THE UNIVERSITY OF HULL

The effect of structured exercise training on endothelial function in patients with coronary artery disease

being a Thesis submitted for the Degree of MSc Sport and Exercise Science

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by

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Abstract

Background: Endothelial function and arterial stiffness have shown to be predictive of cardiovascular risk.

Aim: The study sought to investigate the effect of a current 8-week exercise based cardiac rehabilitation (CR) programme in the UK on endothelial function. Blood-borne biomarkers known to affect endothelial function including intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and C-Reactive Protein (CRP) were measured and correlated with measures of pulse wave velocity (PWV), a surrogate marker of arterial stiffness.

Methods: Patients were randomised to either the intervention (exercise training) or control group (patients who were eligible but refused to participate in the CR programme) following a cardiac event 4-6 weeks prior to the start of CR. All patients were on optimal pharmacological therapy. The intervention consisted of 8 weeks x 2 sessions per week (a total of 16 supervised training sessions equivalent to Phase III) at 40-60% of heart rate reserve (HRR), starting with 10 min of exercise training and progressing to 30 min depending on individual progress in week 8. Baseline and 8-week measurements of PWV and blood-borne biomarkers of endothelial function including ICAM-1, VCAM-1 and CRP were measured.

Results: 28 patients (mean age 62 ± 8 years; 86% male) were recruited (n=20 intervention; n=8 controls). There was a significant difference in the prevalence of Type 2 diabetes between the intervention and the control groups (P=0.004) at baseline. After 8 weeks, no statistically significant changes were found between groups for all measures of pulse wave velocity including cf-PWV, ba-PWV and aortic PWV (all P>0.05). Likewise, no changes were found between groups for blood-borne biomarkers including C- reactive protein, ICAM-1 and VCAM-1 (all P>0.05). There were no significant associations between PWV measures and blood biomarkers after 8 weeks. Weak associations were found between ba-PWV and ICAM-1 (r = -0.090, P=0.706), ba-PWV and VCAM-1 (r =0.304, P=0.192), and ba-PWV and CRP (r =0.317, P=0.174). In addition, there were no significant differences in cardiorespiratory fitness changes as a result of CR between groups (P=0.891).

Conclusions: A short-term CR programme consisting of 16 supervised training sessions does not improve markers of adhesion, inflammation or arterial stiffness in patients with cardiovascular disease. Further investigation is required to determine the appropriate training volume to induce favourable adaptations in endothelial function.

Dedication

To my cousin Dean Petersen, who was taken from us far too young, you had one of the biggest, kindest hearts and a sense of humour that left everyone around you smiling and laughing. You were not only an inspiration to me, but to everyone who was blessed enough to have you in their lives.

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Abbreviations List

ACS- Acute Coronary Syndrome

ADMA- Asymmetric Dymethylarginine

BACR- British Association of Cardiac Rehabilitation

ba-PWV- Brachial Ankle Pulse Wave Velocity

BP- Blood pressure

CAD- Coronary Artery Disease

cf-PWV- Carotid Femoral Pulse Wave Velocity

CG- Control group

CHD- Coronary Heart Disease

CHF- Congestive Heart Failure

CPET- Cardiopulmonary exercise test

CR- Cardiac Rehabilitation

CRF- Cardiorespiratory Fitness

CRP- C-Reactive Protein

CV- Cardiovascular

CVD- Cardiovascular Disease

DBP- Diastolic Blood Pressure

DHD- Degenerative Heart Disease

ECG- Electrocardiogram

ECM- Extracellular Matrix

EDTA- Ethylenediaminetetraacetic acid

eNOS- Endothelial Nitric Oxide Synthase

EPC- Endothelial Progenitor Cells

FMD- Flow Mediated Dilation

HSCRP- High Sensitivity C-Reactive Protein

ICAM-1- Intracellular Adhesion Molecule- 1

IG- Intervention group

IL- Interleukin 6

LDL- Low Density Lipoprotein

LDL-C- Low Density Lipoprotein Cholesterol

NO- Nitric Oxide

NOS- Nitric Oxide synthase

PWV- Pulse Wave Velocity

RAMIT- Rehabilitation after Myocardial Infarction Trial

SBP- Systolic Blood Pressure

SD- Standard deviation

SMC- Smooth Muscle Cells

SST- Serum separating tubes

TNF-α- Tumour Necrosis Factor Alpha

UK- United Kingdom

USA- United States of America

VCAM-1- Vascular Cell Adhesion Molecule- 1

CHAPTER 1- Problem Clarification

1.1 Introduction

Coronary artery disease (CAD) remains the main cause of mortality in developed countries. The role of cardiac rehabilitation (CR) after acute coronary syndrome (ACS) is well established. Interventions are designed to improve cardiovascular (CV) risk factor profiles by increasing compliance to positive lifestyle modifications such as structured exercise training (Cesari et al, 2013). The most substantial evidence-base for the benefits of CR exercise training includes improvements in cardiorespiratory fitness (CRF). Improvements in CRF are strongly linked with improved morbidity and mortality rates, as a consequence of risk factor reduction leading to improved endothelial function.

Endothelial dysfunction and low-grade vascular inflammation play a key role in CAD and other manifestations of atherosclerosis (Cho et al, 2009), (Hansson, 2005). Atherosclerosis is a dynamic and gradual process of endothelial dysfunction and chronic low-level inflammation (Ribeiro et al, 2010). Atherogenesis occurs when a complex interaction of the cells within the vascular wall, monocytes, T-lymphocytes, pro-inflammatory cytokines, chemokines and growth factors stimulate acute phase protein production. This process causes a proliferative process of plaque production and ultimately plaque rupture. The development of atherosclerotic plaque includes the over-expression of cellular adhesion molecules, which promote the recruitment of blood mononuclear cells, and the enhancement of the endothelial layer permeability, in turn, facilitating the diffusion of low-density lipoproteins (LDL) to the intima (Hansson, 2005). Circulating leucocytes do not adhere to the healthy endothelium; but during lesion formation the endothelium expresses cell adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), to which circulating leucocytes selectively attach (Ribeiro et al, 2010).

In the same way, chemoattractant stimuli originating in the vascular wall promote migration of leukocytes into the intima, where the macrophage colony-stimulating factor stimulates the differentiation of monocytes into macrophages. The macrophages express scavenger receptors that allow them to engulf and modify oxidized lipoproteins and become foam cells. These lipid-laden phagocytes secrete a number of inflammatory mediators, such as several

cytokines (for instance, interleukin [IL] -1, IL-6 and tumour necrosis factor-α [TNF-α]), that amplify inflammation in the vessel wall and can contribute to additional leukocyte accumulation, smooth muscle cell proliferation, and extracellular matrix remodelling (Aldous, 2013), (Ribeiro, 2010). Therefore, the increased circulating levels of IL-6 will further induce the hepatic synthesis of acute-phase proteins, including fibrinogen, C-reactive protein (CRP) and serum amyloid A (Figure 1.) IL-6 also acts as an amplifier of the acute-phase response, which is accountable for the augmentation of the expression/production of adhesion molecules, other cytokines, LDL uptake by macrophages, and for the diminution of nitric oxide (NO) bioavailability (Aldous, 2013), (Ribeiro et al, 2010).

Simultaneously, the inflammatory response contributes to the pro-thrombotic blood environment nearby the areas of endothelial dysfunction and may consequently lead to the formation of thrombi that could lead to acute coronary events (Ribeiro et al, 2010). Atherothrombosis is recognised as a dynamic chronic inflammatory process of the vessel wall in which phases of inflammatory and thrombotic activity underlie the clinical presentation of acute coronary syndromes (ACS). Research has suggested that inflammatory markers, such as high-sensitivity C-reactive protein (hs-CRP), provide an alternative method for global assessment of cardiovascular risk (Figure 2.) (Slater and Rill, 2004). Several prospective studies have shown that plasma levels of hs-CRP are a strong independent predictor of risk of future vascular events among individuals with and without known cardiovascular disease (Aldous, 2013), (Milani *et al*, 2004).

Nitric Oxide (NO) is released by the endothelium as a potent vasodilator and inhibits attachment of neutrophils to endothelial cells and the expression of adhesion molecules. NO in high concentrations inhibits the proliferation of smooth muscle cells. Therefore, under all conditions where an absolute or relative NO deficit is encountered, the process of atherosclerosis is being initiated or accelerated (Sydow & Münzel, 2003), (Anderson, 2006). In the L-arginine–NO pathway, NO synthase (NOS) converts L-arginine to NO and L-citrulline. Asymmetric dimethylarginine (ADMA) is an endogenous competitive NOS inhibitor, which can modulate NO production. Several studies have indicated an association between high ADMA and endothelial dysfunction suggesting that ADMA may be a circulating marker of endothelial dysfunction and early atherosclerosis (Päivä et al, 2006).

Since ADMA competes with L-arginine for the NOS binding site, a decrease in L-arginine concentration relative to ADMA may decrease the ability to produce NO and increase arterial stiffness which can be measured by flow mediated dilation (FMD) and pulse wave velocity (PWV) (Cho et al, 2009). Since PWV is considered a feasible method to estimate arterial stiffness it is thought it may be able to predict cardiovascular events and mortality risk (Masaki et al, 2014).

The understanding that endothelial dysfunction and vascular wall inflammation play a key role in atherogenesis and in the stability of the established plaques provides insight into novel therapeutic targets. Thus, endothelial dysfunction and inflammation biomarkers become promising targets for prevention of CAD and exercise training has emerged as an effective non-pharmacological means of improving endothelial function (Ribeiro et al, 2010). The benefits of exercise based cardiac rehabilitation on CAD risk factors, exercise tolerance and cardiac morbidity and mortality have been widely established, however, there is still controversy regarding the optimal exercise characteristics to bring about the most beneficial effects in patients with CAD (Conraads et al, 2015). Recent studies have concluded that UK based CR is only one third the volume of prescribed CR typically received in the USA and Europe (Sandercock et al, 2012). A multi-centre trial that observed the effect of comprehensive CR following acute MI in a sample from hospitals across the UK found that although they do contribute to patient care, no benefits to secondary prevention has been found (West et al, 2012). RAMIT (rehabilitation after myocardial infarction trial) conducted by West et al (2012) still represents the most detailed randomised control trial of CR in the UK to date (Sandercock et al, 2012). The current study sought to investigate the effect of a typical UK based CR programme on specific markers of endothelial function.

CHAPTER 2 -Review of Literature

2.1 Coronary Artery Disease

In the developing nations, CAD is rapidly increasing, and ischemic heart disease is currently the leading cause of mortality worldwide (Conraads et al, 2015). As the population of the industrialised world ages, the impact of CAD on morbidity and mortality is expected to increase, reducing the rate of decline of the last 40 years (Slater and Rill, 2004). During the last decade, there has been considerable advancement in our understanding of the pathophysiology of atherosclerosis as well as major developments in the treatment of the disease. The realisation that the primary prevention (treatment of individuals before there is a clinical manifestation of illness) effectively reduces the impact of the disease has made the value of early disease detection even more important (Slater and Rill, 2004). The new pathophysiological pattern that has emerged in recent years is now leaning towards assigning the mediators of inflammation a much larger role in the disease process. This pattern has helped explain the unpredictable nature of many adverse consequences of CAD. The long latent phase of the disease as well as the sudden initial presentation, makes the efforts at finding a means of early detection extremely important (Slater and Rill, 2004). Considerable research efforts have been dedicated towards identifying, as well as influencing the predisposing risk factors that result in the development of arteriosclerosis. Novel markers of inflammation such as C-reactive protein have been identified and compared to traditional risk factors (Figure 2). In addition, new imaging modalities introduce the possibility of screening for subclinical disease (Slater and Rill, 2004). Also the appreciation of arterial remodeling (compensatory enlargement) has also expanded attention beyond stenosis evident by angiography to encompass the biology of non-stenotic plaques.

Revascularisation can effectively relieve ischemia, but we now recognise the need to attend to the non-obstructive lesions as well. Aggressive management of modifiable risk factors reduces cardiovascular events and should accompany the appropriate revascularisation (Libby, 2005). It is also currently believed that most unpredictable cardiovascular events arise from the fracturing of a fibrous cap that separates the lipid core in the wall of the coronary artery from the flowing blood in the lumen. A large amount of scientific investigations have focused on the composition of this fibrous layer, which has been shown

to be undergoing continuous remodelling. It has been hypothesised that a dynamic exists between synthetic and degradative processes in the vessel wall mediated by cytokines, which are secreted peptides and proteins that mediate local interactions between various cells (Slater and Rill, 2004).

These molecules are involved in the dynamic changes in arterial wall anatomy and plaque morphology, which are part of the long-term accommodation of the arterial lumen to the accumulation of lipids. Most cytokines are believed to be released by cells participating in inflammatory reactions set in motion by the presence of lipid particles in the vessel walls. At sites where plaques have ruptured, causing fatal coronary thrombosis, there are typically fewer smooth muscle cells as well as a relatively thin fibrous cap. Recent work has shown that elastolytic cathepsins expressed in atheroma and regulated by inflammatory mediators can weaken the protective fibrous cap. It has been known for many years that intramural plaque accumulation renders endothelial function abnormal and previously vasodilatory stimuli become vasoconstrictors. In addition, the normally protective endothelial cells begin to secrete prothrombotic tissue factor in response to inflammatory mediators, which alters the balance unfavourably between normal blood flow and thrombosis (Slater and Rill, 2004).

In addition when the arterial endothelium encounters certain bacterial products or risk factors as diverse as dyslipidemia, vasoconstrictor hormones involved in hypertension, the products of glycoxidation associated with hyperglycemia, or pro-inflammatory cytokines derived from excess adipose tissue, these cells augment the expression of adhesion molecules such as ICAM-1 and VCAM-1 that promote the attachment of blood leukocytes to the inner surface of the arterial wall (Figure 1.)Transmigration of the adherent leukocytes depends in large part on the expression of chemoattractant cytokines (mentioned previously) regulated by signals associated with traditional and emerging risk factors for atherosclerosis. Once resident in the arterial intima, the leukocytes, mainly mononuclear phagocytes and T lymphocytes, communicate with the endothelial and smooth muscle cells (SMCs), which are the endogenous cells of the arterial wall. Major messages exchanged among the cell types involved in atherogenesis depend on mediators of inflammation and immunity, including small molecules that include lipid mediators such as prostanoids and other derivatives of

arachidonic acid, e.g., the leukotrienes. Other autacoids, such as histamine, classically regulate vascular tone and increase vascular permeability. (Libby, 2005)

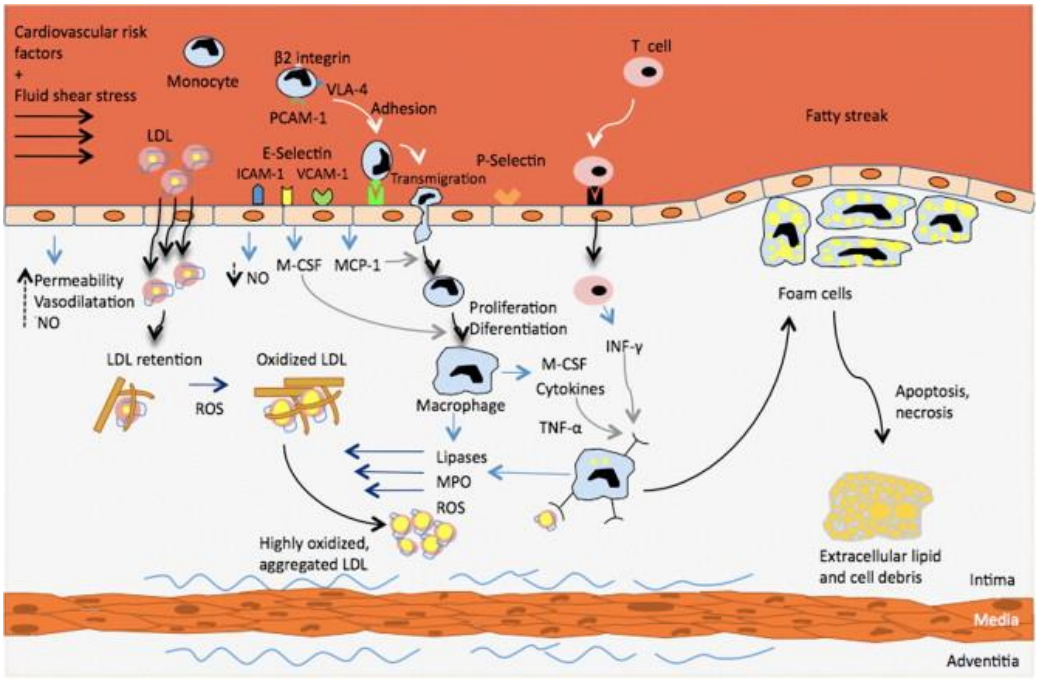

Figure 1. A Schematic representation of the atherosclerotic process. (Ribeiro et al, 2010).

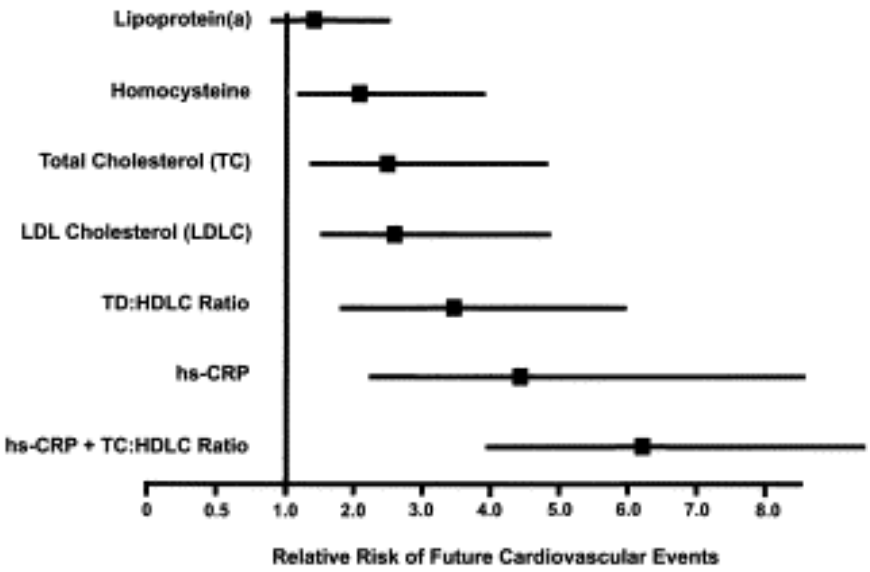

Figure 2. Direct comparison of magnitude of relative risk of future cardiovascular events associated with HSCRP (hs-CRP), cholesterol levels, lipoprotein(a), and homocysteine among apparently healthy women (Slater and Rill, 2004)

2.2 Endothelial function

Endothelium is a monolayer of cells covering the inner surface of the blood vessels acting as a functional and structural barrier between blood and the vessel wall (Marti et al, 2012). The interface prevents platelet and leukocyte adhesion and aggregation, controlling permeability to plasma components and modulating flow (Marti et al, 2012). Healthy endothelium is responsible for regulating vascular tone by balancing the production of vasodilators and vasoconstrictors, such as nitric oxide (NO) and prostacyclin as well as other biochemical mediators, in response to various stimuli, to regulate vascular structure and function (Marti et al, 2012), (Tousoulis et al, 2012).

Endothelial dysfunction (ED) is characterised by a shift of the actions of the endothelium toward reduced vasodilatation, a pro-inflammatory state, and demonstrating pro-thrombic properties. It is associated with most forms of CVD, such as hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure (Endemann and Schiffrin, 2014). ED observed in these conditions, is characterized by decreased NO bioavailability and increased oxidative stress. Specifically, the increased oxidative stress up-regulates the expression of many adhesion molecules, such as vascular adhesion molecule-1, intracellular adhesion molecule-1, E- and P-selectin, which are all redox sensitive (Tousoulis et al, 2014). As a result, ED cintributes to the development of artherosclerosis by promoting inflammation, oxidation of lipoproteins, platelet aggregation and thrombus formation (Tousoulis et al, 2014). The focus in the past decade has been on the endothelial layer of the large conduit vessels and the vascular lumen. However, the vascular bed extends into the vascular wall and the adventitial vasa vasorum, which is also considered an active intravascular microcirculation and is lined by endothelium. Thus, the concept of endothelial function should be extended to the vascular wall and the adventitia (Lerman and Zeiher, 2005). Upregulation of adhesion molecules, generation of chemokines such as macrophage chemoattractant peptide-1, and production of plasminogen activator inhibitor-1 participate in the inflammatory response and contribute to a prothrombic state (Endemann and Schiffrin, 2014). Vasoactive peptides such as angiotensin II and endothelin-1; the accumulation of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide inhibitor; hypercholesterolemia; hyperhomocysteinemia; altered insulin signalling; and hyperglycaemia can contribute to these different mechanisms. Detachment and apoptosis of

endothelial cells are an associated phenomena. Endothelial dysfunction is an important early event in the pathogenesis of atherosclerosis, contributing to plaque initiation and progression. Reductions in circulating endothelial progenitor cells that participate in regeneration of the endothelium participate in endothelial pathophysiology. The severity of endothelial dysfunction is strongly associated with cardiovascular events. Improvement in endothelial dysfunction may be associated with reduced cardiovascular risk. Circulating endothelial progenitor cells may represent a potential therapeutic approach for endothelial dysfunction (Endemann and Schiffrin, 2014).

Circulating markers of endothelial function that can be used to represent the therapeutic approach, are molecules that associate with endothelial injury and/or repair, or products of endothelial cells that change after impairment of endothelium, such as adhesion molecules and cytokines. ADMA is an endogenous NOS inhibitor that increases in patients with cardiovascular risk factors and is related to reduced NO bioavailability (Tousoulis et al, 2014). Both intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are important in various immunological processes early in inflammation. ICAM-1 is a ligand for lymphocyte function associated antigen-1 (LFA-1) and Mac-1, and VCAM-1 is a ligand for α4β1. Respectively; these are found on lymphocytes, monocytes and polymorphonuclear leucocytes (Ino et al, 1997). Circulating leucocytes do not adhere to the healthy endothelium; but during lesion formation the endothelium expresses these cell adhesion molecules (ICAM-1 and VCAM-1), to which circulating leucocytes selectively attach (Ribeiro et al, 2010). In the same way, chemoattractant stimuli originate in the vascular wall and promote migration of leukocytes into the intima, where the macrophage colony-stimulating factor stimulates the differentiation of monocytes into macrophages. The macrophages express scavenger receptors that allow them to engulf and modify oxidized lipoproteins and become foam cells (Ribeiro et al, 2010). These lipid-laden phagocytes secrete a number of inflammatory mediators, such as several cytokines. For example, IL-1, IL-6 and TNF-α which amplify inflammation in the vessel wall and can contribute to additional leukocyte accumulation, smooth muscle cell proliferation, and extracellular matrix remodelling (Ribeiro et al, 2010). This inflammatory response sustained by primary pro-inflammatory cytokines (IL-1 and TNF-α) further stimulate the production of IL-6 in several cell types including smooth muscle cells and endothelial cells which magnify the

inflammatory response beyond the original focal area of endothelial dysfunction (Ribeiro et al, 2010). Therefore, the increased circulating levels of IL-6 will further induce the hepatic synthesis of acute-phase proteins, including fibrinogen, C-reactive protein (CRP) and serum amyloid A (Ribeiro et al, 2010). CRP which is produced in the liver in response to IL-6, is also produced in very limited concentration by non-hepatic cells like neurons, atherosclerotic plaques, monocytes, Kupffer cells, lymphocytes and also upon stimulation by inflammatory cytokines in human coronary artery smooth muscle cells (Chandrashekara, 2014). CRP represents a clinical marker of inflammation and has been demonstrated as a strong predictor of cardiovascular events in initially healthy subjects, and increased CRP is associated with atherosclerosis measures such as higher carotid intima-media thickness and complex plaque in different research populations (Huang et al, 2015).

Bone marrow–derived endothelial stem cells and endothelial progenitor cells (EPCs) may contribute to the repair of vascular injury and play a role in the restoration of tissue repair. The bone marrow contains vascular progenitor cells that can mobilise to the injury site and complement the repair afforded by pre-existing endothelium. Despite experimental evidence demonstrating the contribution of bone marrow–derived progenitor cells to tissue revascularisation, the importance of these cells in repairing vascular damage in the clinical setting remains unknown. (Lerman and Zeiher, 2005)

A wide range of methods are used to evaluate endothelial function, both non-invasive and invasive. Some of these non-invasive measures include flow mediated dilation (Conraads et al, 2015), pulse wave velocity, gauge-strain plethysmography, laser Doppler flowmetry (Anderson et al, 2015), magnetic resonance imaging, peripheral arterial tonometry (Hedetoft and Olsen, 2014) and positron emission tonometry (Tousoulis et al, 2014). Anderson et al (2015) conducted a review, which discussed a variety of measures of peripheral artery function to assess both conduit and resistance vessel function measures. They concluded that FMD, pulse arterial tonometry, laser Doppler techniques, venous occlusion plethysmography and PWV are well established surrogate markers of atherosclerosis and have an important place for clinical research (Anderson et al, 2015). Invasive methods include methods such as quantitative coronary angiography with intracoronary infusion of vasoactive agents,

intrabrachial infusion of vasoactive agents and circulating markers of endothelial function (Tousoulis et al, 2014).

Of the non-invasive methods used to measure endothelial function flow mediated dilation (FMD) is considered to be the "Gold Standard" (Currie et al, 2013), in this study however PWV as a surrogate marker of endothelial function. Flow mediated dilation uses ultrasound to measure flow-mediated changes in arterial diameter in relatively superficial arteries, such as the brachial, radial or femoral vessels. This technique is able to measure endothelial function in conduit arteries rather than resistance vessels. Endothelial function assessed by FMD has been found to correlate strongly with invasive testing of coronary endothelial function as well as with the severity and extent of coronary atherosclerosis (Raitakari and Celermajer, 2000). There are many reasons that this methodology has become widely used. The testing is non-invasive, safe and relatively inexpensive however, it does involve a high degree of operator skill to obtain reproducible results (Anderson et al, 2015). Despite being the most frequently used technique, a closer look at the literature reveals that there are wide variations in mean FMD when different studies in similar populations are compared (Arrebola-Moreno et al, 2012). With regards to normative data for FMD, Currie et al (2013) established that increased cardiovascular mortality and morbidity has been shown to be associated with a relative FMD values estimated at <5.5%.

Although FMD is considered to be the "Gold Standard", in this study PWV was the non-invasive method used to establish arterial stiffness in participants with CAD. It has been established that oscillometric data obtained from an arm cuff inflated to suprasystolic pressure has been validated, showing a strong correlation (Pearson's correlation 0.91; P<.001) with measurements obtained during cardiac catheterization (Arrebola-Moreno et al, 2012). Equipment costs are modest and the procedure is non-invasive. The test is highly reproducible with a co-efficient of variation of 5- 10% (Anderson et al, 2015). However, depending on the method used, results regarding the reproducibility of PWV are still controversial according to Arrebola-Moreno et al (2012).

It has been well established that PWV is age dependent, increasing steadily with advancing age. The relationship of PWV with carotid intima medial thickness is generally positive and there was not a consistent relationship with brachial FMD (Anderson et al, 2015).

According to the AHA, the 6 phases of evaluation of a novel risk marker include: whether the novel biomarker levels differ between subjects with and without outcome; whether the novel biomarker predicts development of future outcomes in a prospective cohort or nested case-cohort study; whether it adds predictive information over and above established, standard risk markers; whether it changes predicted risk sufficiently to change recommended therapy; whether the use of the novel biomarker improves clinical outcomes especially when tested in a randomized clinical trial and whether the use of the biomarkers improves clinical outcomes sufficiently in order to justify the additional costs.
Vlachopoulos et al (2015) suggested that 3 additional steps that are not currently present in the AHA scientific statement should be added in the assessment of vascular biomarkers to qualify as clinical surrogate endpoints. These 3 steps include the ease of use which would allow widespread application, methodological consensus meaning the necessity to allow comparisons between studies and the availability of reference values or cut-off values (Vlachopoulos et al, 2015). Following these steps when reviewing several methods for evaluating vascular biomarkers Vlachopoulos et al (2015) found that on the basis of these criteria, vascular biomarkers can be classified into three groups:
1. Those that fulfil most of the 9 essential criteria and, therefore, are close to being considered a clinical surrogate endpoint (carotid ultrasonography, cf-PWV).
2. Those that fulfil some, but not all of the 9 essential criteria (ba-PWV, central haemodynamics/wave reflections, CRP) and
3. Those that do not at present fulfil the 9 essential criteria (FMD, EndoPAT)

Thus Vlachopoulos et al (2015) with the use of the AHA criteria established that even though FMD is considered to be the most widely used surrogate marker of endothelial function, both cf-PWV and ba-PWV are strong surrogate markers of endothelial function and that CRP can be considered and important putative marker of endothelial function when taking these criteria into account.

2.3 Cardiac Rehabilitation in the United Kingdom

The World Health Organisation's most recent definition of cardiac rehabilitation reads as follows:

"The rehabilitation of cardiac patients is the sum of activities required to influence favourably the underlying cause of the disease, as well as the best possible physical, mental and social conditions, so that they may, by their own efforts preserve or resume when lost, as normal a place as possible in the community. Rehabilitation cannot be regarded as an isolated form of therapy but must be integrated with the whole treatment of which it forms only one facet." (Coats, 1995, pg. xi)

It is clear from this that in order to provide a service which may encompass so many factors a multidisciplinary team of healthcare professionals must be prepared to examine a varied and flexible approach to the provision of CR (Coats, 1995, pg. xi). In the United Kingdom, CR has been slow in its initial development however is now rapidly expanding speciality. The first large scale controlled trial of CR in the UK was published in 1982 by Carson and colleagues and subsequently there was a gradual growth in exercise based cardiac rehabilitation programmes which are usually led by nurses and physiotherapists. By 1992, the British Association for Cardiac Rehabilitation (BACR) was developed. The aims of this association are to:

- Promote a greater awareness and understanding of cardiac rehabilitation throughout the healthcare system.
- Facilitate communication and support among multidisciplinary professionals concerned with the rehabilitation of cardiac patients.
- Set national standards for cardiac rehabilitation and monitor the evaluation of the standards.
- Develop training programmes encompassing a multidisciplinary philosophy.
- Promote and facilitate research
- Liaise with other national and international organizations working in this field.

(Coats, 1995, pg. xii-xiii).

Although the BACR has grown since 1992 (now called the British Association of Cardiovascular Prevention and Rehabilitation; BACPR) there is still a great deal to be done if a high standard of CR service is to be made available to all UK patients who might benefit from it. There needs to be more service providers willing to establish comprehensive programmes and for purchasers to be willing to pay for them as well as sufficient training for CR staff. In order to achieve structure in CR it is important to establish guidelines for good practice and of maintaining ongoing research to ensure service development is well recognised. Since CR is a rapidly developing service, the issue of standards of practice is of considerable importance (Coats, 1995, pg xiii).

CR structure in the UK takes into consideration four stages when creating a CR programme, the in-patient stay, immediate post-discharge period, intermediate post-discharge period, and long term maintenance. Also only certain conditions in the UK make a patient eligible to be considered for CR these conditions include: angina pectoris, cardiac arrhythmias, cardiac failure, hyperlipidaemia and dyslipidaemia, hypertension, transient ischaemic attacks and diabetes mellitus. The recommended time scale to achieve exercise goals set with in the CR guidelines is the attendance of 36 sessions over a three to six month period, although it should be noted that these principles are flexible and an individually orientated time frame should be applied in general. (Coats, 1995). Cardiac rehabilitation teams in the UK are comprised of a multidisciplinary healthcare professional team. When it comes to adherence it is said that as many as 40-50% of patients drop out of there exercise rehabilitation programme within the first 6-12months (Coats et al, 1995, pg 35) of referral due to a variety of different reasons, therefore motivation, accessibility and flexibility are important factors when it comes to improving adherence.

In a survey by Brodie et al (2006) which was compared with the recommendations of the National Service Framework (NSF) for CHD and the Scottish Intercollegiate Guidelines Network (SIGN) guidelines for CR observed that only 30% of the UK population eligible for CR were enrolled in a CR Programme. The survey showed that even for a number as low as 30% there were serious deficiencies in the provision of CR programmes in England (Brodie et al, 2006). They noted that the deficiencies included inadequate staff numbers with a mean shortfall of 37% of that recommended by the SIGN Guidelines; lack of key staff, particularly

with regards to psychologists, pharmacists and dieticians; inadequate premises, with only 57% considered adequate; insufficient exercise sessions, with 79% offering less exercise than recommended by the SIGN guideline; poor record keeping systems and failure to tailor the CR to the patients needs (Brodie et al, 2006). Due to these deficiencies not being in line with the SIGN guidelines they found that English patients were not receiving the benefits of cardiac rehabilitation which might be expected (Brodie et al, 2006).

More recently guidance from the UK Department of Health refers to a seven stage pathway of care that begins with diagnosis of a cardiac event and is followed by assessment of eligibility, referral, clinical assessment, and core delivery of cardiac rehabilitation before progressing to long term management (Dalal et al, 2015).

The BACPR sets out the seven standards and seven core components in support of promoting guidelines for high quality care in the provision of structured programmes for cardiovascular disease (CVD) prevention and rehabilitation in the UK (Bacpr.com, 2015).
The seven standards for cardiac rehabilitation are:
1. The delivery of the seven core components employing an evidence-based approach.
2. An integrated multidisciplinary team consisting of qualified and competent practitioners, led by a clinical coordinator.
3. Identification, referral and recruitment of eligible patient populations.
4. Early initial assessment of individual patient needs in each of the core components, ongoing assessment and reassessment upon programme completion.
5. Early provision of a cardiac rehabilitation programme, with a defined pathway of care, which meets the core components and is aligned with patient preference and choice.
6. Registration and submission of data to the National Audit for Cardiac Rehabilitation (NACR).
7. Establishment of a business case including a cardiac rehabilitation budget which meets the full service costs.

In the UK, formal rehabilitation is predominantly provided to supervised groups in outpatient hospital clinics or community centres, starting 2–4 weeks after percutaneous coronary intervention or myocardial infarction and usually 4–6 weeks after cardiac surgery. The

BACPR standard recommends delivery of the seven core components of cardiac rehabilitation after clinical assessment which is described below (Dalal et al, 2015).

The BACPR seven core components for Cardiac rehabilitation include:

1. Health behaviour change and education
2. Lifestyle risk factor management

- Physical activity and exercise
- Diet
- Smoking cessation

3. Psychosocial health
4. Medical risk factor management
5. Cardioprotective therapies
6. Long-term management
7. Audit and evaluation

(Bacpr.com, 2015).

Exercise-based CR programmes in the UK and specifically in Hull currently comprises of 2 sessions per week at 40-60% of HRR (according to Karvonen estimation). Usually starting with 10min total CV time and increases each week until individuals reach 20 to 30min. Figure 3. shows the general class structure currently being given in the UK.

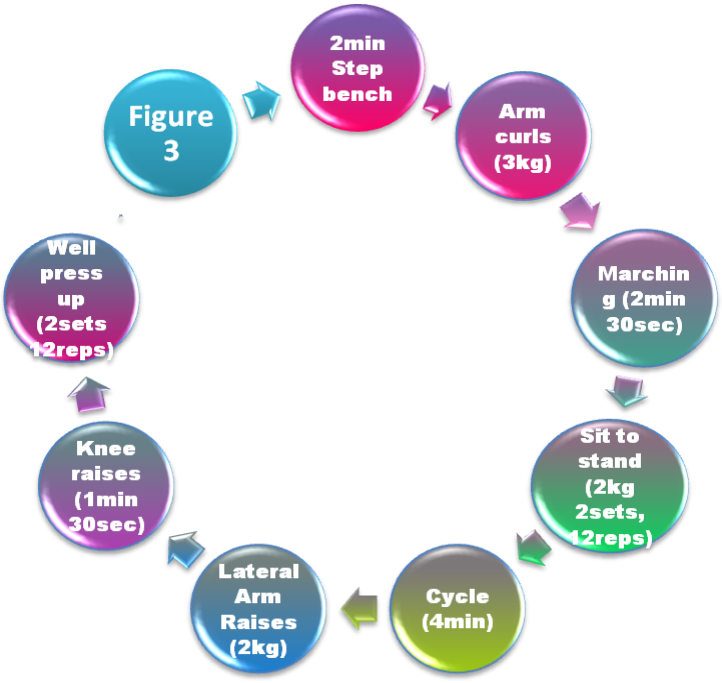

Figure 3. An example of a typical "interval" exercise class structure in the UK

According to Dalal et al (2015) programmes are typically delivered by specialist nurses or physiotherapists supported by exercise therapists, although ideally an integrated multidisciplinary team led by an experienced clinician with a special interest in cardiac rehabilitation should deliver the programme. Most programmes involve weekly attendance at group sessions for an average of 56 (SD 3.6) days or approximately 8 weeks. Centre based sessions involve graduated exercise training, education (covering coronary risk factors and diet), common cardiac misconceptions, preventative medication, and stress management. Ideally, patients should be given information about the cardiac event and lifestyle advice including the importance of smoking cessation (if appropriate), healthy diet, and physical activity to encourage progressive mobilisation (Dalal et al, 2015). Prior to discharge, clinicians should ensure that patients are prescribed drugs for secondary prevention and drugs that are beneficial for those with systolic heart failure such as angiotensin-converting enzyme (ACE) inhibitors and beta-blockers. Good communication between secondary and primary care after discharge can improve uptake of cardiac rehabilitation and optimise secondary prevention (Dalal et al, 2015).

In a study by Almodhy et al (2012) Data was obtained from a UK National Health Service (NHS) Hospital from which the conducted a retrospective analysis of 117 patient (69.7±9.5 years) records. These patients underwent a 6week CR programme with assessments of CRF and standard clinical outcomes at pre- and post-rehabilitation. The primary outcome measure that was used to measure CRF was distance walked during an incremental shuttle-walking test (ISWT). The results observed differences in CRF gain according to age, which may suggest that treating CR patients as a homogenous group in the UK may be misleading. They found that differences in younger patients (≤65 years) may have suggest that it would benefit younger CR patients to do more frequent exercise training as younger patients retain a greater ability to adapt to exercise stimuli or there may be practical or social differences which account for between-age group differences (Almodhy et al, 2012).

According to Sandercock et al (2012) the national guidelines for CR cite evidence from systematic reviews that showed a 20% reduction in mortality of patients who completed an exercise based cardiac rehabilitation programme. However, Sandercock et al (2012) also noted that the studies from which these statistics were derived were conducted 20 to 30 years

ago and were unrepresentative of pharmacological practice. The fundamental difference between UK CR services and the practice reported in many international trials which make up the body of evidence supporting the efficacy of CR is the exercise dose being prescribed. According to evidence, UK patients are only being prescribed one third the amount of exercise that patients in Europe and the USA are receiving (Sandercock et al, 2012). The publication by the RAMIT group posed doubts about the ability of UK CR to reduce patient mortality and morbidity (West et al, 2012).

2.4 Exercise and Endothelial Function

Numerous interventions that improve cardiovascular risk factors and reduce cardiovascular morbidity and mortality also enhance endothelial function (Goanvec et al, 2015). In recent years, the impact of structured exercise training on endothelial dysfunction has received some attention (Higashi and Yoshizumi, 2004). Endothelial dysfunction is a pro-inflammatory state characterised by a shift of the action of the endothelium toward reduced vasodilatation. Endothelial dysfunction is associated with most forms of CV disease, such as hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure (Endemann and Schiffrin, 2014). Several investigators have observed that exercise training improves endothelial function in animal models of hypertension and in patients with hypertension. Regular physical exercise is associated with beneficial changes in blood pressure, lipid metabolism, glucose metabolism, neurohormonal factors, body weight, and shear stress (Higashi and Yoshizumi, 2004). Certain studies have highlighted the importance of exercise in the mitigation of endothelial dysfunction and vascular inflammation, thus new sets of potential explanations for beneficial outcomes of regular exercise in CAD patients (Ribeiro et al, 2010). Exercise training is also said to lead to an improved bioavailability of the endothelial nitric oxide and partially attenuates endothelial dysfunction. Further effects of exercise are the improvement of ventricular function and a reduction of cardiovascular risk factors. In clinical studies exercise training was associated with a decreased total and cardiovascular mortality and a reduced angina pectoris threshold (Wienbergen and Hambrecht, 2013). Regular physical exercise has recently been shown to mobilize EPC from bone marrow. Although eNOS dependency of EPC mobilization was clearly demonstrated in small animal models, the relationship between improvement of NO-

dependent vascular function and the mobilization of EPCs from bone-marrow in response to exercise has not been studied in humans (Steiner et al, 2005).

Reduced bioavailability of nitric oxide (NO) is an important consequence of endothelial damage and is thought to contribute to the development of atherosclerotic vascular disease. Physical exercise augments blood flow and shear stress, resulting in increased NO production and upregulation of endothelial NO synthase activity. Short periods of regular, localised exercise training have been shown to restore flow-dependent dilatation of the systemic arteries of patients with chronic heart failure. (Clarkson et al, 1996)

2.5 Adhesion Molecules

Local accumulation of monocytes (the largest of all leukocytes) in the vascular wall occurs as a result of initial marginalisation within the lumen and rolling of monocytes along the endothelium. Immunohistochemical studies have identified ICAM-1 and VCAM-1 to be involved in monocyte adherence and are usually shown to be upregulated in atheromatous lesions, particularly in the intimal neovasculature (Tousoulis et al, 2001). These adhesion molecules can support the adhesion of various leukocytes including monocytes.

2.5.1 ICAM- 1

ICAM-1 has been significantly associated with the risk of future myocardial infarction (Tousoulis et al, 2001). ICAM-1 has a wide distribution on hematopoietic and non-hematopoietic cells. Expression is moderate on activated T cells, B cells and monocytes and on hematopoietic progenitors. It is constitutively expressed on certain epithelia and endothelia, but expression is high in activated endothelial cells. The ligands (which are substances that form a complex with a biomolecule to serve a biological purpose.) for ICAM-1 are integrins; which are transmembrane receptors that are the bridges for cell to cell, and cell to extracellular matrix (ECM) interactions, such as erythrocytes infected with p.falciparum (a protozoan parasite) and fibrinogen. ICAM-1 binds to integrins through a large extended surface (Greer et al, 2003).

ICAM-1 is associated with ezrin; protein-tyrosine kinase substrate in microvilli; which is a linker between membrane molecules with actin cytoskeleton and regulates cell adhesion and

cortical morphogenesis. The ezrin-ICAM-1 association is regulated by signalling involving phosphoinositides. In small blood vessels in the brain, ICAM-1 ligation induces tyrosine phosphorylation (which is the addition of a phosphate (PO_4^{3-}) group to the amino acid tyrosine on a protein) of the cytoskeletal protein, contactin, which leads to cytoskeletal changes and transmigration of T cells. ICAM-1 is a member of the group of cells that orchestrates leukocyte transendothelial migration (Greer et al, 2003). The VLA-4 integrin functions early in leukocyte adhesion to the endothelium and through cross-talk to β_2 integrin's αL/M β_2, triggers clustering. Initial interaction with ICAM-1 assembles a docking structure, built through ezrin activation by cytoskeleton, on which leukocyte adheres firmly before transmigration. During this process ICAM-1 is exclusively localised on the apical membrane of the activated endothelial cell. ICAM-1 plays a role as an adhesion molecule cell-cell interaction during the induction as well as in effector functions of the immune response, such as T-cell mediated cytotoxicity. (Greer et al, 2003).

The metabolic response to exercise is dependent upon type, duration and intensity, intensive physical activity is associated with oxidative stress. Reactive oxygen species excessively generated during exhausting exercise are capable of reacting with membranes, causing damage to cells and tissues and thus result in an increase of ICAM-1 expression by the endothelial cells (Witkowska, 2005). However regular physical activity is beneficial to certain disease states and is a factor in the reduction of ICAM-1, according to Witowska (2005) this is evident in a randomised cross-over study done on patients with chronic heart failure; they underwent 12 weeks of bicycle training for 30min, 5 times per week at 70 to 80% maximal heart rate which produced a significant fall in ICAM-1. Therefore this evidence may suggest that levels of ICAM-1 concentration in plasma and serum may depend on type, regularity and intensity of training (Witkowska, 2005). A systematic review conducted by Palmefors et al. (2014) supports these findings that type, duration and intensity affect ICAM-1 levels, also suggesting that resistance training alone has no effect on ICAM-1 levels and that there is a high quality of evidence to suggest the decrease in ICAM-1 due to physical activity.

2.5.2 V-CAM 1

VCAM-1 is an immunoglobulin-like adhesion molecule expressed on activated endothelial cells (Ley and Huo, 2001). VCAM-1 binds to $\alpha_4\beta_1$ integrin, which is essentially expressed on lymphocytes, monocytes, and eosinophils. Interestingly, VCAM-1 can mediate both rolling-type adhesion and firm adhesion, depending on the avidity status of $\alpha_4\beta_1$ integrin. According to Ley and Huo (2001) it was initially unclear whether VCAM-1 was simply a marker for atherogenesis or whether it acted in this disease pathway. Evidence does however suggest that circulating levels of VCAM-1 are highly correlated with extent of atherosclerosis and can independently be a predictor of maximum intima-thickness at the carotid bifurcation in patients with peripheral artery disease (Saxton et al., 2008). Tousoulis et al. (2001) noted in their study that VCAM-1 was strongly correlated with the severity of heart failure. Studies with cytokine-activated cultured endothelial cells and reconstitution assays with purified recombinant VCAM-1 protein suggested that VCAM-1 could mediate robust adhesion of $\alpha_4\beta_1$ expressing cells, even under shear flow. However, it remained unclear whether this adhesion could occur in vivo in large arteries of atherosclerosis-prone animals or patients (Ley and Huo, 2001).

Highly suggestive studies have found that the interactions between α4β1 and VCAM-1 can slow down rolling monocytes and that VCAM-1 promotes monocyte adhesion and accumulation on the vessel wall at the sites that are prone to developing atherosclerotic lesions. Other studies reviewed by Ley and Huo (2001) have also shown that VCAM-1 is critical for the development of atherosclerotic lesions in LDL receptor knockout mouse models. This is important to consider due to the LDL receptor in the knockout mouse being a good model of human atherosclerosis and can also develop significant lesions when fed a proatherogenic diet (Ley and Huo, 2001), (Swardfager et al, 2012). Swardfager et al (2012) showed a reduction in VCAM-1 was evident after exercise training in patients with CAD. Although many studies have measured VCAM-1 little is mentioned about the effect of exercise on this marker (Rankovic et al, 2009).

2.6 C-reactive protein

Studies have shown that measurement of C-reactive protein (CRP) is a powerful predictor of coronary events (Slater and Rill, 2004). This protein is made by the liver in response to inflammation elsewhere in the body for example lipoprotein entry and modification in the vessel wall triggers the release of cytokines, followed by leukocyte infiltration, more cytokine release, and smooth muscle migration and proliferation in the intima. Cytokines such as IL-6 travel to the liver and incite increased production of "acute phase reactants," including fibrinogen, CRP and serum amyloid A (Lilly, 2003). Infections can also cause the levels of CRP to increase by up to a 1000-fold the normal value; but in the absence of any obvious infections, baseline levels of CRP can also be quantified and the bell-shaped curve described in Figure 2 can be used to predict coronary events. As can be seen in (Figure 2.) the highest quartile (within the normal range) of CRP is associated with an increased number of coronary events even in people with low cholesterol. The combination of high cholesterol and high CRP is associated with the highest event level in most populations studied. Interestingly, recent work has shown CRP to be a more powerful independent predictor of adverse cardiac events such as, sudden death, myocardial infarction, and angina; and can be a more accurate predictor than low density lipoprotein cholesterol (LDL-C) (Slater and Rill, 2004).

Thus, the mechanisms described above that allow coronary arteries to enlarge in response to intramural plaque accumulation, paradoxically, might also weaken the protective fibrous cover and make those sections of the coronary anatomy, which are in the process of remodelling, most vulnerable to rupture and the onset of sudden, unpredictable coronary events (Slater and Rill, 2004). There are currently proven therapies to reduce high risk CRP levels, these include weight reduction and in particular statin therapy (Milani, Lavie and Mehra, 2004), (Marcell et al, 2005). Even though CR and exercise training are a proven method for reducing cardiac risk factors, and can be effective in reducing overall body fat as well as improving exercise capacity after a major cardiac event, the main effects of these therapies on CRP are not well established (Milani, Lavie and Mehra, 2004), (Kasapis and Thompson, 2005). A study done by Buyukyazi et al, (2010) that focused of different intensity walking programs in post-menopausal women found that increased cardiorespiratory fitness was related to lower CRP levels and found this result to be consistent with previous studies.

The study conducted by Milani, Lavie and Mehra (2004) followed the results of a 3-month cardiac rehabilitation program in New Orleans, USA, reporting the favourable effects of CR on CRP independent of Statin therapy and weight reduction. In this study Milani, Lavie and Mehra (2004) reported that directly following major cardiac events, CRP levels were elevated to far above the healthy norms and as a result of their intervention program found a median reduction of 41%. This reduction was similar or greater than multiple studies using statin therapy where the median reduction reported was between 15% and 20% (Milani, Lavie and Mehra, 2004), (Marcell et al, 2005). How exercise training reduces CRP levels is not well defined (Kasapis and Thompson, 2005). In athletes the effects of exercise varies according to the type of exercise, also directly after exercise CRP levels are greatly elevated, however the long term effects of exercise depend on duration, intensity and frequency (Kasapis and Thompson, 2005).

2.7 Effect of Cardiac Rehabilitation on blood-borne biomarkers of endothelial function

Research has recently explored the effect of exercise Programs on blood-borne biomarkers that are known to be indicators of endothelial dysfunction. It has been noted that increased levels of biomarkers such as ICAM-1, VCAM-1 and CRP independently predict early risk of myocardial infarction or cardiovascular disease (Roberts et al, 2006).

Lim et al (2006) researched the effects of intermittent exercise on biomarkers of cardiovascular risk in night shift workers in South Korea, one of the markers that they focused on was VCAM-1. It was found that levels of VCAM-1 did significantly drop within the exercise group however they did not improve beyond normal levels. Overall results showed that intermittent exercise consisting of 30 min for a brisk walking (10minutes-three times a day), resulted in decreased levels of cardiovascular risk biomarkers, the intensity of these intermittent sessions was between 60 to 79% HR max. (Lim et al, 2006).

A study focusing on reducing blood pressure in patients with type 2 diabetes included measurements of endothelial function. Barone Gibbs et al (2012) focused on FMD mentioned earlier as a measurement of vascular function as well as looking at blood borne biomarkers such as ICAM-1 and VCAM-1. Their research found that participation in 6-months of exercise training improved fitness, decreased BMI and body fat, however they did not

improve vascular function measured by FMD or the endothelial biomarkers ICAM-1 and VCAM-1 even at aerobic exercise intensities of between 60 to 90% HR max for 3 sessions per week consisting of 45min of aerobic training per session followed by 7 resistance training exercises (Barone Gibbs et al, 2012).

Several of the studies in the table 2.7.1 focused on the effects of CR on CRP, Jae et al (2006) and Parrinello (2010) both observed decreases in levels of circulating CRP in participants who underwent the intervention and increases in circulating levels of CRP in the control group. Cesari et al (2013) conducted a 4week aerobic exercise intervention programme which included 3 sessions per week with a 5min warm up, followed by 30min of continuous aerobic exercise and a 5min cool down at 60-70% VO^2max. Their research demonstrated that baseline levels of CRP are independent predictors of EPCs' alterations at the end of the training program, which suggested that an interrelationship between EPCs' system and inflammatory pathway was present in the participants in this study, it was also found that the decreases in CRP were related to the multifactorial intervention of the CR program prescribed (Cesari et al, 2013).

The effect of diet and exercise intervention on inflammatory and adhesion molecules were investigated in postmenopausal women that were on hormone replacement therapy and at risk for CAD by Wegge et al (2004). Similar to the current study Wegge et al (2004) included CRP, VCAM-1 and ICAM-1. It was observed that diet and exercise reduced CRP by 45%, they also hypothesized that levels of both ICAM-1 and VCAM-1 would decrease in response to the diet and exercise intervention. Although ICAM-1 did decrease significantly, VCAM-1 showed no significant changes, it was suggested that lack of response of VCAM-1 in the their study may have been due to the short duration of the intervention which was a 14day intervention involving exercise every day for 45-60min at 70 to 85% HR max (Wegge et al, 2004).

When looking at the research that has been done to date it is evident that exercise duration, frequency, intensity and type could effect changes in adhesion and inflammatory markers. It can be noted that CRP may be a strong indicator of risk associated with future cardiac events,

however ICAM-1 and VCAM-1 may require further observation as research has shown varying responses.

Table 2.7.1. Studies observing the effect of exercise on blood borne biomarkers

Authors	Study Design	Population	Condition	CR Duration	Frequency and Intensity	Observed effect
Lim et al (2015)	Randomised control trial	N=30 male night shift workers (IG 56.80 ± 1.82years and CG 58.33 ± 1.88years)	Night shift workers presenting with history of CVD, hepatectomy, hypertension and diabetes.	10 weeks aerobic exercise	3 sessions per week at 60-79% HR max for 30min per session	VCAM-1 IG= 513.87 ± 21.36 to 497.20 ± 20.40ng/mL CG= 531.53 ± 31.40 to 539.60 ± 32.55ng/mL *P*=0.001
Barone Gibbs et al (2012)	Randomised control trial	N=140 Adults (40-65years)	Sedentary with type 2 diabetes and untreated pre- or stage 1 hypertension	6months aerobic exercise and resistance exercise	3 sessions per week, 45min with 10-15 min warm up and a cool down, 60-90% HR max, followed by 7 Resistance exercise for 2x15reps at 50% 1RM	ICAM-1 IG= 203±63 to 201±56ng/ml CG= 228±90 to 228±80ng/mL *P*=0.215 VCAM-1 IG= 692±196 to 713±220ng/mL CG=692±184 to 675±217ng/mL *P*=0.623
Marcell et al (2005)	Randomised control trial	N=51 Middle aged participants (45.3 ± 8.3 years)	Overweight, insulin-resistant, nondiabetic.	16 weeks of moderate aerobic, intense aerobic or no exercise	5 sessions per week for 30min. Moderate exercise training range not specified, Intense exercise group was 80-90% HR max	CRP Moderate IG= 4.9±3.2 to -1.0 ± 0.5 mg/L Intense IG= 3.4±3.5 to -0.4 ± 0.5mg/L CG= 5.7±5.1 to -1.0± 0.5mg/L
Jae et al (2006)	Non-randsomised control trial	N=47 healthy sedentary individuals (IG 49.6 ±7.5years and CG 49.7 F 6.4	Overweight	12 weeks home based aerobic exercise	5 sessions per week, 50-60min at 60-80% HR max	CRP IG= 0.75±0.4 to 0.56± 0.34mg/dL CG= 0.60±0.3 to 0.70±0.4mg/dL *P* = .01
Parrinello et al (2010)	Experimental design	N=22 Caucasian patients, (62.7±4.8 years) 15 men, 7 women	Compensated CHF (mean left ventricular ejection fraction 38.9±3.6%)	10 weeks aerobic exercise.	5 sessions per week, 30min of mild to moderate walking per session	CRP IG= 0.69±0.34 to 0.39±0.32mg/dL CG= 0.67±0.35 to 0.72±0.34mg/dL
Cesari et al (2013)	Experimental design	N=112 patients (58.2 ±9.5years)	Acute coronary syndrome	4 weeks aerobic exercise	3 sessions per week, 5min warm up, 30min continuous, 5min cool down at 60-70% VO^2	CRP 3.1(0.3–11.8) to 2.1(0.3–10.1)mg/L
Buyukazi et al (2010)	Experimental design	N=26 females (30-49years)	pre-menopausal woman	12 weeks aerobic exercise	5 sessions per a week, for 1st 6 weeks aimed to walk for 30 building to 45 min and 2nd 6 weeks aimed to	CRP High intensity IG= 3.96 ± 6.56 to 1.95 ± 3.37mg/L, *P*< 0.01 Moderate intensity IG= 2.18 ± 1.65 to 1.75 ± 1.39mg/L, *P*= < 0.05

					walk 45 building to 60min, moderate or high intensity (50—55%, 70—75% max HRR)	CG= 2.11 ± 1.79 to 4.32 ± 4.80mg/L, *P*= not specified
Saxton et al (2008)	Randomised control trial	N=104 patients, of which 92 patients supplied biomarker data (Leg Cranking IG 68 (50-85)years, Arm Cranking IG 66 (54-82)years and CG 72 (56-84)years)	Stable intermittent claudication	24 week aerobic exercise	2 sessions per a week, exercised in cycles of 2min at a crank rate of 50 revs/min, followed by 2 min rest resulting in 20 minutes exercise in a 40-minute session.	Leg Cranking IG ICAM-1= 1028 (896-1178) to 997 (852-1167)ng/ml VCAM-1= 1327 (1160-1519) to 1310 (1155-1486)ng/ml CRP= 3.16 (2.14-4.72) to 2.73 (1.86-4.00)mg/L Arm Cranking IG ICAM-1= 1017 (893-1158) to 1019 (894-1161)ng/ml VCAM-1= 1542 (1365-1742) to 1422 (1264-1600)ng/ml CRP= 2.53 (1.76-3.63) to 1.88 (1.28-2.74)mg/L CG ICAM-1= 991 (838-1171) to 943 (827-1076)ng/ml VCAM-1= 1453 (1263-1671) to 1344 (1192-1515)ng/ml CRP ICAM *P*= 0.68 VCAM *P*= 0.72 CRP *P*= 0.07
Adamop-oulos et al (2001)	Experimental design	N=12 patients (59.6 ±2years)	Stable chronic heart failure	12 week home based aerobic exercise	5 sessions per week, 30min at 50rpm to keep HR between 70% to 80% HR max	ICAM-1= 367±31 to 314±29ng/ml, *P*<0.01 VCAM-1= 1247±103 to 1095±100ng/ml *P*<0.01
Conraads et al, (2015)	Longitudinal, randomised prospective clinical study	N=200 participants (40-75years)	Angiographically documented CAD, previous acute MI, LVEF >40%	12 weeks Aerobic interval training (AIT) and Aerobic continuous training (ACT)	3 sessions per week at intensity of 90-95% Peak HR for AIT and ACT at 70-75% Peak HR	CRP AIT= 0.21 ± 0.44 to 0.12 ± 0.52mg/L ACT= 0.24 ± 0.57 to 0.07 ± 0.52mg/L
Wegge et al, (2004)	Experimental design	N= 20 Female participants (51 to 79 years)	Post-menopausal women	14 days aerobic exercise and dietary intervention	Every day, 45-60min at 70 to 85% HR max	CRP= 2.62 ± 2.3 to 1.43 ±0.9 mg/L, *P*< 0 .01. ICAM-1= 158.8 ±48.3 to 145.6 ±38.2 ng/mL, *P*<0.05 VCAM-1= 670±245.7 to 678.8±205.6 (no change in VCAM-1 *P*-value not reported)
Hammet et al (2006)	Random Control trial	N=152 participants taking part in inflam-matory marker study (IG 38 ±12years,	smoked at least 5 cigarettes per day for the last 2 years	12 weeks aerobic exercise	3 sessions per week for 45min at 60-70% HR max	CRP IG= 1.9 (0.5-3.8) to -0.1 (-0.7 to 0.2)mg/L CG= 1.3 (0.8-2.2) to 0.0 (-0.6 to 0.4)mg/L Multivariate *P*-value =0.71 ICAM-1 IG= 182 ± 51 to 38 (-13 - 82)

		CG 39 ±11years				CG= 191 ± 82 to 26 (-14 - 85) Multivariate *P*-value= 0.77
Zoppini et al (2006)	Experimental design	N= 16 sedentary patients (66 ±6years)	Type 2 diabetes	6 month aerobic exercise	2 sessions per week, 10min warm up, 40min brisk walk, 10min cool down at 50-70% HRR	CRP 3.54± 3 to 3.48 ±3mg/L *P* –value not reported ICAM 363 ±124 to 318±132 ng/mL; *P*=0.015
Roberts et al (2006)	Experimental design	N=13 patients (55-74years)	Overweight, obese, diabetic	21days diet and aerobic exercise	Daily 45-60min sessions at 70-85% HR max	CRP ±3.78 to ±3.06mg/L *P*<0.05 ICAM ±389.0 to ± 295.8µg/L *P*<0.05 VCAM Δ after intervention *P*<0.001
(Goldha-mmer et al., 2005)		N=28 patients (64.7 ±7.1years), 18 male, 10 female	Stable CAD	12 weeks aerobic exercise	3 sessions per week, 45min at 70-80% HR max	CRP 7.5±4.2 to 3.9±3.5 mg/l, *P*<0.001

2.8 Pulse wave velocity

Blood pressure has long been known to be an important risk factor for coronary and cerebrovascular disease. While initial work concentrated on diastolic pressure, more recently, it has been recognised that systolic and pulse (difference between systolic and diastolic) pressures play a more important role, particularly with advancing age (Anderson, 2006). The functioning of the systemic circulation depends on the interaction between the left ventricle (LV) and the systemic arterial system (Yu et al, 2007). Stiffening of the large arteries is a common feature of aging and disorders such as hypertension, diabetes and renal failure. The heart adapts so that it is able to cope with the arterial stiffening resulting in higher and later systolic loads by both hypertrophy and ventricular systolic stiffening (Yu et al, 2007). The combined ventricular arterial stiffening has clinical implications in the decreased ability to tolerate physical activity in the elderly, heart failure with preserved ejection fraction and enhanced volume sensitivity of blood pressure in haemodialysis patients (Yu et al, 2007). Yu et al (2007) also suggested that arterial stiffening contributes to the process of atherosclerosis and arterial disease by influencing the sheer stress that is placed on the blood vessel walls and endothelial function.

The most obvious consequences of arterial stiffening are increased pulsatile BP caused by higher systolic BP (SBP) and lower diastolic BP (DBP), thereby causing increased left ventricular afterload and altering coronary perfusion. High SBP and pulse pressure as well as low DBP and left ventricular hypertrophy have been identified as independent factors of cardiovascular morbidity and mortality in the general population (Blacher et al, 1999).

When the arterial wall becomes stiffer or less elastic in cardiovascular disease, for example atherosclerosis, diabetes and hypertension; vascular compliance or windkessel function is reduced. Pulse wave velocity (PWV) is an acknowledged time-domain approach to the windkessel effects in arteries (Bertaux et al, 2012). In the time-domain theory of blood pressure, pressure and flow waveforms are separated into mean and oscillatory components. This leads to the concept of reflected (backward-going) waves that can reinforce a forward-moving pressure wave (Anderson, 2006). Acting as an elastic chamber behind the heart; the aorta and some of the proximal large vessels, store about 50% of the left ventricular stroke volume during systole (Belz, 1995). In diastole the elastic forces of the aortic wall forward this 50% of the volume to the peripheral circulation, thus creating a nearly continuous peripheral blood flow (Belz, 1995). This systolic-diastolic interplay represents the Windkessel function which has an influence not only on the peripheral circulation but also on the heart, resulting in the reduction of left ventricular afterload and improvement in coronary blood flow and left ventricular relaxation (Belz, 1995). Windkessel effect can best be defined in medicine to account for the shape of the arterial blood pressure waveform in terms of the interaction between the stroke volume and the compliance of the aorta and large elastic arteries (Windkessel vessels).

The peripheral tonometry method, which is easy to perform and is non-invasive, has been widely used as a screen for arterial stiffness and vascular damage. Among a variety of peripheral PWV measures, carotid-femoral PWV (CF-PWV) by tonometry has been the most frequently used and studied to date (Kim et al, 2015). CF-PWV is well correlated with aortic PWV obtained using a catheter-tip manometer and is significantly higher in individuals who have cardiovascular risk factors. Nevertheless, CF-PWV has not been fully included in routine clinical use because the transducer used has to be attached with constant pressure on target arteries and carefully adjusted to obtain an accurate pulse wave (Kim et al, 2015).

Thus, it can be technically complex and inconvenient for routine clinical examinations. Additionally, exposure of the inguinal area during acquisition of the femoral pressure waveform can be psychologically invasive for some patients (Kim et al., 2015). Brachial-ankle PWV (BA-PWV) is an easier method of measuring arterial stiffness. This is a simple method of measuring PWV by recording pulse waveforms from an oscillometric sensor attached to a blood pressure cuff (Kim et al., 2015). The potential benefits of BA-PWV screening for vascular damage, especially for outpatients, include its safety, technical simplicity, and short sampling time. However, there is a paucity of data supporting the validity and reliability of BA-PWV. Although a few studies have reported the validity of BA-PWV by comparing it with the catheter-tip manometer method, the number of participants in those studies was too small to draw definite conclusions (Kim et al., 2015).

A study by Yu et al (2007) found a significant correlation between both cf-PWV, ba-PWV and the parameters of LV and arterial structure and function. They also found that the correlation was stronger with ba-PWV than that of cf-PWV. As ba-PWV covers both central and peripheral arterial territories and cf-PWV mainly reflects the stiffness of the central arteries, the results found by Yu et al (2007) suggested that the peripheral muscular arteries contribute independently of the central elastic artery to the ventriculo-arterial interaction.

Several studies have shown habitual exercise to improve arterial health by reducing arterial stiffness, improving endothelial function and intima media thickness (Edwards et al, 2012). According to Edwards et al (2012) the underlying atherosclerosis that is associated with cardiovascular disease develops in a non-uniform fashion affecting the risk factors that contribute to the arterial parameters differently in the central and peripheral arterial trees; due to this the suggest taking into account both cf-PWV as well as brachial artery dispensability. Taking that into consideration both cf-PWV as well as ba-PWV will be measured and reported in this study.

2.9 The effect of Cardiac rehabilitation on Arterial Stiffness and PWV

Regular physical activity improves endothelium-dependent vasodilation in healthy human and animal models as well as individuals with endothelial dysfunction (Goanvec et al, 2015). According to current research exercise can have a positive effect on arterial stiffness, however as can be observed by this table it may be possible that duration, frequency and intensity play a role in this positive effect, as well as the type of exercise prescribed. Exercise training improves mainly the NO-related vasorelaxation (Goanvec et al, 2015). The harmful effects of arterial stiffness derive from hemodynamic changes, as increases in systolic and pulse pressures, which are related to cardiac overload and a reduction in coronary perfusion that can lead to myocardial ischemia. Additionally, inflammatory and endothelial dysfunction biomarkers which are cardiovascular risk predictors in CAD patients, have been associated with arterial stiffness. However, the effects of aerobic exercise training on arterial stiffness have been understudied in CAD patients (Oliviera et al, 2015). Oliviera et al (2015) also observed that only patients who attended 80% of their exercise sessions and above showed improvements in arterial stiffness, those with lower adherence levels did not show any significant improvements.

Yang et al (2013) found there to be a close correlation between the circulating endothelial progenitor cells and ba-PWV. Regular physical exercise can significantly increase the number and activity of circulating endothelial progenitor cells of sedentary healthy individuals and decrease their ba-PWV. The research discovered that regular physical exercise induced increased number and activity of circulating epithelial progenitor cells which attenuated age-related decline in arterial elasticity. These findings supported the use of physical exercise in lowering cardiovascular risks and contribute to prevention of vascular diseases by reducing arterial stiffness (Yang et al, 2013).

Findings by Yang et al (2013) have been supported in several studies that suggest the risk reduction associated with physical fitness, may be attributed to endothelial function, arterial remodelling and compliance; and that an increase in arterial stiffness in turn increases the risk of coronary artery calcification (Lessiani et al, 2015). Research conducted by Lessiani et al (2015) which conducted an exercise intervention of 2 sessions per week, for 55min per session at 60-75% VO^2max. This study indicated that a high amount of high-intensity

exercise training improved the cardiovascular risk profile in these subjects by lowering oxidative stress and thereby decreasing the degree of arterial stiffness.

In contrast, meta-analysis was conducted by Montero et al (2014) focusing on 14 trials involving hypertensive patients, indicated that overall aerobic exercise training did not reduce arterial stiffness in pre-hypertensive subjects. However results also suggested that arterial stiffness could effectively be improved in pre-hypertensive subjects by aerobic exercise training interventions that are associated with systolic blood pressure reduction and/or relatively prolonged duration (Montero et al, 2014). However it should be noted that the average intensity for these studies was approximately 58% VO^2max, a similar intensity to the intensity levels currently used in the UK, at an average of 3.4 sessions per a week for 42.8 min per a session at an average of 13.5weeks, when compared to the study by Oliviera et al (2015), even though these trials may have been longer in duration and frequency it can be noted that the intensity had an average VO^2max of 20% lower than that of the trial by Oliviera et al (2015) this could indicate once again that intensity may play a vital role in improving arterial stiffness.

Montero et al (2015) conducted a meta-analysis which compared aerobic versus combined aerobic and resistance exercise trials and concluded that further research would be needed to elucidate the prognostic relevance of the different impacts of combined vs. aerobic training on arterial stiffness (Montero et al, 2015).

Rossow et al (2014) unlike other research studies, focused on the effects of resistance training on arterial stiffness in pre- and post-menopausal woman and the main findings of the study were that, following training, cf-PWV mean values did not change significantly in either young or older subjects. Their hypothesis that high-intensity resistance training would not increase arterial stiffness in either young or older women was supported by mean values found, however it was only partially supported when individual responses of the participants were examined (Rossow et al (2014).

Laskey et al (2013) however, suggested that arterial PWV has withstood the test of time as a measure of arterial stiffness and observed that arterial stiffness, reflected by PWV,

significantly decreased in subjects with established CHD who participated in an exercise based CR programme. Data in this research suggested that changes in PWV may serve as a sensitive measure of altered arterial stiffness in patients with advanced atherosclerotic arterial disease, that abnormal arterial stiffness may be beneficially modified by exercise and that changes in stiffness despite presence of ongoing pharmacologic treatment are detectable (Laskey et al, 2013). They concluded that improvements in PWV were linked to the number of CR sessions attended (Table 2.8.1) suggesting that adherence to an exercise based CR programme as well as the number of sessions (in this study 60sessions), does ultimately reduce arterial stiffness (Laskey et al, 2013).

Table 2.9.1. Studies observing the effect of exercise on PWV

Authors	Study Design	Population	Condition	CR Duration	Frequency and Intensity	Observed effect
Oliviera et al (2015)	parallel-group trial	N= 96 patients (56 ± 10 years)	Myocardial infarction (MI).	8 weeks of aerobic exercise	70 to 85% of maximal heart rate during 3 sessions a week,	cf-PWV IG 8.0 ±2.2m/s to 7.7 ±1.7m/s CG 8.4±2.1 to 8.5±2.3
Yang et al (2013)	Cross-sectional study	N=40 healthy men. (young = 21 to 33yrs) and (older = 59 to 72yrs)	None. Groups based on age and habitual Exercise status: young (sedentary and endurance trained) and older (sedentary and endurance trained)	12 weeks of aerobic exercise	4 sessions a week 30min/session	Ba-PWV (m/s) Older sedentary: Baseline 13.95 ±1.07 12wks 13.24±1.16 Young sedentary Baseline 11.79±5.1 12wks 11.47±3.9 (no specific values provided for the endurance group)
Lessiani et al (2015)		N=18 subjects (12 males) 54 (48–66)years	Sedentary, healthy individuals	8 week aerobic exercise	2 sessions per week 55min per session at 60-75% VO^2max	Cf-PWV decreased 6.28 (5.44–7.86) m/s vs 5.31 (4.59–6.67) m/s, *P*<0.0001; a 9.9% decrease vs baseline
Montero et al (2014)	Systematic review and meta-analysis	N=472 Patients (over a total of 14 articles)	Hypertension	Mean= 13.5 weeks aerobic exercise	Mean= 3.4 sessions per week, ±42.8 min per session	IG (14 trials, mean difference: -0.19; 95% CI=0.39, 0.01, *P* = .06) CG (8trials, mean difference: 0.10; 95% CI=0.34, 0.14, *P*=.43) Between group difference (7 trials, mean difference: -0.14; 95% CI = -0.45, 0.16, *P*= .36).
Montero et al (2015)	Systematic reviews and Meta-Analyses	N=752 Patients	Cardiovascular disease, Diabetes type 1 and 2, hypertension, hyperlipidaemia, renal failure	8 to 103 weeks of combined resistance exercise	Mean= 2.6 sessions per week ±39.2 min. Average 60–90% HR max for aerobic	The mean difference in PWV did not differ between aerobic and combined Training trials (*P* = 0.12).

					Resistance= Between 1-5 sets, 8-20 reps, mean= 2.4 sessions per week ±31.3 min Intensity = 50 -80% 1 rep max	No significant heterogeneity found among aerobic or combined training trials mean difference in PWV (I^2 = 0%, P = 0.76; I^2 = 41%, P = 0.08, respectively.
Rossow et al (2014)	Experimental design	N= 29 participants 16 young, 22±2years (Pre) and 13 older, 57±3years (post) females	Pre- and postmenopausal females who have not done resistance training in >6months or >5hours a week of endurance training.	8 weeks resistance training	3 sets 60s rest between, 10 reps at 80% 1 rep max	Cf-PWV group effect (P= 0.00) with 'post' having higher cf-PWV than 'pre' No significant cf-PWV interaction (P= 0.55) or time effect (P = 0.08) Change in cf-PWV values significantly (P= 0.001) correlated (r = −0.602) with baseline cf-PWV values.
Laskey et al (2013)	Experimental design	N= 48 Participants 26 men and 22 women 60.5 ±10.8years	Established CHD	20 weeks aerobic exercise	40-50% max HRR increasing to 70-85%, 3 sessions per week	cf-PWV= baseline 7.2±1.4 m/s; final, 6.5±1.3 m/s, (P=0.02)

2.10 Hypothesis

In this study it is hypothesised that the current exercise training recommendations (duration, intensity and frequency) for a typical UK-based CR programme are insufficient to elicit significant improvements in endothelial function (based on improvements in levels of adhesion molecules, inflammation and arterial stiffness) in cardiac patients undergoing a standard 8-week programme.

2.11 Aims

The aim of the study is to investigate the effect of current exercise based cardiac rehabilitation in the UK on endothelial function. The study will focus on blood-borne biomarkers known to affect endothelial function. Biomarkers including ICAM-1, VCAM-1 and CRP will be implicated as increased levels are suggested to be associated with decreased endothelial function. To support the evidence of changes in endothelial function these blood-borne biomarkers will be correlated with pulse wave velocity (PWV), a surrogate marker of arterial stiffness.

2.12 Objectives

In order to address the aim of the study, over an 8 week period (16 supervised training sessions), in individuals diagnosed with pre-existing CAD, the following objectives were set:

- Determine the effect of exercise on ICAM-1 VCAM-1 and CRP.
- Determine the effect of exercise on arterial stiffness using PWV
- Determine biomarker levels and correlate these levels with PWV results in order to determine these blood borne biomarkers can be linked with arterial stiffness

2.13 Scope of the Study

This study will look to investigate whether the training volume (intensity, frequency, and duration) of a typical CR programme in the UK is ineffective in promoting physiological changes that have been noted to be associated with cardiac disease risk.

CHAPTER 3 – Methods and Procedures

3.1 Research design

The study will be quantitative in nature and will utilise an, experimental study design. The experimental approach determines whether or not a predicted result occurs when specified action is taken (Hoskins and Mariano, 2004). The experiment is conducted under a controlled situation, some factors are held constant, other factors are manipulated (Hoskins and Mariano, 2004). The study design separates the participants into two groups; the results in the manipulated group are evaluated and compared to those observed in the control group. For the purpose of this study the tested group of participants who fall within the inclusion criteria as stated below, serve as the participants.

3.2 Participants

The sample relevant for this study must meet the following inclusion criteria:

- Individuals diagnosed with existing CAD
- Aged between 30 and 85.
- The patient has agreed to participate in exercise based cardiac rehabilitation programme (intervention group) or while being eligible have chosen not to participate in the structured CR programme (control group).
- The patient is able to or has given informed consent to be part of the study.

3.3 Sampling Technique

A minimum total number of participants that meet the above criteria will be included in this study with the use of a non-probability sampling technique, in particular, that of purposive sampling. According to De Vos et al (2005), in purposive sampling, a particular case is chosen because it illustrates some feature or process that is of interest for a particular study. A pre-test and post-test design will be used as the intervention group will be tested at baseline prior to the start of the CR programme and 8 weeks later. The control group only undertake the pre- and post-testing phase and are not involved in any training based intervention.

3.4 Measuring instruments

Blood samples will be taken via venepuncture and stored in a -80°C freezer once separated into serum and plasma. Blood samples will be analysed by Tecan plate reader (Infinite M200Pro, Männedorf, Switzerland) using ELISA kits in the Department of Sport, Health & Exercise Science. Height was measured using a Leicester Height Measure (SECA, Birmingham, United Kingdom) and body mass (kg) was measured using a Tanita Body Composition Analyser MC – 180MA (Tanita, Amsterdam, The Netherlands). Pulse wave velocity was measured using the Enverdis Vascular Explorer (Model N2A6P2U-A; Enverdis GmbH Medical Solutions, Jena, Germany). The Cardiorespiratory fitness test (CPET) was conducted using a treadmill (General Electric [GR]) driven by a GE Case system (GE Healthcare, Buckinghamshire, UK) and the Oxycon Pro (Jaeger, Hoechburg, Germany) breath-by-breath metabolic cart to perform the gas exchange analysis. Statistical Package for Social Scientists (SPSS) version 22 (IBM, NY, USA) will be used in order to conduct the statistical analysis of the study.

3.5 Measuring protocol

This study was conducted as part of a larger trial being done on the effects of exercise rehabilitation on patients with coronary artery disease. The particular protocol for this study followed the following test procedural pattern:

- Introduction to the study, explanation of protocol and tests followed by consent and patient history.
- Patient then has stature and body mass measured followed by waist and hip measurements.
- Once completed blood pressure is measured with the patient still in the supine position.
- Jugular symphysis is then measured and pulse wave velocity testing is conducted.
- Venous blood is then drawn using venepuncture for future analysis.
- Blood is separated into 4 gold SST (1 to be used for general blood screening by Castle Hill Hospital), 4 purple EDTA (1 to be used for general blood screening by Castle Hill Hospital), 1 Red SST and 1 fluoride oxilate vacutainers.
- After each vacutainer is filled it is inverted gently 8 times.

- Vacutainers were then spun in a Thermo Electron Corporation Centrifuge (CR3i Multifunction Centrifuge, Waltham, MA, USA) using the serum and plasma protocols, with fluoride oxilate and EDTA being spun 15min after sampling and the SST vacutainers being spun 30min after the sample was taken.
- Once separated, plasma and serum were aliquoted and frozen at -80°C in the Castle Hill Hospital academic cardiology freezer to be analysed once full patient samples size is reached.
- While this was being completed patients went on to have a CIMT test followed by an Echocardiogram.
- Once the Echocardiogram was completed the patient underwent a CPET using the modified Bruce Protocol.
- Once full patient sample was reached samples were transferred to the freezers in the exercise physiology laboratory in Department of Sport, Health and Exercise Science at the University of Hull.
- ICAM-1, VCAM-1 and CRP were then analysed using the following Abcam ELISA kits (Cambridge, USA): ab174445- ICAM-1 (CD54) Human Simple Step ELISA® kit, ab46118-VCAM1 (CD106) Human ELISA® kit and ab181416- C-Reactive Protein (CRP) Human Simple Step ELISA® kit.
- Results were analysed using the TECAN microplate reader (Infinite M200Pro, Männedorf, Switzerland).

3.5.1 Pulse wave velocity testing:

Before starting the measurement, the patient lies down in a room at a comfortable temperature for at least 10 min. To ensure optimal examination conditions, it is essential that the patient not: smoke or use nicotine, eat any food, or drink any alcohol or hot drinks such as coffee or tea, for three hours before the examination. Investigators then take the measurement of the jugular symphysis which is the distance between the jugular notch and the symphysis pubis which is entered into the patient data along with height, weight and resting blood pressure. A NIBP inflatable cuff is then attached to the right arm and right ankle and photoplethysmographic sensors are attached to the right index finger and right hallux. Care must be taken that the red arrows attached to the cuffs are located above the brachial artery on the arm and above the posterior tibial artery on the leg. The lower edge of the arm cuff is

positioned 2.5 cm above the elbow, while the ankle cuff should be positioned with the red arrow pointing towards on the inside of the foot, above the ankle. Once the right side measurements are taken, photoplethysmographic sensors and NIBP cuffs are changed over to the lefts side and measurements are repeated. Aortic blood pressure is the last measurement taken. Once these have been completed the patient is then given the option to remain supine or seated in order to take the blood samples. It is important that the patient should not move, speak or cough during the recording as this may result in malfunctions that could lead to misleading findings.

3.5.2 CPET to determine level of Cardiorespiratory Fitness

A baseline cardiopulmonary exercise testing (CPET) was performed as part of the larger study following the ATS/ACCP Statement on Cardiopulmonary Exercise Testing (2003). Exercise was performed using the modified Bruce protocol on a treadmill (General Electric [GR]) driven by a GE Case system (GE Healthcare, Buckinghamshire, UK). Breath-by-breath measurements of oxygen uptake, carbon dioxide production, and respiratory flow and volume parameters were obtained by applying a Hans Rudolph mask, connected to a pneumotach device. The Oxycon Pro (Jaeger, Hoechburg, Germany) breath-by-breath metabolic cart was used to perform the gas exchange analysis. Oxygen, carbon dioxide and flow sensors were calibrated immediately before each test. Arterial pressure was measured manually every 2 to 3 min by cuff sphygmomanometer. The reasons for test termination were those established by international guidelines. Exercise capacity was expressed as maximal workload (Watt); peak oxygen uptake (VO_2 peak) was averaged over the last 30seconds of exercise, and expressed relative to body weight ($mL/kg^{-1}/min^{-1}$). CPET was repeated at the end of the CR program.

3.5.3 Anthropometric Measurements.

Body Mass

Body Mass (Kg) was measured using a Tanita Body Composition Analyser MC – 180MA (Tanita, Amsterdam, The Netherlands). Patients were instructed to remove footwear, jackets and items from their pockets before standing in the centre of the scales.

Height

Height was measured using a Leicester Height Measure (SECA, Birmingham, United Kingdom) with patients standing, without footwear, in the Frankfort plane. Participants were asked to stand with their back to the height rule and try to place the back of their head, back, buttocks and heels against the upright rule, with their feet together. The participant was then asked to look straight ahead. The examiner then ensured that the top of the external auditory meatus was level with the inferior margin of the bony orbit. The head piece of the stadiometer or the sliding part of the measuring rod was lowered so that the hair is pressed flat. The participant was then asked to take a deep breath and the measurement was then recorded.

BMI

BMI was calculated as the weight (kg) divided by the square of the height (m).

Waist to hip ratio

Waist circumference (cm) was taken with a tape measure as the point midway between the costal margin and iliac crest in the mid-axillary line, with the subject standing and breathing normally. Hip circumference (cm) was measured at the widest point around the greater trochanter. The waist-to-hip ratio was calculated as the waist measurement divided by the hip measurement.

3.5.2 Blood Sampling

Whole blood is obtained by using a collection tube with an anticoagulant to prevent clotting of the sample e.g. blood collected into tubes with a lavender stopper that contains the anticoagulant ethylene diaminetetraacetic acid (EDTA). Immediate inversion of the tube 8 times gently mixes the blood and anticoagulant to prevent clotting and ensure accurate blood counts. (King et al, 2011).

Serum is a clear yellowish fluid that remains from blood plasma after clotting factors (fibrinogen, prothrombin etc.) that have been used in the formation of a clot. The cells are usually glued together by the clot formation. In serum separator tubes the serum stays above the gel while the cells are forced below the gel after centrifugation using Thermo Electron Corporation Centrifuge (CR3i Multifunction Centrifuge, Waltham, MA, USA).

Plasma is a clear yellowish fluid that still contains all of the clotting factors and have not been solidified into clot. The cells are also not glued together and can be easily re-suspended by gently rocking the plasma tube from side to side. In plasma separator tubes the plasma stays above the gel while the cells are force below the gel after centrifugation. Plasma is obtained by centrifuging the tube approximately 15 minutes. Certain test(s) require the plasma to be carefully separated from the red blood cells, making sure the specimen does not contain red blood cells. The use of a transfer pipette is the preferred method, versus tilting the original tube and pouring the plasma into an aliquot tube. The presence of cells may give spurious results.

Blood samples were stored in a -80°C freezer once separated into serum and plasma. Blood was most commonly obtained from the medial cubital vein which lies within the cubital fossa anterior to the elbow, blood was taken from the posterior area of the hand when there was not sufficient flow to enable a full sample collection from the medial cubital vein. Once the needle was inserted into the vein, the vacutainers were pushed into the barrel which was attached to the needle and the vacuum in the vacutainer withdrew blood from the vein, this process was repeated until all samples required were collected. Blood was separated into 10 vacutainers, which included 4 gold SST, 4 purple EDTA, red SST and 1 grey fluoride oxilate vacutainers. 1 gold SST and 1 purple EDTA were sent off for a standard blood check for patient records. Vacutainers were then spun using the serum and plasma protocols, with fluoride oxilate and EDTA being spun 15min after sampling and the SST vacutainers being spun 30min after the sample was taken. Once separated, plasma and serum were aliquoted and frozen at -80°C to be analysed once full patient samples size is reached.

3.6 Use of ELISA Kits for testing blood borne biomarker levels

ELISA is a popular format of "wet-lab" type analytic biochemistry assay that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample. Antigens from the sample are attached to a surface. Then, a further specific antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a colour change in the substrate. The stability of certain ELISA kits is determined by the loss rate of activity. To minimise extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end. For this particular study commercial ELISA kits were sourced from Abcam, particular kits used were the ab174445-ICAM-1 (CD54) Human Simple Step ELISA® kit, ab46118-VCAM1 (CD106) Human ELISA® kit and ab181416- C-Reactive Protein (CRP) Human Simple Step ELISA® kit. (www.abcam.com).

3.7 ELISA kit procedures

For CRP and ICam-1 Elisa procedures were as follows:

The kit's provided well strips that coated with the appropriate antibodies. Before the testing procedures began all reagents as well as blood samples were then equilibrated to room temperature. Guidelines were then followed for each of the kits and ratios were calculated to prepare the correct amount of reagents and standards appropriate for the number of samples. The first two strips of wells on each tray were used to create sample standards to ensure the reliability of the sample readings. The correct ratio of sample and antibody cocktail were then added to each of the wells, two wells were filled for each sample to ensure accuracy of the readings from that sample. Once all samples and antibody cocktail had been added to the wells they were sealed and incubated at room temperature on a plate shaker at 400rpm. Once incubation was complete, the wells were aspirated and then each well was individually washed and aspirated 3 to 4 times, ensuring that the liquid was removed completely after each wash to ensure the best results. After the last wash the tray was inverted and blotted onto paper towels to remove any excess liquid, all bubbles were then removed with a pipette,

which was essential for ensuring accurate results. Once this was completed a TMB substrate prepared earlier was added to each well and incubated in the dark on a plate shaker at 400rpm for 10min. Once incubation was completed, 100 µl Stop Solution was added and set to shake on a plate shaker for 1min to mix. The results were then recorded at an optical density of 450nm (Abcam.com)

VCam1 Elisa Kit procedure

As with CRP and ICAM-1 Elisa kits the appropriate number of antibody coated well strips were place on a well strip tray. Once all the reagents and samples were equilibrated to room temperature before preparation and testing could begin. Following the dilution guidelines all reagents and standards were prepare to the calculated ratios and amounts required for the number of samples. Two well strips were used to ensure reliability of the test. Standards and samples were then added to each of the wells followed by the prepared biotinylated anti-VCAM-1 antibody. The wells were then covered and incubated at room temperature for an hour. Once this was completed the cover was removed and the wells were aspirated, the wells were then washed 3 to 4 times and aspirated after each wash, once the washes were compete all bubbles were then removed with a pipette.

The prepared streptavidin-HRP mix was then added to the wells including the blank wells, covered and incubated at room temperature for 30min. Once incubation was complete, wells were aspirated and once again washed 3 to 4 times, aspirating after each wash, bubbles were once again removed by pipette. The Chromogen TMB solution was then added to each well and incubated in the dark at room temperature for 10-20min, the plate was also covered to avoid direct light exposure. Once this was completed 100µl of stop solution was added to each of the wells (Abcam.com). Results were then analysed using the TECAN microplate reader (Infinite M200Pro, Männedorf, Switzerland).

3.8 Data collection

Patients with pre-existing CAD were recruited to the study. Patients were actively engaged in the local CR service led by City Health Care Partnerships in Kingston-upon-Hull. Participants were tested pre- and post- an 8-week structured exercise-based CR programme in the Department of Cardiology, Castle Hill Hospital, Hull. Venous blood samples and PWV were taken prior to and following 8 weeks of CR. Blood samples were stored in a -80°C freezer once separated into serum and plasma. Samples were analysed by a TECAN microplate reader (Infinite M200Pro, Männedorf, Switzerland) using ELISA kits in the Department of Sport, Health & Exercise Science. Pulse wave velocity was measured using the Enverdis Vascular Explorer, Model N2A6P2U-A (Enverdis GmbH medical solutions, Jena, Germany). Data for each patient was recorded using system software.

3.9 Data Analysis

Descriptive statistics such as means, modes, standard deviations, variances and frequency distributions, were used and graphically represented. Descriptive statistics is statistical procedures that are used to summarise, organise and simplify raw data, to produce more logical, understandable and manageable information (Gravetter, & Wallnau, 2002). Furthermore inferential statistics, including Spearman or Pearson correlation coefficients, will be performed to determine the type of association (i.e. weak, moderate and strong) between adhesion molecule levels and PWV results. Correlation coefficients are defined as a quantitative value of the relationship between two or more variables that can range from -1.00 to 1.00 (Thomas et al 2005).

3.10 Statistical analysis

Statistical analyses were performed by using the Statistical Package for Social Scientists (SPSS) version 22 software (IBM, NY, USA). Due to the fact that there were two groups being compared in a pre- and post- design; a 2 (group) x 2 (time points) repeated measures analysis of variance (ANOVA) was used to analyse the data. The primary purpose of a 2x2 repeated measures ANOVA is to understand if there is an interaction between these two factors on the dependant variable and to see if there is a main effect for time. All data are expressed as mean ± S.E. unless otherwise noted. A $P<0.05$ was considered to be statistically significant.

Repeated measures ANOVAs (within-subject factors) are susceptible to the violation of the assumption of sphericity. Sphericity is the condition where the variances of the differences between all combinations of related groups (levels) are equal. Violation of sphericity is when the variances of the differences between all combinations of related groups are not equal. Sphericity can be likened to homogeneity of variances in a between-subjects ANOVA. If the data does not violate the assumption of sphericity, there will be no need to modify the degrees of freedom In SPSS, this result is presented in the "sphericity assumed" row (statistics.laerd.com). Not violating this assumption means that the *F*-statistic that has been calculated is valid and can be used to determine statistical significance (statistics.laerd.com). If, however, the assumption of sphericity is violated, the *F*-statistic is positively biased rendering it invalid and increasing the risk of a Type I error. To overcome this problem, corrections must be applied to the degrees of freedom *(df)*, such that a valid critical *F*-value can be obtained. The corrections that you will encounter to combat the violation of the assumption of sphericity are the lower-bound estimate**,** Greenhouse-Geisser correction and the Huynh-Feldt correction. These corrections rely on estimating sphericity (statistics.laerd.com). In addition to these results Chi square test was also used to determine if there is a relationship between variables between the two groups, as well as the independent-samples t-test to compare the means between the groups on the same continuous, dependent variable, these tests were mainly used to determine if differences in the clinical characteristics of the groups may have a significant effect on the overall results (statistics.laerd.com).

3.11 Ethical Approval

Institutional ethical approval was provided by the Faculty of Science and Engineering Ethics Committee at the University of Hull. A separate integrated research application system (IRAS) approval for studies involving NHS patients and services was also obtained (See appendices).

CHAPTER 4 - Results

4.1 Clinical Characteristics of Patients

28 patients (mean age 62 ± 8 years; 86% male) were recruited to the study (n=20 intervention; n=8 controls). Participants presented with various cardiac complications including myocardial infarction, percutaneous coronary intervention, and coronary artery bypass graft, hypertension and hyperlipidaemia (Table 1).

Table 1. Clinical Characteristics of Patients

							Between
	Control (n=8)			**Intervention (n=20)**			**Group**
	Baseline	8 weeks	*P*- value*	Baseline	8 weeks	*P*- value*	*P*-value**
Age (years)	63 ±9			61 ±8		0.906	0.919
Body mass (kg)	86 ±12	86 ±11	0.630	87 ±15	88 ±16	0.479	0.386
Height (cm)	173 ±8			170 ±8		0.800	0.821
BMI (Kg/m²)	28.4 ±3	29.0 ±3	0.245	29.7 ±4	29.8 ±4	0.462	0.207
Waist to Hip Ratio	0.98 ±0.04	1.00 ±0.05	0.195	0.95 ±0.08	0.95 ±0.07	0.802	0.172
Resting Blood Pressure (mmHg)	127(±16)/ 83(±7)	118(±22)/ 75(±16)	0.188/ 0.252	128(±19) /81(±12)	119(±19)/ 77(±13)	**0.001**/ 0.058	0.636/ 0.251
Resting Heart Rate (bpm)	58 ±8	63 ±9	**0.031**	58 ±9	57 ±7	0.260	0.436
Previous MI?	50%			25%			0.371
Previous PCI?	37.5%			35%			0.114
Previous CABG?	12.5%			5%			0.486
HTN	12.5%			0%			0.107
Hyperlipidemia	87.5%			90%			0.306
Diabetes	75% (type 2)			15% (type 2)			**0.004**
Smoker	50% (ex-smokers) 25% (active smokers) 12.5% (stopped smoking) 25% (never smoked)			60% (ex-smokers) 5% (stopped smoking) 40% (never smoked)			0.104
Aspirin	100%	100%		100%	100%		0.099
Ticagrelor	87.5%	87.5%		45%	45%		0.122
Clopidogrel	12.5%	0%		40%	40%		0.183

Beta Blocker	100%	75%	100%	100%	0.462
ACE inhibitor	50%	50%	55%	55%	0.474
ARB	12.5%	12.5%	10%	10%	0.814
Statin	100%	100%	100%	100%	0.462
Fibrate	12.5%	12.5%	0%	0%	NS
Diuretics	12.5%	12.5%	0%	0%	NS
Nitrates	25%	12.5%	25%	25%	0.947
GTN	100%	100%	100%	95%	0.530

*Main effect for time difference between baseline and 8weeks. **Baseline comparison between intervention and control groups. NS= Not significant.

Changes in medication were observed in the control group during the intervention including a reduction in clopidogrel from 13% to 0%; beta blockers were reduced from 100-75%; and nitrates from 25-13%. Changes in medication in the intervention group were negligible, only a reduction in GTN spray from 100-95% was reported.

There were no statistically significant changes in body mass, BMI or waist to hip ratio across both the control and intervention groups. The intervention group however observed a significant decrease in systolic blood pressure (P=0.001), with the control group observing a statistically significant increase in resting heart rate (P=0.031).

T-tests and Chi square tests were conducted to determine whether there was any statistical significance with regards to the baseline clinical characteristics between the intervention and control groups. It was found that with regards to type 2 diabetes, the difference between the groups was statistically significant (P=0.004), as 75% of the control group and only 15% of the intervention group presented with type 2 diabetes.

Checks to determine if data was normally distributed for key variables was undertaken using Kolmogorov Smirnov tests. Normal distributions for key variables was identified (all P>0.05) and a 2x2 repeated measures ANOVA was conducted for ICAM-1, VCAM-1 and CRP. Pulse wave velocity variables including ba-PWV (a direct measure of PWV), the estimated value (cf-PWV), and aortic PWV was reported.

Table 2. Influence of CR on CRF, arterial stiffness and blood borne biomarkers

	Control		Intervention		Interaction Effect	Time Effect
	Week 0 (baseline)	Week 8	Week 0 (baseline)	Week 8	*P-value*	*P-value*
CRF	23.109 ± 4.25	23.144 ± 5.16	24.815 ± 5.29	24.994 ± 4.79	0.891	0.839
cf-PWV	6.442 ± 2.35	6.164± 1.28	6.064 ± 2.05	6.452 ± 1.83	0.411	0.892
ba-PWV	19.943 ± 16.89	16.886 ± 5.86	14.194 ± 2.31	14.1 ± 2.17	0.220	0.193
Aortic PWV	6.442 ± 1.58	6.257 ± 0.85	6.2 ± 1.36	6.465 ± 1.22	0.406	0.883
ICAM-1	239.94 ± 55.40	252.25 ± 44.72	262.36 ± 80.53	265.38 ± 75.86	0.666	0.478
VCAM-1	1.435 ± 0.45	1.554 ± 0.59	1.643 ± 0.47	1.87 ± 0.599	0.751	0.112
CRP	3.372 ± 2.51	3.564 ± 2.70	3.452 ± 2.298	2.654 ± 1.74	0.177	0.402

Units of measurement: CRF= Peak VO_2 mL/kg^{-1}/min^{-1}, ICAM-1, VCAM-1 and CRP= µg/ml and cf-PWV, ba-PWV and aortic PWV= m/s

4.2 CRF

The Δ (change) in CRF was not significantly different between the intervention (24.91 ± 95% CI 22.69 -27.11) and the controls (23.13 ± 95% CI 19.64 -26.62) following 8 weeks of CR. The intervention group at baseline showed a mean Peak VO_2 of 24.82 ±5.29 mL/kg^{-1}/min^{-1}, with a non-significant improvement at 8 weeks with a mean time of 24.99 ±4.79 mL/kg^{-1}/min^{-1}. The interaction between Peak VO_2 mL/kg^{-1}/min^{-1} and group was not significant ($F(1,26)= 0.019$, $P <0.891$). This may have been an issue of study power and a large percentage of the participants being diabetic, a larger sample size may have led to more significant findings.

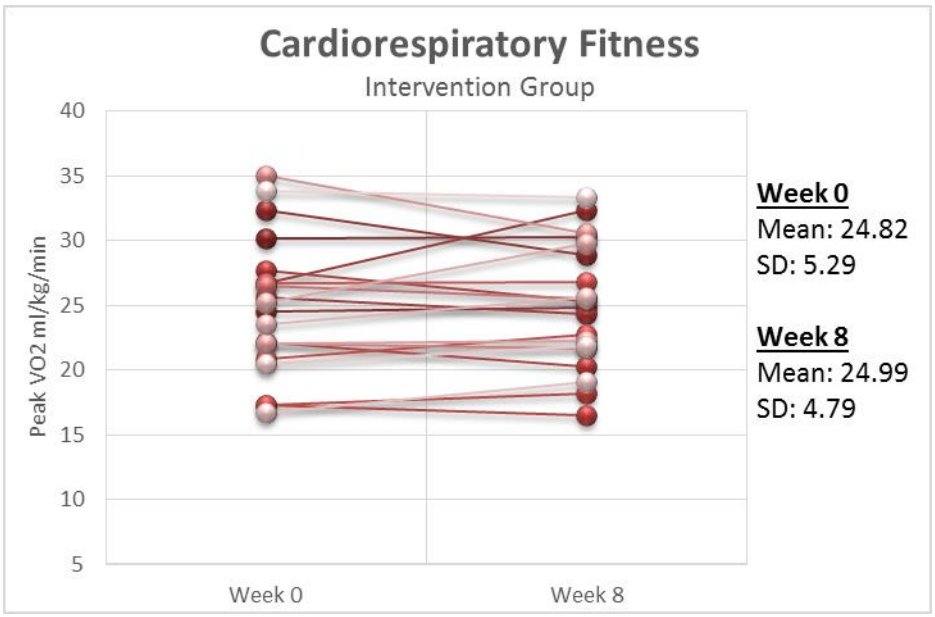

Figure 4.2.1 The difference in individual results for CRF for the intervention group at baseline (Wk0) and re-test (Wk8)

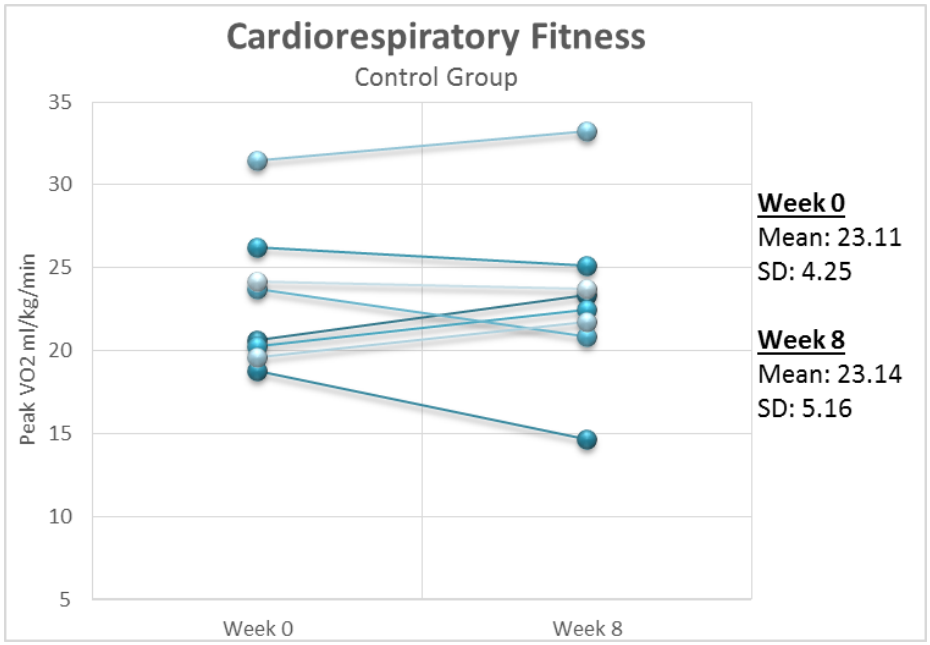

Figure 4.2.2 The difference in individual results for CRF for the control group at baseline (Wk0) and re-test (Wk8)

4.3 cf-PWV

The Δ (change) in cf-PWV was not significantly different between the intervention (6.26 ± 95% CI 5.39 -7.12) and the controls (6.30 ± 95% CI 4.96 -7.65) following 8 weeks of CR. The intervention group at baseline showed a mean cf-PWV of 6.06 ±2.05m/s, with a non-significant improvement at 8 weeks with a mean time of 6.45 ±1.83m/s. The interaction between time and group was not significant (F (1,22) = 0.703, P <0.411). This may have been an issue of study power, and a larger sample size may have led to more significant findings.

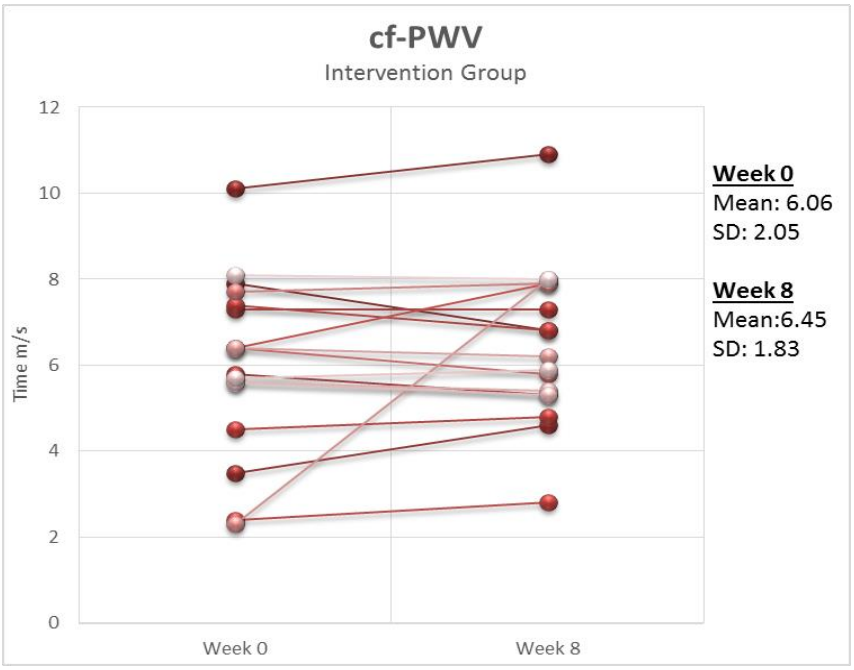

Figure 4.3.1 The difference in individual results for cf-PWV for the intervention group at baseline (Wk0) and re-test (Wk8)

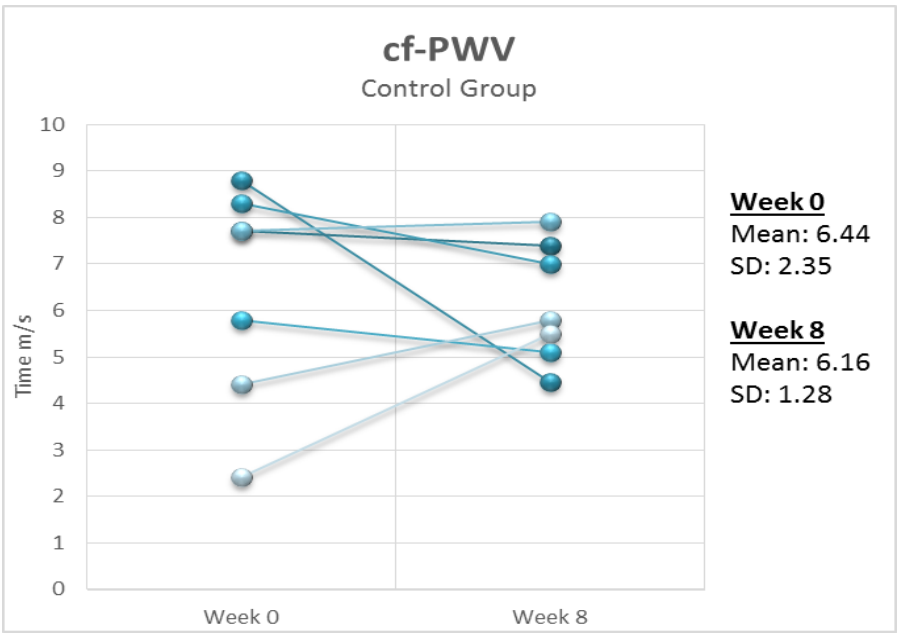

Figure 4.3.2 The difference in individual results for cf-PWV for the control group at baseline (Wk0) and re-test (Wk8)

4.4 ba-PWV

The Δ (change) in ba-PWV was not significantly different between the intervention (14.15m/s ± 95% CI 11.3-16.99m/s) and the control groups (18.41m/s ± 95% CI 13.98 - 22.85m/s) following 8 weeks of CR. The intervention group at baseline showed a mean ba-PWV of 14.19 ±2.31m/s, in comparison to 8 weeks with a mean time of 14.1 ±2.17m/s showing no change. The interaction between time and the groups was not significant [F (1,22) = 1.597, P < .220].

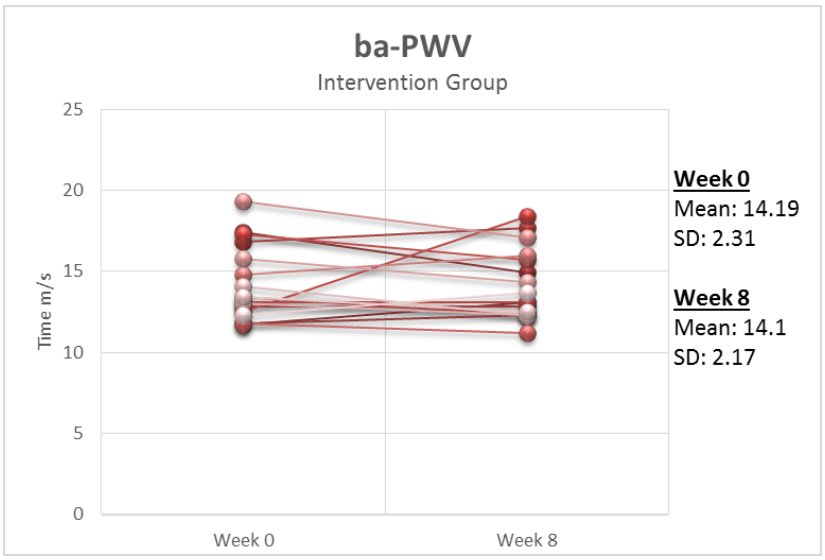

Figure 4.4.1 The difference in individual results for ba-PWV for the intervention group at baseline (Wk0) and re-test (Wk8)

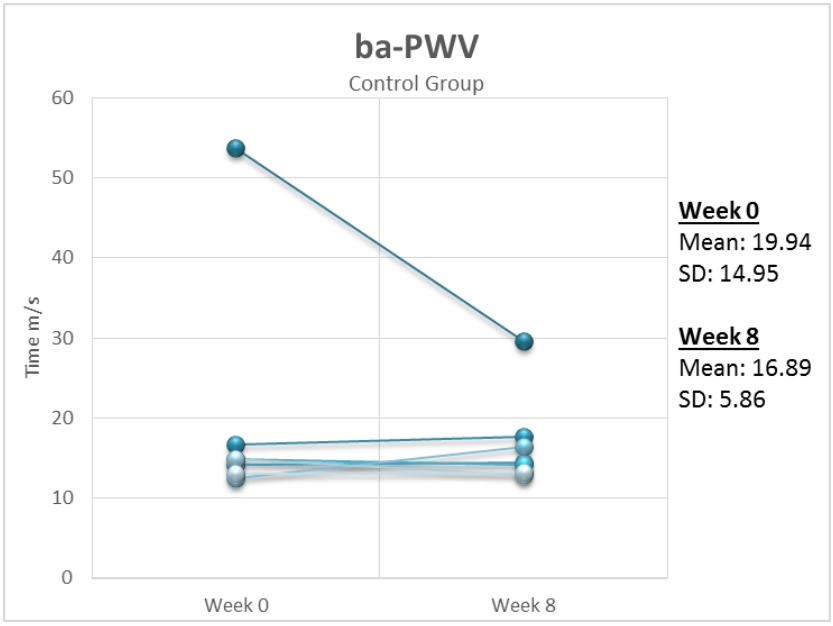

Figure 4.4.2 The difference in individual results for ba-PWV for the control group at baseline (Wk0) and re-test (Wk8)

4.5 PWV aorta

The Δ (change) in PWV aorta was not significantly different between the intervention (14.15m/s ± 95% CI 11.3-16.99m/s) and the control groups (18.41m/s ± 95% CI 13.98 - 22.85m/s) following 8 weeks of CR. The intervention group at baseline showed a mean PWV aorta of 14.19 ±2.31m/s, in comparison to 8 weeks later with a mean time of 14.1 ±2.17 m/s showing no significant change. The interaction between time and group was not significant [F (1,22) = .718, P < .406]. Therefore, there is no difference in PWV aorta between the intervention and control group following 8 weeks of CR.

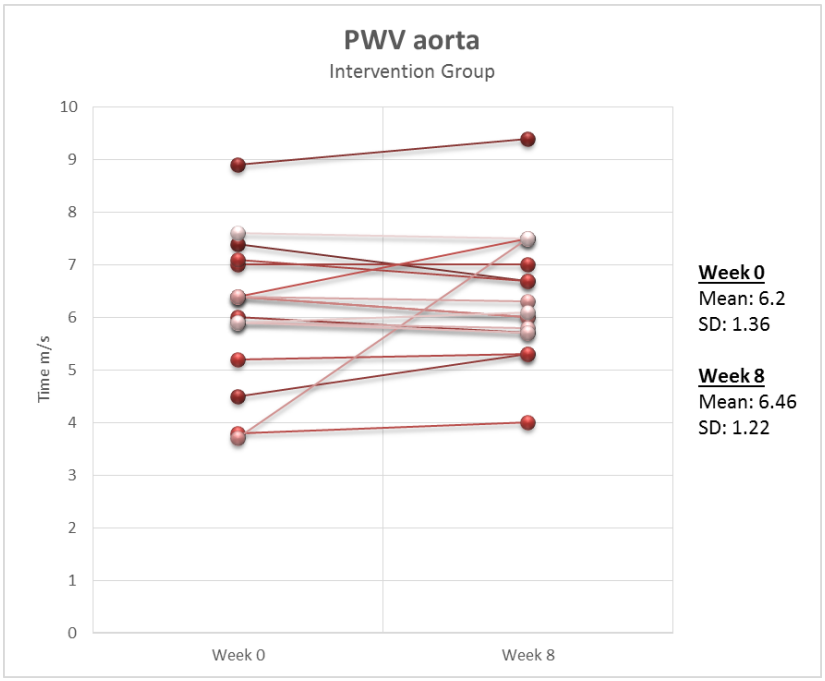

Figure 4.5.1 The difference between baseline (Wk0) and re-test (Wk8) in the intervention group for Aortic PWV

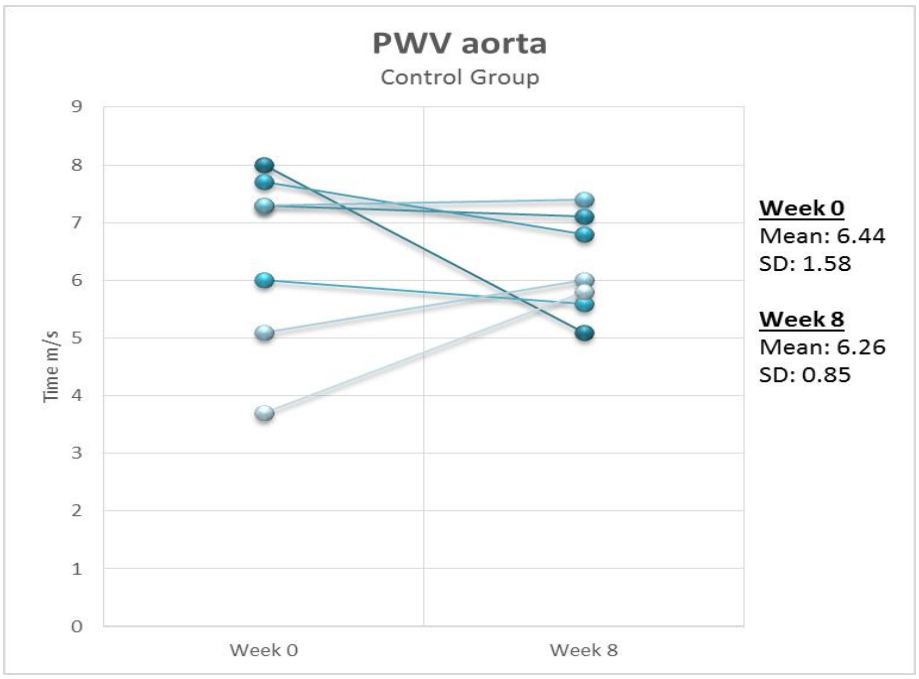

Figure 4.5.2 The difference between baseline (Wk0) and re-test (Wk8) in the control group for Aortic PWV

4.6 ICAM-1

The Δ (change) in ICAM-1 concentration was not significantly different between the intervention (263.870µg/ml ± 95% CI 233.01-294.732µg/ml) and the control group (246.096µg/ml ± 95% CI 197.299 -294.893µg/ml) following 8 weeks of CR. The intervention group at baseline showed a mean ICAM-1 concentration of 232.362 ±80.532µg/ml, in comparison to 8 weeks with a mean concentration of 265.378 ±75.865µg/ml showing a slight increase within the intervention group, however not one of statistical significance. The interaction between time and the groups was not significant (*F* (1,26) .191, *P* <0.666). Therefore, there is no difference in ICAM-1 between the intervention and control group following 8 weeks of CR.

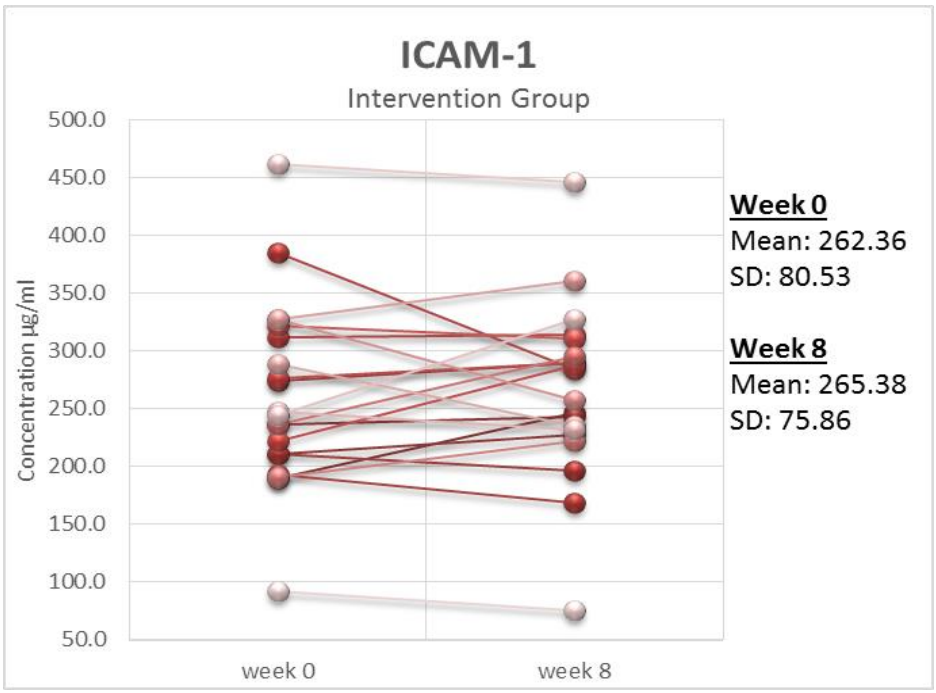

Figure 4.6.1. The difference in individual results for ICAM-1 in the intervention group at baseline (Wk0) and re-test (Wk8)

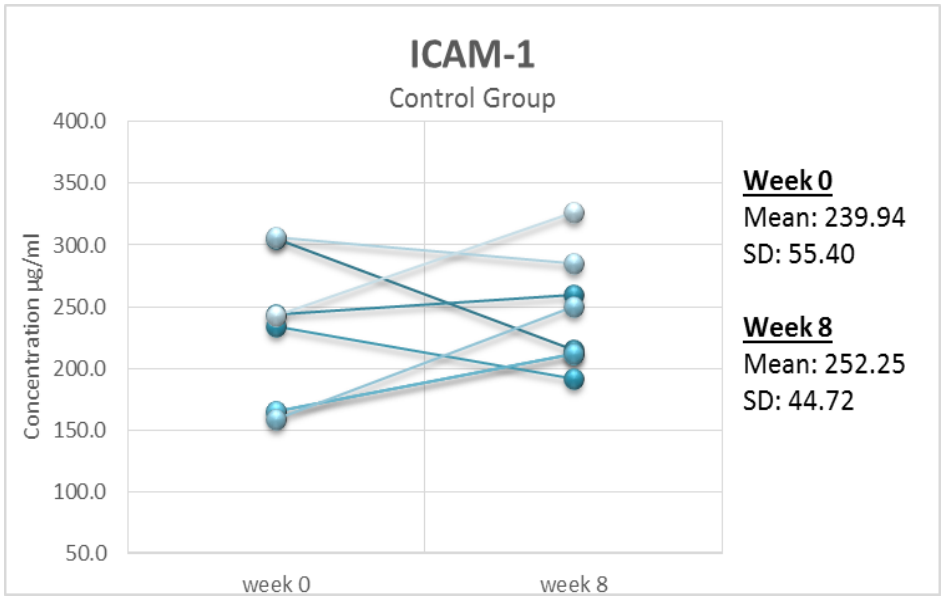

Figure 4.6.2. The difference in individual results for ICAM-1 in the control group at baseline (Wk0) and re-test (Wk8)

4.7 Effect of CR on VCAM-1

The Δ (change) in VCAM-1 was not significantly different between the intervention (1.757 µg/ml ± 95% CI 1.539µg/ml -1.976µg/ml) and the controls (1.413µg/ml ± 95% CI 1.067µg/ml -1.759µg/ml) following 8 weeks of CR. The intervention group at baseline showed a mean VCAM-1 concentration of 1.662 ±.454µg/ml, in comparison to the 8-week retest which showed a mean concentration of 1.853 ±.554µg/ml showing a slight increase but with no statistical significance. The interaction between concentration and the groups was not significant (F (1,26) = 0.011, P <0.751). Therefore, there is no difference in VCAM-1 between the intervention and control group following 8 weeks of CR.

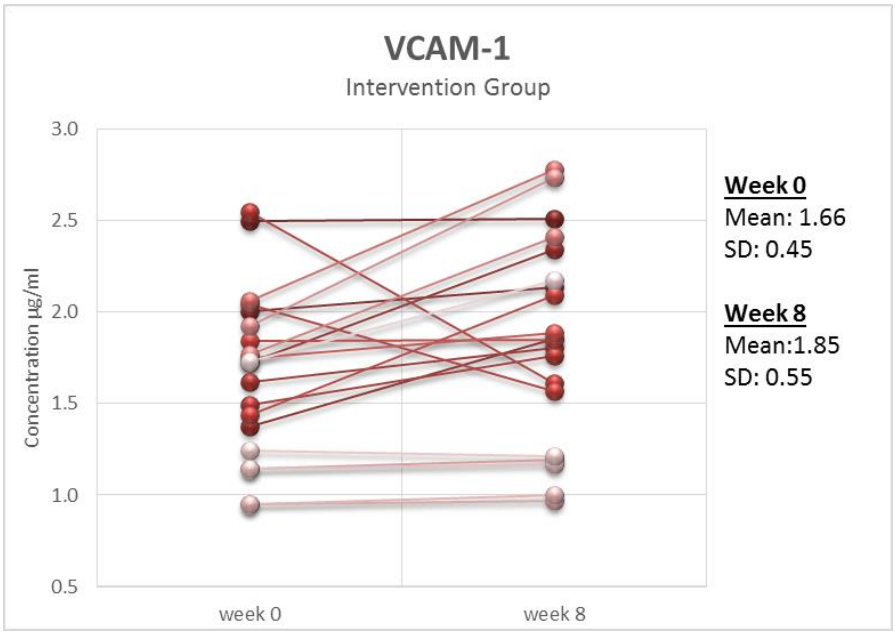

Figure 4.7.1. The difference in individual results for VCAM-1 in the intervention group in patients at baseline (Wk0) and 8 weeks later.

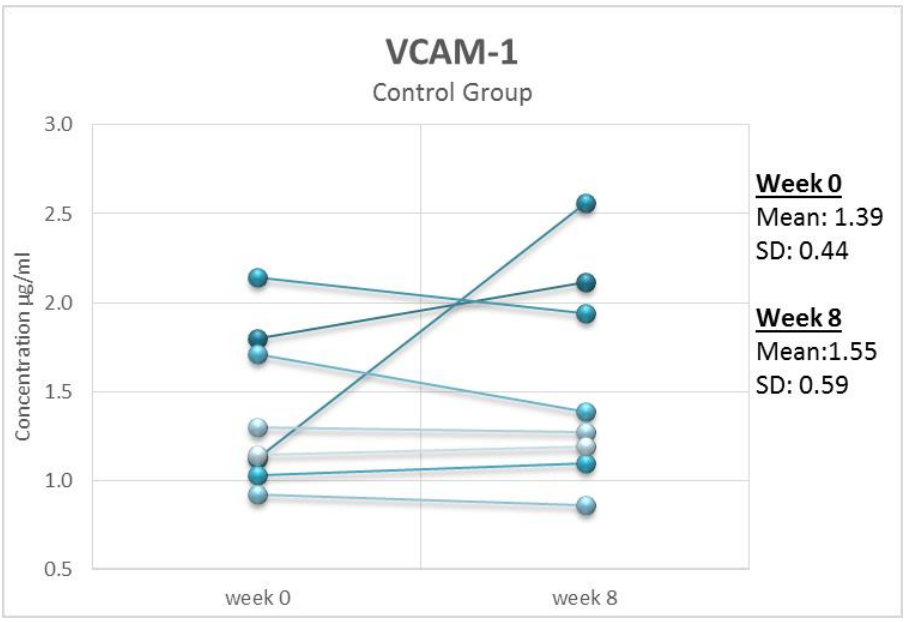

Figure 4.7.2. The difference in individual results for VCAM-1 in the control group in patients at baseline (Wk0) and 8 weeks later.

4.8 C-Reactive Protein

The Δ (change) in CRP was not significantly different between the intervention (3.75µg/ml ± 95% CI 2.119µg/ml -3.989µg/ml) and the controls (3.468 µg/ml ± 95% CI 1.99 - 4.946µg/ml) following 8 weeks of CR. The intervention group at baseline showed a mean CRP concentration of 3.453 ±2.298 µg/ml, in comparison to the results at 8 weeks that showed a mean concentration of 2.655 ±1,736 µg/ml. The between-group interaction was not significant (F (1,26) = 1.927, P =0.177). Therefore, there is no difference in CRP between the intervention and control group following 8 weeks of CR.

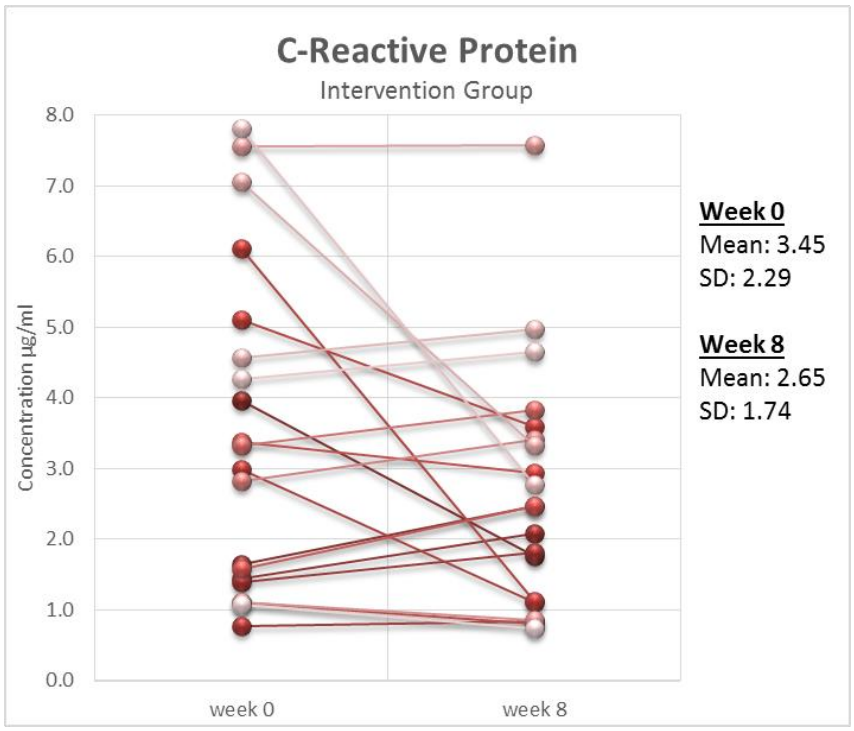

Figure 4.8.2. The difference in individual results for CRP in the intervention group at baseline (Wk0) and re-test (Wk8)

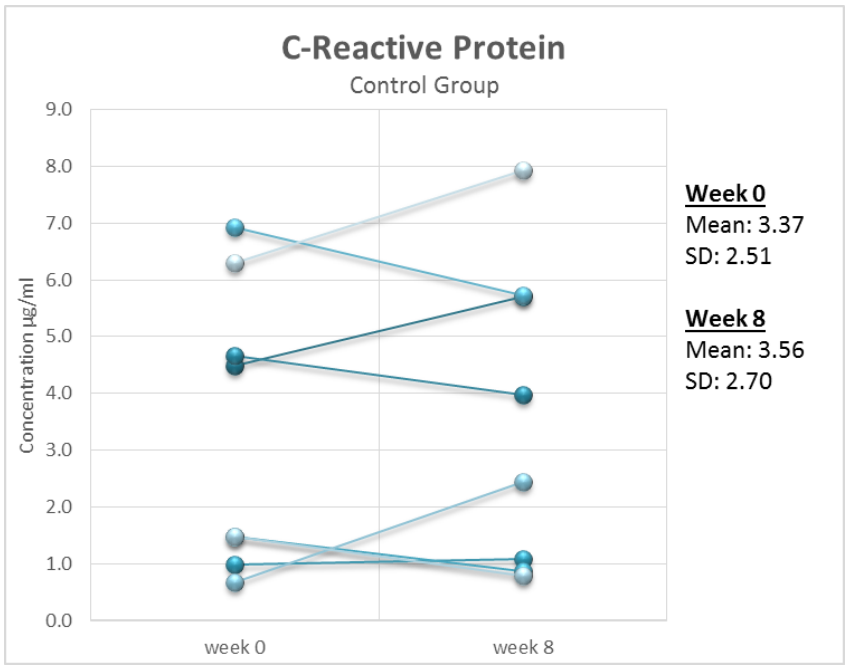

Figure 4.8.2. The difference in individual results for CRP in the control group at baseline (Wk0) and re-test (Wk8)

4.9 Correlations between PWV and blood biomarkers

Table 3. Correlation matrix between measures of PWV and blood biomarkers

		ICAM-1 week 0	VCAM-1 week 0	CRP week 0	ICAM-1 week 8	VCAM-1 week 8	CRP week 8
ba-PWV week 0	r-value	0.168	0.062	0.114	-0.101	0.174	0.296
	P-Value	0.478	0.797	0.632	0.672	0.463	0.205
cf-PWV week 0	r-value	0.052	0.265	-0.029	-0.131	0.415	-0.106
	P-Value	0.826	0.259	0.904	0.583	0.069	0.658
Aortic PWV week 0	r-value	0.059	0.267	-0.028	-0.128	0.416	-0.110
	P-Value	0.805	0.254	0.906	0.592	0.068	0.646
ba-PWV week 8	r-value	0.124	0.058	0.171	-0.090	0.304	0.317
	P-Value	0.602	0.81	0.456	0.706	0.192	0.174
cf-PWV week 8	r-value	-0.214	0.201	-0.077	-0.052	0.397	-0.038
	P-Value	0.364	0.395	0.745	0.827	0.083	0.874
Aortic PWV week 8	r-value	-0.224	0.193	-0.086	-0.055	0.398	-0.042
	P-Value	0.342	0.416	0.718	0.817	0.082	0.859

A correlation analysis between cf-PWV, ba-PWV, and PWV aorta and blood biomarkers including ICAM-1, VCAM-1 and CRP was conducted. The results were compared both at baseline and re-test via bivariate correlation using Pearson's correlation coefficient. Correlations at baseline and re-test showed very similar results and therefore results at the 8 week stage are presented for comparative purposes. Results at 8 weeks showed that there was no correlation between cf-PWV and ICAM-1 (r = -0.052, 95% CI [-0.333, 0.286], *P* =0.827). The correlation between cf-PWV and VCAM-1 showed a moderate association (r = 0.397, 95% CI [0.125, 0.679], *P* =0.083). The results for cf-PWV and CRP yielded no association (r = -0.038 95% CI [-0.361, 0.362], *P*=0.874). The correlation between ba-PWV and ICAM-1 showed no association (r = -0.090, 95% CI [-0.415, 0.413], *P*=0.706). The correlation between ba-PWV and VCAM-1 yielded a low association (r =0.304, 95% CI [0.092, 0.669], *P*=0.192). The association between ba-PWV and CRP showed a low association (r =0.317, BCa CI [-0.109, 0.692], *P*=0.174)

CHAPTER 5 – Discussion

5.1 Discussion

The results of the study show that 8 weeks (16 supervised sessions) of structured CR had no effect on endothelial function in patients with coronary artery disease. Endothelial function was evaluated through a variety of measurements including indices of pulse wave velocity as well as blood borne biomarkers (ICAM-1, VCAM-1). It is plausible that a typical UK-based CR programme is not sufficient in terms of training volume, to elicit positive changes in arterial stiffness.

Exercise training consistently improves nitric oxide (NO) function in patients with cardiovascular risk factors and disease, implicating a direct effect of exercise on the vasculature, mediated through intermittent increases in endothelial shear stress. Whilst the mechanisms responsible for mediating vascular structural change remain largely undefined, there is strong evidence that NO plays an important role in arterial remodelling and thus endothelial function (Green et al., 2004). Asymmetric dimethylarginine or ADMA (an endogenous competitive inhibitor of NO synthase) plasma concentration levels have been examined using several methods to identify the association between various disease states and endothelial function and it has been identified that elevations of ADMA levels in the blood are associated with endothelial dysfunction (Horowitz and Heresztyn, 2007) The link between plasma ADMA levels and established as well as emerging risk factors for progression of vascular disease has been investigated and it may be worth observing how ADMA levels in the blood change with regards to exercise based CR (Sibal L, et al 2014).

In cardiac rehabilitation there is still controversy regarding the exercise characteristics that are most effective for improving peak VO_2 in CAD patients, however it seems that intensity may be an important predictor of the effectiveness of a CR programme since a higher intensity leads to larger improvements in peak VO_2, even after adjustment for other training-related variables. However, a higher intensity is difficult to maintain for a longer period (Conraads et al, 2015).

Studies conducted observing the effects of CR on diabetic populations found that diabetic participants had a lower overall exercise capacity in comparison to non-diabetic participants. In the present study there was a significant difference (P= 0.004) between the control and intervention groups, with 75% of the control group presenting with type 2 diabetes compared to only 15% in the intervention group.

This difference between diabetic and non-diabetic participants was evident in a study conducted by Hindman et al (2005) comparing the effectiveness of a CR programmes on clinical outcomes after MI and revascularization procedures. Their results found that even though at baseline the diabetic group had lower CRF levels than the non-diabetic group which was recorded in METs (1 MET is equivalent to an oxygen uptake of 3.5mL/kg^{-1}/min^{-1}) with the diabetic group 5.7 ±2.3 METs (mean peak VO_2 of 19.95mL/kg^{-1}/min^{-1})at baseline in comparison to the non-diabetic group with a baseline mean of 7.1 ±2.6 METs (mean peak VO_2 of 24.85mL/kg^{-1}/min^{-1}), the diabetic and non-diabetic groups improvements were found to be similar in proportion. The diabetic group showed a significant improvement in cardiorespiratory fitness with an end mean of 7.3 ±2.4METs (25.55mL/kg^{-1}/min^{-1}) and the non-diabetic group improved with an end mean of 8.9 ±2.7METs (31.15 mL/kg^{-1}/min^{-1}) (Hindman et al, 2005). According to Hindman et al (2005) several studies have reported a high dropout rate among diabetic patients possibly due to poor exercise tolerance, comorbid conditions, a greater incidence of intercurrent illnesses, as well as other unknown factors.

Similarly, Carroll et al (2011) conducted a 15month comprehensive CR programme in the UK which involved combination aerobic and resistance training sessions of 45 to 60 minutes for 3 non-consecutive sessions per a week (Carroll et al, 2011). In addition to these sessions patients were encouraged to walk 30 minutes per day. The results showed that patients with diabetes had significantly lower exercise capacity than patients without diabetes at baseline and also had similar relative improvements with exercise training between the two groups, as had been observed by Hindman et al (2005). Although the study did not measure maximal oxygen uptake, when calculated, they observed a mean submaximal oxygen uptake of 3 to 4 mL/kg^{-1}/min^{-1} which is estimated to be a decrease of approximately 27% to 36% in cardiovascular related mortality in secondary prevention settings (Carroll et al, 2011).

The significant difference between the control and intervention groups in the present study, with regards to type 2 diabetes, may have influenced the results of the peak VO_2 at baseline for the control group, with a mean of 23.12 $mL/kg^{-1}/min^{-1}$ ± 4.25 in peak oxygen uptake compared with the intervention group who had a mean baseline peak oxygen uptake of 24.82 $mL/kg^{-1}/min^{-1}$ ± 5.29. However, as observed in previous studies there was a similar improvement in CRF between the two groups (control group increased by a mean of 0.035$mL/kg^{-1}/min^{-1}$ and the intervention group increased by 0.179 $mL/kg^{-1}/min^{-1}$), although the improvement in CRF was not significant (*P*=0.402). According to Carroll et al (2011) an increase of 1$mL/kg^{-1}/min^{-1}$ in peak oxygen uptake is equivalent to a 9% decrease in cardiovascular related mortality, therefore it can be noted that CRF in this study did not yield any beneficial decreases in cardiovascular related mortality.

Conraads et al (2015) conducted a parallel-group trial consisting 96 patients with a mean age of 56 ± 10 years. Participants were randomized into a CR intervention group or control group, 4 weeks after suffering acute MI. CR consisted of 12 weeks of aerobic exercise of either interval training (90–95% of peakHR) or continuous training (at least 70–75% of peak HR). They also used FMD to determine the endothelium-dependent vasodilation and observed improvements after both interval and continuous training, the absolute change in FMD correlated inversely with baseline FMD and positively with the increase in peak VO_2 (Conraads et al, 2015). After the 12 weeks of intervention Peak VO_2 increased with 5.06 ± 4.06$mL/kg^{-1}/min^{-1}$ after interval training from a baseline mean of 23.3 ± 5.78$mL/kg^{-1}/min^{-1}$ and by 4.35 ± 3.21mL/kg-1/min-1 after continuous training with a baseline mean of 22.2 ± 5.56$mL/kg^{-1}/min^{-1}$ (Conraads et al, 2015). It has also been suggested that there are greater aerobic and cardiovascular adaptations after high intensity exercise than with low and /or moderate levels in patients with coronary artery disease, chronic heart failure or left ventricular dysfunction and also in healthy individuals (Wisloff et al., 2007). Also according to Wisloff et al (2007) it is suggested that exercise training at an intensity of 90% VO_2peak is in the upper range of current guidelines for humans and this level of exercise can only be achieved in an interval training format.

According to Sandercock et al (2013) UK rehabilitation programmes do not elicit a large enough training response to provide secondary prevention of cardiac disease which was

evident in the present study. The RAM-IT trial conducted by West et al (2011) was a randomised control trial of comprehensive rehabilitation in 1813 patients following acute MI as provided in typical programmes, in representative hospitals in England and Wales in 1997 to 2000. This trial found that rehabilitation had no effect on all-cause mortality of the population after 1, 2 or 7 to 9 years (West et al, 2011). Other findings included that there was an absence of effect on lifestyle change which suggests that the rehabilitation programmes added little to patients knowledge or motivation to bring about necessary preventative lifestyle changes (West et al, 2012). The changes observed in longer programmes may point to the suggestion that UK programmes were not sufficiently intense also there were no previous trials suggesting current dose-response (West et al, 2012). Current UK national service guidelines for CR cite evidence from systematic reviews that showed up to a 20% reduction in mortality for patients completing exercised based CR, however the studies from which the figures mentioned were retrieved were mostly completed 20-30years ago and are not necessarily representative of modern CR as they also do not include current pharmacological practice (Sandercock et al, 2013).

According to the review conducted by Sandercock et al (2013), UK CR did not produce cardiorespiratory changes in fitness that were comparable to international studies. A comparison between a meta-analysis of international studies undertaken by Sandercock et al (2011) showed an increase in METs of 1.55; 95% CI 1.21–1.89 METs, whereas the multicentre study of UK CR by Sandercock et al (2013) only reported an increase of 0.52; 95% CI 0.51-0.53 METs. Therefore improvements in cardiorespiratory fitness in the UK when compared to international studies may suggest that the 8 week (between 6 and 16 supervised sessions) CR programme in the UK may not be sufficient in reducing cardiac risk factors Sandercock et al (2011). It is suggested that a 1.5MET increase in cardiorespiratory fitness can equate to a 16-54% reduction in cardiac mortality dependant on the estimate used (Sandercock et al 2011). The optimal features of a CR programme to promote such changes is suggested to be >36 sessions of aerobic exercise or a mixed aerobic and resistance exercise delivered over a 12-week period (Sandercock et al, 2011). Therefore it can be noted that exercise does improve cardiorespiratory fitness (CRF) and increased CRF has the ability to prevent cardiac events partially due to its significant effect on maintaining vascular homeostasis (Joyner and Green, 2009).

Regular physical activity, such as walking, jogging or cycling are known to reduce stiffness of central and peripheral arteries in healthy adults (Montero et al, 2014). The most frequently studied index to date among a variety of PWV measures is cf-PWV (Tanaka et al, 2009). An emerging measure of PWV that has been widely used in Japan and other east-Asian countries in the past 10 years is ba-PWV (Tanaka et al, 2009). Although these two PWV measures are widely used, associations between the two are not clear, additionally, it is not known how each of the arterial stiffness measures are associated with coronary heart disease (CHD) risk factors (Tanaka et al. 2009). Moreover, how both techniques are comparatively related to clinical events is not currently known therefore Tanaka et al (2009) attempted to systematically address these issues by conducting a multicentre study to determine associations between cf-PWV and ba-PWV. According to Kim et al (2015) the threshold for cf-PWV of >10m/s proposed by the European Society of Hypertension and the European Society of Cardiology, as a conservative estimate of significant alterations in aortic function (2013 ESH/ESC Guidelines for the management of arterial hypertension, 2013). It is also suggested in these guidelines that hypertensive patients with a PWV above 10m/s should be considered for CR therapeutic strategies. Japanese data (Tanaka et al, 2009) demonstrated a correlation between CF-PWV and BA-PWV to be 14.84 m/s for ba-PWV vs 12.56m/s for cf-PWV. The reason for reviewing the comparison for these two measures of PWV is to better understand how the results found in this study may impact the patient risk for further cardiac events. Therefore when looking at the mean results for PWV in the current study when comparing ba-PWV, 14.19 ±2.31m/s at baseline and 14.10 ±2.17m/s after 8 weeks in the intervention group it can be suggested that these values are similar to those found by Tanaka et al (2009) (14.84 m/s for ba-PWV) with regards to measurements of arterial stiffness that indicate a significant change in arterial function. The control groups mean results observed were 19.94 ± 14.95m/s at baseline and 16.89 ±5.86m/s after 8 weeks, these results are higher than the ba-PWV results observed by Tanaka et al (2009). It should be taken into consideration that Tanaka et al (2009) did not include an exercise intervention and that the aim of the study was purely to observe the correlation between ba-PWV and cf-PWV in patients with conditions including CAD and stroke.

Training studies have been conducted using changes in PWV as an outcome measure. Oliveira et al (2015) conducted a study involving 96 patients being separated into a control and exercise intervention groups, 4 weeks post MI, and evaluated the effects of exercise on both arterial stiffness and inflammation in CAD patients in Portugal. After 8weeks of CR there were no changes in arterial stiffness, endothelial dysfunction and inflammatory biomarkers, despite improvements in cardiorespiratory fitness. In the current study, cf-PWV for the intervention group was 6.06 ±2.05m/s at baseline and 6.45 ±1.83m/s at 8 weeks. The control group had a baseline measurement of 6.44 ±2.34m/s and 6.16±1.28m/s at 8 weeks. Oliviera et al (2015) conducted an 8-week study of aerobic exercise at 70 to 85% of maximal heart rate for 3 sessions per week with a 10min warm up, 30 min on a bicycle or treadmill and a 10 min cool down, which is already longer than a typical UK CR programme as it involves 24 training sessions as opposed to 16 sessions. They observed mean changes in cf-PWV in the intervention group of 8.0 ±2.2m/s to 7.7 ±1.7m/s and 8.4±2.1 to 8.5±2.3 for the control group and found these changes to be statistically significant (P=0.008). The changes observed by Oliviera et al (2015) are slightly higher than the results observed in this study overall, however there were decreases in mean cf-PWV in the intervention group and an increase in m/s in the controls. These observed differences between the intervention and control group in the current study may be attributed to the sample size (n=28 v n=96) in comparison with the larger intervention conducted by Oliviera and colleagues (2015).

Another exercise training study which focused on the trainability of PWV was conducted by Laskey et al., (2013) involving 20 weeks of CR in 26 men and 22 women (N=48), starting at 40-50% max HRR and building up to 70-85%, 3 times (60 trainings sessions in total) per week using treadmill walking, stationary cycling and upper body ergometry. This study showed improvements in cf-PWV from baseline measures of 7.2±1.4 m/s; final, 6.5±1.3 m/s, p=0.02) after the 20 weeks thus reiterating the need to re-evaluate the effectiveness of an 8 week CR programme being able to bring about positive physiological changes. In a systematic review and meta-analysis performed by Montero et al (2015) they compared the effect of combined aerobic and resistance training versus resistance training on its own on PWV and found that aerobic training exhibited a significantly lower ba-PWV after the intervention than the combined resistance and aerobic training combined (P= 0.002). In addition to ba-PWV, cf-PWV also showed a significantly lower result than that of the

combined training therapy (P=0.007). It may be noted that the mean number of training sessions in these studies was 2.6 sessions per week for an approximate mean duration of 39.2 min. On average training intensity for aerobic training was 60-90% of HR max (higher than the current UK guideline of 40-60% of HRR) with a mean resistance training intensity of 1-5sets of between 8-20reps for a mean number of 2.4 sessions per week for mean of 31.3 min at 50-80% 1 rep max (Montero et al, 2015).

When taking into consideration these findings it may be suggested that the inability of the 8 week exercise based CR to bring about significant changes in PWV may be attributed to the fact that the exercise sessions utilize combined resistance and aerobic training. Therefore both 'dose' and modality can be considered as possible reasons for the lack in changes observed in this study. Furthermore Montero et al (2015) concluded that no other moderating factors such as population size, age, gender, blood pressure, health status, duration of training or pre-intervention PWV modified the comparison between aerobic and combined training with regards to mean difference in PWV. Table 4 represents the current evidence base of trials that were conducted using PWV as a key outcome measure in exercise training interventions. It should be noted that the trials which demonstrate an improvement in PWV markers all exceed current UK guidelines for exercise training dose (duration, frequency and intensity) for CR programmes.

Table 4. Studies observing the effect of exercise on PWV

Authors	Study Design	Population	Condition	CR Duration	Frequency and Intensity	Observed effect
Oliviera et al (2015)	parallel-group trial	N= 96 patients (56 ± 10 years)	Myocardial infarction (MI).	8 weeks of aerobic exercise	70 to 85% of maximal heart rate during 3 sessions a week,	cf-PWV IG 8.0 ±2.2m/s to 7.7 ±1.7m/s CG 8.4±2.1 to 8.5±2.3
Yang et al (2013)	Cross-sectional study	N=40 healthy men. (young = 21 to 33yrs) and (older = 59 to 72yrs)	None. Groups based on age and habitual Exercise status: young (sedentary and endurance trained) and older (sedentary and endurance trained)	12 weeks of aerobic exercise	4 sessions a week 30min/session	Ba-PWV (m/s) Older sedentary: Baseline 13.95 ±1.07 12wks 13.24±1.16 Young sedentary Baseline 11.79±5.1 12wks 11.47±3.9 (no specific values provided for the endurance group)
Lessiani et al (2015)		N=18 subjects (12 males) 54 (48–66)years	Sedentary, healthy individuals	8 week aerobic exercise	2 sessions per week 55min per session at 60-75% VO^2max	Cf-PWV decreased 6.28 (5.44–7.86) m/s vs 5.31 (4.59–6.67) m/s, P<0.0001; a 9.9% decrease vs baseline

Montero et al (2014)	Systematic review and meta-analysis	N=472 Patients (over a total of 14 articles)	Hypertension	Mean= 13.5 weeks aerobic exercise	Mean= 3.4 sessions per week, ±42.8 min per session	IG (14 trials, mean difference: -0.19; 95% CI=0.39, 0.01, *P* = .06) CG (8trials, mean difference: 0.10; 95% CI=0.34, 0.14, *P*=.43) Between group difference (7 trials, mean difference: -0.14; 95% CI = -0.45, 0.16, *P*= .36).
Montero et al (2015)	Systematic reviews and Meta-Analyses	N=752 Patients	Cardiovascular disease, Diabetes type 1 and 2, hypertension, hyperlipidaemia, renal failure	8 to 103 weeks of combined resistance exercise	Mean= 2.6 sessions per week ±39.2 min. Average 60–90% HR max for aerobic Resistance= Between 1-5 sets, 8-20 reps, mean= 2.4 sessions per week ±31.3 min Intensity = 50 -80% 1 rep max	The mean difference in PWV did not differ between aerobic and combined training trials (*P* = 0.12). No significant heterogeneity found among aerobic or combined training trials mean difference in PWV (I^2 = 0%, *P* = 0.76; I^2 = 41%, *P* = 0.08, respectively.
Rossow et al (2014)	Experimental design	N= 29 participants 16 young, 22±2years (Pre) and 13 older, 57±3years (post) females	Pre- and postmenopausal females who have not done resistance training in >6months or >5hours a week of endurance training.	8 weeks resistance training	3 sets 60s rest between, 10 reps at 80% 1 rep max	Cf-PWV group effect (*P*= 0.00) with 'post' having higher cf-PWV than 'pre' No significant cf-PWV interaction (*P*= 0.55) or time effect (*P* = 0.08) Change in cf-PWV values significantly (*P*= 0.001) correlated (r = −0.602) with baseline cf-PWV values.
Laskey et al (2013)	Experimental design	N= 48 Participants 26 men and 22 women 60.5 ±10.8years	Established CHD	20 weeks aerobic exercise	40-50% max HRR increasing to 70-85%, 3 sessions per week	cf-PWV= baseline 7.2±1.4 m/s; final, 6.5±1.3 m/s, (*P*=0.02)

In a study by Hoffmann et al, (2014), cf-PWV values were compared between healthy volunteers (n=318) and patients with CAD (n=155). The study aimed to analyse how cf-PWV in CAD patients correlated with reference values for healthy, normotensive volunteers and whether cf-PWV values reflect the extent of CAD. They reported that patients with CAD had a higher cf-PWV to that of healthy volunteers. Cf-PWV results were separated into age group as shown in the results in table 5.

Table 5. Results of Hoffmann et al (2014) showing the association between CAD patients and healthy volunteers.

Age group (years)	CAD Patients cf-PWV, m/s	Healthy Volunteers cf-PWV m/s
40–49	7.9±1.5 (n=8)	7.1±0.9 (n=36)
50–59	8.3±1.5 (n=21)	7.9±1.1 (n=36)
60–75	9.6±1.9 (n=100)	8.2±1.2 (n=18)
40–87	9.3±1.9 (n=155)	7.7±1.1 (n=90)

(Hoffman et al, 2014)

Findings in the current study showed lower values for cf-PWV compared to Hoffman et al (2014), when taking into account the average age of the sample study (63±9 for the control group and 61±8 for the intervention group) we can look at the age groups 50-59 and 60-65 in table 5 and note that the results found by Hoffmann et al (2014) for cf-PWV are higher than those found in this study (IG= 6.06 ±2.05m/s at baseline and 6.45 ±1.83m/s at 8 weeks, CG= 6.44 ±2.34m/s at baseline and 6.16±1.28m/s at 8weeks) The reason for this may however be due to differences in sample size as Hoffmann et al (2014) had a much larger sample size, a larger sample size may improve the accuracy of results. Although there have been studies as mention in table 4 that have evaluated PWV as a primary or secondary outcome measure there is insufficient evidence to determine normative values for PWV. Ranges for normative data would then need to be stratified by confounding variables such as ethnicity, age, sex, and disease risk factors. Therefore, the current picture does not allow us to draw any meaningful conclusions. Also evidence in table 4 suggests that exercise as an intervention strategy may result in decreases in PWV and therefore arterial stiffness.

Another observation that could be made is that the 8 week programme comprising of 16 training sessions, conducted in this study may not be a sufficient time period to conduct CR and observe sufficient changes in arterial stiffness and endothelial function. In particular, measurement of PWV may not be a sensitive enough marker to measure these changes. Flow mediated dilation (FMD) is a "gold standard" measure for measuring endothelial function. Studies conducted observing the effect of exercise on endothelial function may also assist in interpreting the most sufficient dose of CR in order to improve endothelial function. It was noted that percentage changes in FMD may seem small however according to Currie et al (2013) increased cardiovascular mortality and morbidity has been shown to be associated

with a relative FMD value estimated at <5.5%. When looking at aerobic training as an intervention modality, Wisloff et al (2007) showed great changes in FMD for interval aerobic training than for continuous aerobic training over a 12week period (P<0.05). The trial consisted of 27 patients randomised into groups of 9 interval and 9 continuous training, with 9 individuals as control subjects. The exercise duration used was 38min, 3 times per week for interval training at 90-95% with active rest periods of between 50-70% peak heart rate; and 47min, 3 times a week for continuous aerobic training at an intensity of just 70-75% peak heart rate. Since Wisloff et al (2007) used both Continuous and Interval training and showed the most significant changes in FMD during interval training at high intensities, along with results from studies such as Conraads et al (2015) it can be noted that one of the main findings is that studies comparing interval and continuous training, found that interval training showed the greatest improvements in endothelial function. Edwards et al (2004) however reported an improvement of 3.3% using only endurance training for 15 to 50min 3 times a week for 12 weeks (36 sessions compared to the UK's 16) not exceeding 50% HR max as an intervention strategy. Therefore difference in results in FMD may be a result of differences in the exercise modality, duration and intensity suggesting that dose of exercise plays a role in the ability of a CR programme to produce positive changes in endothelial function. Another observation from these results may suggest FMD to be a more sensitive method of measuring the effect of exercise on endothelial function than the results observed by PWV and blood borne biomarkers and endothelial function.

The current study showed that there were no statistically significant changes in ICAM-1, VCAM-1 or CRP after the 8 week CR programme. The current exercise prescription of 16 sessions at 40-60% of HRR is less than what may be required to bring about changes in endothelial function. As mentioned previously, UK patients receive approximately one third the amount of exercise that patients in Europe and the USA will receive (Sandercock et al., 2012). Table 6 displays a sample of studies that have been conducted that compare the effect of exercise intervention on blood borne biomarkers of endothelial function. The majority of these programmes exceed 16 supervised training sessions, however, only 8 studies include patients with some form of heart disease.

Table 6. Studies observing the effect of exercise on blood borne biomarkers

Authors	Study Design	Population	Condition	CR Duration	Frequency and Intensity	Observed effect
Lim et al (2015)	Randomised control trial	N=30 male night shift workers (IG 56.80 ± 1.82years and CG 58.33 ± 1.88years)	Night shift workers presenting with history of CVD, hepatectomy, hypertension and diabetes.	10 weeks aerobic exercise	3 sessions per week at 60-79% HR max for 30min per session	VCAM-1 IG= 513.87 ± 21.36 to 497.20 ± 20.40ng/mL CG= 531.53 ± 31.40 to 539.60 ± 32.55ng/mL *P*=0.001
Barone Gibbs et al (2012)	Randomised control trial	N=140 Adults (40-65years)	Sedentary with type 2 diabetes and untreated pre- or stage 1 hypertension	6months aerobic exercise	3 sessions per week, 45min with 10-15 min warm up and a cool down, 60-90% HR max, followed by 7 Resistance exercise for 2x15reps at 50% 1RM,	ICAM-1 IG= 203±63 to 201±56ng/ml CG= 228±90 to 228±80ng/mL *P*=0.215 VCAM-1 IG= 692±196 to 713±220ng/mL CG=692±184 to 675±217ng/mL *P*=0.623
Marcell et al (2005)	Randomised control trial	N=51 Middle aged participants (45.3 ± 8.3 years)	Overweight, insulin-resistant, nondiabetic.	16 weeks of moderate aerobic, intense aerobic or no exercise	5 sessions per week for 30min. Moderate exercise training range not specified, Intense exercise group was 80-90% HR max	CRP Moderate IG= 4.9±3.2 to -1.0 ± 0.5 mg/L Intense IG= 3.4±3.5 to -0.4 ± 0.5mg/L CG= 5.7±5.1 to -1.0± 0.5mg/L
Jae et al (2006)	Non-randsomised control trial	N=47 healthy sedentary individuals (IG 49.6 ±7.5years and CG 49.7 F 6.4	Overweight	12 weeks home based aerobic exercise	5 sessions per week, 50-60min at 60-80% HR max	CRP IG= 0.75±0.4 to 0.56± 0.34mg/dL CG= 0.60±0.3 to 0.70±0.4mg/dL *P* = .01
Parrinello et al (2010)	Experimental design	N=22 Caucasian patients, (62.7±4.8 years) 15 men, 7 women	Compensated CHF (mean left ventricular ejection fraction 38.9±3.6%)	10 weeks aerobic exercise.	5 sessions per week, 30min of mild to moderate walking per session	CRP IG= 0.69±0.34 to 0.39±0.32mg/dL CG= 0.67±0.35 to 0.72±0.34mg/dL
Cesari et al (2013)	Experimental design	N=112 patients (58.2 ±9.5years)	Acute coronary syndrome	4 weeks aerobic exercise	3 sessions per week, 5min warm up, 30min continuous, 5min cool down at 60-70% VO^2	CRP 3.1(0.3–11.8) to 2.1(0.3–10.1)mg/L
Buyukazi et al (2010)	Experimental design	N=26 females (30-49years)	pre-menopausal woman	12 weeks aerobic exercise	5 sessions per a week, for 1st 6 weeks aimed to walk for 30 building to 45 min and 2nd 6 weeks aimed to walk 45 building to 60min, moderate or high intensity	CRP High intensity IG= 3.96 ± 6.56 to 1.95 ± 3.37mg/L, *P*< 0.01 Moderate intensity IG= 2.18 ± 1.65 to 1.75 ± 1.39mg/L, *P*= < 0.05 CG= 2.11 ± 1.79 to 4.32 ± 4.80mg/L, *P*= not specified

					(50—55%, 70—75% max HRR)	
Saxton et al (2008)	Randomised control trial	N=104 patients, of which 92 patients supplied biomarker data (Leg Cranking IG 68 (50-85)years, Arm Cranking IG 66 (54-82)years and CG 72 (56-84)years)	Stable intermittent claudication	24 week aerobic exercise	2 sessions per a week, exercised in cycles of 2min at a crank rate of 50 revs/min, followed by 2 min rest resulting in 20 minutes exercise in a 40-minute session.	Leg Cranking IG ICAM-1= 1028 (896-1178) to 997 (852-1167)ng/ml VCAM-1= 1327 (1160-1519) to 1310 (1155-1486)ng/ml CRP= 3.16 (2.14-4.72) to 2.73 (1.86-4.00)mg/L Arm Cranking IG ICAM-1= 1017 (893-1158) to 1019 (894-1161)ng/ml VCAM-1= 1542 (1365-1742) to 1422 (1264-1600)ng/ml CRP= 2.53 (1.76-3.63) to 1.88 (1.28-2.74)mg/L CG ICAM-1= 991 (838-1171) to 943 (827-1076)ng/ml VCAM-1= 1453 (1263-1671) to 1344 (1192-1515)ng/ml CRP ICAM *P*= 0.68 VCAM *P*= 0.72 CRP *P*= 0.07
Adamop-oulos et al (2001)	Experimental design	N=12 patients (59.6 ±2years)	Stable chronic heart failure	12 week home based aerobic exercise	5 sessions per week, 30min at 50rpm to keep HR between 70% to 80% HR max	ICAM-1= 367±31 to 314±29ng/ml, *P*<0.01 VCAM-1= 1247±103 to 1095±100ng/ml *P*<0.01
Conraads et al, (2015)	Longitudinal, randomised prospective clinical study	N=200 participants (40-75years)	Angiographically documented CAD, previous acute MI, LVEF >40%	12 weeks Aerobic interval training (AIT) and Aerobic continuous training (ACT)	3 sessions per week at intensity of 90-95% Peak HR for AIT and ACT at 70-75% Peak HR	CRP AIT= 0.21 ± 0.44 to 0.12 ± 0.52mg/L ACT= 0.24 ± 0.57 to 0.07 ± 0.52mg/L
Wegge et al, (2004)	Experimental design	N= 20 Female participants (51 to 79 years)	Post-menopausal women	14 days aerobic exercise and dietary intervention	Every day, 45-60min at 70 to 85% HR max	CRP= 2.62 ± 2.3 to 1.43 ±0.9 mg/L, *P*< 0 .01. ICAM-1= 158.8 ±48.3 to 145.6 ±38.2 ng/mL, *P*<0.05 VCAM-1= 670±245.7 to 678.8±205.6 (no change in VCAM-1 *P*-value not reported)
Hammet et al (2006)	Random Control trial	N=152 participants taking part in inflam-matory marker study (IG 38 ±12years, CG 39 ±11years	smoked at least 5 cigarettes per day for the last 2 years	12 weeks aerobic exercise	3 sessions per week for 45min at 60-70% HR max	CRP IG= 1.9 (0.5-3.8) to -0.1 (-0.7 to 0.2)mg/L CG= 1.3 (0.8-2.2) to 0.0 (-0.6 to 0.4)mg/L Multivariate *P*-value =0.71 ICAM-1 IG= 182 ± 51 to 38 (-13 - 82) CG= 191 ± 82 to 26 (-14 - 85) Multivariate *P*-value= 0.77

Zoppini et al (2006)	Experimental design	N= 16 sedentary patients (66 ±6years)	Type 2 diabetes	6 month aerobic exercise	2 sessions per week, 10min warm up, 40min brisk walk, 10min cool down at 50-70% HRR	CRP 3.54± 3 to 3.48 ±3mg/L *P* –value not reported ICAM 363 ±124 to 318±132 ng/mL; *P*=0.015
Roberts et al (2006)	Experimental design	N=13 patients (55-74years)	Overweight, obese, diabetic	21days diet and aerobic exercise	Daily 45-60min sessions at 70-85% HR max	CRP ±3.78 to ±3.06mg/L *P*<0.05 ICAM ±389.0 to ± 295.8µg/L *P*<0.05 VCAM Δ after intervention *P*<0.001
(Goldha-mmer et al., 2005)		N=28 patients (64.7 ±7.1years), 18 male, 10 female	Stable CAD	12 weeks aerobic exercise	3 sessions per week, 45min at 70-80% HR max	CRP 7.5±4.2 to 3.9±3.5 mg/l, *P*<0.001

The findings in the current study are in contrast to responses of physical activity on ICAM-1 as described by Adamopoulos et al (2001) which mentioned the effect of home-based aerobic training in 12 patients with chronic heart failure who undertook 12 weeks of training for 30 min per session, 5 times per week at 70 to 80% maximal heart rate. The findings showed that ICAM-1 fell significantly from 367±31 to 314±29ng/ml, (*P*<0.01). Due to the possible variation in ELISA kits, the concentration reported in the current study is reported in µg/ml, however the changes observed in ICAM-1 were not significant in the intervention group (±263.870 246.096µg/ml; *P* <0.666). VCAM-1 results observed by Adamopoulos et al (2001) also observed a decrease (1247±103 to 1095±100ng/ml, *P*<0.01) in comparison to the non-significant increase observed in the current study (*P* <0.751). The differences in *P*-values observed between the current study and Adamopoulos et al (2001) may be explained by the 'dose' or volume of exercise, as they participated in 60 training sessions (12x5 sessions) compared to the 16 sessions attended in the UK and at a relatively higher intensity (40-60% HRR v 70-80% HRmax) than our patients.

Other training studies that have been conducted in patients with CVD include Rankovic et al (2009) who conducted a study exposing patients (N=52) with stable coronary disease to 6-weeks of CR, 3 weeks within the programme, and a further 3 weeks in home based exercise. CR consisted of 45min of aerobic exercise 3 times per a week at 70-80% heart rate max. ICAM-1 results in the intervention group showed a decrease from 7.8±1.56

to7.48±1.35ng/ml, it was reported that there was no significant effect on ICAM-1 between groups and that the changes were not significant, however no *P*-value for ICAM-1 was reported (Rankovic et al, 2009).

VCAM-1 was markedly reduced 11.1±2.2 to 9.3±1.21 in the intervention group and after 6 weeks of moderate aerobic physical training (Rankovic et al 2009). The authors found a positive reduction in levels of VCAM-1 ($P<0.05$). In the current study however, after the 8 week intervention, non-significant increases in VCAM-1 were observed (1.662 ±.454 to 1.853 ±.554µg/ml, $P<0.751$). Plasma levels of soluble VCAM-1 can be associated with a 4-fold increased cardiovascular risk according to Brevetti et al., (2010), therefore the effect of CR on VCAM-1 can be considered important in reducing risk of future coronary events.

However, a study by Gibbs et al (2012) performed in a larger population (N= 140) contradicts the findings of Rankovic et al (2009). Exercise prescription between the 2 studies were similar: 3 sessions per week, 45min with 10-15 min warm up and a cool down, 60-90% heart rate max, although Gibbs et al (2012) ran an intervention that lasted for 6 months (24 weeks) as opposed to 6 weeks. Gibbs et al (2012) found non-significant changes in ($P=0.215$) ICAM-1 in the intervention group compared to controls. Saxton et al (2008) also conducted a study that included evaluation of changes in ICAM-1, VCAM-1 and CRP. However, the population size that was included in the study was much larger (n=104 of which 92 provided biomarker samples) than the population sample in the current study. Their overall CR included 48 sessions (>16sessions) of interval training which totalled at 20 min of exercise per a session with 20min of rest (population included individuals with stable intermittent claudication). They compared different modalities of aerobic exercise (Table 6) however the observed effect of exercise on biomarkers included ICAM-1 showed non-significant changes ($P=0.68$), non-significant change in VCAM-1 ($P= 0.72$), but a significant improvement ($P= 0.07$) in CRP.

Elevated levels of CRP can be an independent prognostic indicator of increased cardiovascular mortality and morbidity (Rankovic et al., 2009). As mentioned earlier, how exercise training reduces inflammation and suppresses CRP levels is not well defined (Kasapis and Thompson, 2005). A meta-analysis conducted by Kelley and Kelley (2006)

noted that aerobic exercise across a variety of patient and training programmes produced no statistically significant changes in CRP, with only a ±3% reduction (−0.11 ± 0.14 mg/L; 95% CI [−0.39 to 0.17 mg/L] *P*=0.15) which is consistent with our findings (*P* =0.177). Kelley and Kelley (2006) included 6 trials in their meta-analysis, two of which are included in Table 6 (Marcell et al 2005; Hammett et al 2006). Merrill et al. (2008) conducted a study in 348 healthy individuals and found that despite CRP levels being significantly associated with other cardiovascular risk factors at baseline, after the intervention which included dietary changes and walking or exercising at a mild-to-moderate intensity for at least 30 min a day, the percentage of participants with high CRP fell from 46% (154/335) to 41% (127/312) after 6 months. After a post-hoc assessment, Merrill et al (2008), concluded their sample size was insufficient to identify a significant treatment effect on CRP at the small levels of change observed. However despite these low changes and the findings by Kelley and Kelley (2006) as well as the observed results in the current study, the majority of previous studies with >8 week training programmes and intensity levels higher than those currently recommended by UK CR showed significant decreases in CRP concentration. In athletes the effects of exercise varies according to the type of exercise and directly after exercise is performed CRP levels are greatly elevated, however the long term effects of exercise may depend on duration, intensity and frequency (Kasapis and Thompson, 2005). Despite the overlap between factors associated with physical activity and CRP, higher CRP levels persist in more active subjects in most studies even after adjustment (Kasapis and Thompson, 2005). Therefore it may be suggested that active lifestyle should be taken into account at baseline testing, however it is evident in Table 6 that sedentary and individuals of varying disease states may observe a reduction in CRP after physical activity. For example research conducted by Milani et al., (2004) in 277 patients with CHD found a significant decrease in the mean concentration of CRP (5.9 ± 7.7 to 3.8 ± 5.8 mg/l; −36%; $p < 0.0001$) after 12 weeks of CR, 3 sessions per week (modality and intensity not specified), thus reinforcing the value of CRP as an indicator for examining the effect of training studies on endothelial function.

The need for longer term exercise training is evident when comparing the results of the present study, which observed no significant improvements in CRF with studies that had longer durations with higher intensity exercise which observed significant improvements in CRF and in turn a decrease in cardiovascular related mortality. When taking into account all

the studies observed on the effects of exercise interventions on ICAM-1, VCAM-1 and CRP and their ability to observe changes in endothelial function, the evidence from the current study and others would suggest that there is insufficient evidence to suggest that ICAM-1 and VCAM-1 may be effective markers for examining the effects of exercise training on endothelial function, however CRP, which is a marker of systemic inflammation may be positively influenced by exercise training. Measures of PWV in the current study showed no change following 16 exercise training sessions, however evidence suggests that longer term exercise training may be more effective in showing positive changes in arterial stiffness, and as FMD is considered the "Gold Standard" for testing endothelial function, a study evaluating the relationship between results from these two markers may strengthen the validity of the use of PWV as a surrogate marker.

5.2 Limitations of the study

Initially this study would have included Asymmetric dymethylarginine (ADMA) results however due to restrictions in funding, ADMA was excluded from our blood-borne biomarker profile, however it may be noteworthy to look towards ADMA as a future marker of endothelial function as it is well established for determining functional levels of nitric oxide (a potent vasodilator) in blood vessels. Another limitation of the study was that 2 of the 30 plasma samples were not able to be collected or were compromised for either the baseline or follow up visit, blood sampling for one participant did not yield enough plasma or serum in order to make accurate sample measurements and another sample was compromised due to the sample not being able to be accurately separated. In addition due to problems obtaining PWV measurements only 25 out of the 30 participants were able to give before and after results for the PWV measurements.

Sample size was considered to be a limitation to the study, as only 30 participants volunteered for this particular study, due to this a few of the Chi square tests conducted on participants clinical characteristic were invalid due to one or more of the nominal measures having an expected count of less than 5, however p-values were still included to illustrate whether there were statistically significant clinical characteristics between the two groups. Diabetes for example seemed to have a significance effect between the two groups as majority of the diabetics in the study were in the control group and diabetics have a lower exercise capacity than non-diabetics (Carroll et al, 2011).

Therefore it may be worth repeating the research with the use of a larger sample of participants.

5.3 Recommendations and future studies

Future studies should try to determine the optimal levels of exercise training to be incorporated into UK CR guidelines in order to bring about significant improvements in aerobic fitness and to reduce established cardiovascular risk factors in patients with pre-existing CVD.

5.4 Conclusion

In conclusion, 8 weeks (16 supervised sessions) of structured CR had no effect on endothelial function (measured by PWV and blood-borne biomarkers including ICAM-1 and VCAM-1) in patients with coronary artery disease.

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Appendices

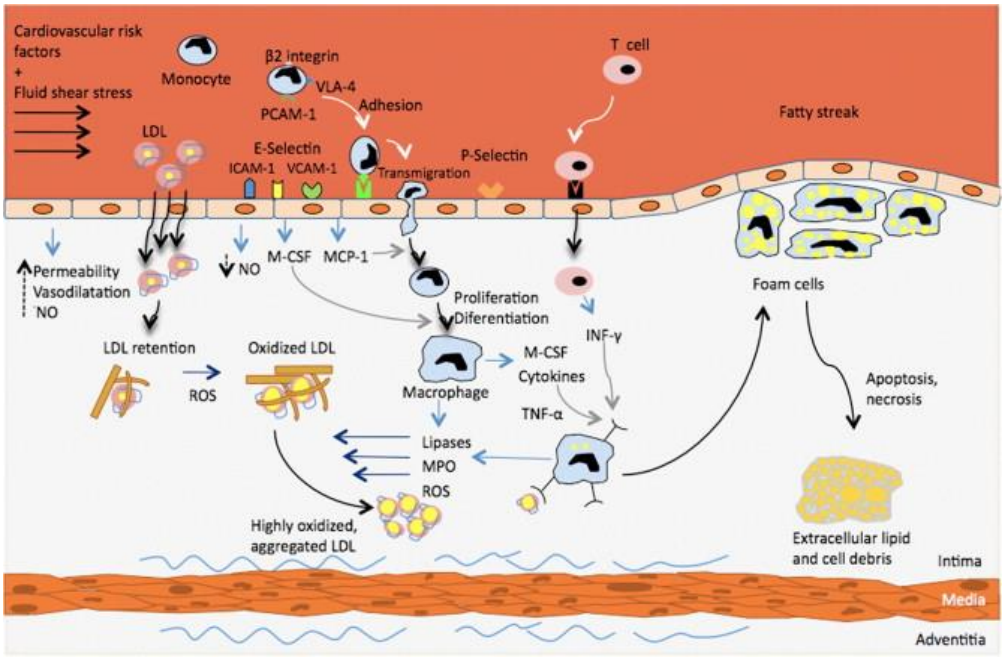

Figure 1. Schematic representation of the atherosclerotic process. (Ribeiro, F.,*et al*, 2010).

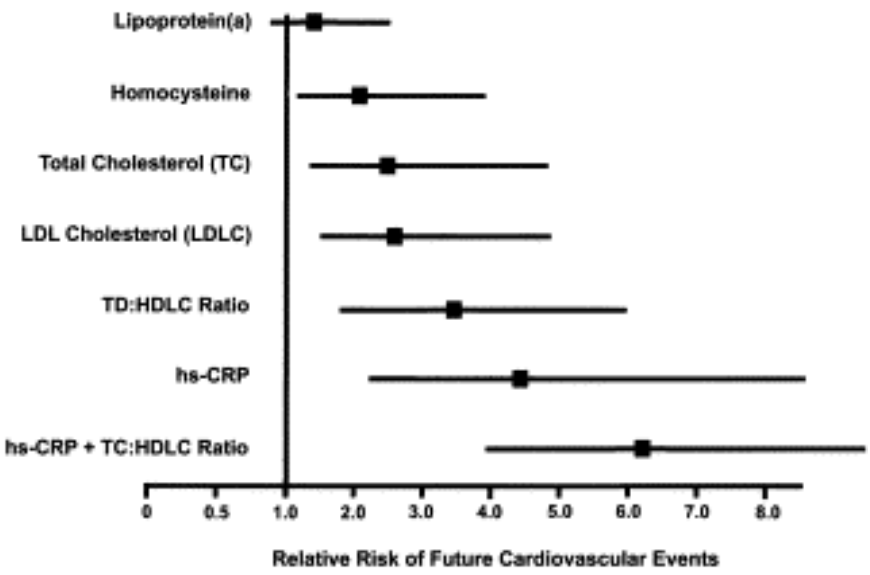

Figure 2. Direct comparison of magnitude of relative risk of future cardiovascular events associated with HSCRP (hs-CRP), cholesterol levels, lipoprotein(a), and homocysteine among apparently healthy women (Slater and Rill, 2004)

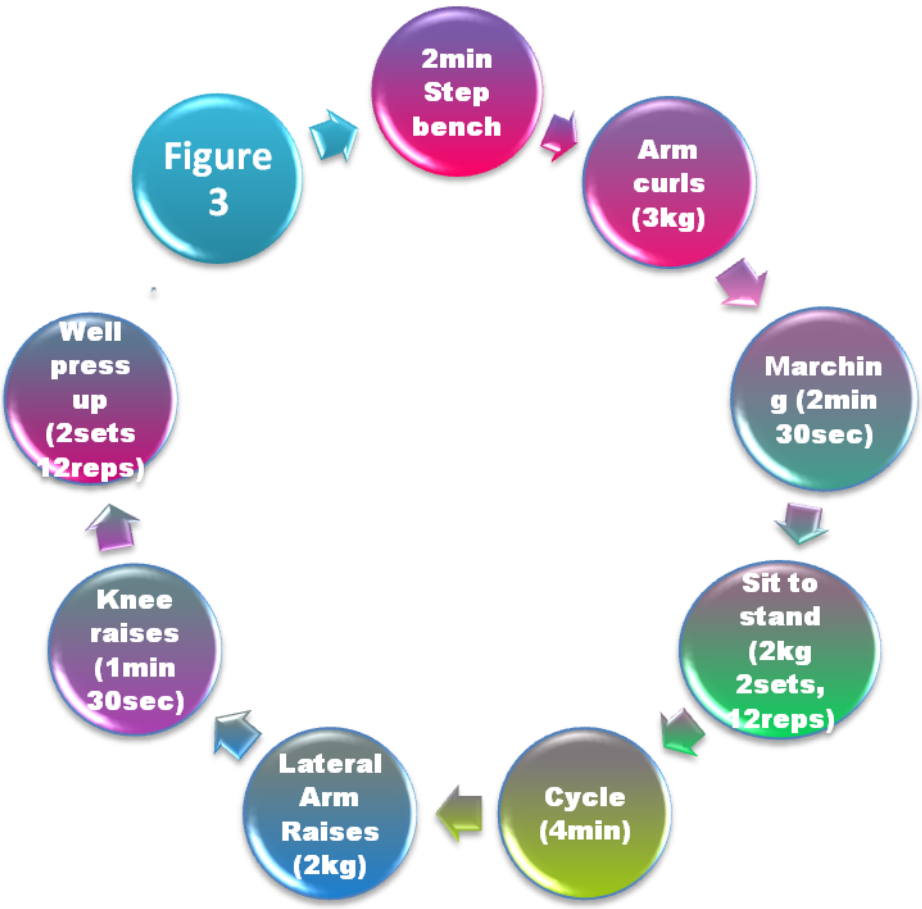

Figure 3. An example of a typical "interval" exercise class structure in the UK

Appendix 1. IRAS approval for studies involving NHS patients

City Health Care Partnership
2 Earls Court
Priory Park East
Henry Boot Way
Hull, HU4 7DY
Tel: 01482 347643
Fax: 01482 347621
Patient Safety & Quality

13 November 2013

Dr Lee Ingle
Senior Lecturer
University of Hull
Sport Health & Exercise Science Dept
Cottingham Road, Hull, HU6 7RX

Dear Dr Ingle

	CHCP CIC Permission to conduct the following research study
Study title:	**Cardiovascular & respiratory adaptations in response to a standard UK exercise based cardiac rehabilitation.**
REC reference:	**13/YH/0278**
Protocol number:	**v2**
IRAS project ID:	***126483***

NRES (Y&H Centre North West): Date of Favourable Opinion (27/09/2013)
CHCP Ref: 2013/003

Thank you for submitting the above application for consideration. The necessary organisational reviews of submitted study documentation have taken place and I can confirm approval from City Health Care Partnership (CHCP CIC) giving you permission to commence this study with CHCP CIC patients from 1st September 2013. You will need to arrange access to CHCP CIC patients and staff via Toni Goodman, Cardiac Rehabilitation Service Manager. CHCP CIC have received all the necessary information about the study. All protocol amendments should be communicated promptly and any incidents or adverse events relating to this study **must** be reported to the local clinical managers who will adopt the CHCP CIC risk management policies in managing the responsive processes.

Please note that in accepting this agreement, you also accept your professional and legal duties to comply with the requirements given by the Ethics Committee; the Department of Health's Research Governance Framework for Health and Social Care and associated legal and statutory duties. CHCP CIC note the researcher and sponsor declarations made within the IRAS R&D form (D1 items 1-11 and D2 items 1-7). These standards are accepted by CHCP CIC as minimum standards for the conduct of research within this organisation. CHCP CIC would welcome mid-stage and end stage reports, especially any findings that could positively contribute to practice and service developments.

Yours sincerely

Dr Louise Girardier,
R&D Manager, on behalf of:
Ms Michelle Smith, Operations Service Director
R&D Management Lead, City Health Care Partnership CIC

Providing Quality Care