	207 ± 39	0.88	0.64	0.76
Moderate activity (min)	29	53 ± 21	57 ± 36	0.97	10	68 ± 21	72 ± 24	0.41	0.09	0.27
Vigorous activity (min)	29	15 ± 17	17 ± 18	0.17	10	27 ± 20	26 ± 23	0.81	0.18	0.38
MVPA (mins)	29	68 ± 31	74 ± 46	0.73	10	95 ± 36	98 ± 38	0.78	0.72	0.82
MVPA bout number	29	0.8 ± 0.9	0.8 ± 0.9	0.19	10	1.4 ± 1.1	1.7 ± 1.7	0.8	0.56	0.79
Time MVPA bouts (min)	29	28 ± 26	33 ± 35	0.21	10	24 ± 18	26 ± 23	0.56	0.29	0.80
Sedentary bouts number	29	1.2 ± 0.8	1.3 ± 1.0	0.47	10	1.8 ± 1.4	1.5 ± 1.1	0.61	0.17	0.20
Time in Sedentary bouts (min)	29	104 ± 77	100 ± 70	0.62	10	144 ± 112	116 ± 90	0.6	0.21	0.11
Sedentary breaks number	29	0.7 ± 0.8	0.7 ± 0.6	0.47	10	1.1 ± 0.8	0.8 ± 1.0	0.11	0.29	0.17
Time in Sedentary breaks (min)	29	104 ± 134	105 ± 100	0.94	10	137 ± 103	87 ± 107	0.16	0.60	0.16

Data are mean ± SD

Table 11: A comparison of PRE-POST cardiac rehabilitation measures of assessments of vascular integrity between the original and improved practice cohort

Vascular integrity	Original cohort				Improved practice cohort					
	n	PRE	POST	p-value (time)	n	PRE	POST	p-value (time)	p-value (time-overall)	p-value (time*cohort)
Brachial arterial diameter (mm)	17	4.13 ± 0.88	4.23 ± 0.84	0.69	9	4.07 ± 0.3	4.00 ± 0.2	0.26	0.78	0.23
Flow-mediated dilatation (%)	16	6.8 ± 1.0	4.4 ± 4.7	0.12	9	7.5 ± 5.7	6.2 ± 4.6	0.57	0.60	0.84
Flow-mediated dilatation (mm)	16	0.22 ± 0.21	0.14 ± 0.23	0.96	9	0.31 ± 0.25	0.25 ± 0.19	0.20	0.50	0.86
Shear stress AUC60 (a.u.)	17	3583 ± 1548	3305 ± 1165	0.4	9	2192 ± 1270	2992 ± 2862	0.39	0.68	0.18
Shear stress AUC90 (a.u.)	17	5338 ± 2269	4962 ± 1746	0.44	9	3442 ± 1811	4701 ± 4665	0.39	0.68	0.18
Time to peak diameter (seconds)	17	59 ± 32	48 ± 30	0.61	9	49 ± 35	65 ± 35	0.07	0.39	0.02†
Peak shear	17	1781 ± 691	1643 ± 569	0.4	9	2335 ± 982	1916 ± 608	0.38	0.33	0.90
Peak hyperaemia	17	87 ± 23	84 ± 26	0.67	9	117 ± 44	96 ± 30	0.36	0.38	0.97
Carotid diameter (mm)	14	7.3 ± 1.0	7.3 ± 1.1	0.33	9	7.8 ± 0.7	7.8 ± 0.8	0.84	0.26	0.87
Carotid intima-media thickness (mm)	14	0.74 ± 0.13	0.68 ± 0.17	0.1	9	0.72 ± 0.09	0.76 ± 0.13	0.13	0.39	0.48
Carotid artery compliance (mm·mmHg·10-3)	14	9.0 ± 3.9	9.6 ± 3.9	0.51	9	8.7 ± 4.3	7.4 ± 2.0	0.25	0.32	0.26

Data are mean ± SD. †denotes a significant time by cohort interaction $p < 0.05$

Table 9 above shows that although significant effects for time were seen in the pooled cohort for ISWT walk distance, speed and increases in peak HR achieved (all $p < 0.001$) as seen in the original cohort, significant main effects for time were not seen in the improved practice cohort for SBP (see Table 8) and peak HR during the ISWT in Table 9 unlike the original cohort).

Significant time by cohort interactions were detected for SBP and time to peak diameter but no other PRE-POST variable was significant (Table 11). The change in SBP showed opposing directions of responses between cohorts with the “improved practice” cohort showing a non-significant increase ($p=0.14$) but the original cohort showing a decrease in SBP. The change in time to peak diameter also displayed opposing directions of change between cohorts (- 11 ± 35 vs 16 ± 49 seconds; original vs improved practice, $p = 0.03$). There was no interaction with time for age, time since cardiac event, pathology or co-morbidities upon changes in outcomes variables ($p > 0.05$) except for a time by age interaction ($p < 0.001$, $\eta^2 = 0.31$) and time by MI interaction negatively affecting changes in peak HR achieved during the ISWT ($p = 0.02$, $\eta^2 = 0.10$) as can be seen in Figure 10.

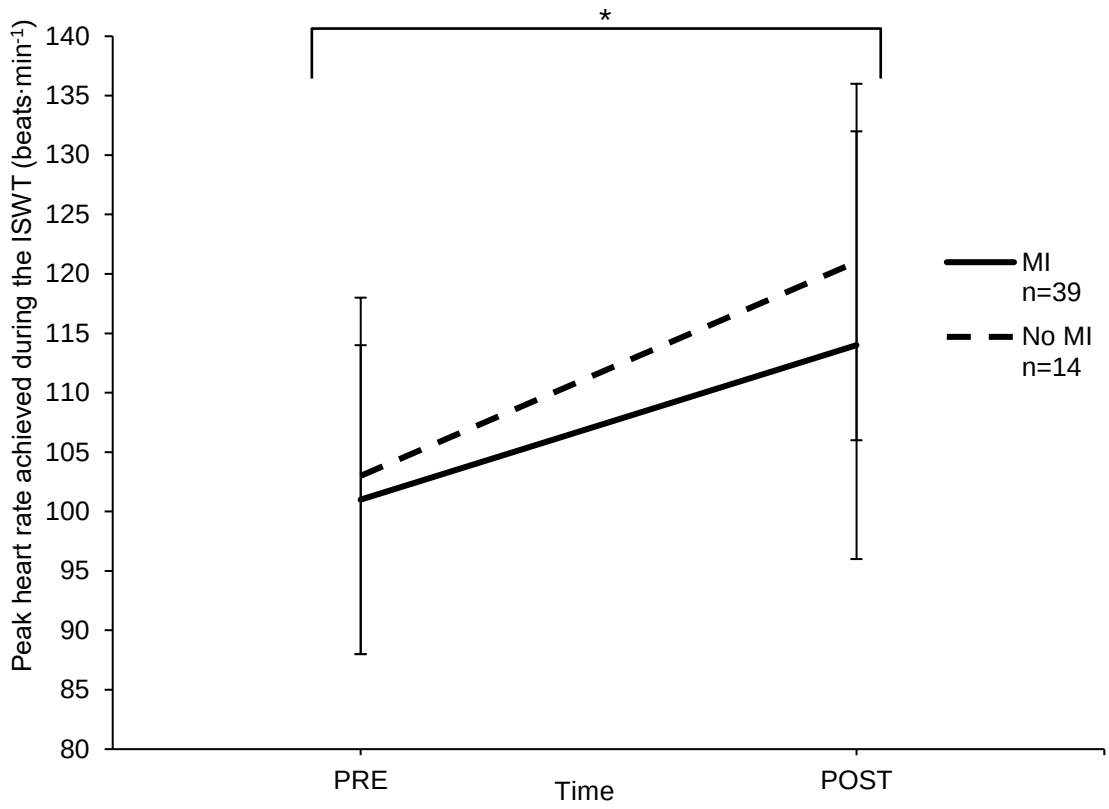

Figure 10: Peak heart rate achieved during the ISWT PRE to POST CR in participants who did and did not suffer a myocardial infarction. Data were adjusted for age and time since event and were evaluated at values of 63 years and 78 days respectively. *p < 0.05 for time by MI interaction.

3.3. **Part 3**

3.3.1. **Introduction**

Parts 1 and 2 of this chapter examined the effects of an outpatient CR programme in two asynchronous cohorts. In part 2, comparisons between the two cohorts revealed no clear indication that the interventions received by each cohort were different or had different effects on participant outcomes. Assuming this is true, further analysis with a larger sample consisting of pooled data from the two cohorts (n = 60) could be used to explore factors that influenced responses to the CR programme. I hypothesised that PRE-POST changes in SBP, FMD, ISWT performance, daily MVPA and daily sedentary time would be related to the intensity/dose of exercise achieved by patients in CR.

3.3.2. **Statistical analysis**

Multiple linear regression analysis was performed with the pooled data from both study cohorts to explore which factors were associated with PRE-POST changes (Δ) in: ISWT speed, systolic blood pressure, FMD, daily MVPA and daily sedentary time. The independent predictor variables included in each model are displayed in Table 12. The number of independent predictors included in each model was limited per 10 observations included of the dependent variable. The outcome variables (i.e. the change in each variable) were described in the model as increases along the regression slope. The

unstandardized β coefficients thus represent both the direction and magnitude of the relationship of each predictor variable. Effect sizes were interpreted as partial η^2 values of greater than 0.02, 0.13 and 0.26 representing small, medium and large effects (Cohen, 1988).

Table 12: Planned multiple regression models displaying dependent and independent variables included in each model

Dependent variable	Independent variables
Change in ISWT speed	Baseline ISWT speed
	Age
	Mean END %HRR
	Baseline daily MVPA
Change in systolic blood pressure	Baseline SBP (mmHg)
	Change in DBP (mmHg)
	Mean MID %HRR
Change in FMD %	Baseline FMD (%)
	Mean END %HRR
Change in daily MVPA	Baseline MVPA
	Mean END %HRR
	Change in ISWT speed
Change in daily sedentary time	Baseline daily sedentary time
	Mean END %HRR
	Change in ISWT speed

3.3.3. **Results**

Tables 13 to 17 display the association of a number of variables with changes in ISWT speed, SBP, FMD, daily MVPA and daily sedentary time, respectively. Multiple regression models that explained a significant proportion of the variance in outcome variables were found for changes in ISWT speed, SBP, FMD and daily sedentary time (all $p < 0.01$) but not for changes in daily MVPA ($p = 0.452$).

Independent predictors that significantly contributed to the regression models include: age and mean HRR achieved in the END session for changes in ISWT speed (Table 13); baseline SBP and change in DBP for changes in SBP (Table 14); baseline FMD and mean HRR achieved in the END session for changes in FMD (Table 15), and baseline sedentary time for changes in daily sedentary time (Table 17) (all $p < 0.05$). No other variables contributed significantly to the models ($p > 0.05$). The magnitudes and directions of these relationships are presented below:

- For every 10 year increase in age the improvements in walk speed were reduced by - 0.07 $m \cdot s^{-1}$ and for every 10% increase in the mean HRR achieved in the END session walk speed increased by 0.05 $m \cdot s^{-1}$ (Table 13).
- For every 1mmg increase in baseline SBP, the CR impact upon SBP was more negative by - 0.33 mmHg (Table 14).
- For every 1mmHg decrease in SBP, the change in DBP became more negative by 0.57 mmHg (Table 14).
- For every 10% increase in the mean HRR achieved in the END session, the change in FMD pre to post CR increased by 1.8 % (Table 15).

- For every 10 minute increase in baseline daily sedentary time, the change in daily sedentary time pre to post CR decreased by 3.8 minutes (Table 17).

Table 13: Linear regression model for change in ISWT speed. N = 41, Adjusted R^2 = 0.33 p = 0.002

Variable	Unstandardized β coefficient (SE)	Standardized β coefficient (95% confidence interval)	p	Partial η^2
Baseline ISWT speed ($m \cdot s^{-1}$)	- 0.131 (0.162)	-0.143 (-0.46 — 0.20)	.427	0.019
Age (years)	- 0.007 (0.003)	-0.395 (-0.01— -0.001)	.026	0.142
Mean END %HRR	0.504 (0.171)	0.423 (0.16 — 0.85)	.006	0.208
Baseline daily vigorous activity (10 min)	0.020 (0.020)	0.149 (-0.002 — 0.005)	.328	0.029

Table 14: Linear regression model for change in SBP. N = 47, Adjusted R^2 = 0.33 p < 0.001

Variable	Unstandardized β coefficient (SE)	Standardized β coefficient (95% confidence interval)	p	Partial η^2
Mean MID %HRR	- 18.8 (13.3)	-0.218	0.080	0.069
Baseline SBP (mmHg)	- 0.333 (0.092)	-0.440	0.001	0.235
Change in DBP (mmHg)	0.571 (0.002)	0.185	0.003	0.182

Table 15: Linear regression model of change in FMD. N = 20, Adjusted R^2 = 0.56 p < 0.001

Variable	Unstandardized β coefficient (SE)	Standardized β coefficient (95% confidence interval)	p	Partial η^2
Baseline FMD (%)	- 0.499 (0.149)	-0.218 (-12.9 — -1.5)	0.004	0.396
Mean END %HRR	17.919 (4.777)	0.185 (7.8 — 28.0)	0.002	0.453

Table 16: Linear regression model of change in daily MVPA. N = 33, Adjusted R^2 = 0 p = 0.452

Variable	Unstandardized β coefficient (SE)	Standardized β coefficient (95% confidence interval)	p	Partial η^2
Baseline MVPA (min)	0.209 (0.142)	0.266 (- 0.080 – 0.499)	0.150	0.259
Mean END %HRR	19.4 (31.7)	0.129 (- 45.3 – 84.2)	0.545	0.064
Change in ISWT speed ($m \cdot s^{-1}$)	- 8.51 (25.9)	- 0.069 (- 61.5 – 44.5)	0.745	0.006

Table 17: Linear regression model of change in daily sedentary time. N = 33, Adjusted R^2 = 0.27 p = 0.007

Variable	Unstandardized β coefficient (SE)	Standardized β coefficient (95% confidence interval)	p	Partial η^2
Baseline daily sedentary time (10 min)	- 3.84 (1.21)	-0.492 (- 0.631 – - 0.137)	0.003	0.259
Mean END %HRR	- 35.2 (85.5)	-0.074 (- 210 – -140)	0.683	0.064
Change in ISWT speed ($m \cdot s^{-1}$)	100 (70.9)	0.257 (- 44.9 – 245)	0.169	0.006

3.3.4. **Discussion**

Part 1 of this chapter showed that the chronic exercise stimulus presented by an outpatient CR programme was variable, likely to be low and had no effect upon parameters of vascular integrity or physical activity behaviours, but it did improve ISWT performance and systolic blood pressure. In part 2, a practicable intervention was employed to increase the dose of exercise achieved by patients through an increase in exercise intensity. It was hypothesised that a greater exercise dose might drive improvements in patients' outcomes. Despite this intervention, there was not a meaningful difference between the exercise intensities achieved by the two cohorts (Figure 9). As a result, the outcome of CR did not differ between cohorts except for SBP (Table 8) and time to peak diameter (Table 11).

There are a number of potential explanations for the lack of effect of the intervention upon patient exercise intensities. Firstly, it could be that despite the provision of a number of education sessions to CR staff and the semi-frequent presence of researchers at CR sessions, the intervention may have been less achievable than anticipated due to competing demands upon staff to preferentially supervise more limited patients who were not participating in the study. Additionally, it is challenging to increase the exercise intensities of all patients undertaking outpatient CR as there remained a notable degree of variability (i.e. little consistency) in the HRs achieved by patients in the improved practice cohort (Table 6 and Table 7). In contrast, many studies in non-UK cohorts report using higher intensity exercise prescriptions (Mitchell, B.L. et al., 2018), though evidence to support the adherence to these

prescriptions is lacking in the overwhelming majority of randomised control trials of exercise-based CR (Powell et al., 2018).

The inadequacy of the present intervention may highlight potential challenges in the implementation of higher exercise intensities intervention in the current format of UK circuit-based outpatient CR programmes. In light of this, the need to trial the implementation of higher intensity CR in the UK cannot be disregarded as the present results also support the case that UK CR may have negligible effects on patients' long-term outcomes (West et al., 2012; Anderson, L. et al., 2016).

Overall, the improved practice cohort was likely to have received a similar exercise stimulus from CR compared to the original cohort, allowing a pooled analysis to be undertaken. This analysis of pooled PRE-POST changes showed largely identical results to the analysis of the original cohort in part 1; two exceptions being that SBP was not reduced and peak HR achieved in the ISWT did not significantly increase. The reasons for these discrepancies are likely related to possibly extraneous observations in the improved practice cohort which opposed the general direction of changes: large increases in SBP (> 25 mmHg) in three individuals and reductions in peak HR achieved in the ISWT in two participants (- 8bpm and -18bpm). In both cases, there were no factors that clearly distinguished these particular individuals. Excluding these two factors, the conclusions and reasoning regarding the lack of effect of the CR programme are unchanged from Section 3.1.5 although including these factors would strengthen the argument of a lack of effectiveness of UK community-based CR.

The pooled analysis revealed an effect of MI and age on changes in peak HR achieved during the ISWT. A higher age was associated with a smaller increase in HR which is unsurprising as the total HRR decreases with age and so a smaller absolute increase in HR in an older individual would be expected for an equivalent %HRR increase in a younger individual.

Having an MI was associated with a smaller increase in the peak HR achieved during the ISWT. This may suggest that training adaptations could have been different in this subgroup. Indeed, all six of the participants who saw a reduction in their peak HR were MI patients. It is well documented that an MI can cause remodelling of the left ventricle (Sutton and Sharpe, 2000) and that with exercise training some of this remodelling can be reversed leading to reductions in end systolic volume (Haykowsky et al., 2011) which in theory should increase stroke volume. Thus, it is conceivable that the increases in cardiac output necessitated by the improvements in walk speed achieved could have been met partly by an increase in stroke volume in patients with an MI and not solely through increases in HR. Alternatively, the lesser improvement in peak HR in the patient sample who suffered an MI could be baroreflex sensitivity mediated (Schwartz, P.J. et al., 1988) or reflect a lesser capacity to improve chronotropic competence as a result of myocardial scar (Hammond and Froelicher, 1985).

The two cohorts responded differently to CR in SBP and time to peak artery diameter in the FMD analysis. In the case of SBP, 4 individuals (33%) from the improved practice cohort saw PRE-POST increases following CR of > 20 mmHg, whereas an increase in SBP of this magnitude was not seen in any

of the participants in the original cohort. This may explain the finding of no overall change in SBP following CR in the pooled cohort despite the observed decrease in SBP in the original cohort. These differences between cohorts could not be explained by changes in medication or other observable factors. This was an unexpected finding and may be attributed to erroneous measurements in the improved practice cohort or (more realistically) this finding is attributable to the large variability of manually assessed blood pressure measures in an outpatient setting of by non-research staff.

As for the difference in changes in time to reach peak artery diameter during FMD PRE to POST CR, whilst some evidence suggests that this could be mediated by changes in artery size (Thijssen et al., 2008), no factors could be discerned that explained this finding other than individual variability.

In part 3, further analysis was performed to explore factors that were associated with CR effects upon a number of outcome measures. The change in FMD following CR was negatively associated with baseline FMD, with higher baseline FMD values resulting in more negative changes. Conversely, participants with a low baseline FMD were more likely to see improvements in FMD. This finding may be explained by the current inadequacy in statistical methods within the research community to account for the effects of changes in baseline diameter upon FMD as there exists a significant negative relationship between changes in baseline diameter and changes in FMD which have been previously identified by Atkinson and Batterham (2013). Statistical methods for rectifying the effect of longitudinal

changes in baseline diameter upon the interpretation of changes in FMD are yet to be developed. This is further complicated by the time course of vascular adaptations to exercise training (if they occur in an individual) as reductions in FMD can occur in the early phases of vascular remodelling as artery diameter increases (Tinken et al., 2008). Otherwise, it may suggest that individuals with endothelial dysfunction may be more sensitive to exercise stimuli and that individuals with better endothelial function may not receive a sufficient stimulus to induce vascular adaptations. Another potential explanation is that, as there was no overall change in FMD, the effect of baseline FMD reflects a regression to the mean effect.

An association between the mean HRR achieved in the END session and changes in FMD was also observed with every 10% increase in HRR resulting in 1.8% higher change in FMD. This is not dissimilar to the finding of 1% increase in FMD for a 10% increase in relative exercise intensity in a meta-regression of the effects of exercise training upon endothelial function in a range of conditions (Ashor et al., 2015). Though the intensity in the MID and END sessions were generally similar, it is uncertain how the exercise intensity achieved in the END session reflects the overall intensity achieved across the programme, which likely determines the overall chronic exercise stimulus. The acute stimulus presented by exercise to the vascular endothelium is mediated by transient increases in shear stress (see Section 2.2.2) that should increase with exercise intensity, driving greater adaptations (Dawson et al., 2018). Additionally, should exercising at a higher

intensity be indicative of increases in CRF with exercise training, this may also contribute somewhat to increases in FMD (Montero, 2015).

The time course of adaptations of endothelial function may again be worth considering as an explanation for the relationship between mean HRR achieved in the END session and changes in FMD. In healthy subjects, Tinken et al. (2008) demonstrate that over the course of an 8-week exercise training programme increases in brachial artery FMD only manifested two and four weeks after the initiation of the programme but not after 6 weeks. It is unknown how the presence of CVD or the use of a number of medications in CVD patients influence the time course of endothelial adaptations to exercise. Saying this, if we are to assume endothelial adaptations follow a similar time course in this cohort, it may not be surprising that those who tended to exercise at higher intensities saw more positive changes in FMD by the 6th week of the programme. These participants had tended to exercise at lower intensities at the start of the programme and may have only achieved a sufficient exercise stimulus to change FMD after a number of sessions. Consequently, the increases in FMD seen in the few individuals who exercised at higher intensities during the END session within the present study may follow a similar time course.

Changes in ISWT speed were also positively related with the achievement of a higher HR in the END session though the effect was not large. An increase in intensity by 10% only led to a 0.05 $m \cdot s^{-1}$ additional increase in ISWT speed. Given this, a one stage additional increase in ISWT performance would require a ~ 30% increase in the mean END session HRR, which likely

constitutes a considerably greater challenge to patients that would require a more effective intervention to increase exercise intensity than was implemented in this study. To put this into terms of exercise capacity, a one stage increase in ISWT performance may represent an increase in exercise capacity of between 0.3-0.7 METs (Buckley et al., 2016).

In Section 3.1.5 doubts regarding the validity of the improvements in ISWT performance to reflect changes in exercise capacity were discussed. Larger increases were observed in walk distance and peak HR PRE to POST CR than typically reported. They are likely in part attributable to a familiarisation effect. In light of this, the influence of age and mean END session HRR upon changes in ISWT speed should not be affected by this bias.

Changes in SBP did not appear related to the exercise intensity achieved in the MID session. This contrasts with reviews that suggest there are exercise intensity-dependent effects upon reductions in SBP with exercise training (Cornelissen, Veronique A and Smart, 2013). Baseline SBP had a notable effect upon changes in SBP. Hypertensive individuals tend to see greater reductions in SBP following aerobic exercise training (Cornelissen, Veronique A and Smart, 2013), presumably because these individuals are more sensitive to the exercise training stimulus or possibly due to regression to the mean effects (Senn, 2003).

Whilst an overall significant regression equation was found that explained 27% of the variance in changes in sedentary time, the only factor that independently predicted changes in sedentary time was daily sedentary time at baseline, which showed a negative association. The most sedentary

individuals may therefore have the most to gain in terms of reducing sedentary behaviours. However, we saw no overall effect of the CR programme on several dimensions of sedentary behaviour. Recent studies also do not the support the efficacy of exercise based CR upon sedentary behaviours in very inactive patients (Biswas et al., 2018) and following a sedentary behaviour change intervention (Prince et al., 2018).

No factors examined were associated with changes in MVPA. These findings were in agreement with a recent meta-analysis and systematic review that showed exercise-based CR was not efficacious for changing indices of physical activity (Dibben et al., 2018). Such a lack of changes in physical activity behaviours calls into question the mechanisms by which cardiac rehabilitation will lead to sustained reductions in CVD risk and act as a tool for secondary prevention of CVD. Moreover, it is at present perplexing as to how to rectify this as physical activity education was provided by a qualified instructor as part of this CR programme. Indeed the current available evidence suggests that patient education surrounding the management of coronary heart disease has no effects upon all-cause mortality, cardiovascular mortality cardiovascular event rate or rehospitalisation rates (Anderson, L. et al., 2017).

The data presented in this chapter suggest that average exercise intensities of 73.6 % (95% confidence interval [71.6-80.0]) of HRR are likely needed to induce a 1% increase in FMD with CR. This represents a higher intensity than > 90% of our sample achieved suggesting that a dramatic change in

practice would be required to enable patients undertaking this 6-week CR programme to improve their endothelial function. An average intensity of this magnitude may be unachievable for many patients, however, performing exercise in an intermittent manner via interval training may provide a means of achieving the same total dose of exercise and providing the physiological stimuli associated with higher exercise intensities. The same argument can be made that higher intensities are likely required to confer sustained benefits to cardiorespiratory fitness. This is in agreement with the findings of Uddin et al. (2016) who show that only higher intensity CR programmes were effective in modifying CRF. Therefore, future research should examine the effects of a higher intensity training paradigm that will allow CR patients to accumulate a sufficient period of time at intensities of > 70% HRR and examine whether this can provide a potent, efficacious and cost-effective CR programme in a 6-week period.

Current evidence suggests that a CR programme that is greater than 12 weeks is more effective than one of less than 12 weeks (Sandercock, G. et al., 2013). In spite of this, the dearth of data relating to the fidelity and adherence to exercise prescriptions makes it difficult to ascertain whether shorter duration, higher intensity programmes could be as effective as longer duration, less intensive programmes, though all other things being equal and resources being unlimited then the longest feasible programmes would be preferable. An increase in session frequency to 3 sessions per week would likely be efficacious but widespread adoption of this prescription is likely limited by resources and local logistics. Should the previously discussed

higher intensity training paradigm for UK CR be unfeasible, unacceptable or ineffective in the current 6-week format then longer duration programmes should be explored.

3.3.5. **Limitations**

Whilst this study provides insight into the characteristics and effects of a practical, community-based CR programme, it is not without its limitations. Due to the nature of performing assessments of endothelial function at the remote venues, it was not possible to control the temperature, lighting or noise levels between assessments of vascular function, which violates a number of the guidelines for performing this technique set out by Thijssen et al. (2011). Additionally, medication use could not be prohibited prior to these assessments and therefore it is unclear what effects fluctuations in the administration of these medications may have had. The duration of the programme did not allow for us to control for menstrual cycle phase in female participants which has been shown to influence FMD values (Hashimoto et al., 1995; Williams, M.R. et al., 2001).

Another factor which could have potentially compromised our findings is the potential inaccuracies using of heart rate monitoring as a proxy for exercise intensity. A reasonable assumption is that the relationship between heart rate and intensity is linear in the ranges we observed, however the assumption that the effect that spending time at higher intensities has

outcome measures is linear is not. Despite this, there is no clear method to improve our estimates of this relationship without further assumptions.

Though small, the variation in day-to-day variability in the response of heart rate to submaximal exercise of 4 bpm (Achten and Jeukendrup, 2003) could account for variability in % HRR values of 4-6 % where errors would be exacerbated in older individuals who will have a lower HRR. As exercise heart rates were not sampled during every exercise session, the true total dose of exercise training achieved cannot be stated, though reasonable estimates can be made from the data presented with the caveat that equal relative heart rates do not necessarily confer an equivalent metabolic strain (Meyer et al., 1999). Moreover, maximal heart rates were estimated based on age and β-blocker use and not determined individually using cardiopulmonary exercise testing, which is standard in most CR programmes worldwide. This introduces two sources of error. Firstly, the estimated heart rates have a standard deviation of 11 bpm (Tanaka et al., 2001). Secondly, the estimated effects of β-blockers on maximal heart rate are a reduction of 19 ± 11 bpm (Wonisch et al., 2003) though the effect of medication dose upon this is unclear. The average HRR estimate in our cohort was 77 bpm therefore a crude suggestion that true deviation of 11 bpm from either of these estimations would result in a ~14% error in estimated % HRR during exercise. The distributions and covariance of these sources of error are not presented in the cited literature, therefore we are unable to know whether there is a systematic bias for higher or lower HR estimations or how these errors may propagate.

The studies in this chapter were lacking in a non-exercising control group, therefore it cannot be stated conclusively that participation in the CR programme has no effect on the short term outcomes of patients. Indeed, it is possible that CR may have the effect of preventing a decline in physiological markers of health such as measures of vascular integrity but this could not be captured with the present experimental design.

3.3.6. **Conclusion**

In summary, the studies in this chapter have provided insights into the exercise characteristics which are achieved by patients undergoing phase III community-based cardiac rehabilitation in the UK and examined the effects of this cardiac rehabilitation programme upon participants' outcomes such including: walking performance, blood pressure, body weight, physical activity behaviours and measures of vascular integrity. In part 1 of this chapter, considerable variability was observed in the heart rates achieved by patients and many patients spent little time above the lower boundary of the prescribed intensity range. Participants subsequently showed improvements in walking performance and reductions in systolic blood pressure but no other outcome measures. The lack of changes in many of these outcomes contrasted with existing literature in CR where higher exercise doses have been successfully employed and changes in patients' outcomes such as to cardiorespiratory fitness and indices of vascular health. In part 2 of this chapter, an intervention was implemented in a second cohort of CR patients with the intention of increasing the exercise dose achieved by patients

undertaking CR via an increase in exercise intensity. This intervention, which was applied without necessitating additional resources, however failed to impact upon the exercise intensities achieved or patient outcomes. Overall, it does not appear that UK outpatient CR may provide a sufficient exercise stimulus to impact vascular integrity in this population. These studies therefore provide a key insight into why current evidence indicates that UK CR is not effective in improving patients' long-term outcomes.

Chapter 4 **Acute effects of exercise intensity upon myocardial perfusion and cardiac dynamics assessed by cardiac magnetic resonance imaging**

4.1.1. **Introduction**

The impact of a chronic exercise stimulus upon peripheral vascular function and structure in a population with advanced CAD was explored in the previous chapter.

Much of the utility of assessments of peripheral vascular integrity, such as brachial artery FMD and carotid arterial stiffness used in the previous chapter, is justified by the close relationships between the function of these vessels and the coronary arteries (see Section 2.2.4). The adoption of these techniques in clinical practice is further proliferated by the reduced need for invasive assessment of these factors in the central circulation. However, there remains some ambiguity as to whether and to what extent changes in peripheral vascular integrity contribute to CV event risk reductions and improvements in CV mortality and morbidity.

On one hand, improved vascular integrity (improved vasodilatory function, reduced stiffness and optimised vessel diameter) in the peripheral vessels may reduce CV risk directly through mechanisms which enhance coronary perfusion. This may happen in the absence of changes in the properties of the central circulation *per se*. Reductions in systemic vascular resistance, for example, contribute to an increase in stroke volume during lower intensity

activities via a decrease in afterload (Suga, H, 1990), which, in turn, reduces heart rate at a given exercise intensity and improves subendocardial perfusion through an increased diastolic proportion of the cardiac cycle (Fokkema et al., 2005). Reductions in afterload will also lead to a leftward shift in the Frank-Starling curve and subsequent reduction in left ventricular end-diastolic pressure leading to a lowered transmural pressure across the myocardium, further enhancing subendocardial perfusion (Duncker et al., 1998). Reductions in peripheral vascular resistance with exercise training have also been seen to improve pulse wave reflection synchronicity in individuals with metabolic syndrome (Donley et al., 2014). This may contribute towards preventing backward pressure wave reflections from reaching the aorta during early systole which would increase central arterial pressure and ventricular work (Nichols, W.W., 1998; Nichols, W.W. et al., 2008). Reduced stiffness of the arteries would also increase the likelihood of backward pulse wave reflections arrival at the ascending aorta during diastole when exercising (Wilkinson et al., 2000; McEniery et al., 2006). This may serve to increase coronary driving pressure to enhance coronary perfusion (Nichols, W.W., 1998). Though this concept is pervasive in pulse wave reflection literature, it has not yet been demonstrated experimentally.

On the other hand, changes in vascular integrity in peripheral vascular beds, following a chronic exercise stimulus, may parallel changes in the central circulation as the effects of whole body exercise are systemic. As exercise-trained individuals exhibit enhanced coronary flow reserves (see Sections 2.3.4 & 2.3.5) and the same is seen in exercise-trained versus non exercise-

trained limbs (Green, D.J. et al., 1996), it is plausible that training effects occur in parallel. This would infer that assessments of peripheral vascular integrity provide a means to assess the effectiveness of treatments that target the central vasculature. Moreover, it would mean that exercise that provides a sufficient stimulus to improve peripheral vascular function creates a sufficient stimulus to drive improvements in central vascular function. However, little is known of the magnitudes of the effects that factors which comprise an exercise stimulus (intensity, duration, pattern etc.) have on the peripheral and central circulation as these are rarely studied in tandem.

Alternatively, should a chronic exercise stimulus differentially affect the central and peripheral circulations, then further work would be required to discern how an exercise stimulus affects the coronary circulation. Specifically, phenomena that may impact upon the exercise stimulus presented to the central circulation would warrant exploration. These include but are not limited to: the existence of threshold effects for exercise intensity; dose-response relationships with exercise intensity, duration and frequency; and the impacts of different exercise modalities. This is particularly relevant with regard to the therapeutic application of exercise training in individuals with impaired coronary endothelial or microvascular functions.

In light of this, in the previous chapter changes in the properties of the central vasculature could only be inferred due to their close correlations with peripheral vascular measures following a chronic exercise stimulus. Assuming that the properties of the central and peripheral vasculature are closely related, this might suggest that central vascular adaptations did not

occur in the UK CR cohorts observed. Knowing that central vascular endothelial function is related to the long-term outcomes of patients with CAD (Schächinger et al., 2000; Al Suwaidi et al., 2000),this fact may explain in part the lack of improvement in the long-term outcomes observed in the RAMIT UK CR cohort following a similar CR programme (West et al., 2012). Whether the exercise stimulus provided by UK CR sessions would stimulate adaptive responses in the central circulation is unclear, particularly as changes in the peripheral circulation appeared absent. The exact mechanisms by which exercise training contributes to reductions in cardiovascular morbidity and mortality are yet to be determined (Linke et al., 2006). Therefore, we cannot rule out the importance of understanding the stimulus provided to the central circulation by exercise training such as that prescribed in UK CR. This includes understanding how elements that comprise the stimulus provided by an acute bout of exercise influence the central vasculature. Moreover, should the true benefits of exercise training in a cardiac rehabilitation context be derived from central vascular adaptations, surrogate measures in the peripheral vasculature may misrepresent the effect of the exercise.

Notably, the concept that coronary microvascular dysfunction may contribute significantly to cardiovascular morbidity and mortality has been investigated far less than macrovascular dysfunction of the coronary arteries (see Section 2.3.4). In particular, the lack of treatments currently available for CMD and the albeit limited evidence for coronary microcirculatory adaptations following exercise training in patients with CVD (Möbius-Winkler et al., 2016;

Hambrecht et al., 2000) (see Section 2.3.5), warrant further investigation into the mechanisms by which exercise may contribute to coronary microvascular health. Specifically, the ability to manipulate elements of the acute stimulus presented by exercise such as exercise intensity, give insight into the mechanisms by which exercise provides a beneficial effect to the coronary microvasculature.

Invasive animal models have demonstrated exercise-induced increases in myocardial blood flow occur and increase linearly with exercise intensity (Duncker and Bache, 2008). Since greater rates of blood flow induce greater haemodynamic forces upon the walls of the vasculature, acting as mechanical stimuli to activate angiogenic and arteriogenic cellular pathways, a greater stimulus for adaptation and preconditioning may be evoked by higher exercise intensities (see Section 2.3).

Myocardial blood flow increases linearly with ventricular work per beat as this drives an increase in the oxygen demand of cardiomyocytes (Suga, H, 1990). Ventricular work per beat increases via an increase in stroke volume and systolic arterial pressure generation, often referred to as the ventricular systole pressure-volume area (Suga, Hiroyuki et al., 1981). Additionally, increases in HR increase ventricular work rate, further increasing myocardial oxygen demand and consequently coronary blood flow. The increasing ventricular pressures lead to an increase in transmural pressure across the myocardium which serve to reduce perfusion of the myocardium during systole by compressing the microcirculation. As a result, perfusion of the myocardium is impaired during systole and is mainly perfused during

diastole (Hess and Bache, 1979). Hence, cardiac dynamics are key determinants of exercise-induced increases in myocardial perfusion.

Much of the basic research into the effects of acute exercise upon cardiac perfusion and dynamics, particularly precise quantification of myocardial blood flow and cavity volumes, has relied on highly invasive techniques. Such methods are unsuitable for clinical investigation and research in low-risk populations as procedural complications can be severe. Therefore, the development of safer non-invasive techniques has been crucial to allow expansion of investigations in the field of cardiac physiology to reach larger cohorts and capture more physiologically relevant data in humans. To date, few studies have explored the application of techniques which employ non-invasive assessments of myocardial perfusion using new technology to the field of (cardiac) exercise physiology.

Currently available technologies capable of non-invasive myocardial perfusion assessment during exercise are limited by accuracy (ultrasound), cost and accessibility (computed tomography, positron emission tomography, and single photon emission computed tomography). Advances in cardiac magnetic resonance (CMR) imaging provide a potential avenue for non-invasive assessment of myocardial perfusion and cardiac function during exercise. A number of imaging pulse sequences have been validated for heart rates above normal resting values (up to 100 beats per minute (Lee et al., 2011)) to accommodate imaging of pathological tachycardia, which may have a similar utility for imaging individuals in the context of exercise-induced tachycardia.

Using CMR imaging, it has previously been proposed that a technique known as T1-mapping can be used to assess changes in intravascular blood volumes in the myocardium following adenosine administration (Liu et al., 2016). In the absence of epicardial coronary artery stenosis, this provides an index of coronary microcirculatory function (Levelt et al., 2017).

T1 time (or simply T1) is a magnetic property of all molecules. It is assessed by first aligning the spin states of nuclei within molecules using a strong magnetic field. At this point the spins of molecules are in state of maximal longitudinal magnetization. Next the orientation of these nuclei are inverted to a point of having no net magnetization via a radiofrequency pulse or series of pulses. Then, with time, the nuclei begin to align with the magnetic field once more causing a logarithmic growth in the longitudinal component of magnetisation back towards its original maximal value - also known as T1 relaxation. T1 therefore reflects the time constant of the rate of growth of longitudinal component of magnetization. Local spatial variations in T1 signal in tissues are primarily dependent upon tissue water content as water molecules are small, polar and mobile. The properties of the surrounding non-water molecules in a tissue interact with the water molecules via weak forces to slow the rate of recovery of orientation of nuclei (longitudinal magnetization). As such, tissues with a higher water content have higher T1 values (blood T1 > muscle T1 > adipose T1). CMR exploits this property of water molecules to characterise tissue properties that can be modified in various physiological states such as stress or disease. For example:

oedema of tissues causes an increase in tissue water content and an increase in T1 of that tissue.

More recently, acute changes in T1 in the myocardium following pharmacologically induced vasodilation have been proposed to reflect an acute increase in the water content which is a direct consequence of increased blood content via increased perfusion. Adenosine administration is known to induce “maximal” coronary vasodilation (Rossen et al., 1991; Belardinelli, L. et al., 1989; Wilson, R. et al., 1990) across the coronary resistance vessels which leads to an increase in myocardial blood flow and also myocardial blood volume. Liu et al. (2016) demonstrated an increase in myocardial T1 in healthy controls following adenosine administration as well as demonstrating reduced T1 reactivity in CAD patients and no reactivity in ischemic and infarcted tissues. This suggests that the response of myocardial T1 to adenosine may largely reflect changes in blood volume and/or flow. Within a given spatial location in the myocardium (demarcated by a voxel), blood is flowing throughout the period of T1 relaxation. T1 is sampled on a voxel-by-voxel basis. Thus it is the mean proportion of blood within a given voxel volume throughout the sampling period that results in the T1.

Exercise does not produce as great a hyperaemic effect as a typical adenosine stress test but arguably provides a more physiologically relevant stimulus than pharmacological stress. However, myocardial blood flow has previously been shown to increase by 2-5 fold during exercise (de Marchi et al., 2011; Heiss et al., 1976; Siegrist et al., 2014) and myocardial blood

volume by 69% (de Marchi et al., 2011). Furthermore, dobutamine stress-induced measures of myocardial blood volume reserve and myocardial $M\dot{V}O_2$ reserve are moderately correlated in healthy canines and strongly correlated in canines with stenosed coronary arteries (Le, D.E. et al., 2002). These studies feature a variety of other modalities that are either invasive or require contrast agents. The clear advantages to using T1-mapping during exercise to assess myocardial perfusion over these studies are that it is neither invasive nor requiring of contrast agent administration. Extensive research has been carried out on the physiological determinants of myocardial perfusion during exercise using invasive techniques. It is not known to what extent more novel, non-invasively determined indices - that are related to known determinants of myocardial perfusion such as cardiac work - influence myocardial T1 (itself a non-invasively determined index of perfusion).

I hypothesised that with exercise myocardial T1 will increase from resting values and myocardial T1 would increase in an intensity dependent manner.

The aim of this study was thus to explore the use of cardiac MRI as a novel, non-invasive method of assessing myocardial perfusion and cardiac dynamics during acute exercise intensities similar to those used in the UK CR cohorts observed in the previous chapter. The novelty of this technique therefore necessitated trialling of the technique in a healthy cohort. Whilst the participants in this chapter were healthy and thus presumably had no central microvascular dysfunction, this novel technique allowed the

examination of the impact of increasing exercise intensity upon myocardial perfusion.

4.1.2. **Methods**

Eleven recreationally active, healthy participants (25 ± 2 years, 7 m/ 3 f) provided written informed consent to take part in the study. Prior to participation, participants were screened for health and physical activity status and completed a standard MR screening form, as per standard laboratory and CMR procedures. Participants were excluded if they had current or historical cardiovascular, pulmonary or metabolic diseases, were a smoker or ex-smoker, were pregnant, suffered from musculoskeletal impairments or injuries which may affect their ability to complete the prescribed exercise or from any other contraindication to exercise or CMR imaging. The procedures and protocols were approved by the School of Biomedical Sciences (University of Leeds) and Yorkshire and the Humber Leeds West (National Research Ethics Service) Ethical Review Committees and were conducted in accordance with the Declaration of Helsinki.

4.1.2.1.1. Experimental Protocol

Participants first visited the exercise physiology laboratory based in the School of Biomedical Sciences. Participants height and body mass were assessed before undertaking a supine cardiopulmonary exercise test (CPET) using a cycle ergometer to measure individual maximum heart rates

(HR_{max}) and peak oxygen uptake ($\dot{V}O_{2peak}$) during supine exercise. A second morning visit, within one week of the first, required the participants to attend the Magnetic Resonance Imaging suite at Leeds General Infirmary where their heart was imaged at rest and under two exercise intensities interspersed with a 2 minute rest period. The work rates applied during each exercise condition were derived from the work rates corresponding to 55% and 75% or the maximum measured heart rate achieved during the supine CPET. Approximately four months later, a repeat of the second visit was performed for all participants using an identical MRI system and exercise protocol to assess reproducibility. Prior to each visit, participants were asked to refrain from participation in strenuous physical activity for 24 hours, alcohol ingestion for 12 hours, and caffeine ingestion for 4 hours and had to arrive at least 2 hours postprandial.

4.1.2.1.2. Cardiopulmonary exercise testing

CPET was performed, prior to CMR, in the human exercise physiology laboratories on an electronically braked supine cycle ergometer (Angio 2000, Lode, Groningen, the Netherlands) which had been modified to replicate the shorter crank lengths of the MR ergometer. The position of the ergometer was adjusted to ensure that during cycling the vertical displacement of the anterior surface of the knee was < 40 cm as this would cause contact with the casing of the MR bore and not represent the cycling action produced in

the bore. With this constraint, participants self-selected a position that minimised distance from this limit to range of motion.

Participants were continuously monitored via 12-lead ECG (Mortara ECG, Milwaukee, US) to measure HR and check for abnormalities that would require premature termination of the test. HR was determined as the highest HR achieved during the test. To assess ventilatory and pulmonary gas exchange variables (volumes of expired air and fractional concentrations of O_2 and CO_2) participants were fitted with a nose clip and a mouthpiece with a pneumotachometer and umbilical sample line which was attached to a breath-by-breath gas analysis system (Medgraphics metabolic cart, Minneapolis, US). Prior to the test, flow sensors were calibrated using a 3 L syringe and O_2 and CO_2 sensors were calibrated using three α-standard gases (BOC gases UK) that spanned the physiological range. For the determination of $\dot{V}O_{2peak}$, breath-by-breath data were filtered for erroneous breaths that were outside a fitted 99% confidence interval. Eight-point rolling averages of 12 breaths, moving backwards from the end of the ramp, were used to determine absolute $\dot{V}O_{2peak}$ (L/min). Relative $\dot{V}O_{2peak}$ (ml/min/kg) was determined by dividing absolute $\dot{V}O_{2peak}$ by body mass (kg).

A ramp-incremental test protocol was used following an initial 2 minute resting period of data collection and a subsequent warm-up period of > 3 minutes cycling at 10 W to a allow for gas exchange variables to reach a steady state. The ramp began at 10 W and increased at a rate of 12 W/min in females and 20 W/min in males and continued until volitional fatigue whereby a cadence of > 60 revolutions per minute could no longer be

maintained despite verbal encouragement. The test concluded with a recovery period of ≥ 4 minutes at 10 W.

4.1.2.1.3. CMR protocol

Participants were imaged using a 1.5 Tesla MRI system (Ingenia, Phillips Healthcare, Best, Netherlands) equipped with a 28-channel coil and a 3-lead ECG. Exercise was performed on a MR compatible cycle ergometer (MR ergometer pedal, Lode BV, Groningen, The Netherlands) whilst inside the MRI system bore (see Figure 11). ECG-derived HR and sphygmomanometer-derived brachial artery blood pressure were monitored continuously throughout the protocol. Images were acquired at rest, when cycling at an intensity of 55% of HR_{max}, and following a 2 minute rest period during exercise at an intensity of 75% of HR_{max}. When expressed as %HRR using the HR_{max} achieved during CPET, the mean exercise intensities represented 25 ± 1% and 58 ± 1% at of HRR respectively. In the event that the work rates applied did not elicit the target heart rates, manual adjustment of the work rate was performed in 5-10 W increments. Each exercise intensity was maintained for 5-7 min (2 minutes to achieve steady-state and ~ 3-5 minutes of image acquisition). Imaging was only performed during steadystate conditions, when HR was maintained at reasonably constant levels. Criteria for premature termination of the test included: significant arrhythmias, a drop in SBP of > 10 mmHg, ST-segment elevation or at the participant’s request.

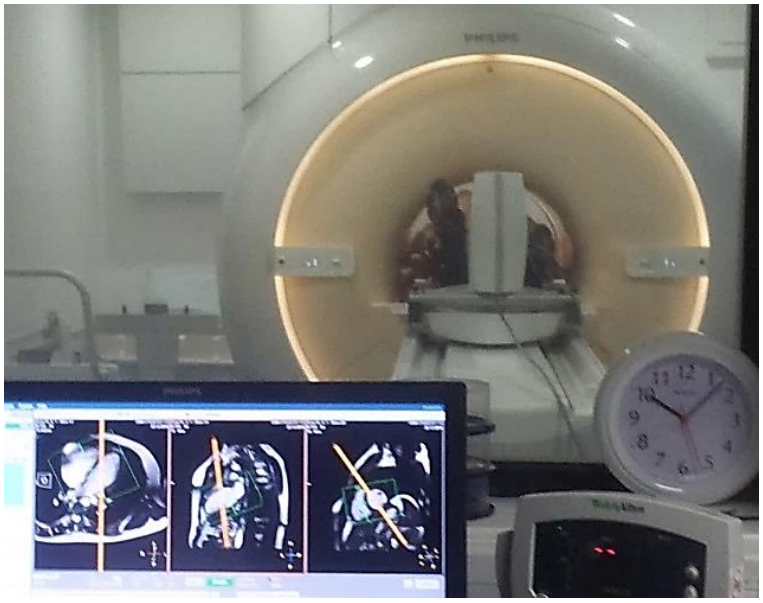

Figure 11: A participant performing cycling exercise within the MR bore

The cardiac long and short axes were determined using standard localizer scans performed in diastole. Biventricular volumes were obtained from single shot navigator-gated cine imaging using 4-chamber, ventricular long and short axis, sagittal-oblique and left ventricular outflow tract oriented views. Cardiac MR parameters were as follows: balanced steady state free precession, typical field of view 320 x 320 mm^2, voxel size 2.4 x 2.4 mm^2, slice thickness 10 mm with 0mm gap, 30 cardiac phases. Volumetric measures were taken under free breathing conditions.

Short axis T1 mapping across apical, mid ventricular and basal slices was obtained via a validated Shortened Modified Look Locker Inversion (ShMOLLI) protocol (Piechnik et al., 2010), ECG-gated with a voxel size of 2.4 x 2.4 mm^2, as was positioned using the 3 of 5 technique where 5 parallel 10mm thick slices are planned intersecting the left ventricle using long axis

and 4-chamber views, slice gaps are adjusted and the outer 2 slices are removed. The ShMOLLI sequence was initiated at rest and at 15 seconds following each exercise bout and to minimise motion artefact allow breath-holds for a 9-second required period.

4.1.2.1.4. Image processing and analysis

Native T1 maps of the apical, mid and basal ventricular slices were generated offline from ShMOLLI source images using commercially available software (Circle Cardiovascular Imaging Inc. Calgary, Canada) following localization using short axis cine images. Native T1 relaxation times of the myocardium and the blood pool were measured from T1 maps of apical, mid ventricular and basal slices. This was achieved by drawing contours around the subepicardial and subendocardial boundaries and adding reference points at the anterior and posterior septal insertions of the RV to the LV. This formed a region of interest comprising the myocardial tissue within each slice (see Figure 12 below). Contouring was performed by a single researcher (this author). Myocardial T1 mapping was performed according to the most recent recommendations of the Society for Cardiovascular Magnetic Resonance (Moon et al., 2013). To avoid contamination of the signal by the blood pool or extra-myocardial structures and to minimise the occurrence of intermediate values caused by partial volume effects, the myocardial contours were artificially inflated and eroded by 10% of segment width.

In brief, image data was performed using a three-parameter nonlinear curve fitting pixel-wise using a Levenberg-Marquardt algorithm and T1 was then calculated in conventional Look-Locker methods (Messroghli et al., 2007). In case of displacement of the left ventricle along the x- or y-axis of the source image due to poor ECG triggering or breath holding of the participant, the software allowed one to manually shift source images along these axes for registration of the left ventricle prior to T1 calculations. Image quality was assessed using a six-segment model of each myocardial slice as shown in Figure 12 below. A segment was declared non-evaluable if ≥ 4 contiguous pixels within that segment were affected by artefact. No non-evaluable segments were found for any participant. Afterwards, segmental T1 of segments across of three slice was noted to give regional T1 values (Figure 12) and mean (global) values were calculated for each participant. In the repeated CMR visit, T1 data were only captured for the mid-ventricular slice due to practical limitations.

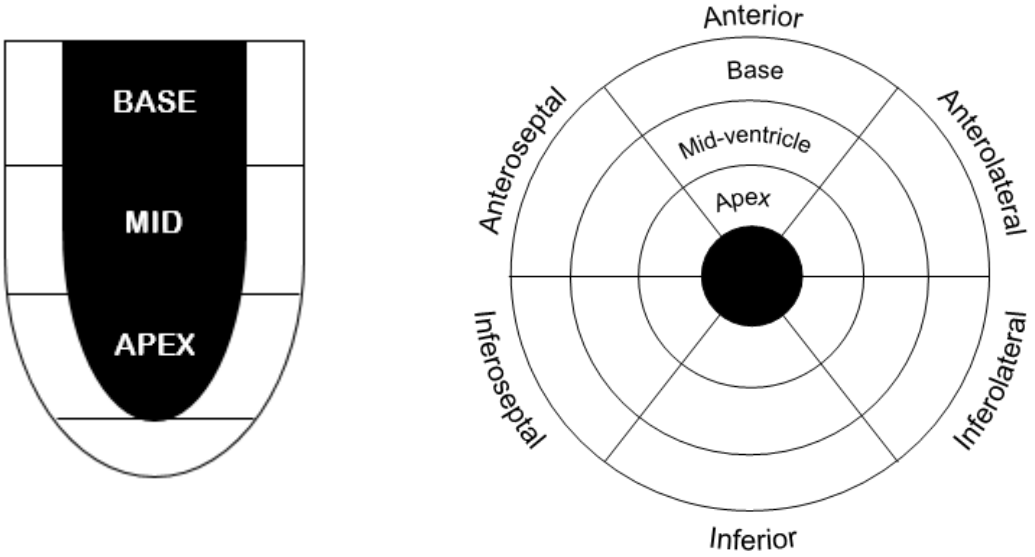

Figure 12: A diagrammatical representation of myocardial T1 demarcations along the length of the left ventricle by slice (left) and cross-sectional segments through each slice (right).

Left ventricular (LV) and right ventricular (RV) end diastolic volumes (EDV), end systolic volume (ESV) and ejection fraction (EF) were assessed via contouring of the endo- and epicardial boundaries of the myocardium from short axis cine images excluding papillary muscles and trabeculations. All volumetric myocardial contouring was performed by an experienced cardiologist.

Stroke volume (SV) is defined as $SV = EDV - ESV$

Cardiac output $\dot{Q}$ was determined as $\dot{Q} = HR \times SV$

Rate-pressure product (RPP) is defined as $RPP = HR \times SBP$

Variables were indexed by dividing by body surface area as calculated as (Mosteller, 1987) $Body\ surface\ area\ (cm^2) = \sqrt{\frac{height(cm)\ \ weight(kg)}{3600}}$

Global longitudinal strain was determined using the feature tagging function in commercially available software (Circle Cardiovascular Imaging Inc. Calgary, Canada). Tissue tracking analysis was manually performed by drawing the endo- and epicardial surfaces in end-diastole using the short axis stacked slices obtained for quantification of biventricular volumes. A reference point in the short axis plane was manually delineated at the upper and lower septal insertion of the RV into the LV for global analysis of strain. The software then automatically drew the endo- and epicardial contours and traced the translation of myocardial voxels throughout the remainder of the cardiac cycle. 2D strain analysis of the 32 stacked short-axis slices was then automatically performed by the software to produce a global longitudinal strain curve across the cardiac cycle. Global longitudinal strain was defined as the maximum of the global longitudinal strain curve and global longitudinal strain rate as the maximum of the second derivative of the curve.

4.1.2.1.5. Statistical analysis

Data analysis was performed using SPSS statistical analysis software (IBM SPSS Statistics for Windows, Version 24. Armonk, NY). Normality of data distributions were assessed using the Shapiro-Wilk test. In the case that data were non-normally distributed an appropriate transformation was

applied based on the distribution of the data and normality re-examined. If data could not be successfully transformed, then analysis proceeded using non-parametric methods. The effect of exercise intensity upon cardiac parameters (mid-ventricular myocardial T1 time, blood pressure, left and right indexed and non-indexed end systolic and end diastolic volumes, global longitudinal strain and strain rate) was assessed using one-way repeated-measures ANOVA performed on the mean data for each participant from the two visits. Assessments of the effects of exercise intensity upon regional myocardial T1 in the initial visit, demarcated by slice and segment, was performed using a three-way repeated measures ANOVA with exercise intensity as the repeated measure and slice and segment as fixed factors. To assess for the effects of factors associated with myocardial T1 upon exercise intensity dependent changes in myocardial T1, factors that correlated with T1 values were included as covariates in this model. Pearson and Spearman correlations were used to assess the relationships of cardiac parameters, heart rate, participant characteristics and segmental myocardial T1. When an effect was observed, post-hoc Bonferroni-adjusted multiple comparisons were performed to assess the effects of exercise intensity. For all analyses an alpha-level of 0.05 was selected. To assess the within-subject reliability of all measures between visits using this novel technique, the coefficients of variation were calculated as a percentage of typical error (Hopkins, W.G., 2000).

4.1.3. **Results**

Participant characteristics and CPET data are displayed in Table 18. All participants completed the CPET test, MRI protocol and the repeated MRI protocol.

Table 18: Participant characteristics

Participant characteristics	
Age (years)	25 ± 2
Sex	7m / 3f
Height (cm)	176 ± 7
Weight (kg)	73 ± 9
Cardiopulmonary exercise testing	
VO_{2peak} ($L \cdot min^{-1}$)	3.2 ± 0.8
VO_{2peak} ($ml \cdot min^{-1} \cdot kg^{-1}$)	44 ± 9
HR_{peak} ($beats \cdot min^{-1}$)	168 ± 19
Peak work rate (W)	192 ± 45

Data are mean ± SD

4.1.3.1. **Cardiac dynamics**

All cardiac dynamics at rest and with increasing exercise intensity are displayed in Table 19. Left and right ventricular cardiac outputs, rate-pressure product (see Figure 14), SBP (see Figure 13) and ejection fractions increased as an effect of exercise at 75% of HR_{max} ($p < 0.05$) LVEDV was unchanged from resting levels with exercise ($p=0.06$) but showed a trend towards a reduction at 75% of HRmax. LVESV decreases with exercise intensity ($p < 0.05$), however, no overall changes in LVSV were seen with exercise ($p = 0.51$). Very similar trends were observed between the left and right ventricles and between indexed and non-indexed left and right ventricular EDV, ESV and SV. Global longitudinal strain per cardiac cycle was unchanged with exercise ($p = 0.15$) (see Figure 15), whilst global longitudinal strain rate increased with exercise at 75% of HR_{max} ($p < 0.001$) (see Figure 16) which was highly correlated with changes in heart rate ($r = -0.80$, $p < 0.001$).

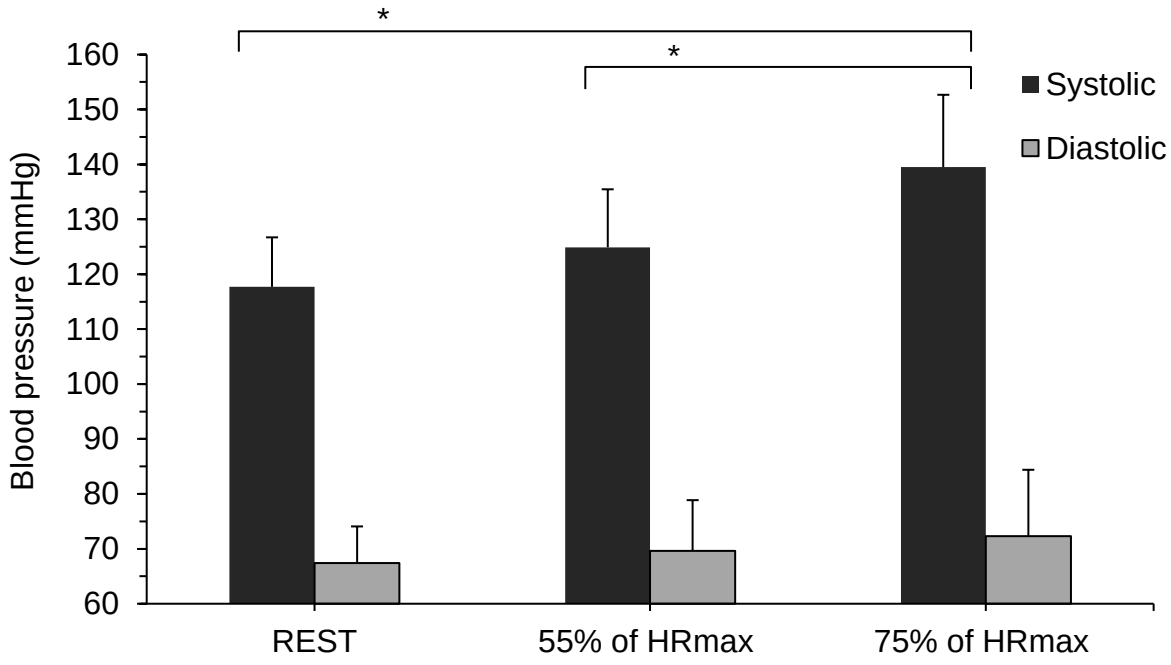

Figure 13: The effect of exercise intensity upon brachial artery systolic and diastolic blood pressure. Data are mean ± SD. *denotes a significant difference in systolic blood pressure ($p < 0.01$).

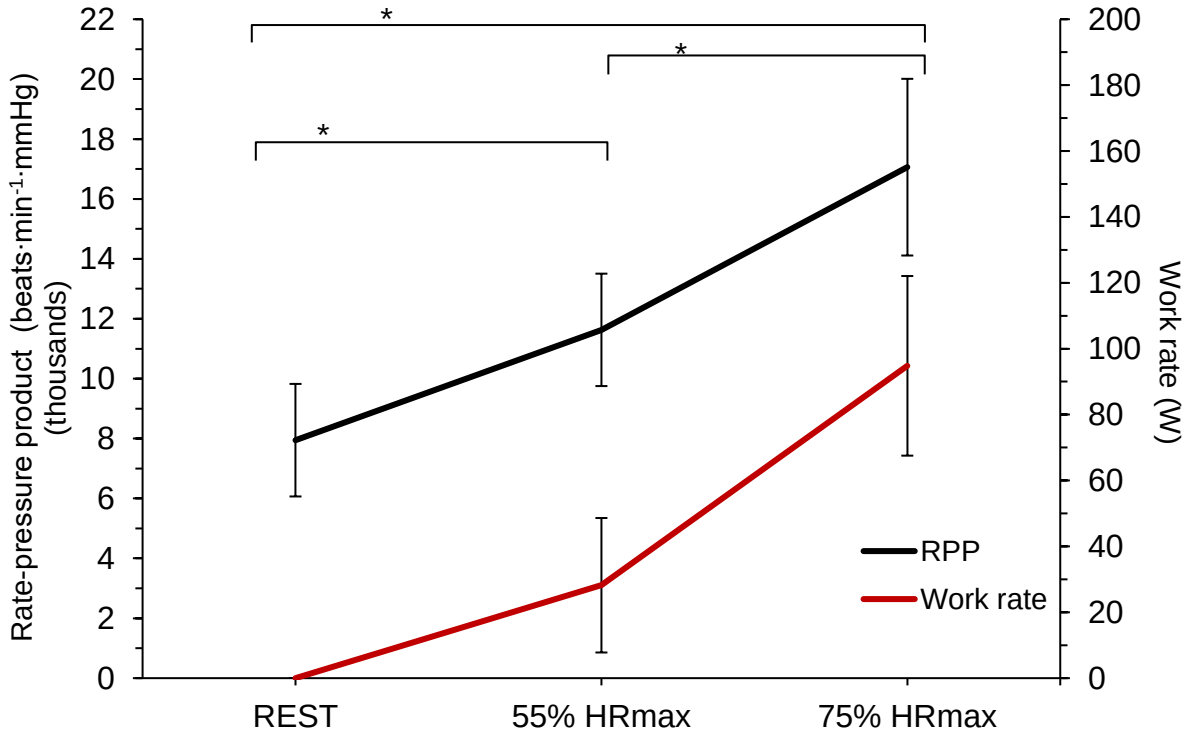

Figure 14: The effect of exercise intensity upon rate-pressure product and the cycle ergometer work rates associated with these intensities. Data are mean ± SD. *denotes a significant difference in rate-pressure product ($p < 0.001$).

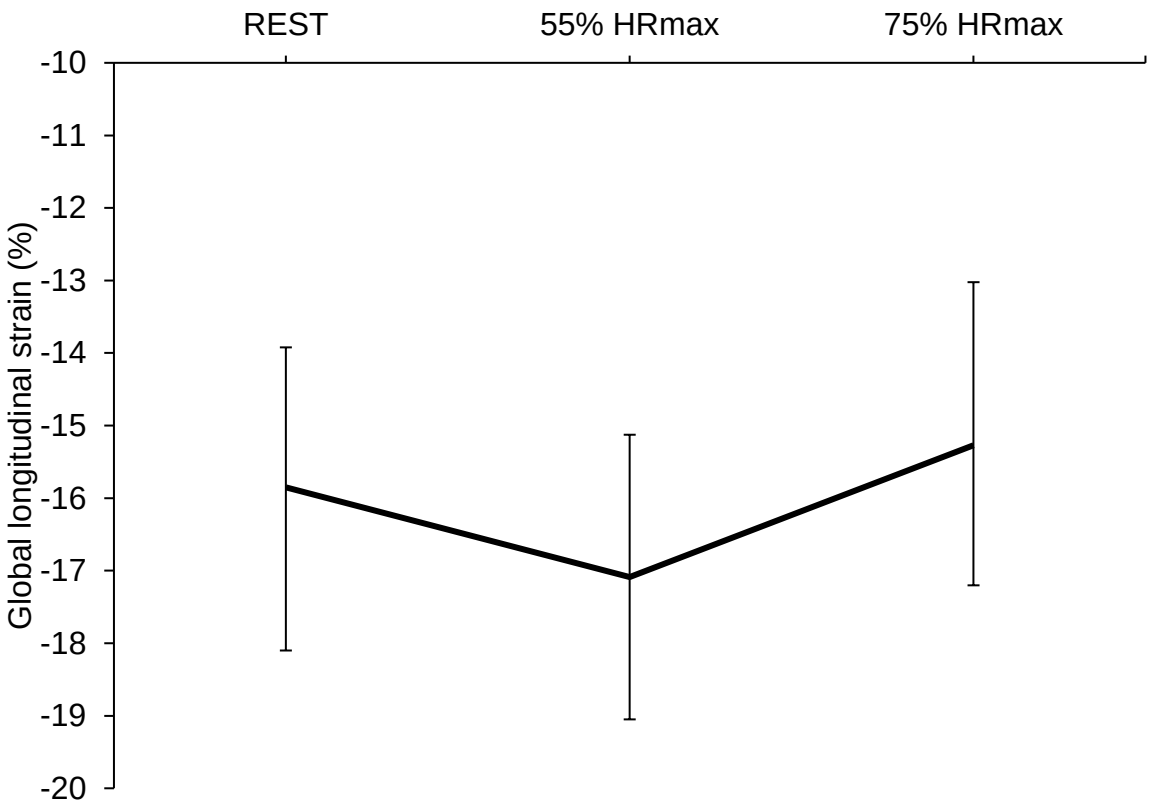

Figure 15: No effect of exercise intensity upon global longitudinal strain. Data are mean ± SD.

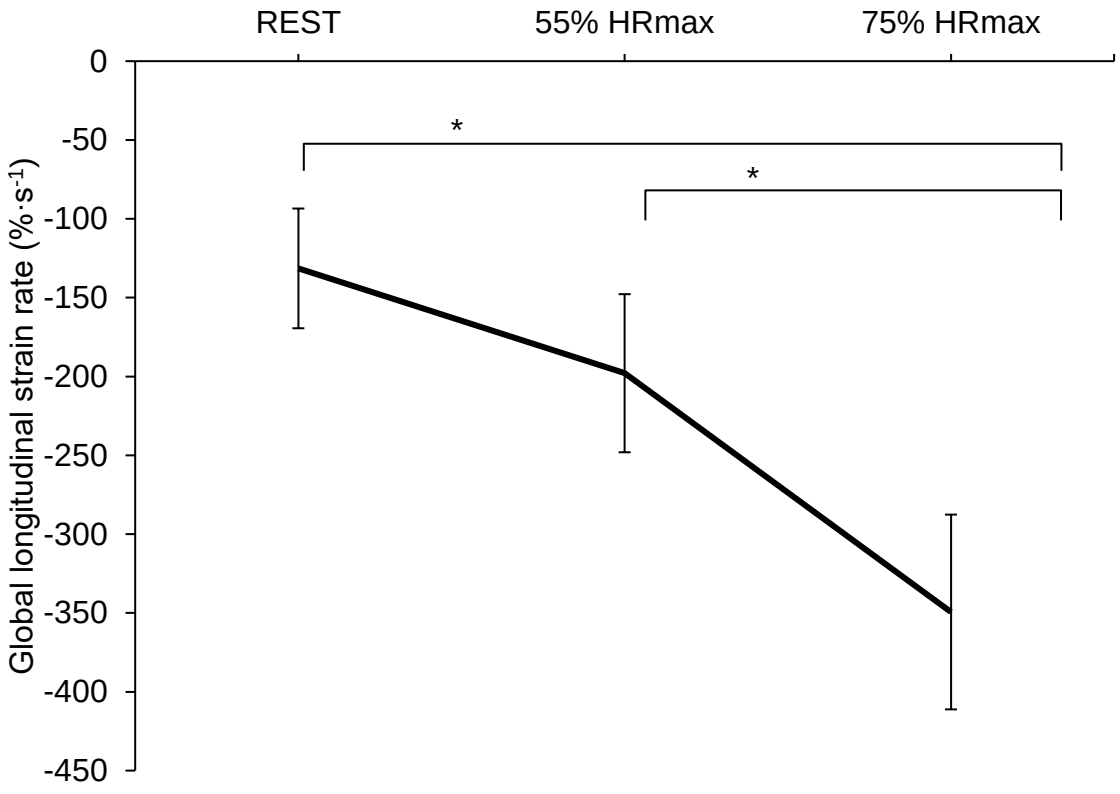

Figure 16: The effect of exercise intensity upon global longitudinal strain rate. Data are mean ± SD.*denotes a significant difference $p < 0.001$

Table 19: Cardiovascular parameters at rest and during two exercise intensities

Variable	REST	55% of HR_{max}	75% of HR_{max}
LVEDV	177 ± 29	175 ± 28	159 ± 20
LVEDVi	90 ± 13	92 ± 14	83 ± 9
LVESV	76 ± 17	69 ± 16	53 ± 8*†
LVESVi	40 ± 8	37 ± 7	29 ± 4*†
LVSV	100 ± 13	106 ± 16	105 ± 14
LVSVi	50 ± 7	55 ± 8	54 ± 8
LVEF	57 ± 4	61 ± 5	66 ± 2*†
LV cardiac output ($L \cdot min^{-1}$)	6.8 ± 1.1	10.2 ± 2.1*	13.8 ± 2.1*†
RVEDV	178 ± 31	175 ± 30	159 ± 21
RVEDVi	94 ± 15	94 ± 17	87 ± 11*
RVESV	80 ± 18	70 ± 20	56 ± 11
RVESVi	43 ± 9	39 ± 11	33 ± 7
RVSV	99 ± 15	106 ± 15	104 ± 13
RVSVi	50 ± 8	55 ± 8	54 ± 7
RVEF	56 ± 4	61 ± 6	66 ± 4
RV cardiac output ($ml \cdot min^{-1}$)	6.7 ± 1.3	10.1 ± 2.0*	13.6 ± 2.0*†

Data are mean ± SD.*denotes a difference from baseline, †denotes a difference from 55% of HR_{max} $p < 0.05$.

4.1.3.2. **Myocardial T1 time**

Global myocardial T1 time in the first visit was unchanged from rest to either exercise intensity (940 ± 24 ms vs 942 ± 14 ms vs 950 ± 20 ms). Regional segment T1 values across the basal, mid-wall and apical slices at rest are displayed in Figure 18. Significant main effects were detected for both slice ($p < 0.001$) and segment ($p = 0.04$) (Figure 20), but not for exercise intensity ($p = 0.65$). A significant slice x segment interaction was observed ($p < 0.01$), however, there were no slice x exercise intensity ($p = 0.27$), segment x exercise intensity ($p = 0.09$) or slice x segment x exercise intensity ($p = 0.82$) interactions.

Post-hoc comparisons revealed the mid-ventricular slice had longer T1 values than the apical slice ($p = 0.046$). Both the inferolateral and anterolateral segments had longer T1 values than the anterior, anteroseptal and infroseptal segments (all $p < 0.05$) and the T1 of the inferior segment was longer than the anteroseptal segment ($p = 0.02$).

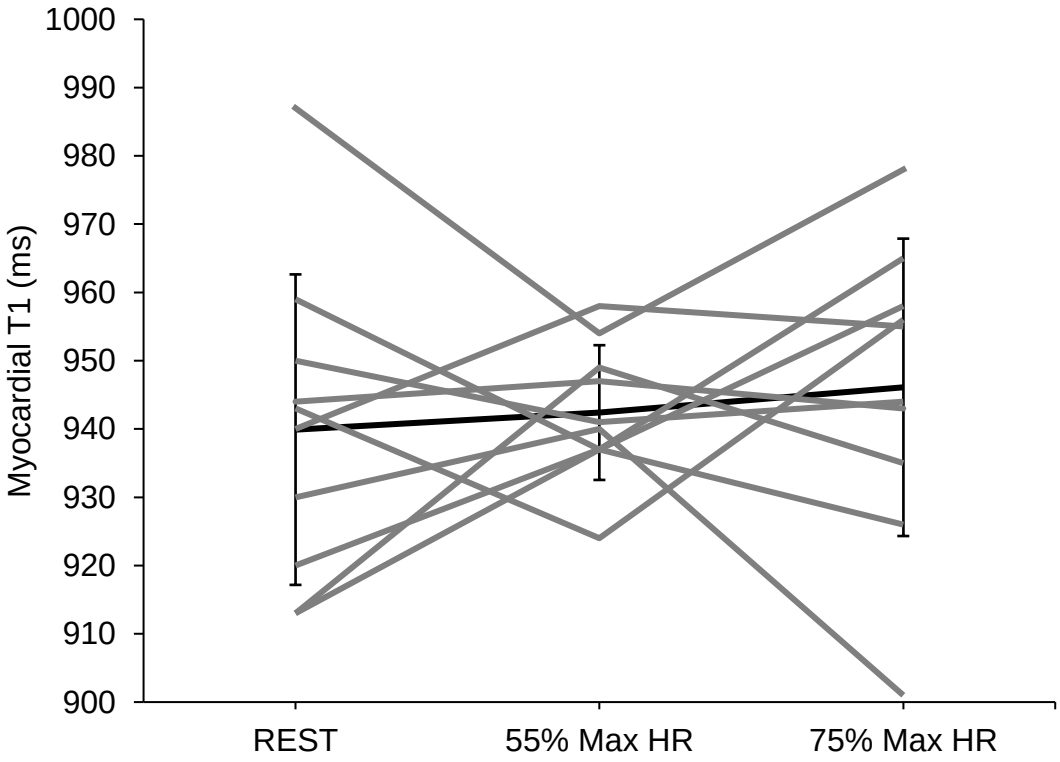

Figure 17: Mid-ventricular myocardial T1 time at rest and at two exercise intensities. Data is individual mean values across both visits. Data are individual T1 time (grey lines) and the group mean ± SD (black).

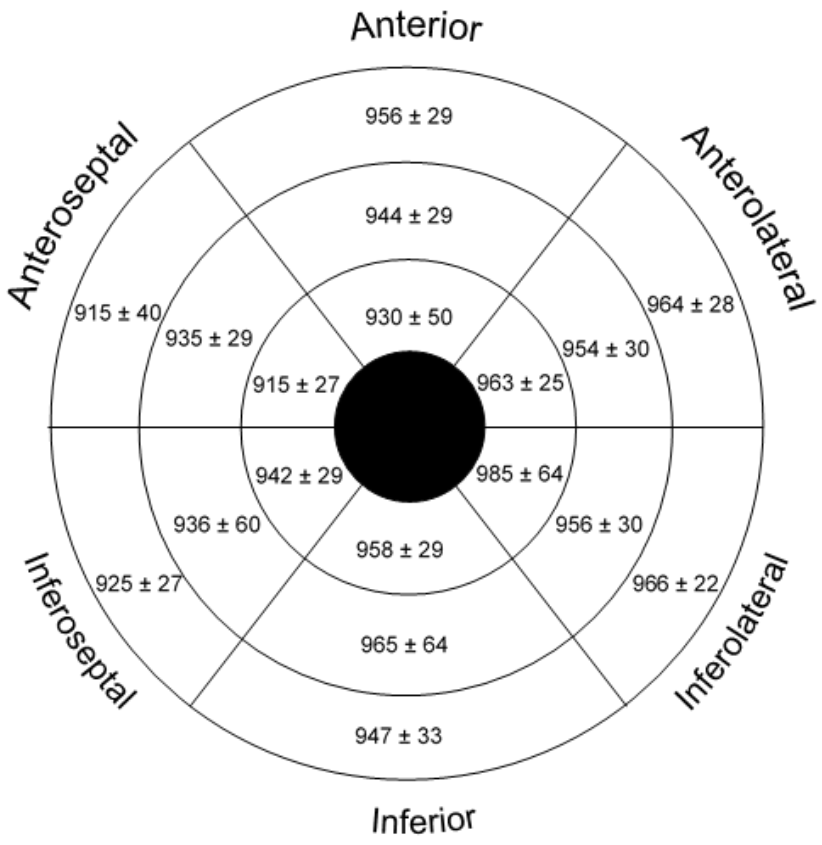

Figure 18: Regional myocardial T1 time (ms) across the base (outer ring), mid-ventricle (middle ring) and apex (inner ring) of the left ventricle at rest. Data are mean ± SD.

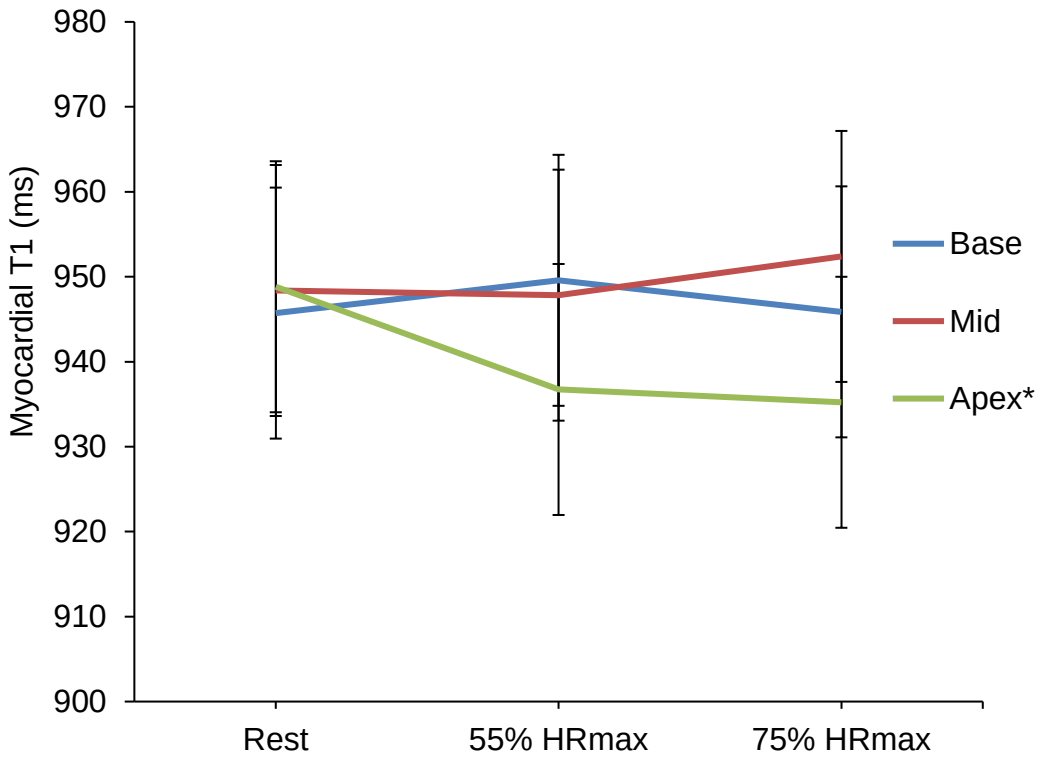

Figure 19: No effect of exercise intensity upon myocardial T1 time in three left ventricular slices. *denotes a significant difference from the mid-ventricular slice $p < 0.05$.

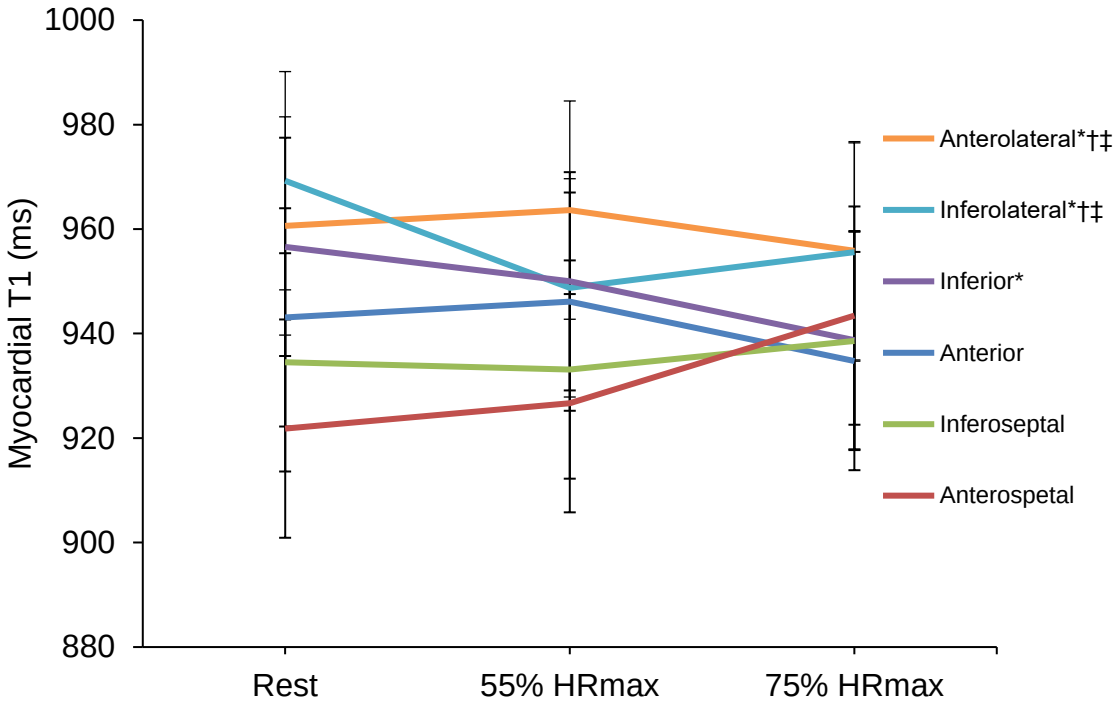

Figure 20: No effect of exercise intensity upon regional myocardial T1 in six cross-sectional segments Data are mean ± SD. *denotes a significant difference from the anteroseptal segment †denotes a significant difference from the inferoseptal segment and ‡denotes a significant difference from the anterior segment $p < 0.05$.

Pooled analysis of the mean T1 in the mid-ventricular slice from both visits also revealed no main effect of exercise intensity (940 ± 23 ms vs 942 ± 10 ms vs 946 ± 22 ms, $p = 0.77$ for rest, 55% of HR_{max} and 75% of HR_{max} respectively).

Myocardial T1 time was correlated with age ($r = - 0.34$, $p = 0.01$) weight ($r = 0.38$, $p = 0.003$), resting heart rate ($r = 0.43$, $p = 0.001$), LVEDV ($r = - 0.35$, $p = 0.007$), LVESV ($r = - 0.38$, $p = 0.003$), RVEDV ($r = - 0.31$, $p = 0.02$), and RVESV ($r = - 0.30$, $p = 0.02$). Inclusion of age and weight as covariates in the ANCOVA model had no impact on any outcomes.

4.1.3.3. **Reproducibility**

Table 20: Within-participant coefficients of variation for MRI protocol parameters

Variable	REST	55% of HR_{max}	75% of HR_{max}
Work Rate (W)	0.0%	84.1%	29.4%
Heart rate (beats·min^{-1})	20.1%	10.0%	12.0%
Systolic blood pressure (mmHg)	4.8%	2.8%	8.4%
Diastolic blood pressure (mmHg)	5.6%	5.5%	30.0%
T1 time (ms)	1.0%	1.5%	2.3%
LVEDV	3.8%	5.9%	7.6%
LVEDVi	5.1%	5.9%	7.7%
LVESV	7.7%	12.7%	13.6%
LVESVi	7.3%	13.0%	13.1%
LVSV	8.9%	14.2%	9.7%
LVSVi	10.2%	13.9%	10.0%
LVEF	6.3%	10.2%	5.5%
LV cardiac output (L·min^{-1})	14.3%	19.2%	10.1%
RVEDV	5.9%	8.8%	12.2%
RVEDVi	7.6%	9.1%	12.5%
RVESV	15.7%	20.0%	34.2%
RVESVi	16.2%	20.1%	34.6%
RVSV	12.0%	9.4%	7.5%
RVSVi	13.1%	9.8%	7.8%
RVEF	10.8%	7.9%	9.3%
RV cardiac output (ml·min^{-1})	18.6%	13.6%	7.8%
Global longitudinal strain (%)	14.3%	20.8%	12.3%
Global longitudinal strain rate (%·sec^{-1})	34.2%	20.7%	51.3%

4.1.4. **Discussion**

This study found that myocardial T1 was unchanged from resting values with exercise. This was disappointing as there were clear exercise-induced and exercise intensity dependent increases in left and right ventricular stroke volume, ejection fractions, cardiac output and global longitudinal strain with increasing work rates.

Extensive literature has demonstrated that myocardial blood flow increases during exercise in an intensity dependent manner (see Sections 2.2.4 & **Error! Reference source not found.**). As exercise intensity increases cardiac output increases via elevated cardiac work. Increases in cardiac work are driven by aerobic metabolism and necessitate compensatory increases in $M\dot{V}O_2$. Augmentations in myocardial blood flow are driven by increases in $M\dot{V}O_2$ which is highly correlated with RPP during exercise (Kitamura et al., 1972). RPP increased ~2-fold with exercise at 75% of HR_{max} in the present study, thus, substantial increases in myocardial blood flow would likely have occurred. However, the index of myocardial perfusion employed in the present study, myocardial T1 relaxation time, measures the inversion recovery of water molecules within the myocardium and not solely myocardial blood flow or even blood volume. Therefore, this technique assesses changes in perfusion as acute changes in tissue water content, which should follow increases in the proportion of blood per voxel of myocardium. Ample evidence shows that increases in myocardial blood flow that occur with exercise are mediated by vasodilation of the coronary

circulation (Duncker and Merkus, 2007) and thus myocardial blood volume. Consequently, increases in myocardial T1 were anticipated with exercise.

In the study of Mahmod et al. (2014), changes in myocardial T1 were induced via adenosine administration (a potent vasodilator) in a healthy cohort which was attributed to increases in myocardial perfusion. Adenosine can induce a ~300% increase in myocardial blood flow in healthy controls (Chan et al., 1992) which for Mahmod et al. (2014) resulted in a 6% increase in myocardial T1. Maximal exercise in healthy individuals has demonstrated increases in myocardial blood flow of ~200% (Heiss et al., 1976).The coronary vasculature is not maximally vasodilated by exercise as further increases can be achieved via adenosine administration in subepicardial regions (Rouleau et al., 1979). Therefore, it would not be expected that submaximal exercise would increase myocardial perfusion (and by extension T1 time) by as a large a magnitude as with adenosine administration. However, myocardial hyperaemia should occur in line with the changes in cardiac dynamics observed with exercise. Despite this, the mean relative increase in myocardial T1 from resting values when exercising at 75% of HR_{max} was only ~0.6%, suggesting a negligible magnitude of increase that is not concordant with the literature on myocardial blood flow. With exercise at 75% of $HR_{max,}$ myocardial perfusion should have increased by less than 2/3 of the amount seen with adenosine-induced hyperaemia resulting in an increase in myocardial T1 of 2-3%, should myocardial T1 increase proportionately with myocardial perfusion. As this finding was not observed, the magnitude of increase in myocardial T1 observed with exercise does not

appear to reflect the expected increases in myocardial perfusion at the exercise intensities used in this study.

Nonetheless, the increases in cardiac work rate in this study indicated by the substantial increases in RPP and global longitudinal strain rate suggest that exercise should increase myocardial perfusion in an intensity dependent manner. The exercise intensities used in this study spanned a similar HRR as the mean HRR observed in CR in the previous chapter (25 ± 1% and 58 ± 1% of HRR was achieved at 55% and 75% of HR_{max} respectively and < 20% of participants achieved mean HRR in the MID session greater than this. It is clear that exercising at these intensities should present an acute hyperaemic stimulus to the central vasculature, which may translate to central vascular adaptations if repeated over time. Even so, the magnitude of this stimulus could not be captured using myocardial T1 mapping. It therefore remains unknown as to whether there are advantages of exercising at higher intensities or a requisite minimum intensity is needed to drive central vascular adaptations.

Though no clear effects of exercise upon myocardial T1 could be discerned, regional heterogeneities in myocardial T1 at under resting and exercise

conditions were identified. Baseline values are reported in

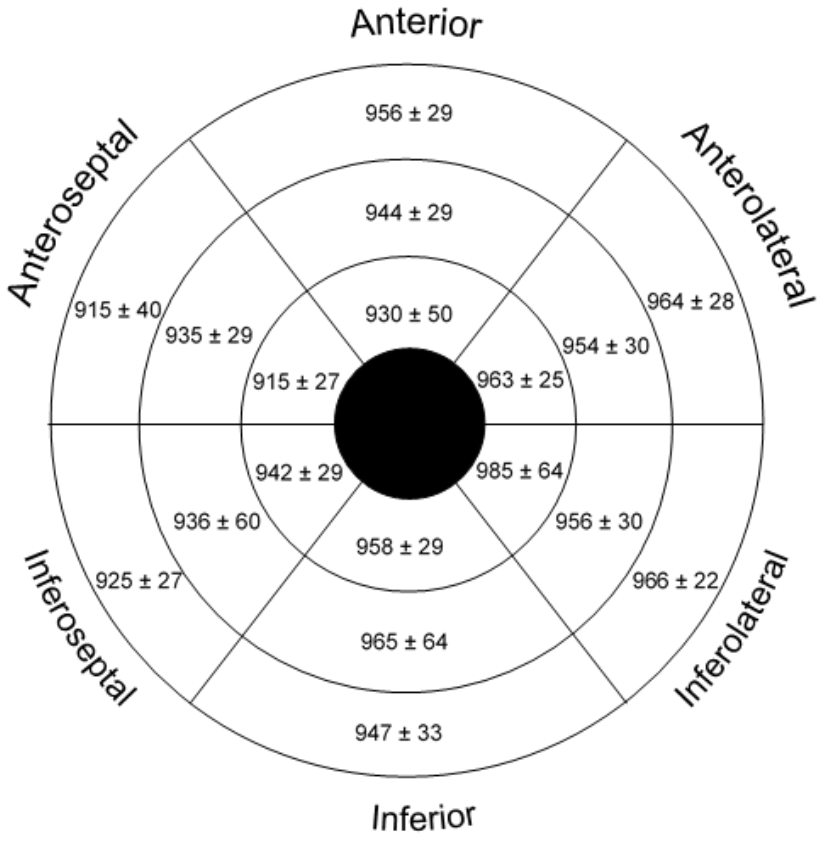

Figure 18. The regional distribution of myocardial T1 in the present study shows greater T1 values in the lateral segments compared to the septal which is an opposite trend to the reference values from Dabir et al. (2014). In light of this, regional differences are small, the T1 times reported here and their variabilities are similar to reference values in the healthy myocardium. That there exists marked spatial heterogeneity of myocardial perfusion reserve (Austin Jr et al., 1990) suggests that exercise may evoke regional differences in perfusion, however, this was not observed using myocardial T1 mapping in the present study. A potential explanation is provided by

Dabir et al. (2014) who conclude that regional differences in myocardial T1 are unlikely to reflect true differences in tissue composition. Therefore, regional perfusion differences may be obscured using the T1 mapping technique.

A valuable finding of this study is that CMR can provide a feasible and reproducible quantification of cardiac dimensions and estimations of myocardial strain during exercise. Our data largely concurs with a similar study from Le, T.-T. et al. (2017) which used a similar CMR approach to quantification of ventricular volumes across a range of exercise intensities. An advantage of our approach was that volumetric measures were taken during continuous exercise whereas previous studies have required the cessation of exercise for image acquisition. As HR begins to decline almost immediately following the cessation of exercise preload is likely to decrease and afterload also decreases, hence the accuracy of measures of SV may be impaired as the recovery dynamics of these variables and their interaction may be non-uniform across individuals. In light of this, the pattern of changes in cardiac dimensions in our data is similar to that of Le, T.-T. et al. (2017). Our cardiac dimension data are somewhat in agreement with a recent meta-analysis of exercise CMR studies that demonstrates changes in stroke volume induced by submaximal exercise are primarily driven by reductions in ESV and that at lower intensities EDV is unchanged (Beaudry et al., 2018). The reproducibility of cardiac dimension data is affected somewhat by exercise intensity, with the coefficients of variation for biventricular EDVs and particularly ESVs increasing with exercise intensity

but not to an unacceptable level. This might be expected as motion artefacts caused by movement of the body or increased heart rate are exacerbated at greater exercise intensities.

Despite there being a fairly good agreement between ventricular volume and functional measurements between CMR and the next most cost-effective modality – echocardiography, CMR is more accurate and reproducible under resting conditions (Gardner et al., 2009) and has a superior spatial and temporal resolution. Though this has yet to be demonstrated in exercise conditions, it may be assumed as echocardiography may be more prone to motion artefacts. Therefore, CMR likely has a greater utility at least in research contexts where a greater ability to detect changes in ventricular structure and function may be needed.

The utility of cardiac strain data is usually confined to exercise echocardiography studies and clinical populations receiving pharmacological stress. Though it is an abnormal finding that global longitudinal strain was unaffected by exercise (Donal et al., 2011), it is a useful indicator of systolic contractile function. Global longitudinal strain rate expectedly increased with heart rate; however the coefficient of variation for global longitudinal strain rate was considerably large. Therefore, measures of global longitudinal strain rate were not reproducible between visits.

The techniques used here are yet to be validated in diseased or older demographics. As these populations will achieve a higher proportion of

individual HRR at the same absolute HRs that have been successfully measured in this study, a greater range of exercise intensities could be employed in future studies in older demographics.

Exercise performed in a supine orientation is known to alter haemodynamics compared to upright exercise at a given workload. Specifically, heart rate and RPP are reduced and stroke volume index and left ventricular end-diastolic pressure are typically greater in a supine versus upright orientation during cycle exercise in healthy individuals at similar heart rates to the present study (128 ± 6 bpm) (Thadani and Parker, 1978). This is likely due to an increased preload that accompanies a greater central venous pressure. Stroke volume augmentation from rest to exercise that occurs during upright exercise may be lessened (Thadani and Parker, 1978) or nullified (Bevegård et al., 1960) in a supine orientation. Left ventricular end-diastolic pressure is positively correlated with the driving pressure at which zero-flow occurs in the coronary arteries (Duncker et al., 1995b). Therefore a greater left ventricular end-diastolic pressure would increase the transmural pressure across the myocardium and reduce the pressure differential between the proximal and distal coronary beds, reducing blood flow as per Poiseuille's law (see Section 2.1.2). To maintain blood flow with increases in left ventricular end diastolic pressure, compensatory reductions in pressure in the distal blood vessels via vasodilation of arterioles are required. Using the estimates of the effect of a supine orientation upon left ventricular end-diastolic pressure by Thadani and Parker (1978) and the effect of left ventricular end-diastolic pressure upon coronary pressure from the data of

Duncker et al. (1995b), we would expect a small increase in the coronary pressure associated with zero-flow by ~ 3.5 mmHg. This would not change mean myocardial blood flow during submaximal exercise, as compensatory vasodilation of the resistance vessels would ensue to maintain an adequate oxygen supply to the myocardium. However, supine exercise may result in impairments to an individual's myocardial perfusion reserve versus upright exercise as the basal level of vasodilation required to maintain flow is elevated when supine. Therefore, when comparing the findings of studies of myocardial perfusion during submaximal exercise in a supine orientation to upright exercise, perfusion but not perfusion reserve can be considered equivalent. How a supine orientation may affect myocardial blood volume and consequently myocardial T1 responses to exercise has not been investigated. Speculatively, if the myocardial blood pressure gradient is maintained to maintain blood flow but at higher absolute blood pressures, then myocardial blood volume should be unaffected. One caveat is that the greater vessel pressures may increase fluid extravasation at the capillaries which could contribute to an elevated T1 although this was not seen in the present study.

4.1.4.1. **Limitations**

A number of technical factors may explain the lack of change in myocardial T1 with exercise in this study. Myocardial blood volume may only change by ~10% during exercise unlike much greater increases in myocardial blood flow (Moir et al., 2005). With dipyridamole- and dobutamine-induced stress

in canines, changes in myocardial blood volume of 30-50% are achievable (McCommis et al., 2009). In humans, using myocardial contrast echocardiography de Marchi et al. (2011) show increases of ~200% in myocardial blood flow and 69% relative myocardial volume when exercising at 80% of maximum heart rate. We must also consider that myocardial blood only represents 8-11% of voxel volume (Jerosch-Herold, 2010; Liu et al., 2016) though these figures quoted in the CMR literature tend not to acknowledge their derivation from animal models (Judd and Levy, 1991; MCanty Jr et al., 1991). Therefore the ShMOLLI technique, with its normal variation of ± 2.5% (Piechnik et al., 2013), may not be sensitive enough to detect the changes in myocardial perfusion induced by submaximal exercise which are comparably smaller to those induced by adenosine administration.

Other sources of error may be the acquisition sequence which was designed with exercise in mind. The ShMOLLI technique takes a series of samples of myocardial T1 as at a fixed time interval of 340 ms following an R-wave across 9 seconds (5 + 1 + 1 with 1 beat pauses) to ensure it is in end-diastole. However, myocardial blood flow is heterogeneous throughout the cardiac cycle and can be ~30% higher throughout diastole than systole (Hiramatsu et al., 1998; Motwani et al., 2015; Judd and Levy, 1991). It has been suggested that tachycardia may cause mis-triggering of the pulse sequence and inaccurate T1 estimation which can be partially remedied by the adoption of a time delay that gives an “end-systolic T1 estimation” (Ferreira, V.M. et al., 2015). It remains to be seen whether this is appropriate for the higher heart rates and lower fidelity R-waves seen during exercise. In

individuals who are exercising at 140 bpm (429 ms per beat), it appears likely that at 340 ms interval following an R-wave should result in an end diastolic image acquisition but conversely this same interval should be exceeded by the duration of systole at lower heart rates (Gemignani et al., 2008; Bombardini et al., 2008).

ShMOLLI relies on breath holding of the participant as image acquisition is susceptible to motion artefacts which may be present during exercise. Therefore, measures were taken shortly following the cessation of exercise and only an estimation of T1 time during the exercise off-transient was achievable. This has a number of implications. First of all heart rate, myocardial oxygen demand and coronary perfusion pressure, all determinates of myocardial perfusion, will decline following the offset of exercise. It has been shown that heart rate and blood pressure offset kinetics over a 15-30 second post-exercise period allow for reductions in these parameters and that the immediate post-exercise reduction are greater when cycling at a greater work rate (Baum et al., 1992). This clearly evidences a reduction in the rate-pressure product which is an index of left ventricular work and strongly correlated with myocardial oxygen consumption which would be expected to decline post-exercise. As ShMOLLI was not performed under steady-state conditions during this study, T1 measures instead represent an integrated average value of myocardial T1 across the off-transient from exercise to rest.

In contrast, Siegrist et al. (2014) demonstrate, using positron emission tomography during supine cycling, exercise-induced increases myocardial

blood flow 168% at comparable workloads (109 ± 18 W) and heart rates (133 ± 14 bpm) to work rate 2 in the present study. They also similarly took measures of myocardial blood flow after exercise cessation but after 1 minute. Unlike the present study, using this technique an elevated myocardial blood flow was observed during recovery (~103% above resting value) following exercise which was discordant with the magnitude of reduction in cardiac workload derived from RPP. This elevation was mainly attributed to a sustained reduction in coronary vascular resistance from exercise to the recovery period suggesting that exercise-induced vasodilation remains elevated in the early recovery period. In light of this, we would expect myocardial blood flow to have remained elevated at the point of sampling.

It is well established that sex has an effect on baseline myocardial T1 from ShMOLLI (Piechnik et al., 2013), however any error this introduces is systematic and does not seem to affect relative changes in T1 under different conditions. One factor that may influence our finding would be acute changes in intravascular or extravascular water content with within the myocardium as a result of exercise. It is known that in fluid overloaded patients myocardial T1 is prolonged under resting conditions (Antlanger et al., 2018). Whilst aerobic exercise causes exercise intensity and duration dependent increase in plasma volume, it is unclear whether intravascular water concentration may have changed in this study. Currently it is unclear what the acute effects of exercise on the fluid content of the extravascular compartment of the myocardium are. Though some observations of post-

exercise fluid accumulation in skeletal muscle exist (Fleckenstein et al., 1988), the limited available data in the myocardium suggests sub-maximal exercise would not alter myocardial water content (Kõrge and Viru, 1971; Maher et al., 1972).

T1 has been assumed to be insensitive to the effects of heart rate when using the ShMOLLI technique (Piechnik et al., 2013), however no data exist regarding heart rates of over 100 bpm. Other potential contributors to T1 include the effects of magnetization transfer and sensitivities to changes in T2 (Piechnik et al., 2017). In brief, T2 is the time constant for the decay of the transverse components of magnetization and magnetization transfer refers to the effects to transfers of magnetization between water and other macromolecules on T1 and T2. Both T2 and magnetization transfer are influenced by blood oxygen-level dependent effects because oxyhaemoglobin is diamagnetic and deoxyhaemoglobin is paramagnetic. Deoxyhaemoglobin in red blood cells distorts local magnetic fields causing accelerated dephasing of the transverse magnetization of water molecules and consequent loss of T2. Though T1 is not typically affected by T2, MOLLI based T1-mapping is biased by T2 of the sample via a positive correlation between T1 and T2 (Robson et al., 2013). The relevance of this to the present study is that exercise associated increases in heart rate and myocardial blood flow will accompany an increased rate of oxygen dissociation and increased appearance of deoxyhaemoglobin in the blood, which could in reduce the mean T2 of the blood in the myocardium. As a result, T1 derived from ShMOLLI should decrease accordingly as

deoxyhaemoglobin levels rise with the onset of exercise and with exercise intensity due to increases in myocardial oxygen extraction (Kitamura et al., 1972). Adenosine administration under resting conditions, whilst causing an increase in heart rate and myocardial blood flow, reduces myocardial oxygen consumption (Sollevi, 1986; Kanatsuka et al., 1989). Hence, we might anticipate that under conditions of adenosine administration increases in myocardial blood flow and volume will occur, but there is little change or a slight decrease on deoxyhaemoglobin levels in the myocardium which results in an increase myocardial T1 (Mahmod et al., 2014; Liu et al., 2016). Thus, during exercise and with increasing intensity we may see divergent effects upon myocardial T1 of reductions caused by increasing deoxyhaemoglobin-level and increases due myocardial blood volume (and therefore water volume) augmentation. Nevertheless, the magnitude of each of these effects on T1 is unclear. For these reasons, changes in myocardial T1 with exercise stress may not have been observed in the present study in spite of changes in myocardial perfusion that almost certainly would have occurred. As such, using myocardial T1 mapping in this study, no changes in myocardial perfusion could be assessed and thus no inferences could be made as to the acute effects that exercising at similar intensities to what were observed in outpatient CR in the previous chapter. Furthermore, regional T1 values exhibited no differences in responses to exercise despite the regional differences in perfusion reserve having been demonstrated in an invasive canine model (Austin Jr et al., 1990). It is likely that this phenomenon was not apparent in the present study for similar reasons to

those discussed above that explain the shortcomings of myocardial T1 as an index of myocardial perfusion. A further implication of this study is that the application of myocardial T1 mapping via ShMOLLI in CR patients is inappropriate and that improved or alternative techniques would be required to assess myocardial perfusion during exercise.

A limitation of this study is that only submaximal intensity exercise could be performed. The primary factor limiting this was excessive motion artefact at higher work rates. A potential remedy for this that has been used by other researchers is to adopt a lower cadence to achieve the desired workloads (Siegrist et al., 2014).

4.1.5. **Conclusion**

In the present study we were unable to detect exercise-induced changes in myocardial perfusion using a novel, non-invasive, contrast free technique. Therefore we are unable to make inferences from our data about the effects that exercise intensity has on myocardial perfusion and the stimulus that acute exercise of different intensities has on the coronary vasculature. In addition, this technique is likely not able to assess whether adaptation of the coronary circulatory response to exercise can occur following a period of exercise training.

There are a number of potential shortcomings of using ShMOLLI for T1 mapping to capture changes in perfusion during exercise, many of which may not be easily remedied by employing a different experimental design.

Chapter 5 **General discussion**

This thesis aimed to examine the role of exercise intensity upon the stimulus that exercise provides to the central and peripheral vasculature. In Chapter 3, the effect that a chronic exercise stimulus has on the peripheral vasculature of patients undertaking an outpatient cardiac rehabilitation programme was investigated. The purpose of this study was threefold. First, cardiac rehabilitation is a routinely prescribed therapy following a cardiac event, the primary therapeutic component of this exercise programme is exercise training. Second, the CR population typically has severe CAD and associated endothelial dysfunction. A stated goal of UK cardiac rehabilitation is to enhance endothelial function. Previous studies indicate that this improvement is achievable using exercise training (see Sections 2.3.3, 2.3.6 & 2.3.7). Third, because endothelial function is prognostic of up to 40% of CV event risk and emerging evidence finds that CR is not effective for improving the long-term outcomes of patients (at least in the UK, at in all but the most potent CR programmes). Therefore, it is arguable that the exercise stimulus presented in UK CR may be inadequate to improve endothelial function by a by a magnitude that confers health benefits. By extension, CR would not contribute to an improvement in any long-term outcomes that are mediated by endothelial function or other aspects of vascular integrity.

The authorreasoned that a dose-response relationship between exercise intensity and vascular adaptations to exercise training exists or at least a minimum effective exercise intensity must exist to confer beneficial effects upon vascular health. The dependence of vascular adaptations upon this exercise intensity is highly likely to be driven by exercise intensity dependent exposure to and mechano-transduction of endothelial shear stress; the primary haemodynamic signal driving vascular adaptations.

The initial findings in Chapter 3 Part 1 appear to indicate that the majority of patients that participated in the CR programme did not accumulate a sufficient dose of exercise to influence vascular health. No changes in assessments of endothelial function or arterial stiffness were observed and the intensities of exercise achieved during CR sessions were inferior to the intensities that were prescribed in studies using comparable cohorts that did observe marked improvements in endothelial function. The intensities achieved were frequently below the lower boundary of the prescribed intensity range in UK CR of 40-70% of HRR. As such, it was hypothesised that through the implementation of an intervention to improve practice then these standards may be met. Should exercise intensity increase with the intervention, the exercise dose received by patients undertaking CR may be sufficient to stimulate improvements in vascular integrity, thus, demonstrating that the current UK CR model can positively impact upon vascular integrity. Additionally, this intervention was implemented without the requirement of extra resource as intensity is the only manipulable element of

exercise dose that does not require additional resource to increase. This is particularly important in resource limited healthcare models.

The intervention failed to have an impact upon the exercise intensities achieved by patients recruited from a second subsequent cohort following the implementation of an educational intervention for CR staff. In addition, this “improved practice” cohort did not display an augmented response to the exercise training. No data were collected that could provide insight as to why the intervention or the implementation of the intervention was unsuccessful. Despite the important portrayal of the resistance of CR to a change in practice, these studies were not designed to and lack the ability to provide insight as to why the chosen intervention failed. There is no doubt that further research is required to understand the implementation of changes in practice in CR contexts.

As the intervention had no apparent effect on the exercise stimulus received by patients in CR and patient responses to CR appeared to be notably variable, further analysis allowed examination of factors that may have influenced patient responses to CR. This analysis revealed that achieving a greater mean exercise intensity in the final week of the programme was associated with more positive changes in FMD and changes in walk test performance. No other associations between exercise intensity and short-term outcomes following CR could clearly be discerned. The achievement of a greater exercise intensity towards the end of the 6-week CR programme cannot be solely attributed to physiological factors as neurobiological and psychological factors will have a bearing on the selection of exercise

intensity, which was largely volitional and evidently not tightly controlled. However, that this intensity selection had some predictive power over changes in walk test performance and FMD signifies that it may indeed reflect a physiological response to the CR programme. Choosing to exercise at higher intensity at this stage of the programme could plausibly be related to improvements in exercise capacity and vascular health. It is unlikely that this denotes a training stimulus *per se* as the time between the session and subsequent outcome measures is too brief (typically < 1 week) to expect overt vascular adaptations to occur.

A major contribution of this study chapter to the existing literature regarding exercise-based CR is to provide insight into the highly variable but generally low exercise intensities achieved by patients in a typical outpatient UK CR programme. Powell et al. (2018) highlight the dearth of information available about the exercise intensities achieved – not simply prescribed – by patients in CR internationally. Further doubts about fidelity to which exercise prescriptions are achieved in CR have been cast by Mitchell, B.L. et al. (2018) in a recent meta-analysis. They found considerable variation in the gains in CRF across CR programmes with similar or identical exercise prescriptions. This finding would be unusual if the exercise dose achieved by patients across all studies analysed was tightly controlled. Extrapolation of the findings presented in Chapter 3 to other CR programmes would suggest that variability in the exercise intensities achieved by patients undertaking CR in part explains a variation in responses to CR programmes.

Furthermore, as habitual physical activity was unaffected by the CR programme, physical activity mediated improvements in endothelial function and other CVD risk factors in the period following CR participation are unlikely to occur. That physical activity behaviours are unaffected by CR is a common finding (Dibben et al., 2018; Anderson, L. et al., 2017). On one hand, the lack of effect of CR upon physical activity should be addressed to improve the effectiveness of CR as a secondary prevention tool. This is emphasized by the findings of Sixt et al. (2009) who showed that in diabetic CAD patients the benefits of an extensive 4-week exercise training programme on coronary artery endothelial function and markers of inflammation were small but following 5 additional months of ambulatory home-based exercise were more pronounced. This suggests that with short duration exercise programmes it may be possible that a sufficient exercise dose to meaningfully impact parameters of health is unachievable and that longer programmes are required. On the other hand, this places an increased emphasis upon the requirement for CR to provide a potent exercise stimulus to augment patients' physiological capacities to the extent required to modify CV event risk and improve long-term outcomes. Hence, another key contribution of these studies is to highlight the impotence of a 6-week UK CR programme to influence vascular health, other CVD risk factors and physical activity behaviours, a finding which is in line with the lack of effect UK CR has upon long-term outcomes (West et al., 2012).

The strongest prognostic marker of long-term outcomes following CR is CRF (Kavanagh, T. et al., 2002; Kavanagh, Terence et al., 2003; Keteyian et al., 2008). Barons et al. (2015) demonstrate in an 11 year follow-up of a phase IIII UK CR programme that baseline CRF and improvements in CRF following CR were the strongest predictors of all-cause and cardiovascular mortality. In this study the mean change in CRF was 3.81 ml/kg/min, which contrasts with more recent UK CR data suggesting a lack of change in CRF (Nichols, S. et al., 2018) or at least more modest improvements (Sandercock, G.R. et al., 2013). Still, this may be due to the use of different exit criteria for the CR programme, which extended programme duration though this was unreported. Similarly, De Schutter et al. (2018) show that ~23% of patients undergoing CR do not improve CRF following a 36 session CR programme and that this was associated with a 2-fold and 3-fold higher all-cause mortality, respectively, than those who improved their CRF by 0-2.5 ml/kg/min or > 2.5 ml/kg/min. Therefore, improving CRF is an focal goal of CR.

Improvements in CRF with exercise training are dose-dependent (Vanhees et al., 1995; Huang, G. et al., 2016). Increases in exercise dose may be achievable through increasing the duration of the CR programme as UK CR is typically half the duration of typical CR programmes (Sandercock, G. et al., 2013), though this would require additional resources to deliver. Increases in exercise intensity would increase the exercise dose delivered without this drawback. Higher intensity exercise programmes tend to cause greater improvements in CRF and also FMD (Ramos et al., 2015). The

observation of greater improvements in CRF with higher intensity training has also been demonstrated in CR (Keteyian et al., 2014). Moreover, the effects of a higher intensity CR programme upon CRF can persist for at least 30 months (Moholdt et al., 2011), though whether any effects upon vascular integrity are sustained for this length of time is unknown. That higher intensity exercise training exerts a greater effect upon FMD than moderate intensity training contrasts with the studies discussed in sections 2.3.1 and 2.3.3, which propose a superior effect of moderate intensity training upon endothelial function. The concurrent increases in CRF and FMD described in the meta-analysis of Ramos et al. (2015) may reflect a causal link between changes in peripheral endothelial function and CRF (Montero, 2015). From a mechanistic perspective, the mediation of CRF by peripheral endothelial function may act through its effect on cardiac output. Maximal cardiac output is a major determinant of CRF (Bassett Jr and Howley, 2000) and peripheral vasodilation is essential for increases in cardiac output (Bada et al., 2012). Hence, improving endothelial function or treating endothelial dysfunction with exercise training may present an important mechanism by which exercise training leads to improvements in CV risk.

Following these studies, in Chapter 4, a novel technique was trialled to assess the acute stimulus that exercise performed at similar heart rates to those achieved in CR would have on the central microvasculature. The reasons for undertaking this study were manifold. Exercise provides a stimulus to both the peripheral and the central vasculature. The chronic stimulus provided by exercise is defined by the characteristics of repeated

acute exercise stimuli. Hence, knowledge of how to manipulate an acute exercise stimulus to the central vasculature is key to influence the chronic stimulus that is delivered by exercise training - as in the context of CR.

Central vascular physiology may have a closer (or at least differing) relationship to patient health and outcomes than the peripheral vasculature, particularly in CVD populations. After all, it is typically when the patency of the coronary vessels is challenged that the majority of CAD related pathological events occur. Indeed, much of the purpose of measuring the physiological properties on the peripheral blood vessels has been to make inferences about the properties and health of the central blood vessels. Though the onus of disease may be on cardiac tissue, the effects of exercise upon the vasculature in humans has largely been explored in the peripheral vasculature, presumably due to the greater availability of non-invasive techniques to assess the peripheral vasculature during exercise. The use of the technique that was trialled in this chapter aimed to provide a non-invasive means of assessing myocardial perfusion with exercise and examine the effects that exercise intensity had upon myocardial perfusion. To date, no therapy exists that has been demonstrated to improve perfusion of the central microvasculature in humans, even though a clear rationale for the potential for exercise training to achieve this goal has been highlighted from highly-invasive experiments in animal models. The rationale for the use of this technique was based upon studies that showed that myocardial T1 (a property of myocardial tissue) increased with the administration of adenosine

(a potent vasodilator) which was reasoned to be caused by an increase in perfusion of the myocardium.

Alas, no changes in the index of myocardial perfusion were observed with exercise at two different intensities in this study despite increases in established factors that are determinants of myocardial oxygen consumption – the major driver of increases in coronary blood flow. Therefore, changes in myocardial blood flow of far greater magnitudes than could be inferred from this data were very likely to have occurred in reality, but the measurement technique employed was unable to detect such changes. Several reasons for this finding are discussed in Section 4.1.4.1. Whilst, on one hand the shortcomings of non-contrast enhanced myocardial T1-mapping during exercise were grounded in technological insufficiencies, the approach used may have also suffered from a lack of accounting for the different physiological effects of exercise-induced versus adenosine-induced hyperaemia upon the magnetic properties of the blood. The substantial motion artefacts that emerge when exercising at heart rates of ~ 140 bpm also present another challenge. Furthermore, myocardial T1 mapping is sensitive to changes in myocardial blood volume rather than blood flow - which is more physiologically relevant. Changes in myocardial blood volume in the absence of changes in myocardial blood volume would be inane. Some techniques such as arterial spin labelling have demonstrated that quantification of myocardial blood flow throughout the cardiac cycle is achievable under resting conditions (Motwani et al., 2015), but the application of these recently developed techniques to exercise CMR may

currently prove challenging. With the advancement of new technologies and processing methods, it may be possible in the future to overcome these limitations; however, at present, alternative modalities necessitating the use of contrast agents may be required.

A contribution of Chapter 4 was the demonstration that left and right ventricular volumes could be assessed during exercise with reasonable reliability, which has not been previously demonstrated with CMR. Since performing this study, the feasibility of capturing left (but not right) ventricular volumes during exercise has been demonstrated for the first time in the published literature by Beaudry et al. (2018) who show very similar changes in left ventricular volumes but lack assessments of reliability. It appears that assessments of global longitudinal strain rate during exercise using current MRI techniques are unreliable but do show increases with exercise intensity as expected. As the predominant modality utilised to assess region strain and other kinetic properties of the heart during exercise is echocardiography - which is far more operator dependent - then a comparison of the accuracy and reliability of the two modalities during cycle exercise is warranted. The findings in Chapter 4 suggest that exercise CMR may allow reproducible assessment of the responses of cardiac output to submaximal exercise. This may provide some insight as to how exercise training may contribute to endothelial function mediated improvements in cardiac output and even cardiac output mediated improvements in CRF.

A strength of CRF as a prognostic marker is that it confers information about integrative physiological systems such as the function of mitochondria in

skeletal muscle, the perfusion of the skeletal and cardiac muscle, the capacity and efficiency of the vasculature to regulate blood flow and neuromuscular function. The same could be said of endothelial function, even though it is a lower level component of CRF because it confers information about CVD risk factors inflammation, thrombotic risk and atherosclerotic risk that are independent of CRF (Hartman et al., 2018). For example, a shortcoming of a single regulatory pathway in the vasodilator systems may be masked through multiple redundant vasodilator mechanisms that compensate and allow normal function (Duncker and Merkus, 2007). A weakness of being limited to measures such as CRF, which reflect higher-level physiological systems, is that it is more challenging to fully understand the mechanisms of improvements in health or long-term outcomes. A hypothetical example of this is that improvements in CRF may reflect the relief of a non-specific system limiting pathology – such as peripheral endothelial dysfunction – resulting in an improvement to the function of the system as whole but little effect on other systems such as skeletal muscle oxidative capacity. There is a difference between improving CRF with exercise through its pluripotent effects on multiple systems and the co-ordination thereof and treating a single physiological systems that is limiting to CRF and observing an equal improvement in CRF. Skeletal muscle weakness or myopathy in the quadriceps, for example, could limit the oxygen demands of skeletal muscle during cycle exercise, which by extension limits exercise hyperaemia and the shear stress stimulus provided to the peripheral vasculature. Thus, an individual with skeletal muscle

weakness requires a lesser cardiac output and a lesser blood flow to the coronary circulation, diminishing the exercise stimulus provided to the central circulation. Likewise, limitations to central perfusion would limit cardiac output during exercise presenting a constraint to the aerobic exercise stimulus that is achievable in the peripheral vasculature and skeletal muscle. The prognostic advantage of a higher CRF may be derived from the lack of a pathology that imparts a physiological bottleneck or rate-limiting step to the delivery or utilisation of oxygen in one or several systems. Exercise training, being a pluripotent therapy, therefore acts upon multiple systems to alleviate defects which are often co-morbid, however the mechanism of gains in CRF may be driven by correction of different systems in different individuals (Houstis et al., 2018). As exercise prescription is highly manipulable, improving the efficacy of CR to enhance CRF may require determination of the physiological limitations to CRF in the heterogeneous CR population and tailoring of exercise prescriptions to enhance specific qualities. Improving peripheral endothelial function may more important than improving myocardial perfusion or cardiac function in an individual whose skeletal muscle has an impaired ability to extract oxygen. Treating an ischemic heart may be less important than treating obesity the associated peripheral microvascular dysfunction in a given individual. Knowledge of patient oxygen uptake pathway limitations would provide insight as to contribution of the vasculature to CRF in healthy and disease diseased populations, as well as allowing prioritisation of exercise therapies that provide maximize individual benefits based on a patient phenotype. This

would serve to enhance the effectiveness of CR programmes, but first more pressing issues concerning the effective delivery of CR programmes need to be addressed.

5.1. **Conclusion**

In summary, this thesis sought to examine the effects of exercise intensity upon the integrity of the central and peripheral vasculature. To date there has been limited available evidence examining the effectiveness of the exercise performed in CR upon vascular health and little attention paid to the potency of the exercise stimulus delivered in CR. Despite the growing prescription of exercise to modify CVD risk, less is known about the determinants of the exercise stimulus presented to the central vasculature, hindering the optimisation of exercise prescription. Furthermore, a dearth of research currently exists surrounding the effects of exercise upon the central vasculature in human subjects, particularly in CVD populations. Therefore, the findings of this thesis shed some light upon these issues. The dose of exercise achieved using the current UK CR model appears inadequate to improve vascular health. A clear potential avenue to remedy this is by trialling higher intensity exercise interventions in this population and assessing the impact of these upon the vasculature. Additionally, it remains to be seen whether the exercise prescribed in the current UK CR model has an impact upon central vascular function. Broadly, the contributions of the elements of the stimulus presented by exercise upon central vascular

adaptations are still unclear and warrant further attention. With advancements in current technology, novel modalities of assessment of the central circulation during exercise may enable these discoveries to be made.

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