PHYSIOLOGICAL AND VASCULAR RESPONSES TO ACUTE AND CHRONIC EXERCISE IN MEN AND WOMEN WITH CARDIOVASCULAR DISEASE

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Submitted for the award of PhD

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work, that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abstract

Study 1: Brachial artery flow-mediated dilation (FMD) using ultrasonography is used as a surrogate measure of coronary artery function. The purpose of this study was to i) assess the reliability of the PhD student in the use of ultrasonography to measure endothelial vasomotor function, and ii) develop and validate a custom-designed software to measure arterial diameter. The sonographer demonstrated excellent reliability in FMD assessment. Compared to in-built ultrasound calipers, the custom-designed software was shown to be a valid measure of arterial diameter.

Study 2: This study evaluated the physiological and vascular responses to self-regulated exercise (SRE) and high-intensity interval training (HIIT) in individuals with cardiovascular disease. Physiological and perceptual responses were significantly higher in response to HIIT than SRE. There was no change in FMD in either group following exercise.

Study 3: This study compared the effects of 4 weeks of traditional cardiac rehabilitation and 4 weeks of HIIT on the physiological and perceptual responses, endothelial function, and blood lipids in individuals with CVD. Treadmill time to exhaustion and percentage change in FMD were significantly higher in the HIIT group at week 4 than week 1.

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Abbreviations

BMI	Body mass index
CABG	Coronary artery Bypass graft
CAD	Coronary artery disease
CVD	Cardiovascular disease
d	Day
DBP	Diastolic blood pressure
EDD	Endothelial-dependent dilation
EID	Endothelial-independent dilation
FMD	Flow-mediated dilation
GTN	Glyceryl trinitrate
h	Hour
HIIT	High-intensity interval training
HR	Heart rate
HRmax	Maximal heart rate
kcal	Kilocalories
METs	Metabolic equivalents
$M\dot{V}O_2$	Myocardial oxygen demand
PCTA	Percutaneous transluminal angioplasty
RER	Respiratory exchange ratio
RPE	Rate of perceived exertion

RR	Respiratory rate
SBP	Systolic blood pressure
sec	Second
$Sp0_2$	Percentage of oxygen saturation
VCO_2	Carbon dioxide elimination
Ve	Minute ventilation
VE	Ventilation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_2max$	Maximal oxygen uptake
VT	Ventilatory threshold
yr	Year
%HRmax	Percentage of maximal heart rate
%HRVT	Percentage of maximal heart rate at ventilatory threshold
% $\dot{V}O_2max$	Percentage of maximal oxygen uptake
%$\dot{V}$O2VT	Percentage of maximal oxygen uptake at ventilatory threshold

Chapter 1

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and premature mortality in men and women in Ireland [1]. Atherosclerosis, the most common form of CVD, involves a complex interplay between oxidative stress, inflammatory stimuli and the infiltration of oxidized low-density lipoproteins (LDL). The endothelium is a biologically active barrier between the blood and the various physiological systems, and plays a crucial role in maintaining vascular integrity. Atherosclerosis is initiated when the vascular endothelial phenotype is modulated by cardiovascular disease risk factors. Damage to the endothelium reduces nitric oxide (NO) bioavailability, leading to the development of pathological inflammatory processes and vascular disease [2].

Assessment of coronary and peripheral artery endothelial vasodilatory function provides useful diagnostic and prognostic information that helps to assess disease burden, and guide therapy in patients with CVD, and individuals with normal coronary arteries and risk factors for atherosclerosis. Brachial artery flow-mediated dilation (FMD) using high-resolution ultrasonography to provide an indirect measure of NO bioavailabilty, is commonly used, and is a surrogate for coronary artery vasomotor function.

Ultrasound image acquisition during FMD assessment is technically challenging and requires specialized training. The practitioner should be deemed proficient in the use of ultrasonography and the FMD technique. Computerised analysis technology has emerged in recent years, and has been shown to significantly improve the accuracy of

arterial diameter measurements compared to manual measurements [3]. Existing general-purpose segmentation algorithms are unable to reliably delineate the artery boundary, and are too computationally intensive to allow for real-time updates and feedback.

Moderate to vigorous intensity physical activity and improved cardiorespiratory fitness, specifically regular exercise training can delay the decrease in endothelial function with ageing [4] and can reverse impairment of endothelial function in individuals with documented CVD [5]. A single bout of exercise can also transiently alter atherosclerotic CVD risk factors and endothelial function.

The culture of exercise prescription can be perceived as highly controlling and aversive. Physiologically based exercise prescriptions often exceed an individual's preferred level of intensity. Using effort perception to self-regulate preferred intensity may allow individuals to complete an activity within their perceptual preference range and without undue physiological strain. Although effort perception is used to self-regulate the pace of many daily activities, no studies have examined the effect of an acute bout of self-regulated exercise on endothelial function in men and women with CVD.

High-intensity interval training (HIIT) involves repeated bouts of high intensity exercise (10 to 300 sec) interspersed with active or passive recovery periods of equal, shorter, or longer duration [6]. Historically, HIIT has been used by athletes, but a number of studies have shown its efficacy and safety in improving exercise capacity and endothelial function in individuals with CAD and heart failure [7,8]. To date, only 4 studies have examined the effect of HIIT on endothelial function in men and women with CVD [8–

[11]. These studies were 8 - 24 weeks in duration and predominantly used exercise intervals of 4 min. Recent studies indicate that 4 - 8 sessions of HIIT involving 30 sec exercise intervals illicit significant physiological and metabolic improvements in healthy individuals [6,12].

Study Aims

The following series of studies will i) assess user reliability in the use of ultrasonography to measure endothelial vasomotor function, ii) develop and validate a custom-designed software to measure arterial diameter, iii) compare the exercise responses to an acute bout of self-regulated and high-intensity interval exercise, iv) compare the physiological and perceptual responses to 4 weeks of high-intensity interval training or community-based cardiac rehabilitation in individuals with CVD.

Objectives

1. To assess the reliability of a practitioner in the use of ultrasonography to measure endothelial vasomotor function
2. To develop and validate a reliable custom-designed analysis tool to measure arterial diameter
3. To evaluate the acute physiological and vascular responses to self-regulated (SR) and high-intensity interval exercise (HIIE) in individuals with CVD
4. To compare the effects of 4 weeks of traditional cardiac rehabilitation and 4 weeks of HIIT on the physiological and perceptual responses, endothelial function, and blood lipids in individuals with CVD

Hypotheses

1. The sonographer will demonstrate reliability in the use of ultrasonography to assess FMD

2. The custom-designed software will be a valid and reliable measure of arterial diameter

3. Acute physiological and vascular responses will be significantly greater following a single bout of HIIE compared to SRE

4. Physiological and vascular responses will be significantly greater following 4 weeks of HIIE compared to 4 weeks of traditional phase IV cardiac rehabilitation

Chapter 2

REVIEW OF LITERATURE

Introduction

Cardiovascular disease (CVD) collectively refers to diseases of the heart and circulatory system, and typically includes coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease, rheumatic heart disease, congenital heart disease, aortic aneurysm, deep venous thrombosis and pulmonary embolism. It is the leading cause of mortality worldwide. According to the World Health Organisation (WHO) roughly 17.3 million people died from CVD in 2004, accounting for 29% of all global deaths. Ireland has the highest mortality rate from CVD in the EU (40%) [13]. In Europe, CVD is the number one cause of mortality accounting for nearly half of all deaths, while in the European Union it accounts for more than 2 million deaths each year [14]. In Ireland, approximately 10,000 people die each year from CVD accounting for 36% of the deaths, making it the number one cause of death. The socio-economic impact of CVD to the EU was €192 billion in 2006. A total of 6% of the Irish healthcare budget in 2009 was spent on cardiovascular disease treatments [1].

Atherosclerosis is the most common cause of CVD, and is considered to be a chronic inflammatory disease resulting from the progressive interaction between modified lipoproteins, monocyte-derived macrophages, and endothelial and smooth muscle cells [15] in the sub-endothelial space of primarily large and medium-sized elastic and muscular arteries [16]. The inflammatory process results in the formation of atherosclerotic plaques, through a slow, insidious process over many years [15]. A

substantial lag period exists between the initial development of atherosclerosis and clinical manifestations such as angina, and myocardial infarction.

The endothelium is a permeable biological barrier between the blood stream and the various physiological systems [17]. It plays a crucial role in maintaining vascular integrity, by releasing a wide variety of substances that regulate leukocyte adhesion, migration and activation, thrombus formation and vessel tone. Atherosclerosis is initiated when the vascular endothelial phenotype is modulated by cardiovascular disease risk factors. Endothelial function can be determined non-invasively by assessing flow-mediated vasodilation (FMD) of the brachial artery using ultrasonography. Brachial artery FMD correlates with endothelial coronary function. Brachial artery ultrasound has emerged as a useful tool for assessing the efficacy of preventive and/or interventional approaches to reducing the burden of CVD. Exercise has been shown to improve endothelial function and decrease cardiovascular risk. The purpose of this chapter is to review i) the biology of the endothelium, ii) the role of the vascular endothelium in the development of atherosclerosis, iii) the assessment of endothelial function and iv) the effect of acute and chronic exercise on endothelial function

Vascular Endothelial - Structure

The endothelium is a 0.2 to 4 μm-thick monolayer of squamous endothelial cells that line the lumen of the entire surface of the vascular tree. It represents a surface area of approximately 4000 to 7000 m^2. Malpighi's discovery in the 17th century of the endothelium as a physical separation between blood and tissue with no substantial functionality persisted through the nineteenth and twentieth centuries. Landmark studies in the 1980's demonstrated the obligatory role of endothelial cells in

acetycholine (ACh)-mediated vasodilation [18] and in the paradoxical ACh-mediated vasoconstriction of atherosclerotic vessels [19]. The endothelium is now viewed as a large endocrine organ system that operates as a dynamic barrier that maintains vascular integrity. The healthy endothelium is anti-atherogenic, through favourable paracrine effects on vasodilation, inhibition of leukocyte adhesion, platelet aggregation and coagulation, and promotion of healing via progenitor cells. However, because of its position in the vascular wall, it is also the target of hemodynamic and biochemical perturbations.

Vascular Endothelial - Functions

The endothelium acts as a selectively permeable barrier, permitting the exchange of solutes between the blood and the subendothelial space to meet the metabolic demands of the surrounding tissue. The movement of glucose and amino acids across the intima-lumen barrier relies on the endothelial derived transporters. For example, glucose transporters, GLUT-1 and GLUT-4, are expressed in endothelial cells. Multiple transport systems also exist for the transport of amino acids across the intima-luminal barrier. One of these transport systems, the y^+ cationic amino acid transporter, is responsible for the transport of L-arginine. Caveolae are invaginations in the cell membrane and are important vesicle carriers, responsible for transcellular transport in endothelial cells. Tight junctions are located between endothelial cells and can selectively regulate the passage of ions, water, and various macromolecules through paracellular spaces, and maintain cell polarity by preventing the movement of charged molecules.

The endothelium provides a non-thrombogenic blood-tissue interface due to the expression of antiplatelet and anticoagulant molecules, and the synthesis of prostacyclin (PGI_2), heparan sulphate and thrombomodulin-thrombin complex. The diatomic gas, nitric oxide (NO), produced by endothelial cells is a fundamental determinant of cardiovascular homeostasis. It is a potent vasodilator, counteracts leukocyte adhesion to the endothelium, attenuates vascular smooth muscle proliferation and migration, suppresses platelet aggregation, and can influence the production of superoxide anion [20]. The antiplatelet activity of endothelial-derived NO is considerably augmented in the presence of prostacyclin (PGI_2). NO also reduces the production by the endothelial cells of endothelin [21], a potent vasoconstrictor and adhesion molecules [22]. These biological actions make NO an important component in the endogenous defence against vascular injury, inflammation, and thrombosis, all key events in the development and progression of atherosclerosis.

Vascular Smooth Muscle - Contraction

Healthy intact endothelial cells respond to humoral, neural and mechanical stimuli by synthesizing and releasing vasoactive substances that regulate vascular tone by relaxing or contracting the underlying smooth muscle [23]. Myosin light chains (MLC) are 20-kD regulatory subunits found on the myosin heads that regulate cross-bridge cycling in vascular smooth muscle (VSM). Myosin light chain kinase (MLCK) is an enzyme that phosphorylates myosin light chain in the presence of ATP. For contraction to occur, MLC-K must phosphorylate the myosin light chain, enabling the molecular interaction of myosin with actin. Energy released from ATP by the action of myosin ATPase activity results in the cycling of the myosin cross-bridges with actin. In some smooth muscle

cells, the phosphorylation of the light chain of myosin is maintained at a low level in the absence of external stimuli (i.e., no receptor or mechanical activation). This activity results in what is known as smooth muscle tone, and its intensity can be varied.

Vascular smooth muscle (VSM) contraction can be initiated by mechanical, electrical, and chemical activation of the actin and myosin contractile proteins and involves a number of signal transduction pathways that converge to increase cytoplasmic calcium [Ca^{2+}]. Cytoplasmic calcium reflects the balance between the calcium that i) enters the cell from the extracellular space ii) is released by intracellular storage sites and iii) is re-sequestered by the intracellular storage sites, or removed from the cell.

Electrical depolarization of the VSM cell membrane elicits contraction, most likely by opening of voltage and ligand gated L-type calcium channels, which increases the intracellular [Ca^{2+}]. Binding of agonists such as norepinephrine, angiotensin II, and endothelin to a Gq protein coupled receptor results in the activation of phospholipase C, and subsequent hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP_2) to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol, (DAG). The binding of IP_3 to receptors on the sarcoplasmic reticulum results in the controlled release of [Ca^{2+}] into the cytosol, where it rapidly increases above the 0.1 mM threshold for contraction. Re-sequestering of calcium in the sarcoplasmic reticulum by an ATP-dependent calcium pump or removal from the cell to the external environment by either an ATP-dependent calcium pump or by the sodium-calcium exchanger (antiport) decreases intracellular [Ca^{2+}]. The antiport uses the energy of the Na^+ gradient for influx.

Cytoplasmic [Ca^{2+}] binds with the acidic protein calmodulin, and the calcium-calmodulin complex activates myosin light chain kinase, and in conjunction with actin, cross-bridge cycling occurs, causing the vascular smooth muscle to contract and the lumen of blood vessels to narrow (Figure 2.1). The calcium concentration within the cell is transient, and the contractile response is augmented at a given intracellular [Ca^{2+}] by a calcium-sensitizing mechanism brought about by the inhibition of myosin light chain phosphatase (MLCPh) [24]. This promotes the contractile state, since the light chain of myosin cannot be dephosphorylated.

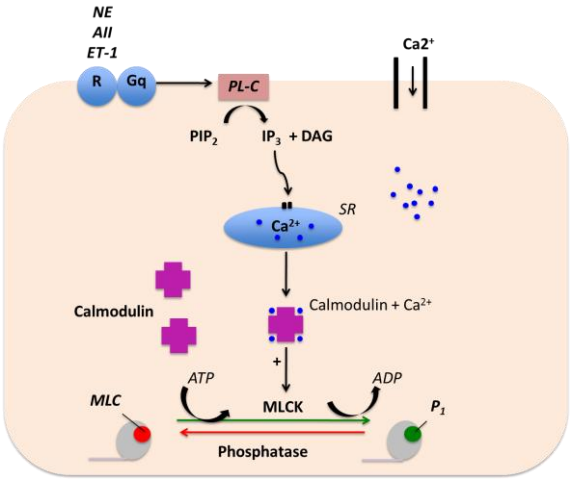

Figure 2.1: Vascular smooth muscle contraction:
R, receptor; Gq, Gq protein ; PL-C, phospholipase C; PIP_2, phosphatidylinositol 4, 5-bisphosphate; IP_3, inositol 1,4,5-triphosphate; DAG, diacylglycerol; Ca^{2+}, calcium; MLC, myosin light chain; MLCK, myosin light chain kinase; P, phosphate; SR, sarcoplasmic reticulum. An increase in free intracellular [Ca^{2+}] can result from increased flux of [Ca^{2+}] into the cell through calcium channels or by release of [Ca^{2+}] from internal stores (e.g. SR). The free [Ca^{2+}] binds to a calcium-binding protein called calmodulin. Calcium-calmodulin activates MLCK, an enzyme that is capable of phosphorylating MLC in the presence of ATP. MLC phosphorylation leads to cross-bridge formation between the myosin heads and the actin filaments resulting in smooth muscle contraction.

Vascular Smooth Muscle - Relaxation

Smooth muscle relaxation requires a decreased intracellular [Ca^{2+}] and increased MLCPh activity. During relaxation, receptor and voltage-operated calcium channels in the vascular smooth muscle plasma membrane close resulting in a reduced [Ca^{2+}] entry into the cell. The sarcoplasmic reticulum and the plasma membrane contain Ca-ATPases that, along with Na^{+}/Ca^{2+} exchangers, remove [Ca^{2+}] from the cell.

The endothelium acts as a complex mechanical signal-transduction interface between the vessel wall and the circulating blood and assist in the regulation of vascular smooth muscle cell relaxation. Furchgott and Zawadzki [18] were the first to demonstrate the obligatory role of the endothelium in the relaxation of isolated rabbit aorta in response to acetylcholine. Subsequent studies revealed that the endothelium-dependent response to exogenously administered acetylcholine (ACh) was attributable to the production and diffusion of NO, a hydrophobic, diatomic gas that is produced by endothelial derived nitric oxide (eNOS) in response to changes in shear forces or via a variety of agonists (histamine, bradykinin) acting on specific endothelial cell membrane receptors.

Shear Forces

Blood flow exerts a frictional force on the endothelial surface of the vessel lumen termed, shear stress. Mechanosensors located on the endothelial cells detect shear stress and alter endothelial cell function in a process called mechanotransduction [25]. The increase in hemodynamic shear results in [Ca^{2+}] efflux from the endoplasmic reticulum into the cytoplasm. Calcium binds to calmodulin and activates endothelial

nitric oxide synthase (eNOS) resulting in the synthesis of nitric oxide (NO) from the terminal guanidino nitrogen of its amino acid precursor, L-arginine. The NO molecule diffuses abluminally into the adjacent vascular smooth muscle, where it binds to the β1 subunit of the heterodemic enzyme guanylyl cyclase, leading to activation of the enzyme, and the production of cyclic guanosine monophosphate (cGMP). This intercellular signaling molecule leads to the inhibition of calcium influx into the SMC, and decreases calcium-calmodulin stimulation of myosin light chain kinase [26]. This in turn decreases the phosphorylation of myosin light chains, decreasing smooth muscle tension development and causing vasodilation.

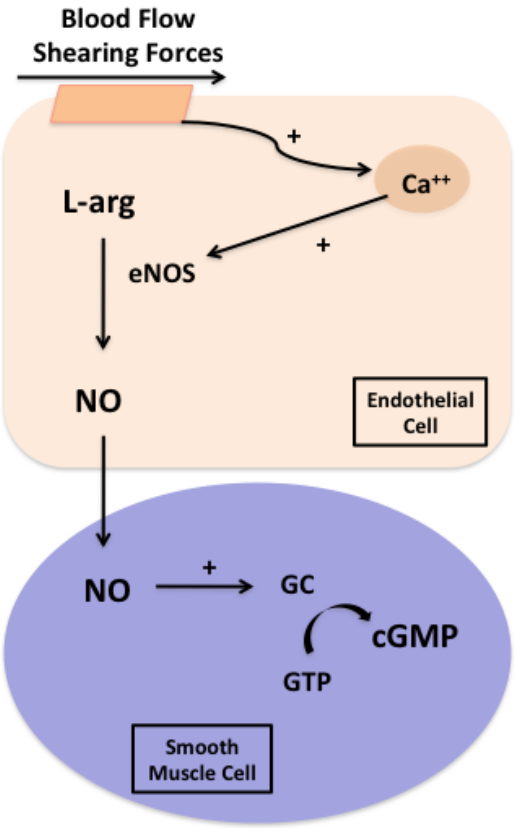

Figure 2.2: Shear stress-induced vascular smooth muscle relaxation: Ca^{2+}, calcium; eNOS, endothelial nitric oxide; L-arg, L-arginine; NO, nitric oxide; GC, guanylyl cyclase; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate. Increases in hemodynamic shear result in [Ca^{2+}] efflux from the endoplasmic reticulum into the cytoplasm. [Ca^{2+}] binds to calmodulin and activates eNOS resulting in the synthesis of NO from the terminal guanidino nitrogen of its amino acid precursor, L-arg. The NO molecule diffuses abluminally into the adjacent vascular smooth muscle, where it binds to the β1 subunit of the heterodemic enzyme GC, leading to activation of the enzyme, and the production of cGMP. This intercellular signaling molecule leads to the inhibition of [Ca^{2+}] influx into the smooth muscle cell, and decreases calcium-calmodulin stimulation of myosin light chain kinase. This in turn decreases the phosphorylation of myosin light chains, decreasing smooth muscle tension development and causing vasodilation.

The significance of NO as a regulator of coronary vasomotor tone can be demonstrated experimentally by inhibiting its synthesis. N^G-monomethyl-L-arginine (L-NMMA) is a relatively non-selective inhibitor of all NOS isoforms that competes with L-arginine as the substrate for NO synthesis by the eNOS, but cannot be oxidized to form

NO [27]. It increases basal coronary vascular resistance and reduces the vasodilator response to the endothelium-dependent vasodilator agonists acetylcholine (ACh) and bradykinin in isolated perfused hearts [28].

There is no clear consensus as to how the frictional forces in response haemodynamic shear are sensed by endothelial cells. Adams *et al*., [29] proposed that changes in membrane potential, associated with the activation of K^+, Na^+, and Cl^- ion channels, alter the electrochemical gradient for Ca^{2+} transport across the endothelial cell membrane. The serine/threonine protein kinase B (Akt) can directly phosphorylate and activate eNOS in response to shear stress, and the effect is independent of intracellular [Ca^{2+}]. The stimulation of Akt phosphorylation by shear stress appears to be mediated by triphosphoinositide 3-OH kinase. Repetitive episodes of increased hemodynamic shear causes an up-regulation of eNOS, mRNA and protein, permitting the endothelial cell to increase NO production [30].

The NO output elicited by shear stress is approximately a third of agonist-induced NO production, but it is maintained for longer duration. In addition to NO, other endothelial-derived vasodilators, prostacylin (PGI_2) and endothelium-derived hyperpolarizing factors (EDHF), act locally to modulate vascular smooth muscle tone. The endothelium also produces vasoconstrictors such as endothelin and angiotensin II [31]. The balance between vasodilators, vasoconstrictors and the sympathetic nervous system maintains blood vessel tone.

Endothelial Function – Assessment

Invasive Assessment

The realization that endothelial dysfunction precedes the early morphological atherosclerotic changes in the arterial walls, and is significantly correlated with future cardiovascular events has resulted in the development of assessment techniques to monitor coronary and peripheral vascular health. Clinically, coronary endothelial function is commonly assessed by measuring the endothelial receptor–mediated vasomotor response to intracoronary infusion of ACh or other agonists, and measurement of the subsequent change in vessel diameter using quantitative coronary angiography [32].

Angiographically normal coronary arteries dilate in response to ACh, and the vasodilator response is predominantly caused by the release of NO from the intact endothelial cells. In the presence of endothelial dysfunction, intracoronary infusion of ACh causes paradoxical vasoconstriction as a result of a direct muscarinic smooth muscle constrictor effect [19]. Other endothelial-dependent agonists such as bradykinin and substance P are also used as a measure of endothelial function [33]. Exogenous nitrovasodilators such as glyceryl-trinitrate (GTN) and sodium nitroprusside (SNP) can act directly on the smooth muscle to activate guanylyl cyclase [34].

The nitric oxide synthase inhibitor, N-monomethyl-L-arginine (L-NMMA) is a commonly used pharmacological tool for investigating the contribution of NO to endothelial vasomotor responses [27]. Infusion of L-NMMA ameliorates ACh-induced paradoxical vasoconstriction, indicating that ACh-induced endothelial vasodilation is mediated, at least in a large part, by NO [35].

Cold Pressor Test

The cold pressor test (CPT) is another commonly used experimental technique to assess endothelial-dependent coronary vasomotor function. Immersion of an extremity (foot, hand, forearm) in ice water activates the sympathetic adrenergic system resulting in increased heart rate, arterial blood pressure, and myocardial oxygen ($M\dot{V}O_2$) demand. Angiographically normal coronary arteries dilate to accommodate the increased blood required to satisfy increased $M\dot{V}O_2$ demand. In contrast, CPT can also induce paradoxical vasoconstriction in coronary arteries with different degrees of stenosis. The vasodilatory response is thought to be mediated through α-adrenoreceptors with direct stimulation of nitric oxide synthesis and through a flow-dependent release of endothelial-derived nitric oxide [36]. The response to CPT is studied using quantitative angiography.

Venous Occlusion Plethysmography

Venous occlusion plethysmography is a relatively inexpensive, versatile, well-tolerated and reproducible technique used to measure changes in forearm resistance and blood flow in response to arterial infusions of varying concentrations of endothelial-independent and dependent agonists. This procedure is however, invasive due to the arterial cannulation that is required for infusion.

Non-Invasive Assessment

Although the assessment of endothelial function in the coronary artery is clinically important, the invasive nature of these assessments renders them impractical for use in asymptomatic individuals, and also limits repeated testing during serial follow

up. Less invasive methods have been developed that are safe, reliable, and easy to perform.

Pulse Wave Velocity

Compliance refers to the ability of a blood vessel to distend and increase volume with increasing transmural pressure. Stiffness is the inverse of compliance and non-compliant blood vessels have a large transmural pressure for a given volume. Pulse wave velocity (PWV) is a non-invasive, highly reproducible estimate of arterial stiffness. The technique involves measuring the time it takes for a pressure pulse to travel between two points in the arterial system, usually the carotid artery and femoral artery [37].

Brachial Artery Flow-Mediated Dilation

The principle of brachial artery FMD is based on the ability of healthy, intact endothelial cells to detect, and respond appropriately, to changes in shear stress with acute adjustments in vascular tone. This method uses high-resolution ultrasonography to measure the diameter of the brachial artery before and after a brief period of arterial occlusion. As a result of arterial occlusion, downstream resistance vessels dilate via autoregulatory mechanisms. Release of the cuff causes a transient increase in blood flow through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. This subsequent increase in blood flow causes the brachial artery to dilate [38]. The change in artery diameter is expressed as a percentage change from baseline diameter.

It is believed that brachial artery FMD occurs predominantly as a result of NO release from the endothelial cells. As in the coronary circulation, the FMD response of the brachial artery can be compared to the endothelium-independent dilator response to sublingual glyceryl-trinitrate (GTN) [39]. The non-invasive nature of the technique allows for serial assessment of endothelial function in young and old, healthy and diseased, and permits accurate evaluation of lifestyle and pharmacological interventions at an early, preclinical stage when the disease is likely to be reversible [40].

Using both hemodynamic shear and pharmacological agents as a stimulus in the peripheral circulation, and coronary arteries respectively, Anderson *et al*., [41] were the first to report a modest but significant relation between endothelium-dependent vasodilation in the brachial and coronary arteries. Later, Takase *et al.,* [42] used shear stress as the stimulus in both the brachial and coronary arteries to investigate the vasodilatory response in both vascular beds, and found a strong correlation between FMD in the coronary and the brachial arteries. These findings indicate that FMD in the brachial artery can be used as a surrogate measure for coronary artery endothelial function.

Brachial Artery FMD - Methodology

FMD assessment of endothelial function is technically challenging and a strict, standardized protocol is essential to provide accurate and reproducible data [38,43]. In addition to the brachial artery, FMD can also be assessed in the radial, axillary and superficial femoral arteries. A limitation to using these vessels is their small diameter. Vessel diameters < 2.5 mm are difficult to accurately measure [44].

Ultrasound images should be attained by the same, experienced and highly-trained sonographer. A number of factors that have been shown to affect the measurement of FMD should be controlled. These include room temperature [45], time of day [46], diet [47], tobacco [48], exercise [49], caffeine [50], vitamin C [51], menstrual cycle phase [52], medications [47], sympathetic stimuli [53], and current viral illness [54]. Where possible all vasoactive medications should be withheld for at least four half-lives [55].

Occlusion Time

Corretti *et al.,* [38] compared different methodological aspects of the FMD technique in order to determine the most accurate means to assess endothelial function. Similar flow increases have been found in response to 1, 3 and 5 min of cuff occlusion. A statistically significant vasodilation occurred only after the 5 min duration. Since the resultant change in diameter are similar following 5 and 10 min occlusion periods, the more tolerable duration of 5 min is recommended.

Cuff Placement

The sphygmomanometric cuff is inflated to at least 50 mmHg above systolic blood pressure to occlude arterial inflow, resulting in ischemia, and arterial occlusion is sustained for a standardized period of time. Placement of the cuff has varied, with both the upper arm (proximal to the area of insonation) or the lower arm (distal to the area of insonation) being used. Upper arm occlusion is technically more challenging due to the distorted image following the collapse of the brachial artery and the shift in soft tissue [37,56].

A significantly greater peak hyperemic flow and FMD has been found when the cuff is placed on the upper arm, proximal to the area of isonation, than on the lower arm distal to the imaging site [57,58]. This may be due to the greater flow stimulus resulting from recruitment of resistance vessels, or perhaps to the direct effects of ischemia on the brachial artery [37]. The vasodilatory response to upper arm ischemia has also been shown to persist for up to 20 min [57]. In contrast, when the cuff is placed distal to the imaging site, on the lower arm, the vasodilatory response following vasoconstriction, reverses rapidly. Doshi *et al*., [58] investigated how the placement of the cuff, relative to the area of insonation, can influence the degree to which endothelium-derived NO mediates the vasodilator response. Doshi compared FMD with and without prior L-NMMA infusion when the cuff was placed proximal and distal to the area of insonation. Occlusion on the lower arm distal to the ultrasound probe was associated with a 7% FMD response, which was abolished by L-NMMA. When the cuff was inflated proximal to the ultrasound probe, there was a 12% FMD response that was only partially decreased by NO blockade. These results indicate that dilation following lower arm occlusion is exclusively NO-mediated, whereas dilation following upper arm occlusion is only partly mediated by NO [15]. It is possible that the measured vasodilatory response obtained with upper arm occlusion may have been confounded by ischemic mediators other than NO.

Timing Post Occlusion

The standard procedure for assessing endothelial function using FMD is to measure the peak artery diameter approximately 60 s after cuff release, or 45 – 60 s after peak reactive hyperemic blood flow [45,59]. The time to reach post occlusion peak

diameter following 5 min of occlusion may be longer in individuals with CVD, or CVD risk factors compared to healthy individuals [60]. Irace *et al*., [60] measured FMD in the brachial artery at 50, 120, 180, and 300 sec following 5 min of forearm occlusion in apparently healthy men and men with Type II diabetes mellitus (T2DM). Maximum FMD occurred after 50 sec and 120 sec in the healthy and diabetic men, respectively. FMD response in the diabetic cohort was significantly lower than the healthy controls 50 sec following the cuff release.

Using a similar study design Thijssen *et al*., [61] used continuous diameter assessment to examine the time-course of brachial artery FMD after 5 min of forearm ischemia in 12 young, 12 fitness-matched older and 12 older untrained individuals. Arterial diameter was recorded 30 sec before and continued for 3 min after cuff release. Using the 50 - 70 sec or 70 – 90 sec post-cuff deflation time period, the FMD response calculated was significantly lower in all groups compared with the true peak FMD values observed. There was a significant difference in brachial artery FMD between sedentary older subjects and healthy subjects when using the continuous assessment method. However, there was no difference in brachial artery FMD between groups when comparing diameter changes at 60 s post cuff release. A limitation of this study is the fact that the diameter assessments were recorded at 30 sec interval. With the absence of continuous diameter measurement, there is a possibility that the true peak diameter may have fallen between the predetermined time points.

Gating

Brachial arterial diameters vary significantly throughout the cardiac cycle [56]. ECG gating, a technique in which FMD measurements are performed at a pre-

determined time-point during the cardiac cycle (e.g., R-wave or peak of the T-wave) is used to standardize FMD measurements. Failure to use ECG gating may increase the magnitude of error in arterial diameter and FMD measurements [56]. Chuang et al., [56] found that ignoring the temporal phase in the cardiac cycle artificially increased FMD values into the normal range despite impaired 'true FMD' The range of apparent FMD values calculated by ignoring the cardiac cycle was on average nearly threefold the magnitude of 'true FMD'. Investigations into the necessity of QRS gating have yielded conflicting results. Corretti's guidelines in 2002 [38] recommend ECG-triggered measurements for accurate FMD assessment [38]. However, recent data showed FMD measurements calculated with an average of the vessel diameter over the entire cardiac cycle produces equivalent results over a wide range of compliance [62,63].

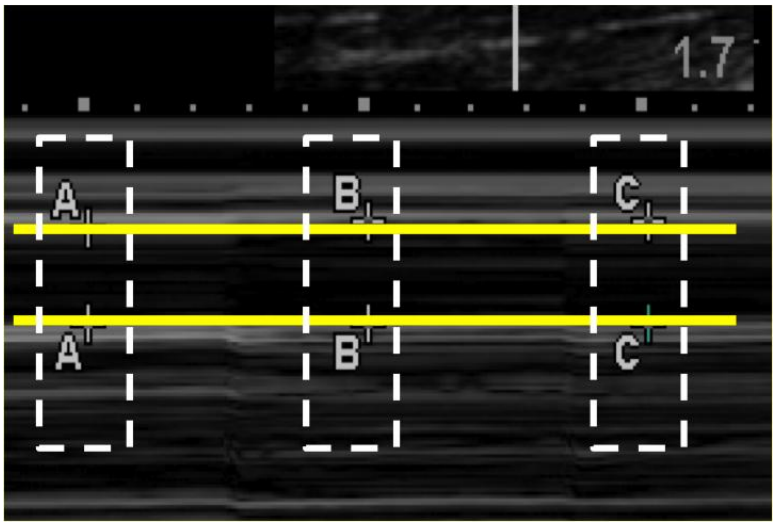

Figure 2.3: ECG Gating: Brachial artery diameter is recorded as an average of diameter measurements coinciding with 2-3 consecutive ECG R waves.

Normalization

The magnitude of the FMD response is related not only to the health of the vascular endothelium, but also to the magnitude of the imposed stimulus between subjects [64,65]. A limitation of the FMD technique is the uncontrolled nature of the evoked shear stress stimulus. Recent evidence suggests that the mechanisms of FMD may vary depending on the rate of onset and duration of the shear stress stimuli.

Normalisation of the dilatory response to shear stress has been recommended as an appropriate way to express FMD [43], although there is no clear consensus as to what is the most accurate method [66]. Equating FMD responses to the underlying stimulus is believed to i) reduce day-to-day variability, ii) account for the magnitude of the stimulus evoking FMD, and iii) allow endothelial vasomotor measurements to be compared between studies and laboratories [67].

Shear stress is a function of vessel diameter, blood flow velocity at the vessel lumen, and blood viscosity, such that shear stress = $\eta V/D$, where V is mean blood flow velocity, D is vessel diameter and η is blood viscosity [68]. Although it does not account for blood viscosity, shear rate (SR) (velocity/diameter) is an accepted surrogate measure of shear stress, as it considers both blood flow velocity and vessel diameter [43]. When mean blood flow velocity is not available, peak blood flow velocity (v_{max}) can be used to calculate shear rate, such that shear rate = $4\ v_{max}$/diameter [69].

The technique of normalizing to the shear stimulus has evolved significantly. Initially, normalizing FMD to the shear stimulus used the peak shear response to occlusion [70]. Measuring the AUC shear response from post-occlusion to the time at which peak diameter occurs is now a commonly used means of normalisation [71].

Cardiovascular Disease Risk Factors and Vascular Health

Non-modifiable cardiovascular disease risk factors such as aging and a family history of CVD, and modifiable risk factors, including active and passive smoking, lipid disorders, hypertension, diabetes mellitus, obesity, and physical inactivity, among others, have been shown to promote the development of atherosclerosis through their deleterious effects on endothelial structure and function [72,73]. The underlying mechanisms through which risk factors initiate vascular injury are multifactorial. The predominant mechanism is believed to be oxidative stress and redox injury resulting in decreased synthesis or increased degradation of NO [74]. The progressive inability of endothelial cells, exposed to risk factors, to generate sufficient NO promotes a vascular phenotype prone to atherogenesis [20].

Reactive oxygen species (ROS) interact with NO to reduce its bioavailability [20] and directly damage cellular structures via the production of peroxynitrate. O'hara *et al*., [75] examined the presence of superoxide anion (O^-_2) in normal vessels and vessels from cholesterol-fed animals and found there was an excess production of O^-_2 in hypercholesteremic vessels. This O^-_2 was found to be endothelial-derived and responsible for accelerated degradation of NO. In contrast, normal vessels were shown to have a protective effect, with reduced production of O^-_2. The excessive production of O^-_2 in diseased vessels is thought to be related to excessive quantities of the enzyme xanthine oxidase.

The underlying pathogenesis of atherosclerosis involves an imbalance of lipid metabolism and a maladaptive immune response entailing a chronic inflammation of the arterial wall [76]. Elevated levels of total cholesterol, LDL-C, triglycerides, low levels of

HDL-C and an elevated ratio of total to HDL-C are inversely related to endothelial function [77], even in the absence of CVD [78].

A large number of clinical studies have demonstrated improved coronary and brachial artery endothelial function in response to both pharmacological [79] and non-pharmacological therapies [80] in men and women, with and without CVD, with markedly elevated or borderline total cholesterol, LDL-C, triglycerides, low levels of HDL-C or an elevated ratio of total to HDL-C. Putative mechanisms for the associated link between improved lipid profile and endothelial function are increased synthesis of NO [81] and improved antioxidant capacity [82].

Hypertension is the most common cardiovascular risk factor in the world [83]. Patients with hypertension are characterized by functional and structural vascular abnormalities. Brachial artery FMD is significantly reduced and the severity is related to the duration of blood pressure elevation. There is conflicting opinion as to whether hypertension precipitates endothelial dysfunction or a defect in endothelial NO activity acts directly or indirectly to increase the risk of developing hypertension.

Type 2 diabetes mellitus (T2DM) is associated with impaired endothelial responses to pharmacological and physiological stimuli [83]. In addition, disturbances in the endothelium are a fundamental component in the development of diabetic microvascular and macrovascular complications [84]. Hyperglycemia is the major causal factor in the development of endothelial dysfunction in individuals with T2DM. Individuals with T2DM tend to have a constellation of other risk factors, including obesity, hypertension, elevated triglycerides and LDL-C and reductions in HDL-C, that also impair endothelial function [85].

Endothelial function declines with age [74] and this can occur independent of other cardiovascular risk factors [86]. Although, the underlying mechanism of age-related development of vascular dysfunction is difficult to assess in humans, numerous contributing factors have been identified. These include reduced bioavailability of NO due to oxidative stress, increased endothelin-1 (ET-1) bioactivity, reduced endothelial production of prostaglandins, vascular inflammation, production of advanced glycation end-products, increased endothelial apoptosis and reduced expression of estrogen receptors [86]. Other contributing factors to the modulation of vascular endothelial function with age include physical activity, diet, body composition, menopause, and vitamin D status.

Studies in children and adults have consistently found a significant relation between obesity and endothelial dysfunction, and weight loss in overweight or obese individuals improves endothelial function [77]. Short-term (8 weeks) weight cycling involving the repeated loss and regain of body weight also impairs endothelial function [78]. Adipose tissue is a proinflammatory organ. There is evidence that visceral fat may contribute to endothelial dysfunction through the direct effect of the adipokines, adiponectin and TNF-α, which are secreted by adipose tissue following the recruitment of monocytes [79].

Endothelial Function - Prognostic Value

The functional and structural integrity of the endothelium is critical in maintaining vascular homoeostasis. The presence of atherosclerosis has been shown to affect the vasomotor responses of the diseased vessel to shear stress, and vasoactive compounds such as ACh, serotonin, thrombin, and ADP [87].

Assessment of coronary and peripheral artery endothelial vasodilatory function may provide substantial diagnostic and prognostic information that may be useful to assess disease burden and guide therapy in patients with CAD and those with normal coronary arteries and risk factors for atherosclerosis. The prognostic significance of endothelial function as a predictor of cardiovascular events in healthy individuals and those with vascular disease has been addressed in a number of studies.

In a retrospective study, Halcox *et al.,* [88] evaluated the relation between coronary endothelial function and the occurrence of cardiovascular death, in patients with and without coronary atherosclerosis. Study participants underwent cardiac catheterisation at baseline and follow-up for a mean of 46 ± 3 months. Patients in the tertile with the best microvascular responses to ACh, and those with epicardial dilation in response to ACh had improved survival among all patients, as well as those in the subset without CAD. Endothelium-independent responses were not predictive of outcome.

Endothelial-dependent coronary vasoreactivity was associated with a significantly higher incidence of cardiovascular events over a 7.7-year period in 147 patients undergoing either routine catheterisation, evaluation of chest pain or percutaneous transluminal coronary angioplasty (PTCA) for single vessel disease [89]. Tests of coronary vasoreactivity were significant, independent predictors of a poor prognosis, even after adjustment for traditional cardiovascular risk factors, or the presence of atherosclerosis. Coronary artery endothelial dysfunction was also found to be associated with a higher rate of cardiac events over a 28-month period in patients with non-obstructive CAD [90]. In patients with a normal coronary angiogram, abnormal

vasoreactivity of epicardial coronary arteries (0 to 30% vasoconstriction) in response to sympathetic stimulation (cold pressor test) was associated with the risk of developing cardiovascular events [91].

Abrogated brachial artery FMD predicts complications in high-risk patients 30 d and 1.2 yr following vascular surgery [92,93], and cardiac events in patients with chest pain and no angiographic evidence of CAD, heart failure (HF) or valvular defects [94]. During 5 years of follow-up, patients with impaired brachial artery reactivity and chest pain were more likely to undergo PTCA, and coronary artery bypass graft (CABG) surgery than patients with normal flow-mediated dilation [94]. Furthermore, normal flow-mediated dilation in the brachial artery of patients with chest pain was associated with a low risk of cardiac events. Brachial artery FMD also predicts future cardiovascular events in older adults aged 72 – 98 years (41.4% men), even after adjustment for conventional risk factors [95].

Forearm endothelial function assessed by intra-arterial infusion of ACh and sodium nitroprusside has been shown to predict cardiovascular events in men and women (35 – 54 yr) with essential hypertension [96]. In a subsequent study, Modena *et al*., [97] found that improvements in brachial artery flow-mediated dilation in response to antihypertensive treatment decreased the risk of cardiovascular events over a mean period of 6 – 7 years in postmenopausal women with mild to moderate hypertension and impaired flow-mediated dilation. Interestingly, there was an increased risk of cardiovascular disease events in the women who showed no improvement in endothelial function in response to hypertension medication [97].

Combining carotid plaque echolucency and endothelial function of the brachial artery has an additive value on the prediction of cardiovascular events during a mean follow-up period of 52 ± 10 months in men and women with stable CAD [98]. Using a similar cohort, Chan *et al*., [99] showed that both brachial artery FMD and carotid atheroma burden were independent predictors of coronary events in 152 men and women. However, endothelial function was significantly more related to outcome in that it provided better discriminatory information with respect to event-free survival and it modulated the outcome for any degree of atheroma burden in patients with CAD.

During a median follow-up period of 21 months, Brevetti *et al*., [100] found that reduced brachial artery FMD was an independent predictor for increased cardiovascular risk, in 131 patients with intermittent claudication who did not require vascular surgery. The predictive value of FMD was independent of classic risk factors and of previous incidence of cardiovascular events. Brachial artery FMD improved the prognostic value of ankle-brachial pressure index, the most powerful marker of cardiovascular risk in PAD.

Fichtlscherer *et al*., studied forearm blood flow (FBF), using venous plethysmography, in 191 patients within 5 days of surviving an acute coronary syndrome, documented by coronary angiography. The incidence of recurring cardiovascular events increased significantly with decreasing vasodilator responses to the intra-arterial infusion of ACh. Improvement of systemic endothelial vasodilator function within 8 weeks of the index assessment during the acute coronary syndrome was associated with significantly fewer cardiovascular events [101].

The majority of prognostic studies have been conducted in individuals who are already at increased risk for cardiovascular events. In contrast, a prospective study by

Shimbo *et* al., [102] evaluated baseline brachial artery FMD in 842 asymptomatic, low risk individuals from a multi-ethnic sample. The individuals were tracked annually for the occurrence of cardiovascular events or deaths, for up to 36 months. Significant association was found between impaired FMD and the risk of experiencing a cardiovascular event. The predictive value of FMD however, was not independent of traditional risk factors (e.g. older age, diabetes mellitus, and history of smoking) in this population-based sample.

Suessenbacher *et al*., [103] examined the efficacy of serial brachial artery FMD measurements to predict cardiovascular events in 68 individuals with CAD (53 years ± 9.1) one day after coronary angiography and 14 ± 12 months later. Individuals were divided into two groups, determined by the FMD-median at baseline. They were further characterized by their FMD results, at baseline and after follow up, as FMD-improvers (absolute improvement $\geq$ 3%) or FMD-non-improvers (absolute improvement $\leq$ 3%). Ten cardiovascular events were recorded 44 ± 12 months after baseline assessment. When using a single FMD measurement (baseline) there was no difference in cardiovascular events between the two groups. The FMD-improvers group had significantly fewer events compared to the FMD-nonimprovers group (1 vs 9) when serial assessments were considered. These findings highlight the benefit of multiple FMD assessments. While a single measurement of FMD may not determine clinical outcome, improvements in FMD over time may be associated with a lower risk of long-term cardiovascular events, and may provide more detailed, prognostic information than a single measurement.

In contrast Frick *et al*., [104] found no relation between FMD and future cardiovascular events after a mean follow-up of 39 ± 12 months in 398 men who underwent coronary angiography due to chest pain (315 had documented CAD). Median FMD value was used to divide the men into two groups. Group 1 had FMD values below the group median, and group 2 had FMD values above the group median. No significant difference in cardiovascular events was observed when the men were divided according to the median FMD value. Moreover, FMD was not significantly different between individuals with and without CAD.

In a prospective study assessing the prognostic value of brachial artery reactivity in 444 (179 women; 58 ± 14 years) individuals at risk of CVD, individuals with the greatest FMD impairment had significantly greater risk of cardiovascular events than those who had normal or mildly impaired FMD response [105].

Endothelial Function - Exercise

There is accumulating evidence that cardiorespiratory fitness delays the decrease in endothelial function associated with ageing [4] and reverses impaired endothelial function in individuals with atherosclerotic CVD [5] and HF [106]. Exercise induced improvements in vascular function appear to occur more readily and with remarkable consistency in vessels with antecedent functional impairment [107]. Improvements in endothelial function induced by exercise training are attributable to a combination of enhanced vasodilatory capacity and arterial remodeling.

Endothelial function and Cardiorespiratory Fitness – Cross Sectional Studies

A large number of cross-sectional studies involving young and old, healthy and diseased have assessed the effect of fitness on endothelial vasomotor function.

Despite a significant difference in $\dot{V}O_2$max, Moe *et al.* [108] found no difference in brachial artery FMD between endurance-trained and sedentary female university students.

Aging is associated with endothelial dysfunction and exercise training can have a protective effect in preserving endothelial function and reducing the risk of CVD development.

Rinder *et al*. [4] found a superior brachial artery FMD in response to hyperemia in 10 endurance-trained older men (68.5 ± 2.3 years) compared to 10 age-matched sedentary controls (64.7 ± 1.4 years). Endothelium-dependent dilation at 1 min post occlusion was significantly related to $\dot{V}O_2$max. Considering that only 2 of the trained individuals performed upper extremity exercise, these results indicate that lower-limb exercise can induce systemic improvements in endothelium-dependent dilation and are not mediated exclusively by local adaptations.

Others [109] have found that brachial artery FMD is reduced in sedentary older men compared to exercising older men and young controls. However, older fit men had similar FMD to the young controls. Exercising older men and young controls had lower expression of the $p47^{phox}$ subunit of the oxidant-producing enzyme nicotinamide adenine dinucleotide phosphate (NADP) oxidase and the redox-sensitive pro-oxidant transcription factor nuclear factor kappa B (NFκB), and greater expression of antioxidant enzyme manganese superoxide dismutase (SOD) than older sedentary men.

An underlying gender-related difference in endothelial function may exist. In a recent study Black *et al,* [110] found no difference in brachial artery FMD between healthy young men and fit and sedentary older men. In contrast, the vasomotor response following 5 min of brachial artery occlusion was significantly impaired in older sedentary women compared to both healthy young and older fit women. These finding suggest that women can reduce the age-related decline in vascular function through maintenance of cardiopulmonary fitness.

Tew *et al*., [111] used laser Doppler flowmetry to measure changes in forearm skin blood flow in response to post-occlusive reactive hyperemia, local heating, ACh and SNP administration in young active, older sedentary and older fit individuals. Peak blood flow was greater in the young and older fit group compared to the older sedentary group in response to hyperemia, and 30 min of local heating at 42°C. Reactive hyperemia and local heating responses were moderately correlated with $\dot{V}O_2$max in the older groups. Skin vasodilator responses to ACh were higher in the young than the older sedentary group, and SNP responses were higher in the young group compared to both older groups. There was no relation found between $\dot{V}O_2$max and ACh and SNP responses. Using strain-gauge plethysmography, Taddei *et al*., [112] compared forearm blood flow in young and older trained and untrained men and women in response to infusion of ACh, SNP, L-NMMA, vitamin C, and combined L-NMMA and vitamin C. The vasodilator response to ACh was significantly impaired in the older group compared to the young individuals. While there was a reduced response compared to young group, the ACh response was significantly greater in the elderly trained individuals than the elderly sedentary individuals. Infusion of L-NMMA induced a significant decrease in vasodilation in the young and elderly trained individuals, but was ineffective in the

elderly sedentary controls. In contrast to the young group and the older trained individuals, vitamin C infusion significantly increased vasodilation induced by ACh infusion and also restored the inhibiting effect of L-NMMA on the endothelial agonist. Response to SNP was similar in all groups. These findings indicate that oxidative stress-induced reductions in NO availability may be responsible for the age-related decline in endothelial function in older sedentary individuals, and habitual physical training can preserve NO availability in older men and women.

To assess the putative role of oxidative stress in the age-related decline in endothelial function, Eskurza et al. [113] assessed brachial artery FMD before and after acute infusion, and 30 days supplementation of vitamin C, in young sedentary, older sedentary and older endurance-trained, healthy men. Baseline FMD was significantly lower in the older sedentary men than the young and older exercise-trained men. Vitamin C infusion significantly improved FMD in the older sedentary men, but had no effect on the young or older trained men. In contrast, 30 days of vitamin C supplementation had no effect on FMD in any of the 3 groups. Endothelial-independent dilation in response to NTG did not differ between groups and was unchanged after vitamin C infusion and supplementation.

Endothelial Function - Acute Exercise

Observations that a single bout of exercise can transiently alter atherosclerotic CVD risk factors [114] have led to the notion that perhaps some of the effects of exercise on endothelial function may be attributable to the acute effect of exercise. A number of studies have investigated the effect of an acute bout of exercise on endothelial function

in healthy and disease populations. Table 2.1 provides a summary overview of studies that have examined the effect of acute exercise on endothelial function

Cosio-Lima *et al*., [115] compared the effect of 30 min of treadmill walking at 70-85% HRmax on brachial artery FMD in kidney transplant (KT) recipients and healthy controls (HCs). Brachial artery FMD was significantly higher in HCs than KT recipients after exercise (22% vs 3%). This is perhaps not surprising considering that incidence of hypertension, hyperlipidemia, and T2DM is high among renal transplant recipients and CVD is the principal cause of death in renal transplant patients.

Compared to healthy controls, brachial artery FMD was significantly reduced following treadmill exercise to maximal claudication pain at 30 min and 2 hr post exercise in patients with PAD [116]. An acute bout of treadmill exercise to the onset of claudication pain has no effect on brachial artery FMD measured 5 min post exercise in men and women (62 ± 2 yr) with PAD [117]. In contrast, FMD is significantly impaired following treadmill exercise to maximal claudication pain, demonstrating that exercise-induced ischemia further deteriorates FMD. Intravenous administration of vitamin C ameliorates the impaired FMD response following treadmill exercise to maximal claudication pain only. Others have shown that antioxidant treatment can prevent acute impairment in endothelial function. The benefits of vitamin C on endothelial function are believed to be due to superoxide scavenging, and/or inhibition of LDL-C that takes place during high-intensity exercise. Vitamin C is also an important regulator of the intracellular redox [117].

Brachial artery FMD is significantly reduced in healthy male smokers compared to non-smokers (7.7 v 4.1%) immediately following 40 min of submaximal steady-state

exercise on a cycle ergometer [48]. Endothelium-independent dilation was similar in both study groups. In contrast, 30 min of acute cycling at 50% $\dot{V}O_2$peak increases brachial artery FMD in female smokers (20.6 ± 2.1 years) and non-smokers (20.5 ± 2.3 years) [118]. The different findings may be due to the fact that the men were older (30-51 year) than the women (20.5 yr), and may therefore have a longer history of smoking. Flow-mediated vasodilation at rest is significantly lower in postmenopausal, than premenopausal women (5.3% vs 12.1%). Treadmill exercise for 34 min at 60% $\dot{V}O_2$max almost doubled brachial artery FMD in postmenopausal women and had no effect on FMD in premenopausal women [119].

A study examining diurnal variation on vascular characteristics and function in healthy young men found significantly greater brachial artery blood flow velocity and shear rate following 30 min of semi-supine cycling at 70% $\dot{V}O_2$peak in the morning (0800) compared to evening (1600). Despite higher shear rates in the morning, there was no difference in post exercise brachial artery diameter between the two times of day [120].

Rognmo *et al*., [121] compared the effect of a single bout of high-intensity interval running on brachial artery diameter, peak blood flow and FMD in men with normal and high levels of aerobic capacity. The interval protocol involved 5 x 5 min of high-intensity running, interspersed with 2 min of active recovery. Brachial artery FMD and endothelial-independent dilation (EID) were assessed immediately before, 1 h, 24 h and 48 h post exercise. High fit individuals had significantly larger resting arterial diameters and maximal arterial diameters following cuff release at each time point than the sedentary individuals. High fit individuals experienced a 38% reduction in FMD 1 h after

exercise, compared with baseline and returned to pre-exercise values within 24 h after the exercise bout. There was no difference in EID between the two groups following the acute bout of exercise. There was a significant inverse relation between the pre-exercise resting diameter and FMD.

Llewellyn *et al.,* [49] also found a decrease in brachial artery FMD in healthy young men and women (19-30 years) after 30 min of continuous treadmill exercise at 60% $\dot{V}O_2$max. The percentage change in diameter was not significantly different from rest when normalized for shear rate (SR_{AUC}). Baseline diameter was not altered after exercise, but shear rate was significantly augmented. There was a significant positive relation between FMD and SR_{AUC} at rest, but not after the acute bout of exercise. The absence of a relation between FMD and SR_{AUC} after exercise may be attributable to the fact that the change in SR_{AUC} from rest to post-exercise varied greatly among individuals, e.g., SR_{AUC} decreased in 7 participants and increased in 6 participants. A high degree of variability in the physiological shear rate response to reactive hyperemia post-exercise was observed.

Brachial artery FMD is enhanced 1 h following 45 min acute bouts of low, moderate and high-intensity treadmill exercise in active overweight men, but is attenuated in inactive overweight men [122]

Dawson *et al*., [123] found that resting femoral artery diameter is unchanged while FMD is significantly reduced in non-elite runners 1 h after completing a marathon. Resting and maximal brachial artery diameters were both significantly increased post marathon, but brachial artery FMD was unchanged. Reduced bioavailability of NO resulting from an increased oxidative stress and/or inflammation in the exercising limbs,

may help to explain the impaired femoral artery FMD. Furthermore, the femoral and brachial artery are exposed to different blood flow velocities and shear rate patterns during prolonged, strenuous exercise. The profound changes in blood flow and shear rate in the femoral artery during the marathon may exhaust eNOS. Interestingly, the increased resting brachial artery diameter following the race may account for the unchanged brachial artery FMD.

Both low volume high-intensity interval exercise (HIIT) and moderate-intensity endurance exercise (END) significantly increased absolute and normalized brachial artery FMD 1 h post exercise in CAD patients. Despite differences in exercise intensities and durations, this study demonstrated similar acute increases in brachial artery FMD 1 h after a single bout of END and HIT exercise in men and women with CAD. Although there was only a 10 min difference in exercise duration between the END and the HIT protocols, total work performed was significantly lower for the HIT group, and was equal to 60% of the work performed by the END group [124].

Table 2.1: Studies that examined the effect of acute exercise on endothelial function

Author	Patient Cohort	Mode of Exercise	Duration	Intensity	FMD
Cosio-Lima *et al*., (2006)	Renal Transplant/healthy	TM Walking	30 min	70 - 85% HRmax	—
Gaenzer *et al*., (2001)	Smokers/non-smokers	Cycling	40 min	50% $\dot{V}O_2$peak	↓ vs. non-smokers
Silvestro *et al*., (2002)	PAD	TM Walking	Max claudication	3 km.h^{-1} @ 3%	↓
Harvey *et al*., (2005)	Pre & post menopausal	TM Walking			↑ vs. pre menopausal
Rooks *et al*., (2011)	Smokers vs. non-smokers	Cycling	59% $\dot{V}O_2$peak	30 min	↑ in both groups
Jones *et al*., (2009)	Healthy men (28 y)	Intermittent Cycling	3 x 10 min	70% $\dot{V}O_2$peak	Morning ↑ vs. evening
Jones *et al*., (2010)	Healthy men (28 y)	Intermittent Cycling	3 x 10 min	70% $\dot{V}O_2$peak	— post ex morn vs. eve
Rognmo *et al*., (2005)	Normal/high aerobic capacity	HII Running	5 x 5 min	90% HRmax	↓ high fit, — low fit
Harris *et al*., (2008)	Active/inactive overweight	Intermittent Cycling	45 min	25% $\dot{V}O_2$peak 50% $\dot{V}O_2$peak 75% $\dot{V}O_2$peak	↑ active ↓ inactive
Llewellyn *et al*., (2012)	Healthy (M/F; 19-30 y)	TM Running	30 min	60% v $\dot{V}O_2$max	↓
Currie *et al*., (2012)	CAD	Intermittent vs. Continuous	10 x 1min vs 30 min	80% vs. 55% POpeak	↑ in both ex groups
Dawson *et al*., (2008)	Non-elite Runners	Marathon	42.2 km	n/a	—
Joras *et al*., (2008)	PAD/healthy controls	TM Walking	Max claudication	3.2 km.h^{-1} @ 12%	↓

CAD, coronary artery disease; FMD, flow-mediated vasodilation; TM, treadmill; HII, high-intensity interval

Endothelial Function – Moderate-intensity Exercise Training

Appropriately planned, systematic increases in the training load coupled with optimal recovery will induce adaptive changes in the structure and function of cells, tissues and organs systems that are disturbed during the workout. The physiological adaptations called training effects increase fitness levels, and help to diminish the magnitude of the disruptions to the internal environment from future exercise at the same absolute intensity. This in turn permits an individual to exercise subsequently for a longer duration at the same absolute workrate before the onset of fatigue.

Exercise training involving 30 min of daily home-based cycle ergometry at 75% HRmax, coupled with a 90 min outdoor group session, twice per week, for 6 months was found to significantly improve brachial artery endothelial-dependent dilation in individuals with CAD and pre-diabetes. In contrast, 6 months of rosiglitazone therapy (8 mg/day) had no effect on endothelial function in the same patient cohort. Shorter duration training programs consisting of 4-12 weeks of aerobic training, resistance training or a combination of both reversed endothelial dysfunction in men and women with a recent first MI [125,126]. The training effects are lost after 4 weeks of detraining.

Hospital-based exercise training for 10 min, 6 times per day for 4 weeks significantly attenuates the vasoconstrictive response to 7.2 μg/min of ACh, and increases coronary blood flow above baseline with no change in EID in men with CAD and abnormal baseline ACh-induced vasoconstriction [127]. Glyceryl trinitrate-induced, endothelium-independent coronary vasodilation did not change in response to training, indicating that the response of the vascular smooth muscle to NO was not impaired.

Using a similar study design Hambrecht et al., [128] examined the effects of exercise training on endothelial vasodilator function and eNOS expression in the left internal mammary artery (LIMA) in response to ACh infusions and to increases in blood flow after adenosine administration in men with CAD. Patients were randomized to 4 weeks of supervised, hospital-based, aerobic exercise training or an inactive control group. Exercise training involved 10 min of rowing ergometry, followed by 10 min of cycle ergometry, 3 times per day for 4 weeks. Vasodilation and peak blood flow velocity of the LIMA were significantly enhanced in response to ACh after exercise training. Similarly, adenosine-induced flow-dependent vasodilation of the LIMA was markedly improved after exercise training. There was no change in EID in response to training. Expression of eNOS mRNA expression and eNOS protein content doubled. The rise in eNOS expression is thought to be mediated by shear stress-responsive elements in the promotor region of the eNOS gene, or by stabilization of eNOS mRNA.

The precise mechanism by which endothelial cells sense changes in flow or blood pressure amplitude are not clearly understood. In addition to eNOS protein expression, NO production is also dependent on eNOS phosphorylation. The Ser^{1177} residue seems to function as a sensor of shear stress, because exposure of endothelial cells to laminar shear stress specifically, increases phosphorylation at this site leading to a rise in the enzymatic activity of eNOS and enhanced NO production. The shear stress-induced phosphorylation of eNOS is maintained, whereas the agonist-mediated Ser^{1177} phosphorylation, e.g., bradykinin, is only transient indicating that a sustained eNOS phosphorylation, resulting in an increased NO production or availability mediates the improvement in endothelial function in response to exercise training.

Heart failure is now regarded as a systemic rather than a cardiac disorder involving hemodynamic, neurohormonal and peripheral derangements. Reduced peripheral blood flow at rest, during exercise, and in response to endothelium-dependent vasodilators have been identified as factors contributing to key symptoms of CHF like exercise intolerance. Findings from studies examining the systemic vascular effects of lower limb exercise in HF have been equivocal.

Cycling 14 min per day, 2 to 3 days per week for 3 months significantly increases posterior tibial artery FMD and has no effect on brachial artery FMD in patients with HF [129]. In contrast, 6 daily hospital-based, supervised 10 min bouts of cycle ergometry at 70% $\dot{V}O_2$peak for 4 weeks significantly improved ACh and flow-mediated dilation in the brachial artery [130]. Peak aerobic capacity increased significantly and the improvements in ACh- and flow-mediated dilation were correlated with the increase in aerobic capacity. Radial artery FMD and $\dot{V}O_2$max increased significantly following 12 weeks of daily exercise for 30 min on a cycle ergometer in individuals with HF [131].

In HF patients, femoral artery blood flow increased by 203% in response to 6 months of exercise training, 6 times daily for 10 min on a cycle ergometer at 70% HRpeak. In contrast, peripheral perfusion decreased 174% after L-NMMA infusion, indicating that the improvements in peripheral perfusion may be attributed to an enhanced formation and/or release of NO [106].

Although L-arginine is stored in significant amounts in intracellular depots, oral supplementation of L-arginine has been shown to increase exercise-induced blood-flow in patients with chronic HF. There is evidence that 4 weeks of combined L-arginine

supplementation and daily handgrip training have superior effects to either intervention alone on radial artery endothelium-dependent vasodilation in patients with HF [132].

Endothelial Function – High-intensity Interval Training

High intensity interval training (HIIT) involves repeated bouts of high intensity exercise (10 to 300 seconds) interspersed with active or passive recovery periods of equal, shorter, or longer duration [6]. The rapid repletion of phosphagen stores during recovery coupled with a reduction in blood lactate accumulation allows individuals to undertake a greater volume of high-intensity exercise during a single training session [133]. Historically, HIIT has been used by athletes, but a number of studies have shown its efficacy and safety in improving exercise capacity and endothelial function in individuals with CAD and heart failure [7,8].

Studies have found a significant increase in $\dot{V}O_2$max and indices of cardiac function in men and women with CAD following HIIT on a treadmill, cycle ergometer, stairclimber and combined arm and leg cycle ergometer. In one of the first HIIT studies involving CAD patients, $\dot{V}O_2$max, heart rate, blood pressure and ST-segment changes during maximal exercise testing on a treadmill and cycle ergometer were assessed before and after 12 months of intense exercise training [134]. The double-product threshold for an ischemic ST-segment response was also assessed. The first 3 months of exercise training involved alternate walking and jogging, continuous jogging or cycling at 50 – 70% $\dot{V}O_2$max 3 times per week. After 3 months, exercise sessions were increased to 4 - 5 times per week and the intensity was increased to 70 – 80 % of $\dot{V}O_2$max, interspersed with 2 – 3 intervals at 80 - 90% lasting 2 - 5 min.

Absolute and relative $\dot{V}O_2$max and the double-product threshold for ischemic ST-segment depression were increased significantly, and resting HR and HR at the same submaximal workload decreased significantly at 12 months. Systolic blood pressure was significantly lower at the same submaximal workloads during exercise on the treadmill and cycle ergometer. Left ventricular end-diastolic dimension and posterior wall thickness were significantly increased after training. Training also reduced the extent of ST-segment depression at the same double product and reduced or unchanged maximum ST-segment depression despite a large increase in maximum double product. With the slower heart rate and lower systolic blood pressure, the double-product was significantly lower at the same submaximal work rates. However, after 12 months training, the double product in both treadmill and cycle ergometer exercise increased 20% and 19%, respectively, at maximal exercise.

Using a combination of continuous training and interval training for 12 months Ehsani *et al*., [135] found a significant increase in $\dot{V}O_2$max and left ventricular end-diastolic diameter in individuals with CAD. Resting heart rate, submaximal heart rate and systolic blood pressure at given work rate were lower after training.

Both moderate-intensity continuous training for 41 min at 50 – 60 %$\dot{V}O_2$max and treadmill walking involving four intervals of 4 min at 80 – 90 %$\dot{V}O_2$max interspersed with 3 min of walking at 50 – 60 % $\dot{V}O_2$max, 3 times per week for 10 weeks have been shown to increase $\dot{V}O_2$max in CAD patients. The improvement was significantly greater in the high-intensity aerobic training group than the moderate-intensity group (17.9% vs 7.9%) [7]. In contrast, improvements in $\dot{V}O_2$max were similar in response to 16 weeks of continuous bouts of exercise at 65% HR reserve and HIIT involving 90% HR reserve, and

interval durations of 2 min interspersed with 2 min active recovery bouts. Both groups exercised for a total of 30 min per day, 2 days per week [136]. A 16-week programme of HIIT involving 5 to 10 min intervals (15-18 on the Borg scale) was also found to increase quality of life and performance in a 6-min walk test in HF patients [137].

The metabolic syndrome (MetSyn) is characterized by a group of metabolic risk factors that increase the risk for premature heart disease, stroke, peripheral vascular disease and type 2 diabetes. Risk factors include central obesity, atherogenic dyslipidemia, elevated resting blood pressure and insulin resistance or high fasting glucose levels. A 16-week aerobic interval training (AIT) programme comprised of four 4 min intervals at 90% HRmax, with 3 min active recovery at 70% HRmax, 3 times per week, was superior to 47 min of continuous moderate-intensity exercise (CME) at 70% HRmax in reversing risk factors for metabolic syndrome [138]. After 16 weeks, FMD and $\dot{V}O_2$max were significantly improved in both training groups, but the increases in the AIT group were significantly greater than the CME group. Insulin sensitivity, β–cell function and PGC-1α levels were also significantly greater in the AIT group, than the CME group at week 16.

Interval training (4 x 4 min at 90% HRmax) 3 times per week for 12 weeks partly reversed the impaired age-related diastolic function in healthy men and women (72 ± 1 yr) at rest, and significantly improved LV diastolic and systolic function during exercise [139].

To date, only 4 published studies have examined the effect of HIIT on endothelial function in men and women with CVD (Table 2.2). Wisløff *et al*., [8] found a significantly greater increase in $\dot{V}O_2$max and brachial artery FMD in elderly men and

women (75.5 ± 11.1 years) with post-infarction HF, following 12 weeks of AIT compared to moderate continuous training (MCT), 3 times per week. The AIT involved four 4-min intervals at 90 – 95% HRpeak. The MCT group walked continuously at 70 – 75% HRpeak for 47 min in order to expend an equivalent number of calories to the AIT group. Changes in FMD were significantly related to changes in aerobic capacity in the combined training groups

Munk *et al*., [10] compared the effect of 6 months supervised HIIT, 3 times per week for 1 hour, to a non-exercising control group, on aerobic capacity, degree of restenosis, FMD, and CRP levels in 40 men and women, following percutaneous intervention (PCI). The HIIT involved 4 min intervals at 80 - 90% HRmax on a cycle ergometer or running, followed by 3 min active recovery at 60 – 70%. Changes in $\dot{V}O_2$peak, ventilatory threshold, CRP, heart rate variability and FMD were significantly greater in the HIIT than the control group at 6 months. Endothelium-independent dilation did not change in either group. Restenosis was measured as in-segment late luminal loss of the stented coronary area and was smaller in the HIIT group than the control at 6 months.

Hermann *et al*., [9] found that 8 weeks of HIIT, involving cycle ergometry and running, with interval blocks of 4 min/2 min/30 s at 80%, 85% and 90% $\dot{V}O_2$peak, and 1-2 min recovery periods, significantly improved endothelial function and $\dot{V}O_2$max in stable heart transplant recipients compared to inactive controls. There was a significant relation between the change in FMD and the change in $\dot{V}O_2$peak.

In a recent randomized controlled trial, Moholdt *et al*., [140] found similar improvements in $\dot{V}O_2$peak and brachial artery FMD in post MI patients following 12

weeks of treadmill interval training (n=29) involving 4 min intervals at 85 - 95% HRmax, followed by 3 min active recovery at 70% HRmax, and 12 weeks of cardiac rehabilitation (n=44). All participants met for exercise training in the hospital twice weekly, and exercised once weekly at home. The increase in $\dot{V}O_2$peak was greater in the interval training group than the cardiac rehabilitation group (15% v 8%).

Table 2.2: Summary of studies that evaluated the effect of HIIT on endothelial function in individuals with CVD

Author	Patient Cohort	Exercise Mode	Intervals	Intensity	Frequency (per wk)	Duration	FMD	$\dot{V}O_2$peak
Hermann *et al*., (2011)	HT (active vs. con) (N = 30)	Treadmill	4 min/2 min/30 s	80%/85%/90% $\dot{V}O_2$peak	2	8 weeks	↑ vs. con	↑ vs. con
Munk *et al*., (2009)	PCI (bare metal/drug eluting stent) vs con (N = 30)	Cycling/Running	4 x 4 min	85 - 95% HRpeak	3	8 weeks	↑ vs. con	↑ vs. con
Wisloff *et al*., (2007)	HF (MCT vs. AIT) (N = 20)	Cycling/Running	(4 x 4 min)	80 - 90 HRmax	3	24 weeks	↑ vs. MCT	↑ vs. MCT
Molholdt *et al*., (2012)	HF (AIT vs. UCR) (N = 9)	Treadmill	4 x 4 min	85-95% HRpeak	2	12 weeks	↑ vs. UCR	↑ both groups

HT, heart transplant; Con, non-exercising controls; HF, heart failure; PCI, percutaneous coronary intervention; MCT, moderate continuous training; AIT, aerobic interval training; UCR, usual care rehabilitation

Mechanisms for Exercise-induced Adaptations in BAR

Repetitive increases in blood flow and shear stress that accompany regular exercise elicit an adaptive response that alters the intrinsic responsiveness of the endothelium by increasing mRNA expression of NOS [141]. This in turn increases the synthesis and release of NO, and improves endothelial function. Chronic increases in shear stress can also enhance vascular structure and function due to functional and histological alterations of vascular endothelium [142].

The benefits of exercise training in terms of endothelial function may also be related to secondary effects, mediated through risk factor modification. Lower cholesterol levels were related to an enhanced forearm dilation response to ACh in highly-trained athletes compared with sedentary controls [143]. However, improvements in endothelial function following 10 weeks of combined aerobic–anaerobic training were not related to changes in total cholesterol, high-density lipoprotein (HDL)-C, lipoprotein(a), fibrinogen level or resting blood pressure [144].

Different patterns of shear rate are believed to induce different vascular adaptations [145]. Shear rate can differ significantly in response to different types of exercise. For example, handgrip exercise results in elevated antegrade (forward) shear rate, while cycling can induce large increases in both antegrade and retrograde blood flow and shear rate [144]. Both *in vitro* and animal studies have found that different shear patterns induce different cellular events, varying between proatherogenic and antiatherogenic changes.

Self-Regulated Exercise

Exercise prescription is as much an art as a science, and there is a need to balance physiological effectiveness with enjoyment and pleasure, and positively effect desired biological changes. According to the hedonic theory of motivation [146] people are likely to repeat an activity if they derive pleasure, sense of energy, or enjoyment from their participation in the activity. In contrast, if people derive displeasure, sense of exhaustion, pain, or discomfort from their physical activity, the chances of them repeating the activity would be reduced.

Studies that have evaluated the acute effect of exercise on endothelial function have for the most part prescribed exercise based on objectively measured thresholds of caloric expenditure or percentage of HRmax or $\dot{V}O_2$max. This form of exercise prescription may exceed an individual's preferred level of intensity and may establish a negative attitude toward physical activity [146].

Longitudinal studies report that participants tend to deviate from prescribed levels of intensity in favour of their apparently preferred levels [147]. In contrast, allowing individuals to use effort perception to self-regulate [148] their preferred intensity may encourage the development of intrinsic motivation, a central element in promoting adherence to exercise, and increase enjoyment and participation levels. In a recent study, Johnson et al., [148] found that 86% of women involved in aerobic exercise used effort perception exclusively to determine exercise intensity. This is not surprising considering that exertional feedback is often used unconsciously to regulate the pace of many daily activities. The intensity of home, recreational, and even some occupational activities is often self-regulated, using exertional perceptions that reflect local and

general fatigue and shortness of breath. In most cases, the pace with which these activities are undertaken allows their successful completion without undue physiological strain.

Walking (4.8-6.4 $km.h^{-1}$) is promoted as a moderate-intensity (~3-6 METS) activity that is highly accessible, requires no special skills or facilities, can be easily accommodated into an existing lifestyle and can be undertaken at almost any age with little risk of injury and has been shown to positively impact on physical, psychological and cognitive function. Most individuals prefer to walk alone and unsupervised, self-regulating their own intensity [146].

Although effort perception is used to select exercise intensity during walking, few studies have examined the effect of walking at preferred intensity on endothelial function in men and women with CVD.

In one of the first published studies to examine the physiological, metabolic and perceptual responses to self-regulated exercise, Spelman *et al*., [149] covertly measured the walking velocity during a typical walking session in 29 adult habitual walkers (22 women and 7 men) with a mean age of 35 yr. Participants subsequently performed an 8-min level treadmill walk at the velocity determined in the typical walking session to determine physiological, metabolic, perceptual and treadmill speed responses. The mean walking speed was 1.78 $m{\cdot}s^{-1}$ and the effort was perceived to be "fairly light" (10.9) on Borg scale. The average metabolic cost of walking at a self-selected pace was 5.2 METS and corresponded to 52% $\dot{V}O_2$max and 70% HRmax. Spelman estimated that the subjects expended approximately 257 kcal, or 3.8 kcal/kg of body weight, during a typical walking session, reaching a level associated with improvements in health and

longevity. Using a similar design Murtagh *et al*., [150] covertly observed the walking velocity in 11 women with a mean age of 40 years. They found a mean walking velocity of 1.56 $m.s^{-1}$ which corresponded to 59 %$\dot{V}O_2$max and 67% HRmax. The RPE was 11.5 (fairly light). When asked to demonstrate their interpretation of "brisk" walking over the same course the velocity increased significantly to 1.79 $m.s^{-1}$, which corresponded to 69% $\dot{V}O_2$max and 79% HRmax and an RPE of 13.6. These findings indicate that when walking for exercise, adults self-select speeds and intensities that meet definitions of moderate intensity activity, and instructing them to walk briskly encourages vigorous exercise.

A number of studies have shown that when allowed to self-regulate their exercise intensity healthy young men and women and middle-aged men and women [146] select an intensity that equates to 55-65% $\dot{V}O_2$max, 65-75% HRmax, and RPE 11-13 (fairly light to somewhat hard on the Borg RPE scale).

Lind *et al*., [151] examined the intensity of physical activity that sedentary middle-aged women self-select during 20 min of treadmill exercise. They found that the %HRmax, %$\dot{V}O_2$max, and RPE during the 20 min walk was 81%, 66% and 13.4 respectively and was similar to the intensity corresponding to the ventilatory threshold. The RPE values ranged from 12-14 on the 12-point Borg RPE Scale. The ratings of affective valence (decreases in pleasure or increases in displeasure) remained unchanged, and were within the positive (or nonnegative) range. Rose and Parfitt *et al*., [152] found that exercise at a self-regulated intensity resulted in more positive affective responses compared with exercise above and at lactate threshold in sedentary women (39.4 ± 10.3 years). When allowed to self-select their exercise intensity, sedentary

middle-aged women (age 43. 7 ± 4.8 years $\dot{V}O_2$max 23.0 ± 5.7 mL.kg^{-1}.min^{-1}) choose a level that approximates their ventilatory (or lactate) threshold in order to allow them to maintain a stable and positive affective state [151].

Chapter 3

STUDY 1

USER RELIABILITY AND DEVELOPMENT AND VALIDATION OF A CUSTOM-DESIGNED ARTERIAL DIAMETER MEASUREMENT SOFTWARE

Brachial artery flow-mediated dilation (FMD) using high frequency ultrasonography is commonly used to non-invasively assess endothelial function. Image acquisition and analysis are technically challenging and are reliant on the skill level of the practitioner. Vigilant attention to image acquisition and analysis is required in order to ensure reproducible FMD measurements. Typically, 6 months of training, and > 100 supervised scans is required before a practitioner is deemed proficient [153].

Brachial artery diameter is calculated by measuring the distance between the arterial walls at one or more cross-sections. Manual measurement of arterial diameter, using B-mode or M-mode ultrasound images, is typically performed in real-time using electronic or virtual calipers built into the ultrasound hardware. The major disadvantage of this technique is the time-consuming nature of the manual measurements, which are prone to error and variance among practitioners. In addition, manual, real-time measurements require the presence of the patient, and limits the number of time-points at which the diameter can be assessed.

Computerised analysis systems utilizing edge-detection and wall tracking software allow for multiple, objective measurements along the vessel wall and can markedly improve the precision and accuracy of the FMD technique [3]. Studies

comparing the validity and reproducibility of computerised edge-detection and wall tracking software have found significantly lower intraobserver variation for the automated systems than for the manual technique [66]. It has been recommended that validated, accurate, and reproducible edge-detection and wall-tracking systems should be used to assess FMD measurements [66].

Existing general-purpose segmentation algorithms, such as seeded region growing, are inadequate for segmenting an ultrasound image of an artery into arterial lumen and arterial tissue for two reasons [154]. First, many general purpose segmentation algorithms are unable to reliably delineate the artery boundary. This is often caused by the inherent noise in ultrasound images. In particular, unconstrained region growing algorithms often generate incorrect segmentations, since they do not incorporate prior knowledge about the shape of the region of interest [154]. Algorithms that incorporate prior shape knowledge, like active contour models [155] and active shape models [156], require careful initialization and significant user interaction. Second, most existing segmentation algorithms are too computationally intensive to allow for real-time updates and feedback.

Off-the-shelf ultrasound analysis software tends to be considerably expensive. There is a need for an ultrasound image processing tool that is low cost, can be implemented with minimal investment and development time, will allow sonographers to quickly and robustly assess the diameter and wall thickness of arteries from ultrasound images whilst requiring minimal input or interaction from a sonographer. Moreover, there remains a need for segmentation methods that facilitate rapid and

reliable segmentation of tubular structures such as vascular tissues so as to aid the processing steps discussed above.

Study Purpose

The purpose of this study was to assess the reliability of the present investigator in the use of ultrasonography in the measurement of endothelial vasomotor function and to develop and validate a reliable, custom-designed arterial diameter measurement (ADM) software.

Specific Aims

1. To assess the reliability of the investigator in the use of ultrasonography to assess brachial artery diameter (Study 1A)
2. To assess the reliability of the investigator in the use of ultrasonography to assess Doppler blood flow (Study 1A)
3. To develop and validate a custom-designed analysis software (ADM software) that will semi-automatically calculate brachial arterial diameter (Study 1B)
4. To assess the reliability of the ADM software to assess brachial artery diameter by comparing off-line brachial artery diameter measurements using the ADM software and manual measurements using in-built ultrasound calipers (Study 1B)

Hypothesis

1. There will be no significant difference in brachial artery diameter measurement recorded on two separate occasions by the same sonographer
2. There will be no significant difference in Doppler blood flow measurements recorded on two separate occasions by the same sonographer
3. The custom-designed ADM software, utilizing segmentation methods, will allow sonographers to obtain similar brachial artery diameters as when using in-built ultrasound calipers
4. The custom-designed ADM software developed will provide valid and reliable measurements of the brachial artery diameter

STUDY 1A

USER RELIABILITY OF ULTRASOUND IMAGE ACQUISITION AND ANALYSIS

METHODOLOGY

Participants

Twenty-five healthy men (n=12) and women (n=13) (mean age 32.2 yr) volunteered to participate. Exclusion criteria included smoking and currently taking prescribed medication for cardiovascular disease.

Study Overview

The study involved assessing brachial artery flow-mediated dilation (FMD) on 2 separate occasions, no more than 5 d apart. FMD was assessed using high-resolution ultrasonography, by the same investigator, in a quiet, temperature-controlled room (VRU). Ultrasound measurements were performed on a SonoSite MicroMaxx® (SonoSite Inc., Bothell, Washington, US) ultrasound system with a linear array transducer (Figure 3.1), operating at a frequency of 12.0 MHz.

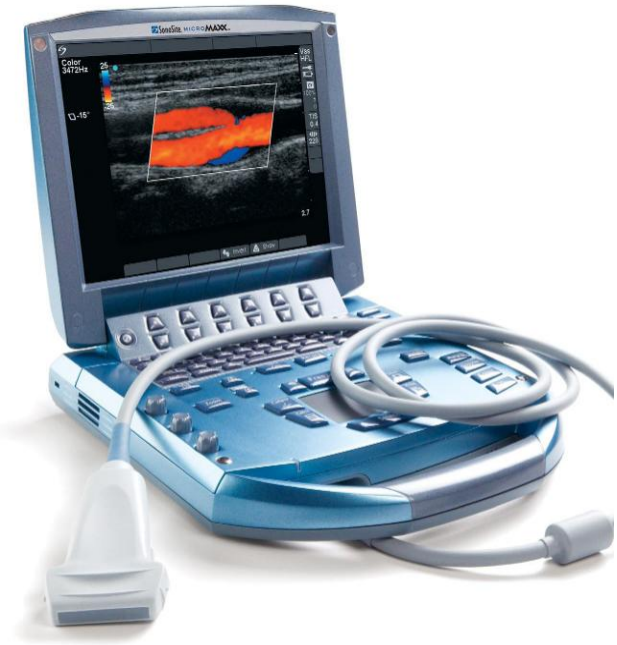

Figure 3.1: SonoSite MicroMaxx® Ultrasound system and 12.0 MHz linear array transducer

Overview of Endothelial Function Assessment

All brachial artery images were acquired with the participants in a supine position. A baseline brachial artery image was acquired following a 10 min rest period and was immediately followed by assessment of FMD. Ultrasound images were analysed in real time, using in-built ultrasound calipers (SonoSite MicroMaxx®).

The right arm of the participant rested on an examination table perpendicular to the bed, and was extended and externally rotated to permit imaging of the right brachial artery. An automated blood pressure cuff was placed on the right forearm, distal to the brachial artery (Figure 3.2) and electrodes for a 3-lead ECG were placed on their chest. The ECG tracing was activated and settings adjusted to ensure clear identification of the R wave which corresponds to the end of diastole in the cardiac cycle.

Participant Preparation

Participants were tested between 7 and 10 am, by the same experienced investigator, in the same, quiet, temperature-controlled room (Vascular Research Unit). Participants arrived to the Vascular Research Unit, DCU after an overnight fast (from 10pm the previous night). Only water consumption was permitted. Participants were not permitted to exercise, or ingest substances that might affect FMD such as caffeine and vitamin C or use tobacco for at least 6 h before the study.

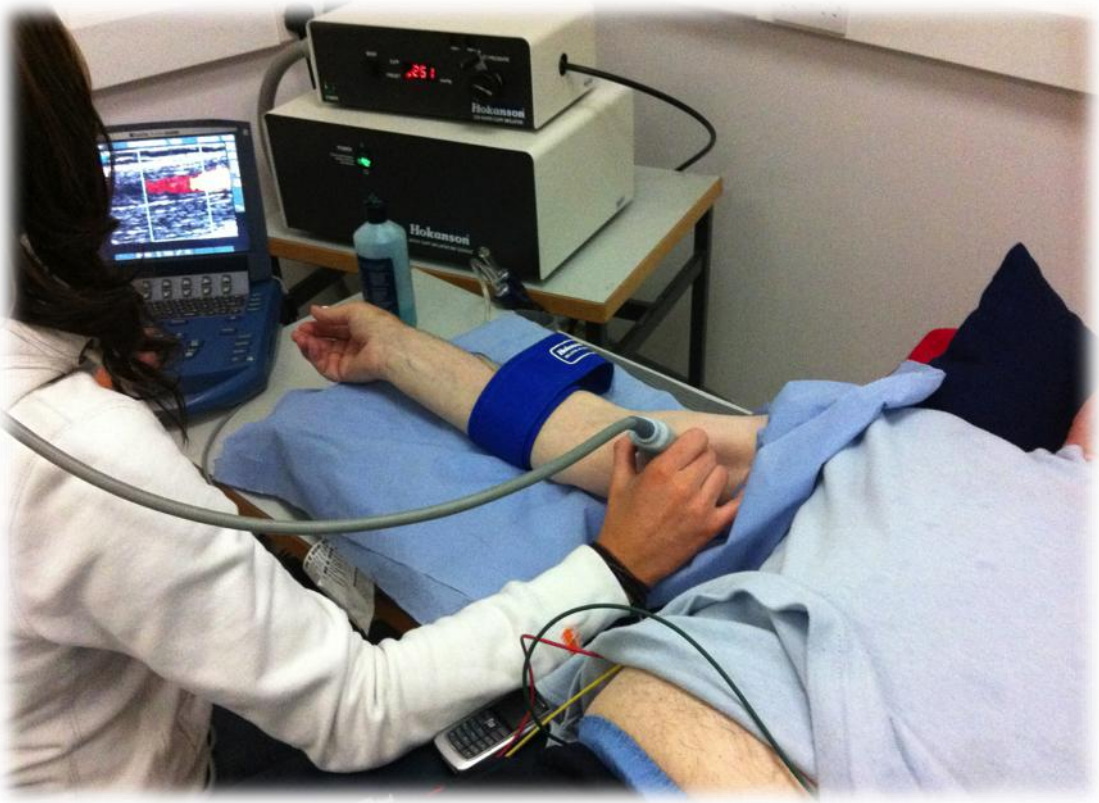

Figure 3.2: Arm position and cuff placement.

Ultrasound Technique and Image Acquisition

Anatomic landmarks such as veins and fascial planes were noted and used to ensure that all M-mode images and Doppler measurements were recorded at the same site. A longitudinal image of the brachial artery was obtained using B mode ultrasound. The brachial artery was insonated 3-7 cm above the antecubital crease. Great care was taken to ensure that the anterior and posterior intimal interfaces between the lumen and the arterial wall were clearly visible. Depth and gain settings were optimized to delineate the lumen-arterial interface optimally on both the near (anterior) and far

(posterior) wall. The boundaries were clearly visualized with the angle of insonation perpendicular to the vessel. It is recommended that the imaging plane should bisect the vessel in the longitudinal direction to ensure that diameter measurements obtained from the images reflect the true diameter of the vessel [38]. Images were magnified using a "zoom" function.

M mode Imaging

The brachial artery was imaged using M mode function to facilitate arterial diameter measurements at appropriate time points (Figure 3.3). Brachial artery diameter was assessed in real time using the in-built ultrasound calipers (SonoSite MicroMaxx®). Each image acquired incorporated a minimum of 2 and a maximum of 3 consecutive ECG R waves. The brachial artery diameter measurements were obtained at cross sections corresponding to the R waves, which represent the end of diastole in the cardiac cycle.

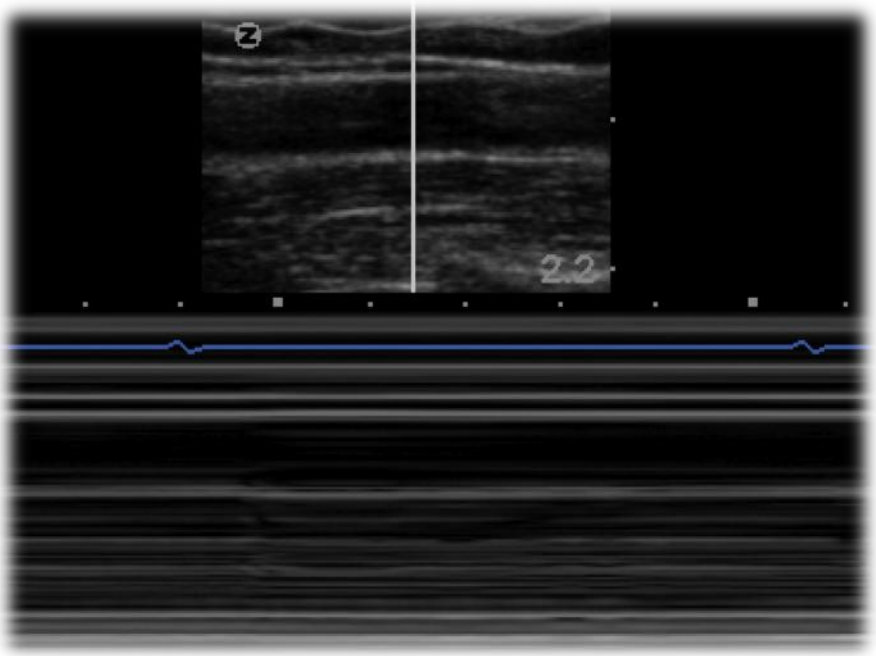

Figure 3.3: M mode ultrasound image of the brachial artery

Doppler Imaging

Doppler imaging was used to measure blood flow velocity (cm/s) in the brachial artery. The Doppler scale was adjusted to accommodate the spectral signal and the expected increase in blood flow following cuff release. The scale was maintained at the minimum range to decrease measurement error. The Doppler gate was set to minimum (1.5 mm) and was positioned in the centre of the artery lumen. The Doppler gate was aligned with the direction of flow and the transducer was adjusted to achieve an angle of insonation of 60° [157]. The insonation angle between the pulsed-wave Doppler beam and the vessel walls was adjusted by manipulation of the transducer, to allow the beam to be steered and the angle corrected in alignment with the vessel orientation/parallel, and blood flow axis at a discrete segment of vessel 60° [157]. The Doppler function traced the spectral wave-form (Figure 3.4). The image was frozen and peak systolic velocity was manually measured using the in-built ultrasound calipers (SonoSite MicroMaxx®).

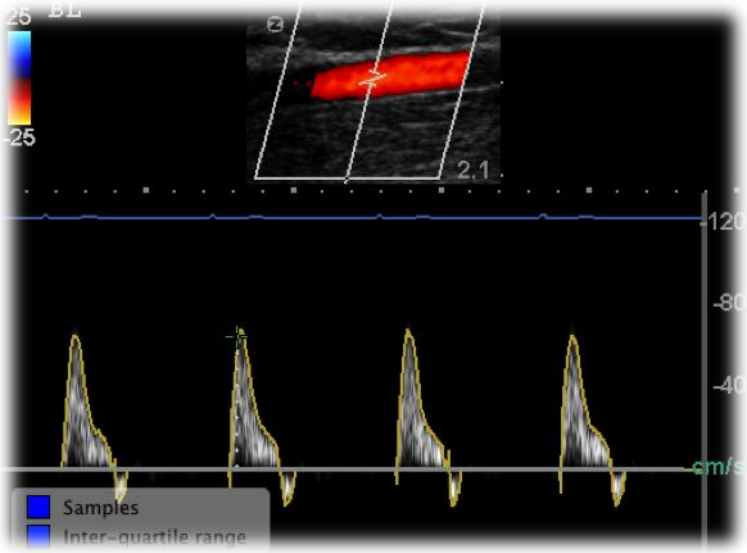

Figure 3.4: Frozen screen shot of a Doppler image

Baseline Assessment

Once a satisfactory image was obtained the investigator and the participant remained in the same position throughout the study (anatomic landmarks such as veins and fascial planes were noted). The baseline brachial artery image was named and saved for subsequent off-line analysis of arterial diameter using a custom-designed, semi-automated ultrasound arterial measurement software. The saved M-mode images incorporated at least 2 and a maximum of 3 R waves on an ECG. The "Doppler" function traced the spectral wave form. The investigator froze the image and manually measured the peak systolic velocity using in-built calipers on the ultrasound system.

Endothelial-Dependent Dilation (EDD)

Figure 3.5 illustrates the endothelial-dependent dilation assessment protocol. Following a 10 min rest period a pneumatic cuff was inflated to 250 mmHg for 5 min [38].

Following 5 min of occlusion, the cuff was rapidly released resulting in reactive hyperemia and a subsequent increase in brachial artery blood flow. Post-deflation peak systolic velocity was measured using Doppler within 15 sec of cuff release [107]. M-mode images of the brachial artery were recorded every 60 sec post-deflation for 5 min.

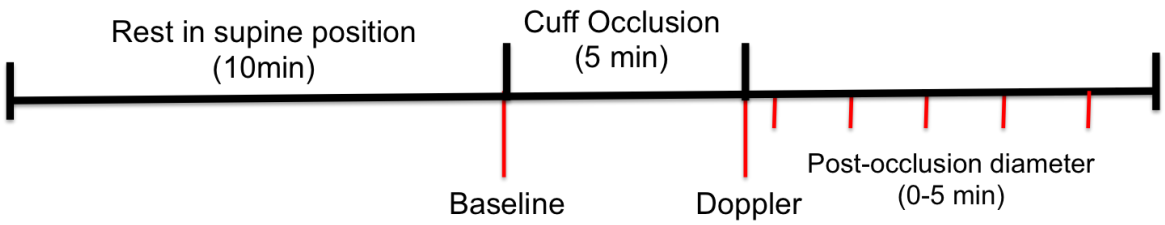

Figure 3.5: Overview of the protocol used to assess endothelial-dependent dilation

Real-time analysis of the ultrasound images was performed using in-built ultrasound calipers. The virtual calipers were placed at the anterior and posterior intimal interfaces between the lumen and the arterial walls to provide an estimate of the arterial diameter. The diameter of the brachial artery was measured at a minimum of two and maximum of three consecutive R waves on the ECG, a process referred to as "gating". The R wave represents the end of diastole in the cardiac cycle. Gating allows diameter estimates to be taken at specified vertical cross-sections of the artery. The mean of the 2-3 measurements was taken as the brachial artery diameter.

Statistical Analysis

Pearson product-moment correlation was used to assess the reliability in brachial artery measurement between day 1 and day 2 ($p<0.001$). Data was analysed using SPSS (V19.0, SPSS Inc, IL).

Results

Scattergrams for FMD and Doppler blood flow are illustrated in Figure 3.6 and Figure 3.7. There was a significant correlation between the two brachial artery diameter measurements ($r=0.78$, $p<0.001$) and the two Doppler blood flow measurements ($r=0.83$, $p<0.001$)

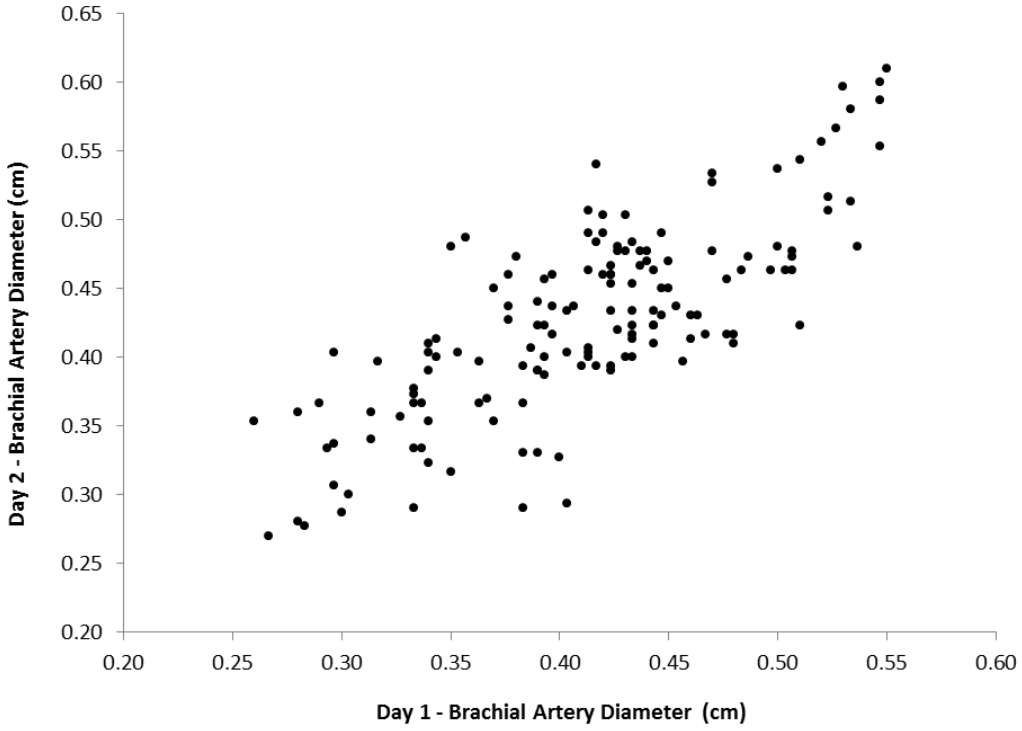

Figure 3.6. Scattergram of brachial artery diameter measurements (cm) on two separate occasions.

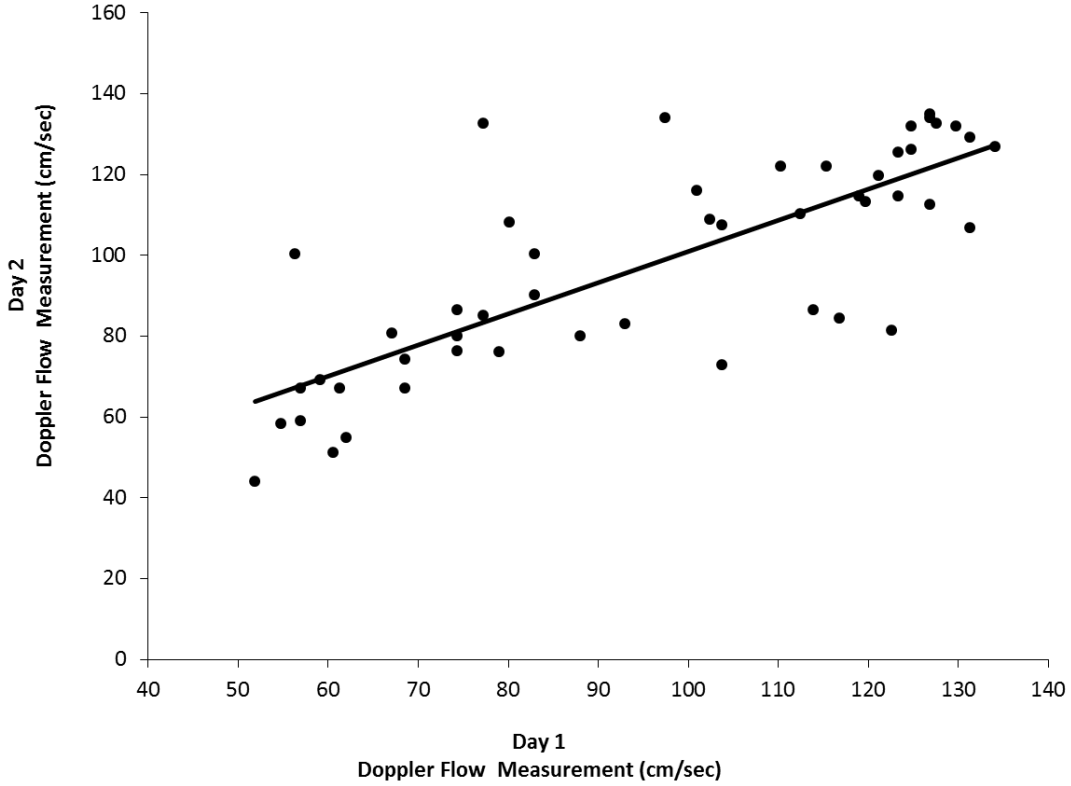

Figure 3.7. Scattergram of Doppler blood flow measurements (cm/sec) on two separate occasions.

Summary

The reproducibility of the FMD measurement was confirmed as there was a significant correlation between the repeated measures.

STUDY 1B

IMAGE DETECTION SOFTWARE DEVELOPMENT - VALIDITY AND RELIABLITY

METHODOLOGY

Image Detection Software Development

The new segmentation algorithm was developed by Dr. Kevin McGuinness, a postdoctoral researcher in CLARITY: The Centre for Sensor Web Technologies. The algorithm operates in two stages: horizontal expansion, and vertical expansion. In the horizontal expansion stage the seed point is expanded to a single pixel thick horizontal connected path. This is achieved by growing the seed point in both the left and right directions using a simple greedy strategy. This strategy begins by setting the current pixel to the seed pixel. Assuming that the line is growing in the correct direction, at each step, the three neighboring pixels to the right of the current pixel are examined. The current pixel is updated to be the darkest of these and is added to the line. This process continues until the expansion has reached a predefined boundary.

In the vertical expansion stage the region is grown upwards and downwards using a dynamic programming approach. The region is expanded upwards by examining each pixel above the topmost part of the previous region in turn. If the pixel brightness is below a certain threshold, and the three neighboring pixels behind it are already part of the front, the pixel is added to the new front. This process is repeated recursively until the front is fully expanded. A similar strategy is followed to expand the front downwards.

Image processing

Image processing is divided into the following stages, i) image acquisition, ii) calibration, iii) pre-processing, iv) segmentation, v) post-processing, vi) robust statistics, vii) refinement and viii) additional tools

Image acquisition

The practitioner selects one or more images of a large artery for analysis using a standard dialog box. For each image, the practitioner locates the artery and clicks the area between the arterial walls. The software can handle images stored in various file formats including BMP, PNG, JPEG, and GIF.

Calibration

A device specific calibration step establishes the number of pixels per centimetre in each ultrasound image. The implementation is based on a Sonosite MicroMaxx® ultrasound system. Calibrating this device requires first detecting the mode, zoom level, and depth, followed by counting the number of pixels between the measurement bars on the right-hand-side of the image. The mode and zoom-level are recognized by template matching at pre-defined areas of the image. The depth is recognized by template matching using prototypes of the digits zero through nine in the bottom right hand corner of the image. The distance between the measurement bars in cm is a function of the mode, zoom-level, and depth. As such, counting the number of pixels

between each measurement bar may be used to determine the number of pixels per cm in the image.

Preprocessing

The image is cropped to remove irrelevant information according to the device setup. Excessive noise can diminish the effectiveness of medical image processing algorithms. As such, a pre-smoothing step is optionally performed to reduce the noise in the image. In this step, the image is filtered with a Gaussian bilateral filter. This helps ameliorate noise without disturbing significant edge information in the image. Preprocessing can be disabled in the user interface if desired.

Segmentation

The practitioner identifies the artery of interest, and notifies the application of its position by clicking inside it. A constrained seeded region-growing algorithm is used to delineate the arterial boundary based on the position indicated by the practitioner and a live adjustable threshold (algorithm protected by invention disclosure). The result is depicted visually in that the segmented arterial lumen is highlighted using grey shading.

Post-processing

The region boundary is smoothed by convolving it with a 1-dimensional Gaussian kernel.

Robust statistics

An estimate of the arterial diameter is computed from the segmentation using a subset of the vertical diameters that correspond to the inter-quartile range. The cross-sections that correspond to these diameters are shown in a lighter color so that the practitioner may easily distinguish them.

Refinement

The practitioner observes the feedback and diameter estimate from the previous step and decides if the estimate is satisfactory. At this stage, the practitioner may choose to adjust the threshold used in the segmentation step with a slider or pick a new seed point and start again. As the slider is moved the feedback and diameter estimate are updated in real time.

Additional tools

The application includes built-in software calipers that allow manual measurements to be taken at fixed points in conjunction with the diameter estimates. A gating tool is included to allow measurements to be taken at specific cross-sections of the estimated arterial region.

Validation of ADM Software

Concurrent validity quantifies the relation of one measure to another. To assess the concurrent, criterion related validity of the ADM software, 143 brachial artery ultrasound images were randomly selected and arterial diameter was assessed by the

same investigator using the ADM software and the SonoSite ® virtual calipers. The diameter was assessed at a minimum of 2 and a maximal of 3 ECG R waves. All measurements were performed in a blinded fashion.

Arterial Diameter Assessment Using ADM Software

All images were selected for analysis using a standard dialog box (Figure 3.8). For each image, the artery was located and the area between the anterior and posterior arterial walls was manually selected. The software used this point to segment the arterial boundary using a constrained region-growing algorithm, and the result was depicted visually in that the segmented arterial lumen was highlighted using grey shading. The segmentation of the artery was updated in real-time. The automated values can be overridden by selecting a new seed point or using a slider to change the threshold intensity values of the segmentation.

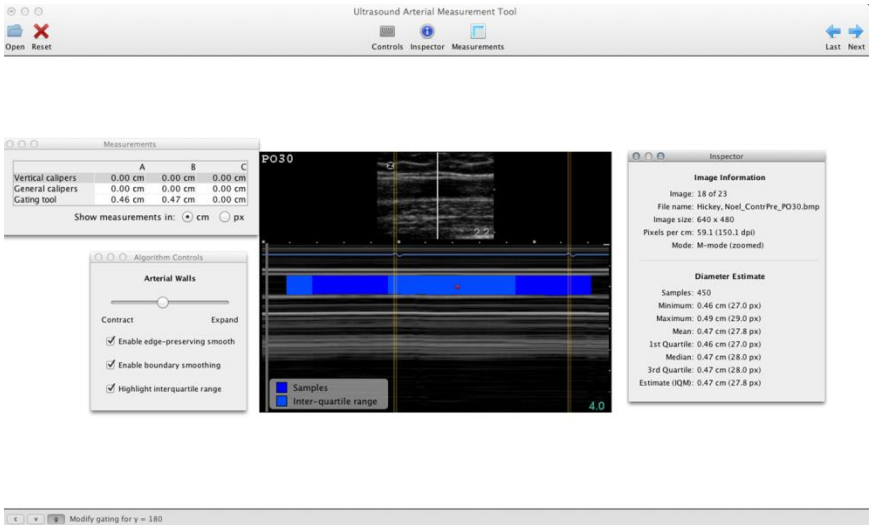

Figure 3.8: Standard dialog box

Gated measurements of the brachial artery diameter were recorded using a minimum of 2 and maximum of 3 consecutive R waves on an ECG. The mean of the 2-3 measurements was taken as the brachial artery diameter.

Statistical Analysis

Bivariate and intraclass correlation coefficients were used to assess the validity of the ADM software. Due to limitations of these statistical procedures, agreement between the methodologies was further assessed using the Bland and Altman procedure. Pearson product-moment correlation was used to assess the reliability of the ADM software Data, using SPSS (V19.0, SPSS Inc, IL).

Results

ADM Software – Validity

The bivariate correlation between techniques was 0.99 ($p<0.001$) (Figure 3.9) and average measurement intraclass correlation coefficient was 0.99 (99% CI = 0.992 – 0.996).

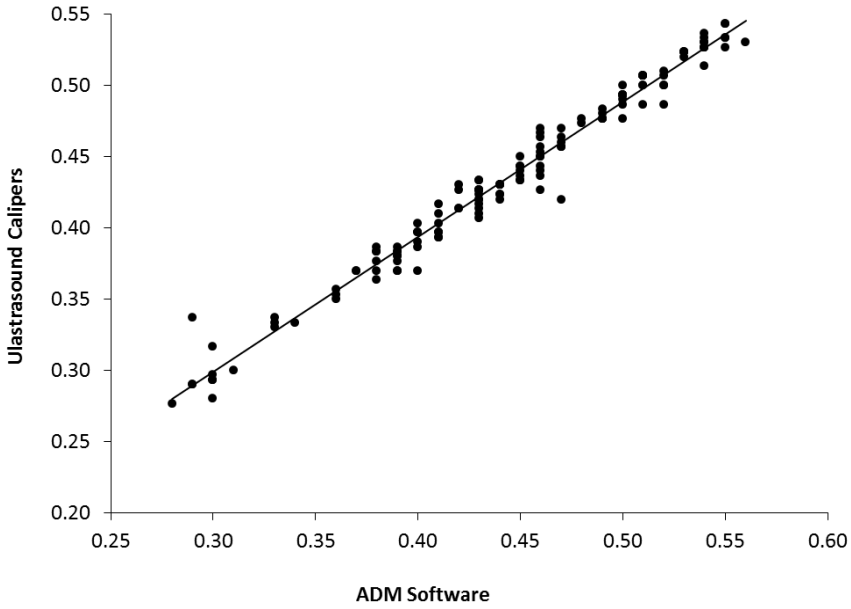

Figure 3.9. Scattergram of brachial artery diameter using ultrasound calipers and ADM software

Using the Bland and Altman analysis [158] there was generally good agreement (i.e. ± 2 SD) between the two measurement procedures thus confirming the results for the bivariate and intraclass correlations (Figure 3.10).

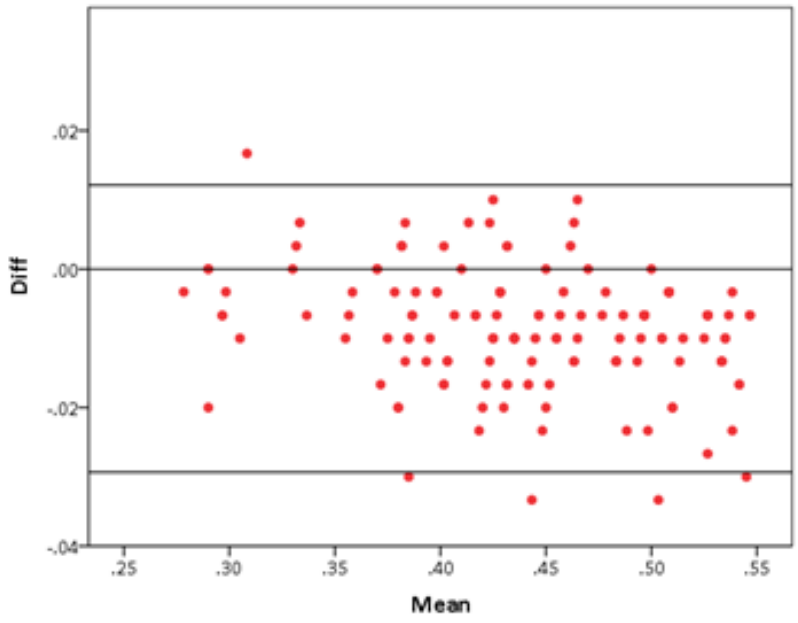

Figure 3.10. Bland and Altman limits of agreement between the arterial diameter measurements using the ADM software and ultrasound calipers

ADM Software - Reliability

A total of 112 brachial artery ultrasound images were randomly selected and arterial diameter was assessed in a blinded fashion by the same investigator using the ADM software on 2 separate occasions. The correlation coefficient was r = 0.98 ($p<0.001$) (Figure 3.11).

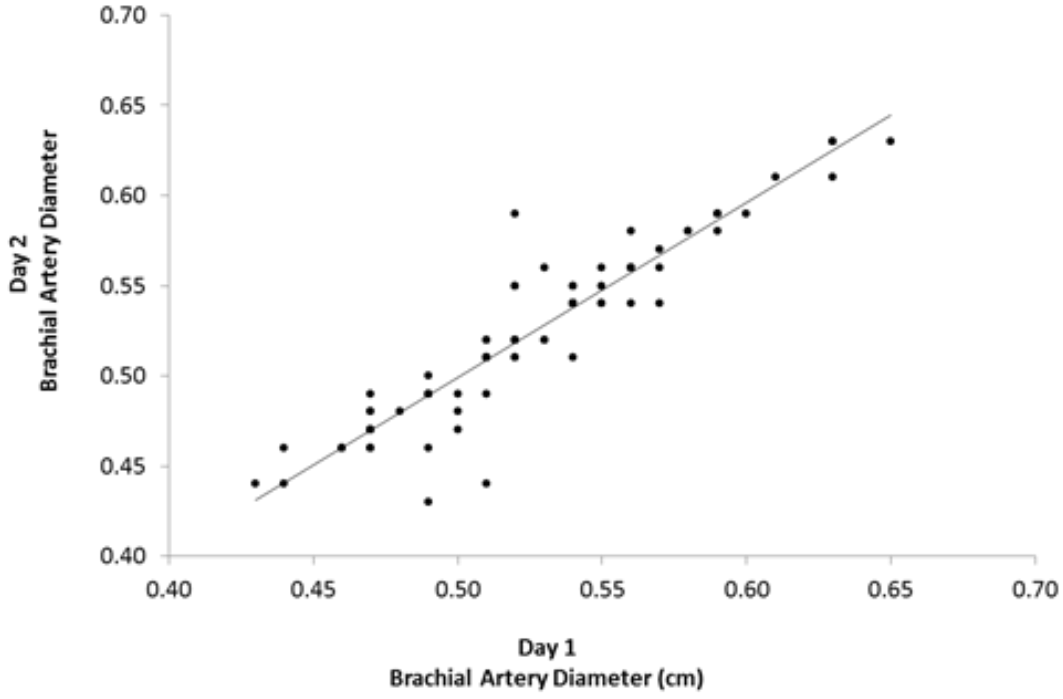

Figure 3.11. Scattergram of brachial artery diameter measurements on two separate days

Summary

Compared to in-built ultrasound calipers, the user-friendly, custom-designed ADM software was shown to be valid and reliable. Its unique feature is the fact that it utilizes a novel algorithm that allows for updating the seed points and parameters while providing real-time feedback to the operator during offline, retrospective analysis. The semi-automated nature of the software reduces operator subjectivity and as a consequence human error. This makes the method more resilient to noise than manual arterial diameter estimation tools, such as virtual calipers. It is low-cost and can be implemented with minimal investment and development time.

Chapter 4

STUDY 2

Effect of an Acute Bout of Self-Regulated and High-Intensity Interval Exercise on Physiological Responses and Endothelial Function in Individuals with Cardiovascular Disease

Rationale

Cardiovascular disease (CVD) refers to coronary heart disease, cerebrovascular disease and peripheral arterial disease, and is the leading cause of death in Ireland. The treatment of individuals with CVD has evolved considerably in the last 50 years [159]. Individuals with CVD are now encouraged to undertake a rehabilitation programme that offers a multi-faceted and multidisciplinary approach to optimize cardiovascular risk reduction, promote adoption and adherence to healthy behaviours, enhance emotional well-being, reduce disability, and encourage an active lifestyle [160]. Lifestyle modification involving diet and exercise has been found to result in a significant reduction in cardiac and total mortality [161].

Traditionally, physiologically based exercise prescriptions for individuals with CVD involve identifying an intensity range that elicits a predetermined $\dot{V}O_2$ or heart rate. In many instances prescribed exercise may exceed an individual's preferred level of intensity and may establish a negative attitude toward physical activity. Longitudinal studies report that participants tend to deviate from physiologically based, prescribed levels of intensity in favour of their apparently preferred levels.

Participation in regular physical activity is influenced by a number of factors. These include demographic, psychological, behavioural, socio-cultural, environmental and activity characteristics [162]. For example, a number of surveys involving women from various racial-ethnic groups have found that they prefer to exercise on their own (autonomously performed physical activity). The reasons are not well understood but may be related to the fact that activity is experienced as pleasant due to the greater sense of perceived autonomy in determining the mode, duration, frequency and intensity of exercise [163].

Allowing individuals to use effort perception to self-select their preferred intensity may encourage the development of intrinsic motivation, a central element in promoting adherence to exercise and increase enjoyment. In a recent study Johnson *et al*., [148] found that 86% of women involved in aerobic exercise used effort perception exclusively to determine exercise intensity. This is not surprising considering that exertional feedback is commonly used to regulate the pace of many daily activities. This is often done without conscious awareness.

Self-regulated exercise intensity may increase enjoyment by allowing individuals complete an activity within their perceptual preference range and without undue physiological strain. Exercise activities of lower-intensities and shorter duration are associated with significant and often substantial shifts toward more pleasant affect [164] and are more likely to be continued [165]. Although effort perception is used to self-regulate the pace of many daily activities, few studies have examined the effect of an

acute bout of short duration (20 min), self-regulated exercise on physiological and perceptual responses in men with CVD.

High-intensity interval exercise (HIIE) involves a series of repeated bouts of short duration (≤ 5 min) high intensity exercise (70 – 100% of maximal effort), alternated with periods of active or passive recovery. A growing body of evidence has found that HIIE can be an effective alternative to traditional continuous exercise interventions. When compared to continuous endurance exercise, interval training has been shown to elicit similar or even superior physiological adaptations, in healthy men and women [6] and those with CVD [8]. The majority of studies that have evaluated HIIE in CVD patients have used cycling and involved exercise interval durations of ≥ 2 min interspersed with ≥ 2 min active or passive recovery (Cornish et al, 2010). Relatively few HIIE studies have involved exercise interval durations of ≤ 1 min and recovery periods ≤ 30 sec.

The vascular endothelium is a single layer of cells which line the inside of blood vessels and forms a biological interface between circulating blood elements and the various organ systems [166]. It is a dynamic tissue that is involved in the modulation of vascular smooth muscle tone, platelet aggregation, monocyte and leucocyte adhesion and thrombosis by synthesizing and metabolizing vasoactive substances [23]. Given its strategic location, the endothelium is a primary target for injury from mechanical forces and processes related to cardiovascular risk factors. Impairment of the normal function of the endothelium, a process known as endothelial dysfunction (ED), represents one of the earliest events in the pathogenesis of atherosclerosis. The ability of large conduit

vessels to dilate in response to a brief period of occlusion and sublingual administration of GTN is termed endothelial-dependent dilation (EDD) and endothelial-independent dilation (EID), respectively, and are commonly used to non-invasively assess endothelial function.

The purpose of this study was to compare the effect of an acute bout of self-regulated exercise (SRE) and short (<1 min) high-intensity interval exercise (HIIE), interspersed with short recovery periods (<30 sec), on physiological and perceptual responses and endothelial vasomotor function in individuals with documented cardiovascular disease (CVD).

Specific Aims

1. To compare the physiological responses during an acute bout of SRE and HIIE in individuals with CVD

2. To compare the effects of an acute bout of SRE and HIIE on endothelial-dependent and endothelial-independent dilation in individuals with CVD

3. To compare the perceptual responses during an acute bout of SRE and HIIE in individuals with CVD

Hypothesis

1. Physiological responses will be significantly greater during an acute bout of HIIE than SRE in individuals with CVD

2. There will be a significantly greater increase in endothelial-dependent dilation (EDD) immediately following an acute bout of HIIE than SRE in individuals with CVD

3. Perceptual responses will be significantly greater during an acute bout of HIIE than SRE in individuals with CVD

METHODOLOGY

Participants

The present investigator volunteered to help with the supervision of two community-based phase IV cardiac rehabilitation classes per week for 18 months prior to the beginning of the study. Men (n = 20) and women (n = 2) with documented cardiovascular disease, who had been participating in the exercise programme (HeartSmart), twice a week for a minimum of 6 months, were informed of the research study and a brief summary and contact details were provided. Following an expression of interest, potential subjects were asked to visit the Vascular Research Unit (VRU) in the School of Health and Human Performance for a screening visit. The natures, benefits, risks and discomforts of the study were explained. Inclusion criteria were stable angina; prior myocardial infarction; prior revascularisation; ability to achieve 30 min of continuous walking without symptoms (cardiac chest pain/discomfort, severe breathlessness, dizziness or palpitations) or be able to undertake activities of at least 5 METS (manually washing a car, digging/turning soil, walking/jogging a mile in less than 15 min) without symptoms; clinically stable and in good health for a minimum of 2 weeks prior to beginning the study. Participants were excluded if they were current smokers, had unstable angina, uncontrolled hypertension (systolic blood pressure (BP) >180 mmHg, diastolic BP >100 mmHg), resting tachycardia or unstable/acute heart failure. Individuals who met the entry criteria and who received medical clearance from a physician to participate were randomly assigned to the SRE (n = 12) or HIIE (n = 12, 2 female) group. Two of the participants in the SRE group withdrew due to illness,

another withdrew due to orthopedic limitations and a fourth withdrew for personal reasons. One participant withdrew from the HIIE group due to illness. Participants' medications are summarized in Table 4.4. Individuals who took part in study 3, did not partake in study 4.

Overview of Study Design

Research Design

Participants in both experimental groups visited the Cardiovascular Research Unit in DCU on two separate occasions. The first visit was used to screen potential participants and measure peak aerobic capacity ($\dot{V}O_2$peak). During the second visit endothelial vasomotor function was assessed before and immediately following an acute bout of SRE or HIIE.

Visit 1

Participants fasted for 4 h, and refrained from strenuous physical activity for 24 h prior to the visit. The nature and risks of the study were explained. A plain language statement was read and informed consent was obtained in accordance with the Research Ethics Committee at Dublin City University (DCUREC 148). Participants then completed a physical activity readiness questionnaire (PAR-Q), a general health questionnaire (Appendix B), had their blood pressure, height and weight measured and performed an exercise test on a treadmill to determine $\dot{V}O_2$peak.

Visit 2

Participants arrived to the Cardiovascular Research Unit, DCU at 7.00 am after an 8 h overnight fast. Water consumption was permitted during the fasting period and where possible, all vasoactive medications were withheld for at least 4 half-lives. Participants were not permitted to exercise for at least 24 h before the visit or ingest substances that might affect FMD such as caffeine and vitamin C or use tobacco for at least 6 h before the study. Women recruited for this study were post-menopausal, therefore menstrual cycle phase was not relevant. Following a 10 min rest period in a quiet, temperature-controlled room, a blood sample was drawn from the antecubital vein, and endothelial function was assessed. Participants then undertook an acute bout of SRE or HIIE on a treadmill. Within 5 min of exercise cessation a blood sample was taken and endothelial function was again assessed (Figure 4.2).

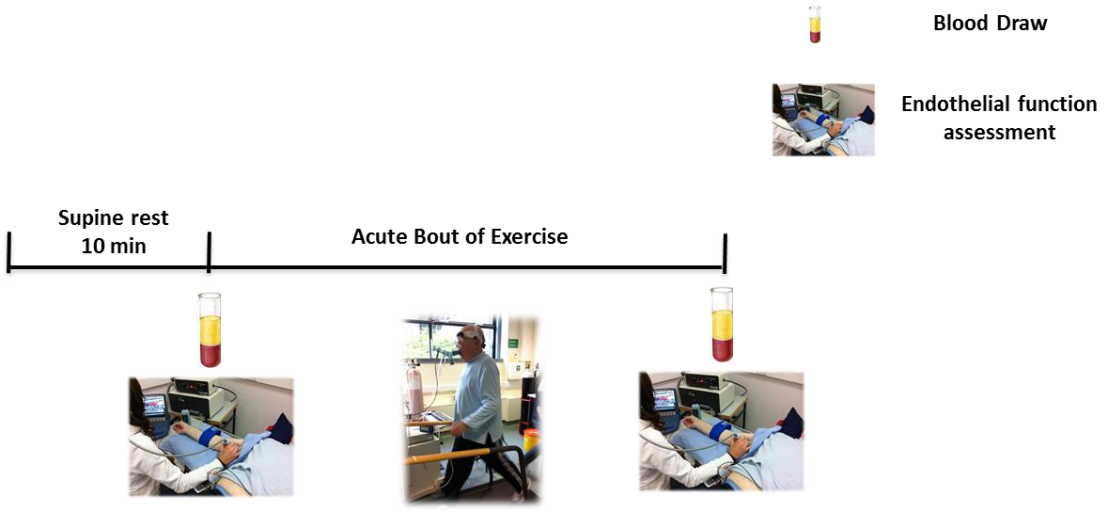

Figure 4.1: Overview of visit 2

Self-Regulated Exercise

The SRE group exercised for 20 min on a treadmill at a self-selected intensity. Participants were allowed to alter the treadmill speed and grade during the first min of exercise and subsequently during the first 30 sec of every 6th min. The velocity control arrows were kept visible to allow participants alter the treadmill grade and velocity when signalled by the research assistant. Metabolic measurements were recorded throughout the exercise bout using open circuit spirometry (Sensormedics Vmax 229 metabolic system, SensorMedics Corp., Yorba Linda CA). Heart rate and RPE were recorded during the last 30 sec of every 5^{th} min using telemetery (Polar Vantage NV™ Polar, Port Washington, NY) and the Borg RPE scale, respectively. Participants were asked to "select an intensity that you prefer…that you feel happy to do regularly".

High-Intensity Interval Exercise

Figure 4.2 illustrates the HIIE protocol. The session began with a 10 min warm-up at 50% HRmax followed by 2 min of seated rest. Participants then performed three blocks of 8 x 45 sec of treadmill exercise at ≥ 85% HRpeak. Grade and/or speed was adjusted to reach target intensity. Each 45 sec interval was interspersed with a 15 sec period of passive recovery. The session concluded with a 5 min cool-down period. Participants were continuously monitored with a 12-lead ECG. Arterial blood oxygen saturation (SpO_2) and heart rate were continuously monitored. Participants were asked to provide subjective estimates of perceived exertion and degree of chest pain at regular intervals during both exercise sessions.

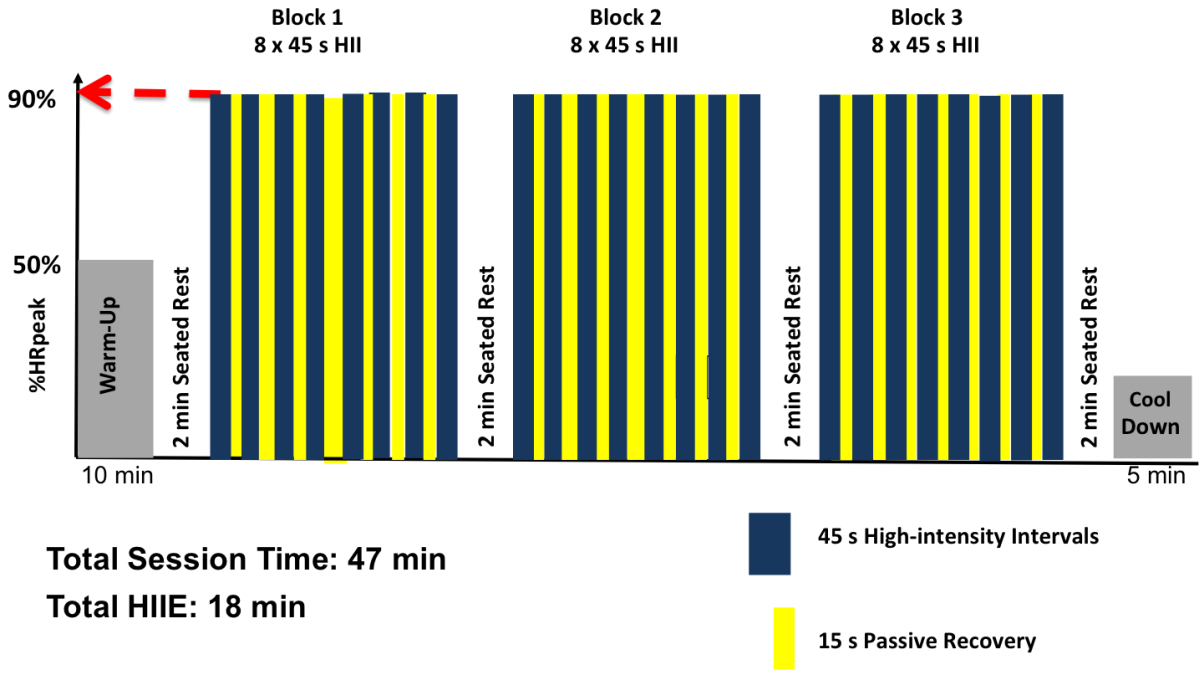

Figure 4.2: High intensity interval exercise session

Anthropometrics

Height and mass were measured using a wall stadiometer and electronic balance (Seca 797, USA) respectively. Footwear was removed prior to the measurement. Height was measured to the nearest cm, and weight was measured to the nearest 0.1 kg.

Blood Sampling and Storage

Blood sample were obtained with participants in a seated position for 5 min with legs uncrossed in order to minimise plasma volume shifts. Serum vacutainers were allowed to stand for 30 min before centrifugation at 3000 rpm (1600 g) for 15 min at 4°C. Sodium citrate vacutainers were pre-chilled in an ice bucket and samples returned

to the ice bucket prior to centrifugation. Blood for microparticles analysis was collected, prepared and stored according to the double centrifugation method of Bernal-Mizrachi *et al*., (2004). Blood was collected in pre-chilled sodium citrate vacutainers and spun first at 160 g for 9 min to produce platelet rich plasma (PRP). This was harvested from the top leaving a 0.5 cm layer of plasma undisturbed close to cell debris. The PRP was centrifuged for a further 9 min at 1000 g to yield platelet poor plasma (PPP). PPP was aliquoted and stored at – 80°C until analysis.

Biochemical Analysis

Serum triglycerides, total cholesterol, HDL-cholesterol (HDL-C), free fatty acids (FFA) and glucose were determined using spectrophotometric assays, performed on an automated bench-top clinical chemistry system (ACE®, Alfa Wassermann B.V., Netherlands) using appropriate reagents, calibrators and controls (Randox Laboratories, UK). Serum insulin was determined by a dissociation enhanced lanthanide fluorescent immunoassay (DELFIA) using a commercially available kit (Perkinelmer, Wellesley MA, USA). Serum concentration of sVCAM-1, sICAM-1 and interleukin-6 (IL-6) were determined in duplicate using a quantitative sandwich enzyme immunoassay technique and commercially available kits (R & D Systems, Minneapolis, USA). Haematocrit values, haemoglobin concentrations and counts of leukocytes were determined from an EDTA whole blood sample using an automated haematology analyser (AcTdiff2, Beckman Coulter, USA).

Peak Aerobic Capacity

Peak aerobic capacity test ($\dot{V}O_2$peak) was assessed on a treadmill (Marquette 2000, General Electric, USA) using 1 of 5 incremental protocols (Appendix E), depending on individual exercise capacity. The Balke protocol was selected and modified to allow participants to reach volitional fatigue in 8 – 12 min. A 2 min warm up preceded each test and the gradient was increased 2.5% every 2 min until the subject reached volitional exhaustion or presented with contraindications to exercise. If necessary, treadmill speed was increased by 1 $km.h^{-1}$ every 2 minutes while the treadmill gradient was maintained at 22.5%. Participants were verbally encouraged to give their best effort.

Respiratory metabolic measures, heart rate and an ECG were monitored continuously throughout the test. RPE was recorded every 2 min. Peak oxygen uptake was determined by averaging the three highest consecutive 20 second values.

Cardiorespiratory and Metabolic Measures

Respiratory metabolic responses were determined using standard open-circuit spirometry techniques (Sensormedics Vmax 229, SensorMedics Corp., CA). Prior to testing, the gas analysers were calibrated with standard gases of known concentration. A mass flow sensor (Sensormedics, Loma Linda, CA, USA) was used to collect breath-by-breath measurement of ventilation. A 3 L volume syringe (Sensormedics, Loma Linda, CA, USA) was used to calibrate the mass flow sensor prior to each test.

Mass Flow Sensor Heated Wire Anemometer-Mode of Operation

The mass flow sensor is a low resistance tube with a tapered internal diameter extending from both ends of a laminar flow throat. A cold and hot stainless steel wire electrically heated to -180°C and -240°C respectively, are centered in the flow stream. These wires are elements in a servo-controller bridge circuit that maintain the resistance ratio of the two wires at a constant value. If only the temperature of the inspired gases changes then both wires lose heat at the same rate and no current change is required to keep the bridge balanced. As air flows across the wires, the hot air loses heat more rapidly than the cold air and current must be added to keep the bridges balanced at a 3:4 ratio. The amount of current required is proportional to the mass flow of the gas. This method ensures that the sensor measures only the heat loss from the molecular convection of the moving gas stream, and not the artifact due to cooling of the gas as it passes through a breathing assembly. The mass flow meter responds to instantaneous flow rates between 0-16 $L.sec^{-1}$ and integrated flow between 0-350 L.min-1 with flow resistance <1.5 $cmH_2O.L\text{-}1.sec^{-1}$. The mass flow sensor was outputted to the analyser module of the Vmax 229 and was sampled at a rate of 125 Hz.

Mass Flow Sensor Calibration

A 3 L volume syringe (Sensormedics, Loma Linda, CA, USA) was connected to the mass flow sensor, and stroked four times in order to measure inspired and expired volumes. The volumes were calculated by expressing 3 L as a fraction of each measured inspired and expired volume achieved during calibration. An average correction factor

was calculated for inspired and expired volumes, and used to fine-tune the volume measurement.

A verification procedure was performed. This involved stroking the 3 L volume syringe four times. Inspired and expired volumes were measured using the newly calculated correction factors. In order to pass the calibration procedure, one of the four strokes had to have an average flow rate < 0.5 $L.sec^{-1}$, and at least one of the four strokes had to have an average flow > 3.0 $L.sec^{-1}$.

Gas Analysers

The Vmax 229 utilizes a rapid response infrared measurement technique. An O_2 and CO_2 analyser is integrated with the Vmax 229. A small sample of inspired air is drawn through a sample cell, and exposed to an infrared light through an optical that is passed through a band pass filter and the sample cell. An infrared detector responds to the amount of infrared light that passes through the sample cell. The amount of light that passes through the sample cell varies according to the concentration of CO_2 in the sample cell. Based on measured levels of infrared light intensity, the analyser computes the PCO_2 in the gas sample. The CO_2 analyser is linearly scaled across the 0-100% range with a resolution of 0.01% CO_2, and a response time of < 130 ms (10-90%) at 500 $ml.min^{-1}$ flow. The O_2 analyser is based on the high paramagnetic susceptibility of O_2. A diamagnetic glass dumbbell suspended in a magnetic field rotates in proportion to the PO_2. The analyser is linearly scaled across the 0-100% range with a resolution of 0.01% O_2 and a response time of < 130 ms (10-90%) at 500$ml.min^{-1}$ flow.

Calibration of CO_2 and O_2 Analysers

The gas analysers were calibrated with standard gases of known concentration (BOC gases, Dublin, Ireland). The first calibration gas contained 26.00 ± 0.02% oxygen and the balance nitrogen (N_2). The second calibration gas contained 4.00 ± 0.02% carbon dioxide, 16.00 ± 0.02% O_2, and the balance N_2. A small bore drying tube connected to the CO_2 and O_2 analysers sampled the calibration gases. The absorption and evaporative properties of the drying tube ensured that the relative humidity of the calibration gas was equilibrated to ambient conditions prior to sampling by the O_2 and CO_2 analysers. The calibration gas was sampled at a rate of 125 Hz. The response time was similar between O_2 and CO_2 analyser.

Ratings of Perceived Exertion

RPE was obtained using the 16-point Borg category RPE scale. Prior to the maximal exercise test participants read a standard set of perceptual scaling instructions. These instructions followed an established format used in previous investigations. Low and high "perceptual anchors" were established during the maximal exercise test. This involved asking participants to assign a rating of 6 (low anchor) to the lowest exercise intensity, and 20 (high anchor) to the highest exercise intensity. Participants were instructed to make their subjective assessments of perceived exertion relative to these minimum and maximum standards (perceptual anchors).

Electrocardiographic Monitoring (ECG)

Heart rate (HR) and electrical activity of the heart were monitored continuously using a 12-lead ECG monitor (GE Case 8000 12 lead ECG). The signal to noise ratio at the skin electrode interface was reduced by cleansing the area with an alcohol saturated gauze pad. The superficial layer of skin was then removed using light abrasion with fine grain emery paper. The electrodes were placed on 10 standard anatomical landmarks.

Ventilatory Threshold

The V-slope method described by Beaver *et al*., [167] was used for the determination of ventilatory threshold (VT). This method involves the analysis of the CO_2 elimination ($\dot{V}CO_2$) in relation to O_2 uptake ($\dot{V}O_2$). By plotting $\dot{V}O_2$ against $\dot{V}CO_2$ the initial slope of 1.0 is followed by a steeper slope when lactic acid is buffered by bicarbonate and CO_2 is formed. When VT could not be determined using V-slope method, the ventilatory breakpoint method was employed [168]. This involved plotting $\dot{V}O_2$ and minute ventilation ($\dot{V}e$). The $\dot{V}O_2$ at which the $\dot{V}e$ increased nonlinearly was used to determine the point of the VT.

Endothelial Function Assessment

Endothelial function was assessed, pre and post exercise, using FMD [39]. Endothelial function was assessed with the participants in a supine position in a quiet, temperature-controlled room. The right arm rested on an examination table, perpendicular to the bed, and was extended and externally rotated to permit imaging of the right brachial artery. An automated pneumatic cuff was placed on the forearm,

distal to the brachial artery and electrodes for a 3-lead ECG were placed on their chest. The ECG tracing was activated and settings adjusted to ensure clear identification of the R wave, which corresponds to the end of diastole in the cardiac cycle.

Endothelial-Dependent Dilation

Figure 4.3 illustrates the brachial artery FMD assessment procedure. Following a 10 min rest period the pneumatic cuff was inflated to 250 mmHg for 5 min. The cuff was rapidly deflated and peak systolic velocity was measured using Doppler within 15 sec of cuff release. M-mode images were named and recorded every 60 sec post-deflation for 5 min. Brachial artery diameter was analysed off-line, using the ADM software. FMD was normalised for the shear stimulus. Shear rate was calculated using the formula; 4 x peak velocity/peak diameter [69].

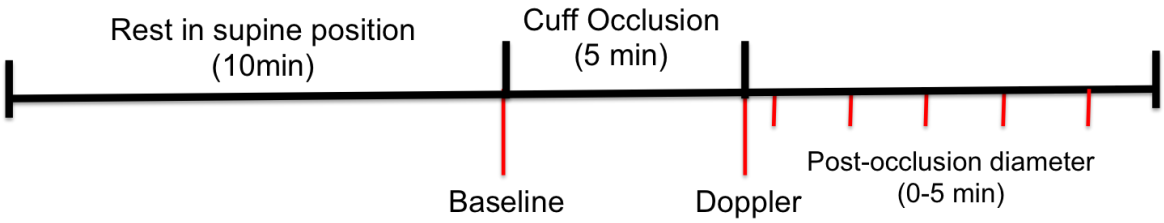

Figure 4.3: Overview of the protocol used to assess endothelial-dependent dilation

Endothelial-independent Dilation

Figure 4.4 illustrates the endothelial-independent dilation assessment protocol. Following a 15 min rest period, a baseline brachial artery image was again recorded. Gylceryl trinitrate (GTN; 400 μg) was then administered sublingually. To assess brachial

artery diameter, M-mode images were named and recorded every 30 sec following 2.5 min of GTN administration.

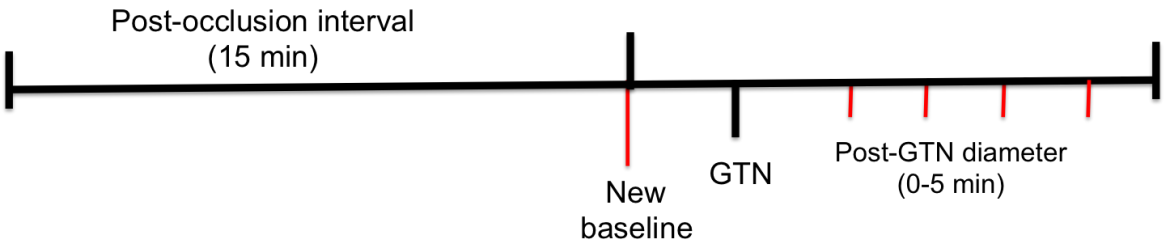

Figure 4.4: Overview of endothelial-independent dilation

Off-line Analysis

Ultrasound images were analyzed off-line, using the ADM software (see chapter 3 for details). The same experienced investigator performed the off-line analysis of the ultrasound images using a custom-designed arterial diameter measurement tool. Image processing is divided into the following stages, i) image acquisition, ii) calibration, iii) pre-processing, iv) segmentation, v) post-processing, vi) robust statistics and vii) refinement. This method of arterial diameter estimation using the ADM software is explained in detail in chapter 3.

Statistical Analysis

Prior to statistical analysis the data was checked for normality using the Shapiro-Wilks test. An independent t-test was used to compare the physiological and perceptual responses between the two experimental groups at maximal exercise. A condition (HIIE and SRE) by time (pre exercise and post exercise) two-way repeated measure ANOVA was used to compare the percentage change in brachial artery diameter in response to hyperemia and GTN administration. A one-way ANOVA was used to compare the physiological and perceptual responses for each block of HIIE and every 5 min during SRE. Significant main effects were probed using a Bonferroni post hoc test. SPSS for Windows statistical software (ver 19.0) was used to perform the statistical analysis. Statistical significance was accepted at the $p < 0.05$ level of confidence.

Results

Participant characteristics

Baseline physical characteristics, blood pressure and blood lipids are summarized in table 4.1. With the exception of LDL-C, there were no differences in any of the measured parameters at baseline between the SRE and HIIE groups. There was no significant difference in any of the measured parameters at maximal exercise between groups (Table 4.2).

Acute Exercise Bout

The mean physiological and perceptual responses during the SRE and HIIE are summarized in table 4.3. Average treadmill velocity, treadmill gradient, $\dot{V}O_2$, %$\dot{V}O_2$peak, METs, %VT, RPE, HR, respiratory rate and ventilation were significantly higher during HIIE than SRE.

The mean physiological and perceptual responses during each block of HIIE are summarized in table 4.4. Heart rate, %HRmax and SpO_2 were significantly higher during block 2 than block 1. Respiratory rate, minute ventilation and %HRmax were significantly higher during block 3 than block 1 and block 2. Relative $\dot{V}O_2$ and %VT, were also significantly higher during block 3 than block 1.

The mean physiological and perceptual responses during each 5 min period of SRE are summarized in table 4.5. Treadmill velocity, RPE, and respiratory rate were significantly higher between 10-15 and 15-20 min than 0-5 min. Oxygen uptake,

%$\dot{V}O_2$peak, METs, and minute ventilation were significantly higher between 10-15 min than 0-5 min. The %VT was significantly higher between 15-20 min than 0-5 min. Compared to 5-10 min, %$\dot{V}O_2$peak and RPE were significantly higher between 10-15 min and 15-20 min respectively.

Endothelial Function

There was no significant difference in endothelial-dependent dilation or endothelial-independent dilation before or after exercise in either experimental group (Figure 4.5). Peak diameter, peak blood flow velocity and endothelial-independent dilation results are illustrated in Figures 4.6 – 4.8.

ECG Changes and Clinical Symptoms

ST-segment changes ranged from 1 – 3 mm, and returned to normal during passive recovery. None of the participants reported angina symptoms during or following the acute bout of HIIE.

Table 4.1: Medication

Main Drug Class	**SRE**	**HIIE**
Statins	6	7
ACE Inhibitor	4	4
Beta Blocker	2	3
Anti-platelet	5	7
Diuretic	2	1
Proton Pump	1	2

Values are means

Table 4.2: Physical characteristics and blood lipids of the participants

	Mean	
	SRE (n = 8)	**HIIE (n = 11)**
Age (y)	65.8 ± 4.5	65.7 ± 7.2
Height (cm)	169.9 ± 9.1	168.7 ± 5.7
Weight (kg)	85.9 ± 13.6	80.8 ± 10.6
BMI ($kg \cdot m^{-2}$)	29.7 ± 3.3	28.3 ± 2.7
Systolic blood pressure (mmHg)	130.9 ± 5.5	138.3 ± 13.6
Diastolic blood pressure (mmHg)	83.1 ± 7.4	80.6 ± 6.9
Total cholesterol (mmol)	3.9 ± 0.8	2.9 ± 1.2
HDL-cholesterol (mmol)	1.3 ± 0.2	1.1 ± 0.1
LDL-cholesterol (mmol)	1.8 ± 0.5	1.3 ± 0.3*
Triglycerides (mmol)	1.3 ± 0.2	0.7 ± 0.3
ApoB	76.9 ± 17.9	74.1 ± 13.0
ApoA	134.1 ± 16.7	137.8 ± 17.9

Values are means ± SD; *p < 0.05 vs. self-regulated

Table 4.3: Descriptive characteristics of the participants in the SRE and HIIE group during graded exercise test

	Mean	
	SRE (n = 8)	**HIIE (n = 11)**
$\dot{V}O_2$peak ($ml \cdot kg^{1} \cdot min^{1}$)	28.1 ± 7.0	29.05 ± 6.9
$\dot{V}O_2$peak ($L \cdot min^{1}$)	2.4 ± 0.6	2.33 ± 0.7
Heart rate max ($beats.min^{-1}$)	122.3 ± 29.7	132.27 ± 13.6
$\dot{V}O_2$VT ($ml \cdot kg^{1} \cdot min^{1}$)	23.2 ± 5.9	24.21 ± 4.6
% $\dot{V}O_2$VT	81.3 ± 8.9	73.29 ± 26.3
Ventilation peak ($L \cdot min^{1}$)	64.1 ± 20.8	64.94 ± 19.2
RER	1.1 ± 0.1	1.13 ± 0.1

Values are means ± SD; *p < 0.05 vs. self-selected

Table 4.4: Average Responses during SS and HIIE

	Exercise Condition	
	SRE (n = 8)	**HIIE (n = 11)**
Treadmill velocity ($km \cdot h^{-1}$)	5.1 ± 0.8	7.5 ± 2.0†
Grade (%)	0.6 ± 1.0	3.6 ± 1.6‡
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	17.6 ± 4.2	23.3 ± 5.1†
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.50 ± 0.5	1.9 ± 0.5*
%$\dot{V}O_2$peak	63.8 ± 12.8	77.9 ± 9.7†
METs	5.0 ± 1.2	6.7 ± 1.5†
% $\dot{V}O_2$VT	74.0 ± 12.8	98.5 ± 13.5†
RPE	11.4 ± 1.9	14.1 ± 2.0†
Heart rate ($beats.min^{-1}$)	96.3 ± 15.7	117.4 ± 18.0†
%HRmax	80.2 ± 12.4	89.2 ± 13.4
Respiratory rate ($breaths.min^{-1}$)	25.9 ± 2.2	32.7 ± 7.1†
Ventilation ($L \cdot min^{-1}$)	33.0 ± 9.1	49.8 ± 16.1†
Systolic blood pressure (mmHg)		160.6 ± 21.7
Diastolic blood pressure (mmHg)		71.3 ± 3.3
SPO_2 (%)		95.4 ± 2.2

Values are means ± SD, *p < 0.05 vs.SS †p < 0.01 vs. SS; ‡p < 0.001 vs. SS

Table 4.5: HIIE

	Exercise Blocks		
	1	**2**	**3**
Treadmill velocity ($km \cdot h^{-1}$)	7.3 ± 1.8	7.5 ± 2.1	7.7 ± 2.2
Grade (%)	3.7 ± 1.6	3.7 ± 1.6	3.5 ± 1.5
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	22.4 ± 4.3	22.6 ± 5.2	25.0 ± 6.5
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.8 ± 0.5	1.8 ± 0.5	2.0 ± 0.6
%$\dot{V}O_2$peak	74.8 ± 8.7	75.9 ± 12.4	83.1 ± 11.9*
METs	6.4 ± 1.2	6.5 ± 1.5	7.2 ± 1.8
RPE	13.4 ± 2.5	14.1 ± 2.1	14.8 ± 2.0
% $\dot{V}O_2$VT	94.2 ± 13.0	95.8 ± 17.1	105.6 ± 15.4*
Systolic Blood Pressure (mmHg)	159.3 ± 19.5	162.0 ± 20.9	160.4 ± 32.8
Diastolic Blood Pressure (mmHg)	70.4 ± 6.5	74.7 ± 7.0	68.7 ± 5.8
Heart rate ($beats.min^{-1}$)	111.0 ± 15.7	117.5 ± 18.2*	115.9 ± 42.1
%HRmax	84.4 ± 12.4	89.2 ± 13.7*	95.2 ± 15.4*¥
Respiratory rate ($breaths.min^{-1}$)	30.3 ± 7.1	32.0 ± 6.2	35.8 ± 8.5‡¥
Ventilation ($L \cdot min^{-1}$)	44.9 ± 14.2	48.0 ± 16.1	56.5 ± 19.7†¥
SPO_2 (%)	96.0 ± 1.8	95.0 ± 2.5*	95.1 ± 2.4

Values are means ± SD; *p < 0.05 vs. Block 1; †p < 0.01 vs. Block 1; ‡p < 0.001 vs. Block 1; ¥p < 0.05 vs. Block 2

Table 4.6: Self-Regulated Exercise

	Time (min)			
	0-5	**5-10**	**10-15**	**15-20**
Treadmill velocity ($km \cdot h^{-1}$)	4.5 ± 0.9	5.0 ± 0.8	5.3 ± 0.9*	5.5 ± 1.0*
Grade (%)	0.2 ± 0.6	0.7 ± 1.1	0.8 ± 1.2	0.7 ± 1.2
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	14.6 ± 2.7	16.7 ± 3.2†	18.7 ± 4.6*	20.3 ± 6.6
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.2 ± 0.3	1.4 ± 0.4†	1.6 ± 0.5*	1.7 ± 0.7
Kcal/min	3.9 ± 0.4	4.0 ± 0.3	4.1 ± 0.3	4.3 ± 0.3*
%$\dot{V}O_2$peak	53.5 ± 11.5	61.1 ± 11.0‡	67.7 ± 13.5†¥	72.7 ± 18.3
Energy Cost (MET)	4.2 ± 0.8	4.8 ± 0.9†	5.4 ± 1.3*	5.8 ± 1.9
RPE	9.9 ± 2.1	10.6 ± 2.1	12.2 ± 1.8†	12.9 ± 2.4*¥
% $\dot{V}O_2$VT	55.5 ± 25.9	63.0 ± 27.8†	68.6 ± 29.8†	72.2 ± 32.2*
Heart rate ($beats.min^{-1}$	86.9 ± 10.9	93.9 ± 13.0	98.9 ± 17.2	105.4 ± 24.4
%HRmax	73.2 ± 14.2	78.6 ± 13.3†	82.2 ± 13.1	86.6 ± 11.5
Respiratory rate ($breaths.min^{-1}$)	23.5 ± 2.5	25.3 ± 3.3	26.6 ± 2.8†	28.4 ± 4.0*
Ventilation ($L \cdot min^{-1}$)	26.6 ± 7.3	30.8 ± 7.6†	35.2 ± 10.0†	39.4 ± 14.4

Values are means ± SD; *p < 0.05 vs. 0-5 min; †p < 0.01 vs. 0-5 min; ‡p < 0.001 vs. 0-5 min; ¥p < 0.05 vs. 5-10 min

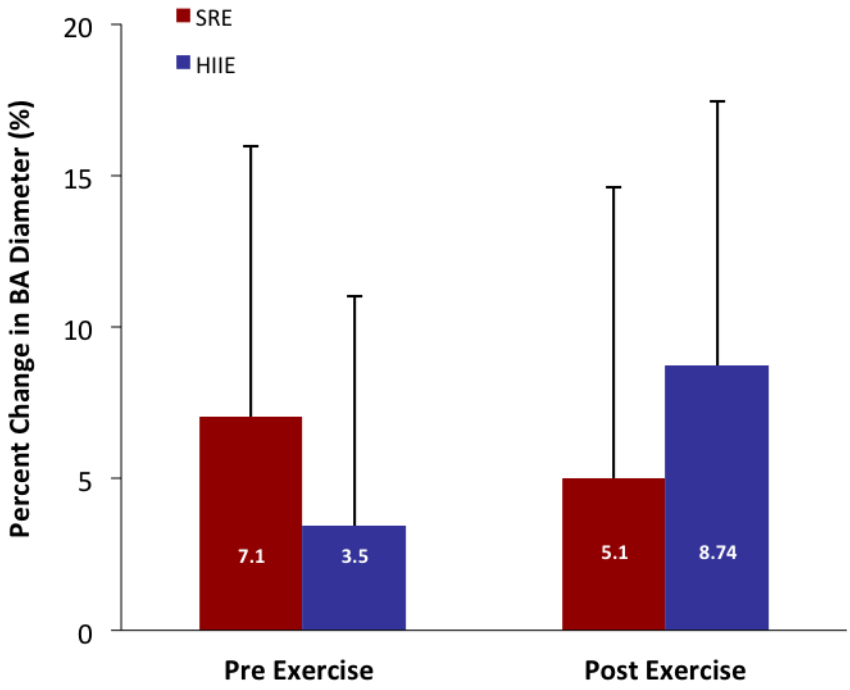

Figure 4.5: Percent change in brachial artery diameter before and after an acute bout of self-regulated exercise (SRE) and high intensity interval exercise (HIIE)

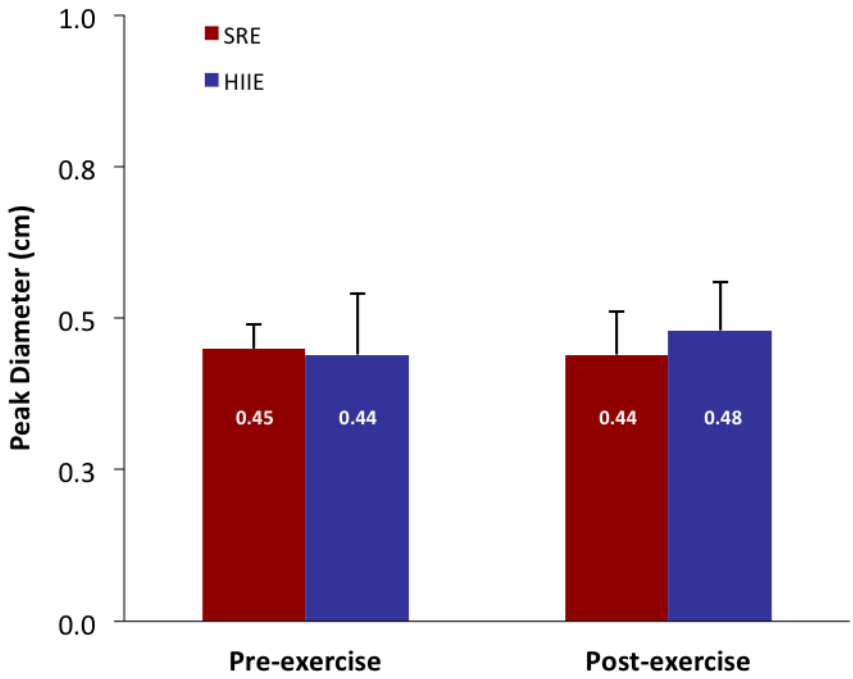

Figure 4.6: Peak brachial artery diameter (cm) before and after an acute bout of self-regulated exercise (SRE) and high intensity interval exercise (HIIE)

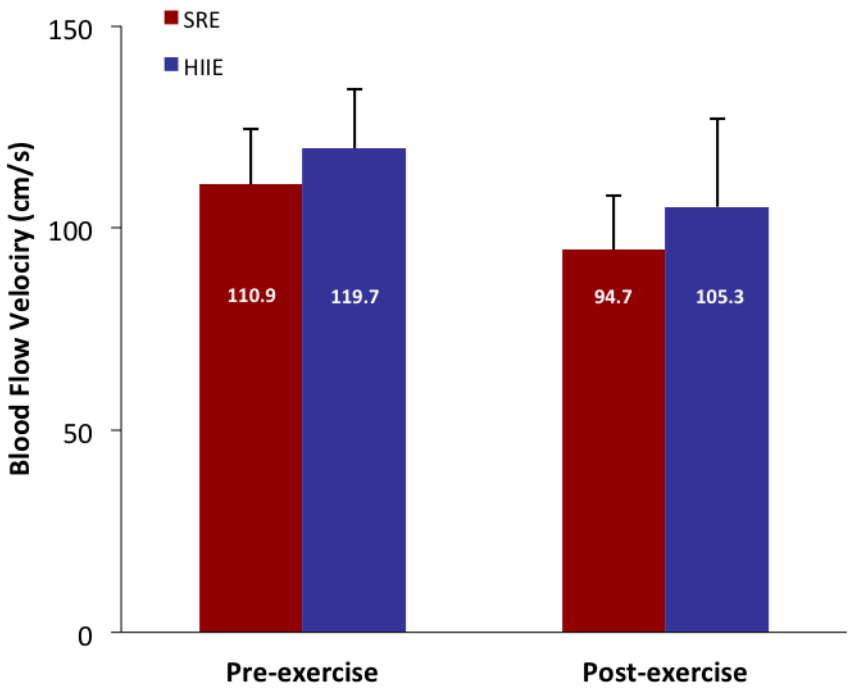

Figure 4.7: Peak blood flow velocity (cm/s) before and after an acute bout of self-regulated exercise (SRE) and high intensity interval exercise (HIIE)

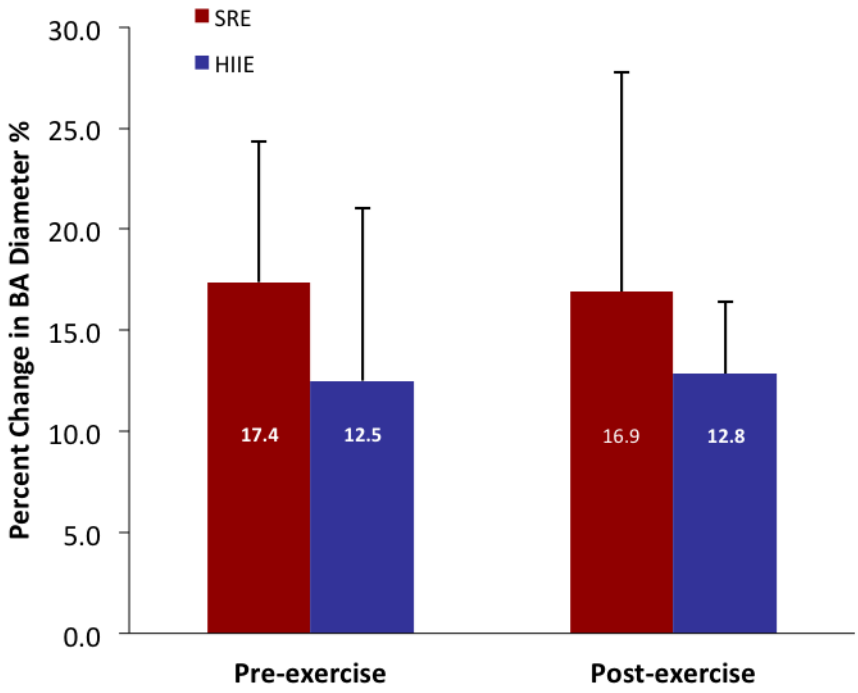

Figure 4.8: Percent change in brachial artery diameter (%) following GTN administration before and after an acute bout of self-regulated exercise (SRE) and high intensity interval exercise (HIIE)

Summary

The SRE group self-selected an average treadmill velocity of 5.1 $km \cdot h^{-1}$, while the HIIE group exercised at 7.5 $km \cdot h^{-1}$, which correspond to 64% and 78% $\dot{V}O_2$peak, and 80% and 89% HRmax, respectively. The RPE values for the SRE were within a narrow range and indicated that when allowed to self-regulate their exercise intensity individuals with CVD will exercise at an intensity that they perceive to be fairly light to somewhat hard. Alternatively, when HIIE is prescribed, individuals with CVD perceive the intensity to be somewhat hard to hard. There was no significant difference in endothelial-dependent or endothelial-independent dilation before or after exercise in either experimental group

Chapter 5

STUDY 3

EFFECT OF 4 WEEKS OF HIIT OR COMMUNITY-BASED CARDIAC REHABILITATION ON AEROBIC CAPACITY, ENDOTHELIAL FUNCTION AND SELECTED CARDIOVASCULAR BIOMARKERS IN INDIVIDUALS WITH CARDIOVASCULAR DISEASE

Rationale

Individuals with cardiovascular disease are now encouraged to undertake a rehabilitation programme that offers a multi-faceted and multidisciplinary approach to optimize cardiovascular risk reduction, promote adoption and adherence to healthy behaviours, enhance emotional well-being, reduce disability, and encourage an active lifestyle [160]. A 20% reduction in overall mortality has been found in patients who undertake an exercise-based cardiac rehabilitation programme [169].

There is still, however, no clear consensus on the precise type, volume, intensity and frequency of exercise to achieve optimum health benefits. Traditionally, individuals with CVD are encouraged to undertake continuous, moderate-to-vigorous intensity exercise as part of their cardiac rehabilitation programme. A growing body of evidence has shown that high-intensity interval training (HIIT) can be an effective alternative to traditional continuous, moderate-to-vigorous intensity exercise interventions.

High-intensity interval training involves a series of repeated bouts of exercise performed between 70 – 100% of maximal effort, and alternated with periods of active or passive recovery. The duration of the high-intensity intervals can range from 10 sec to 5 min. Interval training has been shown to elicit similar or even superior physiological adaptations than continuous endurance exercise, in both healthy and diseased populations.

Compared to usual care cardiac rehabilitation, 12 weeks of treadmill interval training was more effective in improving $\dot{V}O_2$peak in cardiac patients, but resulted in similar increases in endothelial function [140]. Similarly, when HF patients were randomised to either moderate continuous training (MCT; 70% HRpeak), aerobic interval training (AIT; 95% HRpeak) or a control group (recieved standard advice regarding physical activity) for 12 weeks, improvements in $\dot{V}O_2$peak and brachial artery FMD were significantly greater in the AIT group than baseline, and the MCT and control group. Postive left ventricular (LV) remodeling occurred in the AIT group only. A marker of hypertrophy, proBNP declined by 40% in the AIT group [8].

The benefits of aerobic exercise training on blood lipid profiles are well established [170]. The effect of high-intensity interval training on blood lipids, however, have been investigated, but to a lesser extent. A recent study by Lamina *et al*., [171] observed significant decreases in total cholesterol and significant increases in HDL-cholesterol following 8 weeks of moderate-intensity (60 - 79% HRR) interval training in 245 men with mild–moderate hypertension. Each session was 45 – 60 mins, with an

interval to rest ratio of 1:1, 6 min each. An earlier study [172] involving 8 weeks of HIIT (90% HRmax), resulted in significant improvements in HDL-cholesterol, and total cholesterol/HDL-cholesterol but no changes in total cholesterol in young, untrained men with normal total cholesterol. Tsekouras *et al*., [173] found that 2 months of HIIT, involving 3 sessions/week, running at 60 – 90% $\dot{V}O_2$peak in 4 min intervals for 32 min, lowered the rate of very low-density lipoprotein-triglyceride secretion from t she liver in previously sedentary men. The potential role of HIIT as an alternative mode for improving blood lipid profile in individuals with CVD has not been adequately explored.

Studies examining the effect of interval training on endothelial function in individuals with CVD have involved a training period of 8 - 24 weeks, and intervals $\geq$ 3 min in duration. Recent studies found that 4 - 8 sessions of HIIT involving 30 sec exercise intervals, illicit significant physiological and metabolic improvements in healthy individuals. The purpose of this study was to compare the effect of 4 weeks of HIIT (8 sessions; interval duration < 1 min) or community-based cardiac rehabilitation on aerobic capacity, endothelial function and selected CVD risk biomarkers in men and women with CVD.

Specific Aims

1. To evaluate the effect of an individualized 4-week HIIT programme on endothelial-dependent dilation in men and women with CVD

2. To evaluate the effect of an individualized 4-week HIIT programme on endothelial -independent dilation in men and women with CVD

3. To evaluate the effect of an individualized 4-week HIIT programme on $\dot{V}O_2$peak in men and women with CVD

4. To evaluate the effect of an individualized 4-week HIIT programme on circulating levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides in men and women with CVD

Hypotheses

1. There will be a significant increase in endothelial-dependent dilation after a 4-week HIIT programme and no change after 4 weeks participation in a traditional community-based cardiac rehabilitation programme in men and women with CVD

2. There will be no change in endothelial-independent dilation after a 4-week HIIT programme or 4 weeks participation in a traditional community-based cardiac rehabilitation programme in men and women with CVD

3. There will be a significant increase in $\dot{V}O_2$peak after a 4-week HIIT programme and no change after 4 weeks participation in a traditional community-based cardiac rehabilitation programme in men and women with CVD

4. There will be no change in total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides after a 4-week HIIT programme or 4 weeks participation in a traditional community-based cardiac rehabilitation programme in men and women with CVD

METHODOLOGY

Participants

The present investigator volunteered to help with the supervision of two community-based phase IV cardiac rehabilitation classes per week for > 2 yr prior to the beginning of this study. Twenty-five men and five women with documented CVD, who had been participating in a community-based phase IV cardiac rehabilitation programme, twice a week, for a minimum of 6 months, were informed of the research study and a brief summary and contact details were provided. Following an expression of interest, potential subjects were asked to visit the Vascular Research Unit (VRU) in the School of Health and Human Performance for a screening visit. The natures, benefits, risks and discomforts of the study were explained. Inclusion criteria were stable angina; prior myocardial infarction; prior revascularisation; ability to achieve 30 min of continuous walking without symptoms (cardiac chest pain/discomfort, severe breathlessness, dizziness or palpitations) or be able to undertake activities of at least 5 METS (manually washing a car, digging/turning soil, walking/jogging a mile in less than 15 min) without symptoms; clinically stable and in good health for a minimum of 2 weeks prior to beginning the study. Participants were excluded if they were current smokers, had unstable angina, uncontrolled hypertension (systolic blood pressure (BP) >180 mmHg, diastolic BP >100 mmHg), resting tachycardia or unstable/acute heart failure. Individuals who met the entry criteria and who received medical clearance from

a physician to participate were randomly assigned to HIIT (n = 15, 3 female) or to continued participation in cardiac rehabilitation (CPCR) (n = 15). Participants' medications are summarized in Table 5.5.

Research Design

Participants visited the Health and Human Performance Laboratories for a screening visit, and for 2 visits before and after 4 weeks of HIIT or CPCR (Figure 5.1).

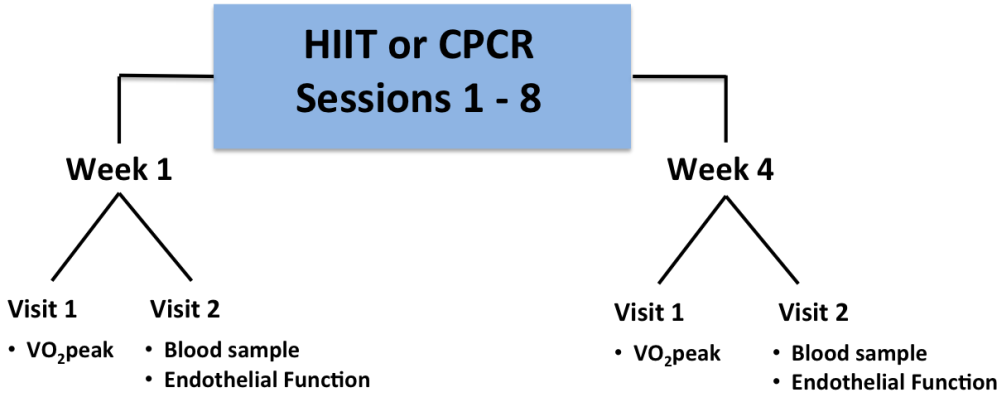

Figure 5.1: Study design

Screening visit: The nature and risks of the study were explained and written informed consent was obtained from each participant (Appendix D). The experimental procedures were approved by the Research Ethics Committee at Dublin City University, Ireland. Participants then completed a general health (Appendix A) and physical activity readiness questionnaire (PAR-Q) (Appendix B).

Visit 1: Participants abstained from strenuous physical activity for 24 h, and abstained from food, alcohol and caffeine for at least 3 h before the visit. During this visit, height, weight, and blood pressure were measured and peak aerobic capacity ($\dot{V}O_2$peak) were assessed using an exercise test on a treadmill.

Visit 2: Participants arrived to the laboratory after an overnight fast. Resting blood pressure was obtained, blood was drawn, and endothelial function was assessed by measuring brachial artery FMD.

HIIT

Participants assigned to HIIT, undertook two supervised high-intensity interval sessions per week for 4 weeks in the cardiovascular research unit (Sch. HHP, DCU), and did not attend the community-based phase IV cardiac rehabilitation for the duration of the study. Following a 10 min warm-up, participants performed three blocks of 8 x 45 sec intervals of high-intensity exercise at ≥85 %HRmax on a treadmill (Figure 5.2). Each interval was interspersed with a 15 sec period of passive recovery and a 2 min interval separated each block. The session concluded with a 5 min cool-down. Participants were continuously monitored via a 12-lead ECG throughout each exercise session. Heart rate, blood pressure, percentage of oxygen saturation (SpO_2), RPE and angina score were obtained throughout each HIIT session. The present investigator and principle investigator, Prof. Moyna, supervised the HIIT.

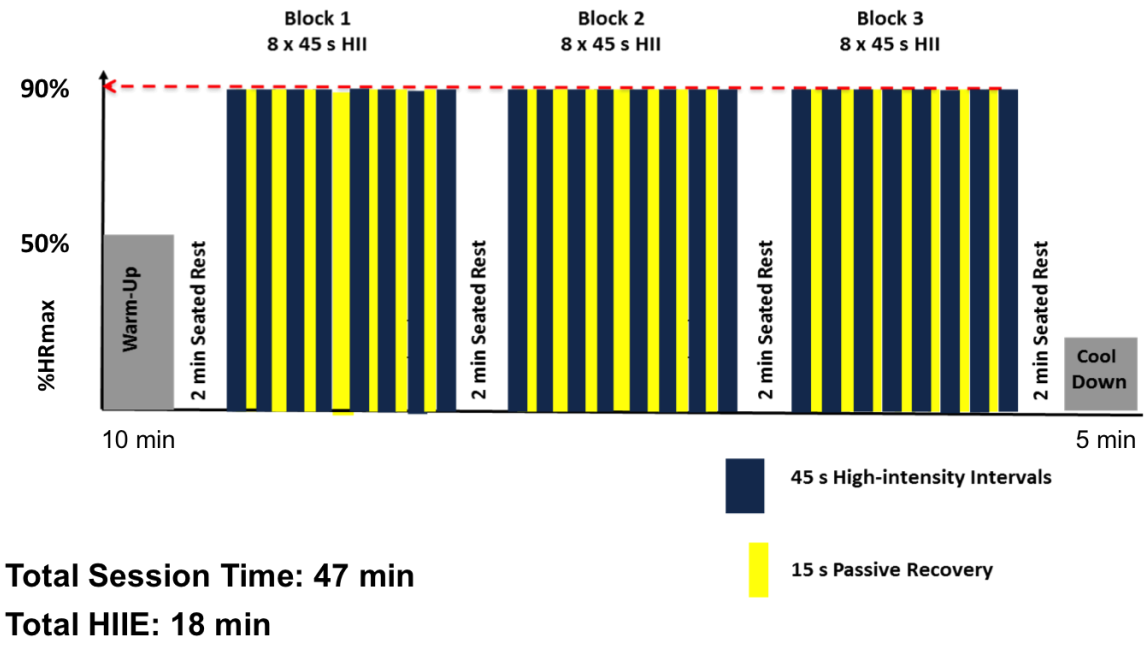

Figure 5.2: Overview of a HIIT session

CPCR

Participants assigned to CPCR attended HeartSmart two times per week. HeartSmart is an established community-based cardiac rehabilitation programme that takes place in DCU sports complex and is supervised by a physician and DCU Sport staff. Cardiac rehabilitation involves highly structured, medically supervised, and electronically monitored exercise regimes. The programme is multi-faceted but predominantly focuses on exercise. Exercise classes are normally 60 minutes in duration and primarily involve aerobic exercise and some form of resistance training. Sessions begin with a 10 min warm-up at a low-intensity. The main exercise phase can range from 15 – 40 min at a moderate-intensity. Participants exercise in small groups and rotate between local muscular endurance circuits, various cardio machines and aerobics, during a class. The class concludes with a 10 min cool-down.

Anthropometrics

Height and mass were measured using a wall stadiometer and electronic balance (Seca 797, USA) respectively. Footwear was removed prior to the measurement. Height was measured to the nearest cm, and weight was measured to the nearest 0.1 kg.

Peak Aerobic Capacity

Peak aerobic capacity test ($\dot{V}O_2max$) was assessed on a treadmill (Marquette 2000, General Electric, USA) using 1 of 5 incremental protocol (Appendix F), depending on individual exercise capacity. The Balke protocol was selected and modified to allow participants to reach volitional fatigue in 8 – 12 min. A 2-min warm up preceded each test and the gradient was increased 2.5% every 2-min until the subject reached volitional exhaustion or presented with contraindications to exercise. If necessary, treadmill speed was increased by 1 mph every 2 minutes while the treadmill gradient was maintained at 22.5%. Participants were verbally encouraged to give their best effort. Respiratory metabolic measures, heart rate and an ECG were monitored continuously throughout the test. RPE was recorded every 2 min. Peak oxygen uptake was determined by averaging the three highest consecutive 20 second values.

Open Circuit Spirometry

Respiratory metabolic responses were determined using standard open-circuit spirometry techniques (Sensormedics Vmax 229, SensorMedics Corp., CA). Prior to testing, the gas analysers were calibrated with standard gases of known concentration.

The calibration gas was sampled at a rate of 125 Hz. A mass flow sensor (Sensormedics, Loma Linda, CA, USA) was used to collect breath-by-breath measurement of ventilation. A 3 L volume syringe (Sensormedics, Loma Linda, CA, USA) was used to calibrate the mass flow sensor prior to each test.

Ratings of Perceived Exertion

RPE was obtained using the 16-point Borg category RPE scale. Prior to the maximal exercise test participants read a standard set of perceptual scaling instructions. These instructions followed an established format used in previous investigations. Low and high "perceptual anchors" were established during the maximal exercise test. This involved asking participants to assign a rating of 6 (low anchor) to the lowest exercise intensity, and 20 (high anchor) to the highest exercise intensity. Participants were instructed to make their subjective assessments of perceived exertion relative to these minimum and maximum standards (perceptual anchors).

Electrocardiographic Monitoring (ECG)

Heart rate (HR) and electrical activity of the heart were monitored continuously using a 12-lead ECG monitor (GE Case 8000 12 Lead ECG). The signal to noise ratio at the skin electrode interface was reduced by cleansing the area with an alcohol saturated gauze pad. The superficial layer of skin was then removed using light abrasion with fine grain emery paper. The electrodes were placed on 10 standard anatomical landmarks.

Endothelial Function Assessment

Endothelial-Dependent Dilation

Figure 5.3 illustrates the FMD protocol. M-mode images were named and recorded every 30 sec post-deflation for 5 min. Shear rate was calculated using the formula; 4 x peak velocity/peak diameter [69].

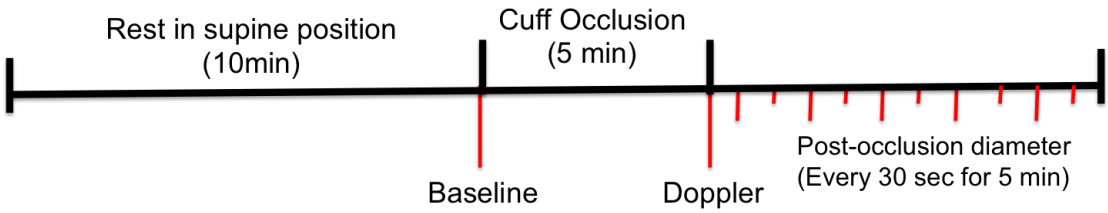

Figure 5.3: Overview of endothelial-dependent dilation

Endothelial-Independent Dilation

To assess brachial artery diameter, M-mode images were named and recorded every 30 sec post-deflation for 5 min (Figure 5.4).

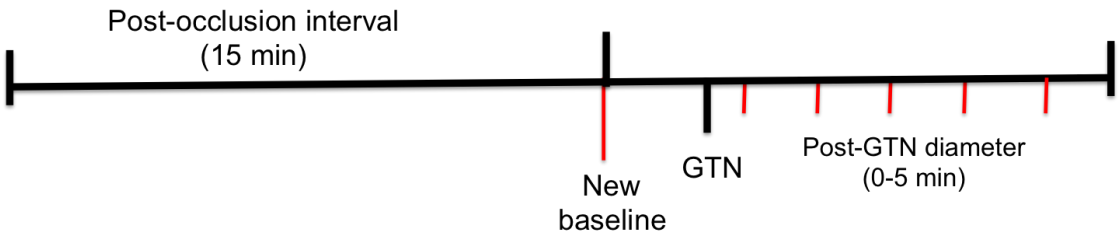

Figure 5.4: Overview of endothelial-independent dilation

Off-line Analysis

The same experienced investigator performed the off-line analysis of the ultrasound images using a custom-designed arterial diameter measurement tool. Image processing is divided into the following stages, i) image acquisition, ii) calibration, iii) pre-processing, iv) segmentation, v) post-processing, vi) robust statistics and vii) refinement. This method of arterial diameter estimation using the ADM software is explained in detail in chapter 3.

Statistical Analysis

Prior to statistical analysis the data was checked for normality using the Shapiro-Wilks test. A group (HIIT and CPCR) x time (baseline and week 4) repeated measures ANOVA was used to compare the mean differences within and between group. Significant main effects were probed using a Bonferroni post hoc test. SPSS for Windows statistical software (ver 19.0) was used to perform the statistical analysis. The criterion for significance (alpha) was set at 0.05 and with a sample size of 12 the study had a power of 80% to show a 50% increase in FMD.

Results

Training compliance was excellent. Four participants dropped out of the CPCR group; 2 due to illness, 1 due to orthopedic problems and 1 due to lack of interest. Two participants were unable to complete one of their HIIT sessions due to illness, but we stopped the exercise and completed the session on another day.

Participant characteristics

Physical characteristics, blood pressure and blood lipids in both the HIIT and SCR groups at week 1 and week 4 are summarized in table 5.2. There were no group differences in height, weight, blood pressure and blood lipids at week 1 and week 4. There were no changes in height, weight, blood pressure and blood lipids in either group between week 1 and week 4.

Maximal Exercise

Participant responses at maximal exercise at week 1 and week 4 are summarized in table 5.3. There was a significant increase in treadmill time to exhaustion in the HIIT group at week 4 compared to week 1. There was no significant difference in any of the other measured parameters at maximal exercise between or within experimental groups at week 1 or week 4.

High-intensity Interval Training

The mean physiological and perceptual responses for each training block of HIIT are summarized in table 5.4. There average treadmill velocity was significantly higher during block 2 than block 1. Average heart rate, %HRmax, %HRR and RPE were significantly higher during block 2 than block 1 of HIIT and during block 3 than block 1 and block 2.

Endothelial Function

There was no significant difference in percentage change in brachial artery FMD between the two groups at week 1. The percentage change in FMD was significantly greater at week 4 than week 1 in the HIIT group only. Brachial artery FMD was significantly higher in the HIIT group than the CPCR group at week 4 (Figure 5.5). There was no significant difference in endothelial-independent dilation between or within experimental groups at week 1 or week 4. Compared to week 1, there was no change in shear rate at week 4 in the HIIT group (951.2 ± 294.1 s^{-1} vs. 1020.5 ± 245.1 s^{-1}) or the CPCR group (1009.5 ± 179.9 s^{-1} vs. 932.5 ± 146.2 s^{-1}). Peak diameter, peak blood flow velocity and endothelial-independent dilation results are illustrated in Figures 5.6 – 5.8.

ECG Changes and Clinical Symptoms

ST-segment changes ranged from 1 – 3 mm, and returned to normal during passive recovery. None of the participants reported angina symptoms during or following the 4 weeks of HIIT.

Table 5.1: Medication

Main Drug Class	**HIIT**	**CPCR**
Statins	9	5
ACE Inhibitor	6	3
Beta Blocker	2	2
Anti-platelet	9	6
Diuretic	1	
Proton Pump	3	2

Values are means

Table 5.2: Physical characteristics and blood lipids of the participants at week 1 and week 4

	Group			
	HIIT (n=15)		CPCR (n=11)	
	Week 1	Week 4	Week 1	Week 4
Age (y)	66.6 ± 6.7		68.6 ± 5.7	
Height (cm)	170.6 ± 6.9		167.6 ± 8.3	167.6 ± 8.3
Weight (kg)	80.8 ± 10.3	80.6 ± 10.3	77.8 ± 12.0	79.3 ± 11.5
BMI ($kg \cdot m^{-2}$)	27.7 ± 2.6	27.3 ± 2.6	27.8 ± 4.4	28.2 ± 4.8
Systolic blood pressure (mmHg)	140.1 ± 14.5	137.0 ± 18.7	122.0 ± 11.3	130.0 ± 26.5
Diastolic blood pressure (mmHg)	81.1 ± 9.6	80.3 ± 2.8	71.0 ± 1.4	78.3 ± 7.6
Total cholesterol (mmol)	3.4 ± 0.7	4.5 ± 1.7	3.6 ± 0.6	3.4 ± 0.3
HDL-cholesterol (mmol)	1.2 ±0.2	1.3 ± 0.2	1.3 ± 0.2	1.0 ± 0.2
LDL-cholesterol (mmol)	1.4 ± 0.3	1.8 ± 0.6	1.8 ± 0.8	1.5 ± 0.3
Triglycerides (mmol)	1.0 ± 30.3	1.3 ± 0.4	1.1 ± 0.4	1.1 ± 0.2
ApoA ($mg.dL^{-1}$)	142.8 ± 19.9	142.1 ± 19.1	140.0 ± 15.2	127.3 ± 19.2
ApoB ($mg.dL^{-1}$)	70.4 ± 12.3	84.5 ± 17.2	84.1 ± 33.0	62.3 ± 16.4

Values are means ± SD; *$p < 0.05$ vs. week 1; †$p < 0.01$ vs. week 1

Table 5.3: Participant responses at maximal exercise

	Group			
	HIIT (n=15)		CPCR (n=11)	
	Week 1	Week 4	Week 1	Week 4
Treadmill velocity ($km \cdot h^{1}$)	5.50 ± 1.16	5.38 ± 1.31	5.30 ± 0.79	5.90 ± 2.04
Treadmill grade (%)	12.00 ± 3.16	12.30 ± 2.59	9.50 ± 2.75	10.54 ± 3.22
Time to exhaustion (s)	524.54 ± 110.96	591.77 ± 97.83†	584.86 ± 180.51	550.00 ± 152.04
$\dot{V}O_2$peak ($ml \cdot kg^{1} \cdot min^{1}$)	30.48 ± 7.90	32.23 ± 7.77	28.52 ± 6.14	29.20 ± 6.47
$\dot{V}O_2$peak ($L \cdot min^{1}$)	2.37 ± 0.81	2.40 ± 0.60	2.03 ± 0.53	2.10 ± 0.65
Heart rate ($beat \cdot min^{1}$)	138.20 ±13.75	135.87 ± 13.75	126.90 ± 14.44	129.38 ±22.51
Ventilation ($L \cdot min^{1}$)	63.95 ± 20.32	66.20 ± 59.63	56.02 ± 16.14	59.63 ± 18.59
RER	1.13 ± 0.07	1.14 ± 0.84	1.09 ± 0.10	1.15 ± 0.08

Values are means ± SD; †$p < 0.01$ vs. week 1

Table 5.4: Average Responses during HIIT

	Exercise Blocks			
	1	**2**	**3**	**Mean**
Treadmill velocity ($km \cdot h^{1}$)	6.5 ± 1.9	6.8 ± 2.1*	6.9 ± 2.2	6.7 ± 2.1
Grade (%)	4.0 ± 1.7	4.0 ± 1.7	4.0 ± 1.7	4.0 ± 1.7
Heart rate ($beat \cdot min^{1}$)	103.6 ± 9.9	109.7 ± 9.6‡	114.1 ± 10.9†‡	109.7 ± 9.6
%HRpeak	72.7 ± 8.2	77.0 ± 8.1‡	79.9 ± 8.2†‡	76.5 ± 7.9
%HRR	53.4 ± 11.6	60.8 ± 11.6‡	65.9 ± 12.2†‡	60.0 ± 11.3
Systolic blood pressure (mmHg)	155.0 ± 40.2	152.5 ± 40.0	148.6 ± 39.8	152.0 ± 39.8
Diastolic blood pressure (mmHg)	70.0 ± 17.4	69.4 ±17.3	67.5 ± 16.8	69.0 ± 17.1
RPE	12.2 ± 3.3	13.1 ± 3.4‡	13.9 ± 3.5†‡	13.1 ±3.3
SPO_2 (%)	90.0 ± 22.7	86.2 ±21.4	89.6 ± 22.6	89.6 ± 22.6

Values are means ± SD, *p < 0.05 vs. block1 †p < 0.01 vs. block 2; ‡p < 0.001 vs. block 1

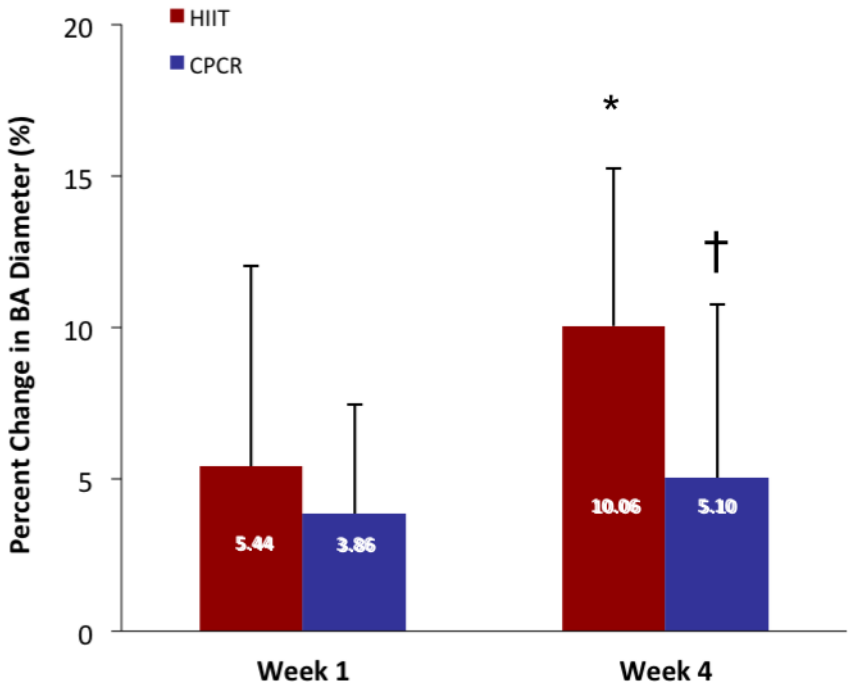

Figure 5.5: Percentage change in FMD in the HIIT and CPCR group at week 1 and week 4

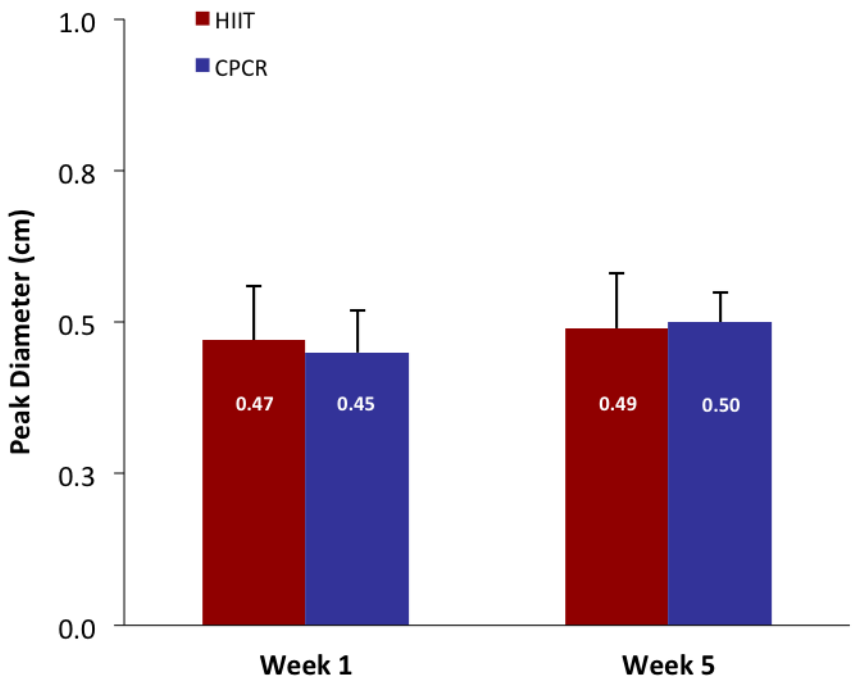

Figure 5.6: Peak brachial artery diameter (cm) in the HIIT and CPCR group at week 1 and week 4

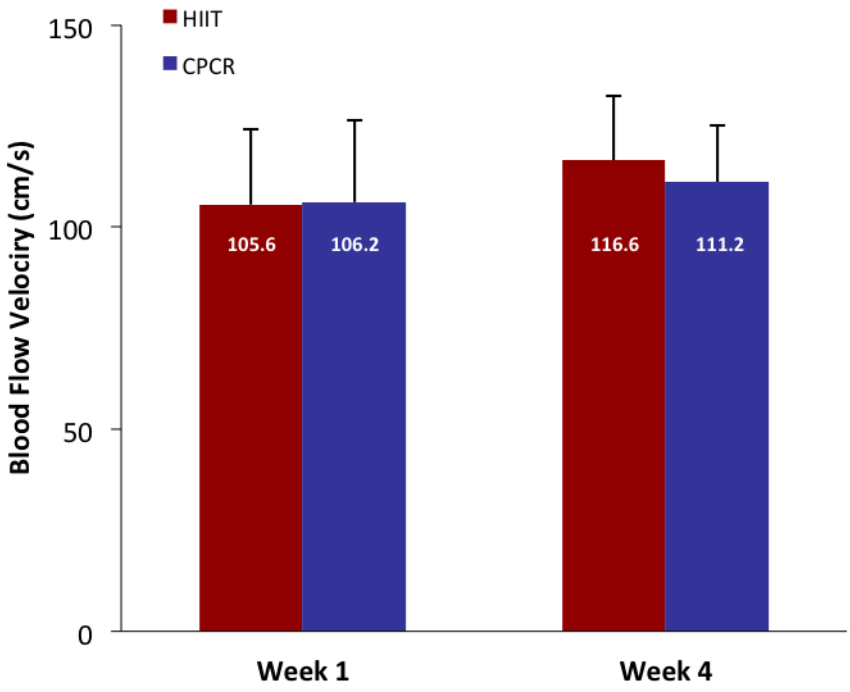

Figure 5.7: Peak blood flow velocity in the HIIT and CPCR group at week 1 and week 4

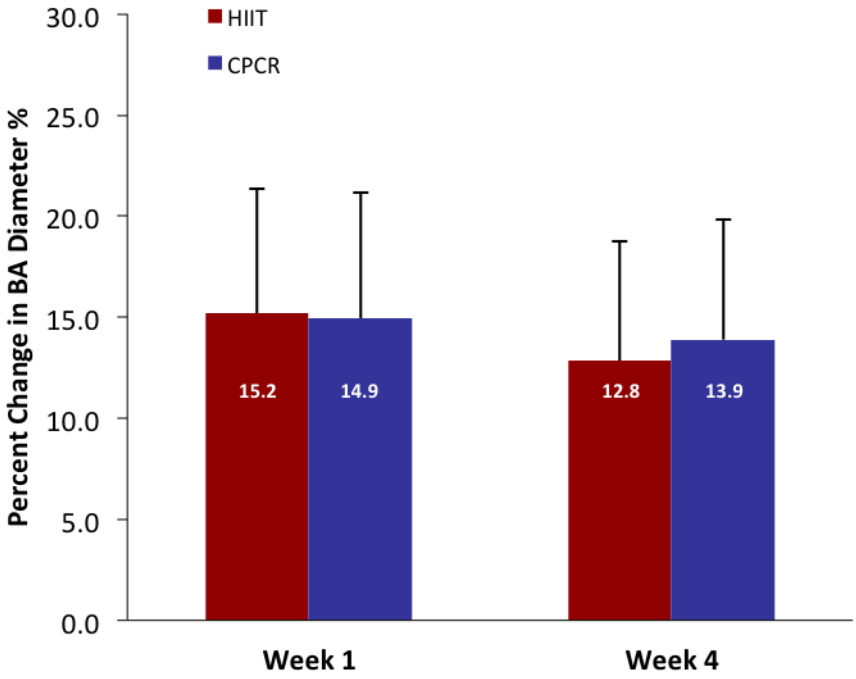

Figure 5.8: Percent change in brachial artery diameter (%) following GTN administration at week 1 and week 4

Chapter 6

Discussion

Overview

The endothelium is a complex dynamic barrier that plays a crucial role in maintaining vascular integrity. It is central to the initiation, development and progression of atherosclerosis [31]. Established CVD risk factors promote the development of atherosclerosis through their deleterious effects on endothelial structure and function, [174] and subsequent reduction in nitric oxide (NO) bioavailability. This is evident as a reduced vasomotor response of the diseased vessels to shear stress, and a variety of vasoactive compounds.

Flow-mediated dilation (FMD) is a commonly used non-invasive procedure to assess endothelial function, and is based on the assumption that healthy, intact endothelial cells can detect changes in shear stress following 5 min of occlusion and dilate in response. The percentage change in arterial diameter in response to shear stress can be measured using ultrasonography, and is believed to be endothelial-dependent and mediated by NO [58]. Diameter changes are also measured after administration of GTN to assess the response of the vessel to endothelium-independent vasodilation. Assessment of brachial artery FMD provides substantial diagnostic and prognostic information that is useful in assessing disease burden and guiding therapy in

patients with CAD and those with normal coronary arteries and risk factors for atherosclerosis.

A series of studies were undertaken to assess user reliability in the use of ultrasongraphy to measure endothelial vasomotor function, develop and validate a custom-designed software to measure arterial diameter, and evaluate the effect of acute and chronic exercise on endothelial function in men and women with CVD.

Study 1

Study 1 assessed the reliability of the practitioner (present investigator) in the use of ultrasonography to measure endothelial vasomotor function, and the development and validation of a reliable, custom-designed arterial diameter measurement tool.

Reproducible FMD measurements require careful attention to training, technique and analysis. The practitioner underwent intensive training in the use of ultrasonography under the guidance of a chief vascular technician from a local hospital. After 6 months of training and > 100 supervised scans, the investigator was deemed proficient in the use of ultrasonography, and reproducibility of the FMD measurement was confirmed on repeated tests in 25 healthy men and women.

The manual method of using ultrasound calipers for blood vessel diameter assessment has several drawbacks. Firstly, because measurements are only taken at a small, fixed number of vertical cross-sections, they are sensitive to small variations,

which may arise from noise in the ultrasound imaging system. Secondly, manual methods are time-consuming. They require the practitioner to carefully examine each image to differentiate the upper and lower arterial boundary. The boundary positions chosen by a practitioner are subjective, and can vary across practitioners based on individual skill and experience. Finally, calipers are often built into the ultrasound hardware, precluding off-line measurements.

Technical modifications and the development of edge-detection and wall-tracking software have evolved to significantly improve the sensitivity of blood vessel diameter measurements during FMD [175] [43]. Existing semi-automatic methods used in ultrasound analysis have two primary drawbacks. Firstly, they can often be more time-consuming than using calipers. The practitioner must select around the boundary of the artery, multiple times, to obtain an accurate estimation. Secondly, although software for delineating the boundary is general, and is usable in various different medical imaging tasks, it tends to be very expensive. The ADM software, developed in the present study addressed many of the problems with in-built calipers. It enables the imaging and recording of brachial artery ultrasound images and supports post-analysis at an increased number of time-points. The measurements are fast, can be performed offline, and are semi-automatic.

The ADM software developed is innovative because it i) uses a novel constrained region-growing algorithm to provide fast and reliable segmentation of M-mode ultrasound images, ii) provides real-time feedback to the practitioner, showing

clearly the cross-sections used to compute the diameter estimate, iii) uses robust statistics to ameliorate the effect of any inaccuracies in the segmentation, iv) allows measurements to be taken at specific cross sections of the estimated arterial boundary using a gating tool, thus accounting for cyclic changes in arterial dimensions at different phases of the cardiac cycle, v) provides for the comparison of boundary estimates with manual measurements using in-built software calipers, and vi) allows measurements to be taken offline, without requiring patient presence.

The algorithm used by the ADM system has a number of advantages over existing alternatives. It is more specific and less expensive, and the computational complexity of the segmentation is low, so live feedback is provided, permitting adjustment of the segmentation in real time. The system requires a single click inside the arterial region, and a possible slider adjustment, and the use of the inter-quartile mean provides a diameter estimation that is robust to inaccuracies in the segmentation of the artery. Visual feedback giving the exact region used in the diameter estimation increases the practitioner's confidence in the results, and prevents the practitioner spending time correcting errors that will have no impact on the final estimation.

Using in-built calipers as the criterion reference, the custom-designed ADM software was found to be a valid and reliable measurement of brachial artery diameter. The use of a semi-automatic arterial diameter estimation algorithm reduces the subjectivity of the results and makes the method more resilient to noise than the manual calipers. Enhanced data collection is provided, using the software, due to the fact that a greater number of ultrasound images can be recorded. In addition, the ADM software permits an increased number of time points and cross-sections at which the vessel diameter can be assessed.

Study 2

Observations that a single bout of exercise can transiently alter atherosclerotic CVD risk factors [114] have led to the notion that perhaps some of the effects of exercise on endothelial function may be attributable to the acute effect of exercise. Study 2 compared physiological and perceptual responses and endothelial vasomotor function following a single bout of SRE and short duration HIIE in men and women with CVD.

Current physical activity guidelines recommend that individuals with CVD should undertake moderate-intensity aerobic (endurance) physical activity for 30 min or more for 5 days per week. This equates to approximately 40-60 % $\dot{V}O_2$max (ACSM Guidelines). Standard exercise prescription procedures normally involve the titration of exercise intensity, to elicit a pre-determined heart rate, or $\dot{V}O_2$. The culture of exercise prescription can however, be perceived as highly controlling and aversive. Allowing

individuals to self-regulate their exercise intensity may provide a sense of control over their behavior, resulting in perceptions of autonomy [146] and greater levels of enjoyment. In addition to potentially improving vascular function, it is also important to ensure that an acute bout of SRE is within the prescribed range for improving health, and reducing the risk for CVD.

HIIE was, until quite recently, used primarily by athletes in preparation for athletic competitions. A number of recent studies have found supervised HIIT to be safe and effective for improving health in men and women with CVD [7,8]. When compared to continuous endurance exercise, HIIE has been shown to elicit similar or even superior physiological adaptations, in healthy men and women [6] and those with CVD [8]. Most of the previous studies that examined the effect of acute HIIE on endothelial function used intervals ≥ 4min in duration. It was hypothesized that an acute bout of high intensity treadmill exercise involving intervals of 45 sec and 15 sec passive recovery would provide a greater hemodynamic shear than SRE, and transiently, improve endothelial function.

On average, participants in the SRE and HIIE group exercised at 64% and 78% $\dot{V}O_2$peak, respectively, and all participants in both groups exercised at an intensity ≥ 50 %$\dot{V}O_2$peak (moderate-intensity). Four participants in the SRE group and all of the participants in the HIIE group exercised an intensity considered to be vigorous (60–85 % $\dot{V}O_2$max) (Figure 6.1). The average metabolic cost was 5.0 and 6.7 METS for the SRE and HIIE group, respectively.

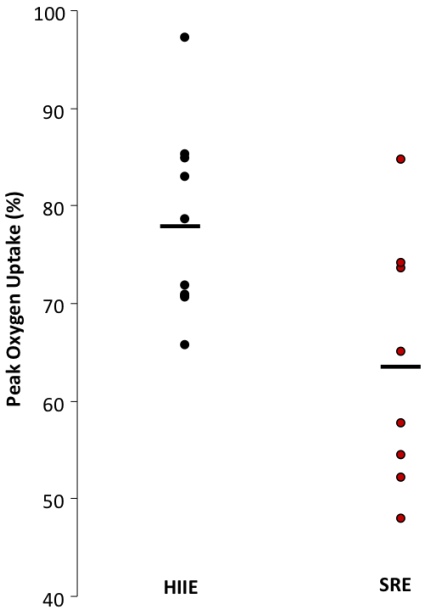

Figure 6.1: Mean individual %$\dot{V}O_2$peak values during the acute bout of HIIE and SRE

An individual's perception of physical exertion can be viewed as a psychophysiological construct that represents the integration of multiple sensory inputs between external stimuli arising from physical work and internal responses reflecting physiological functions and situational and dispositional factors [146]. When allowed to self-regulate their exercise intensity men and women with CVD, on average, exercised at an intensity that they perceive to be fairly light (Figure 6.2). In contrast, the HIIE group perceived the exercise to be hard, and two of the participants perceived the effort to be very hard or very, very hard (Figure 6.2).

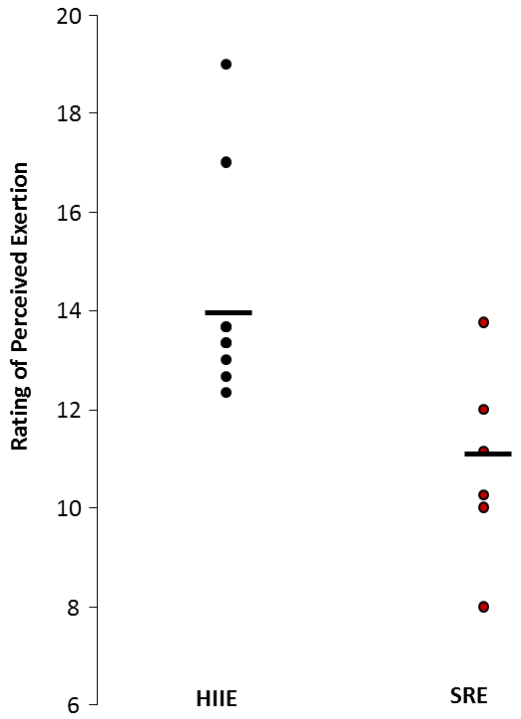

Figure 6.2: Mean individual RPE-O values during the acute bout of HIIE and SRE

Although affect was not measured in the present study, it is worth noting that when given a choice, individuals will generally adjust their effort intensity during exercise to maximize affect. This is important considering that a positive affective response may lead to greater enjoyment of the exercise session, promote a positive memory of that activity, and, consequently, contribute to increased motivation for future physical activity behaviour [146]. In contrast, activities that are perceived to be difficult are more likely to lead to withdrawal from the activity. Although the acute bout of HIIE may have resulted in a significantly higher physiological and metabolic response compared to the SRE exercise, the high RPE would indicate that the exercise experience may be perceived as negative, and may have a deleterious effect on subsequent exercise participation.

Despite exercising within the desired intensity range for cardiovascular benefits, a single SRE session does not appear to provide an appropriate shear stimulus to elicit acute improvements in endothelial function. Similarly, endothelial-dependent dilation did not change significantly after the acute bout of HIIE, compared to pre-exercise values, despite the fact that participants exercised at an intensity, generally deemed 'vigorous' [176], and one that was significantly higher than SRE.

Cycling for 30 min at 50% $\dot{V}O_2$peak increases brachial artery FMD in young female smokers . Treadmill exercise for 30-45 min at low-, moderate- and high-intensity almost doubled brachial artery FMD in postmenopausal women [119] and active overweight men [122]. A recent study [124], found that both low volume high-intensity interval exercise and moderate-intensity endurance exercise significantly increased absolute and normalized brachial artery FMD 1 h post exercise in men and women with CAD. In contrast, Joras *et* al., [116] showed that brachial artery FMD was significantly decreased in men with PAD, 30 min and 2 h following 10 min of treadmill exercise to intolerable ischaemic pain in the affected lower extremity. Similarly, an acute bout of treadmill exercise to the onset of claudication has no effect on brachial artery FMD in men and women with PAD [117]. Indeed, FMD was significantly impaired following treadmill exercise to maximal claudication pain, demonstrating that exercise-induced ischemia further deteriorates FMD.

A number of other studies have found a transitory functional deterioration in FMD in i) healthy young men and women (19-30 years) after 30 min of continuous

treadmill exercise at 60% v$\dot{V}O_2$max [49], ii) fit individuals 1 h after a single bout of high-intensity interval running [121], iii) non-elite runners 1 h after completing a marathon [123], and iv) healthy male smokers compared to non-smokers (7.7 v 4.1%) immediately following 40 min of submaximal steady-state exercise on a cycle ergometer [48].

Previous acute exercise studies have found a significant positive relation between FMD values and shear rate (area under the curve) at rest, but not after an acute bout of exercise. This may be attributable to the fact that the change in shear rate (area under the curve) from rest to post-exercise can vary greatly among individuals. In a study investigating the effect of 30 min of continuous treadmill exercise on brachial artery FMD in healthy young men and women, shear rate (area under the curve) decreased in 7 and increased in 6 participants [49]. FMD was significantly attenuated after exercise compared with rest. When the percent change in diameter was normalized to shear rate (area under the curve) the difference in FMD from pre to post exercise was abolished. As a result of the high degree of variability in physiological shear rate in response to hyperemia post-exercise observed in previous literature, FMD values were not normalized to shear rate in this acute exercise study.

There was no difference in endothelial function within or between groups following the acute bouts of SRE or HIIE. Results from previous studies examining the effect of acute exercise on endothelial function in individuals with CVD have been equivocal. Many of these studies, observing acute improvements in endothelial function following exercise, assessed brachial artery FMD 1 hour after the exercise bout. Currie

et al., [124] observed an increase in FMD following bouts of endurance and interval exercise in men and women with CAD. In this study, FMD was assessed 1 hour after each exercise bout. Similarly, Harris *et al*., [122] observed enhanced FMD following acute exercise in active overweight men when FMD was assessed 1 hour after the exercise bout. In contrast, the present study examined FMD within 5 min of exercise cessation. Exercise-induced alterations in arterial diameter as a result of augmented blood flow in the brachial artery, may result in vasodilation that can exist for up to 1 hour post exercise cessation [177]. This post exercise arterial dilation may be responsible for the relatively low FMD results.

Study 3

Study 3 compared the effect of 4 weeks of HIIT to 4 weeks of CPCR on aerobic capacity, endothelial function and selected cardiovascular biomarkers in CVD patients. Traditionally, exercise guidelines to promote health, and reduce cardiovascular risk factors and all-cause mortality have encouraged continuous, moderate-to-vigorous intensity exercise training (ACSM). The most recent position statement on exercise prescription for individuals with CVD [178] recommends continuous exercise involving large-muscle groups (e.g. walking, jogging, cycling etc.) performed at a moderate-intensity level corresponding to 40-85% heart rate reserve.

Exercise performed at a vigorous intensity (≥6 METs) has been shown to induce a greater reduction in CVD risk, and greater improvements in diastolic blood pressure,

glucose control, and aerobic capacity compared to exercise performed at a moderate intensity [179]. It is thought that exercise guidelines for phase IV cardiac rehabilitation may fall below the threshold to induce physiological and metabolic changes that will improve cardiovascular health.

Historically, HIIT has been used to improve athletic performances. In recent years a number of studies have shown its efficacy and safety in improving exercise capacity and endothelial function in individuals with CAD and heart failure [7,8]. Recent training studies, utilizing a short exercise interval duration and fewer exercise sessions have found similar or superior physiological and metabolic improvements compared to traditional, continuous, high-volume exercise. Gibala *et al*., [6,12] observed similar responses to 2 weeks of HIIT and continuous endurance exercise, despite the fact that the total training volume for the HIIT group was approximately 10% of the endurance exercise group, with the HIIT group exercising for a total of 2.5 h over 2 weeks compared to 10.5 h in the endurance group. The HIIT consisted of 6 sessions of six 30 sec "all out" cycling, whereas the endurance training involved six 90 – 120 min of continuous cycling at 65% $\dot{V}O_2$peak. These findings indicate that HIIT is a time-efficient method of exercise training in healthy population.

To date, 4 studies have evaluated the effect of HIIT on endothelial-dependent dilation in CVD patients (Table 2.2). Study duration ranged from 8 – 24 weeks, and exercise intervals of 4 min in duration were predominantly used. Eight weeks of HIIT cycling and running at > 80% $\dot{V}O_2$peak significantly enhanced brachial artery FMD and

maximal aerobic capacity in heart transplant patients, compared to inactive controls [9]. Using four 4-min intervals at 90 – 95% HRpeak, Wisloff *et al*., [8] found significant improvements in aerobic capacity and endothelial function following 12 weeks of aerobic interval training compared to moderate-intensity exercise in individuals with CAD. A similar 12 week randomised controlled trial also found significantly greater increases in brachial artery FMD and $\dot{V}O_2$peak following HIIT, involving 4 min intervals at 85–95% HRmax, than 12 weeks of standard cardiac rehabilitation [140]. The longest study was 6 months in duration and found that supervised HIIT involving 4 min intervals at 80–90% HRmax on a cycle ergometer or treadmill significantly increased $\dot{V}O_2$peak and FMD [180].

Individuals assigned to CPCR in Study 3 had been attending a local community-based Phase IV cardiac rehabilitation for at least 6 months, and continued attending class 2 times per week for the duration of the study. Due to logistics, it was not possible to collect heart rate data on the participants during their participation in the CPCR group. Participants in the HIIT group exercised at 77% HRmax during each exercise interval, falling below the target intensity of > 80% HRmax. Considering the cohort, participant feedback and RPE values, it was difficult at times to further increase the intensity of the exercise intervals. Although there was no change in $\dot{V}O_2$peak following 4 weeks of HIIT or CPCR group, treadmill time to exhaustion increased significantly in the HIIT group only.

Differences in exercise prescription, study duration and participant characteristics may help to explain the inability to increase $\dot{V}O_2$peak. Gibala et al, [6,12] recruited healthy young college-aged students, and the training was undertaken at a higher intensity (i.e. "all out" cycling against a fixed resistance) than the present investigation. Previous HIIT studies involving CVD patients that have resulted in improvements in $\dot{V}O_2$peak have used training durations of > 8 weeks, have recruited patients with heart failure, a history of transplantation, that are mostly overweight, or individuals 2 – 12 weeks post MI, or immediately post PCI. Participants in study 3 have been regularly participating in cardiac rehabilitations exercise classes for more than 6 months and were excluded from study if they had heart failure.

Endothelial-dependent dilation was significantly greater at week 4 (10.1%) than week 1 (5.4%) in the HIIT group only. Brachial artery FMD was also significantly greater in the HIIT group (10.1%) than the CPCR group at week 4 (5.1%). Despite no change in $\dot{V}O_2$peak, 4 weeks of HIIT significantly improved endothelial function. These results support the potential role of HIIT in phase IV cardiac rehabilitation.

The putative mechanisms responsible for the regulation of endothelial NO bioavailabilty and in turn the improved brachial artery FMD may include flow-mediated i) transcriptional upregulation of the endothelial NO synthase (eNOS), ii) post-transcriptional activation of eNOS, and iii) reduction of reactive oxygen species-mediated breakdown of NO by increaseing the antioxidative defence mechanisms [181].

Study Limitations

- The most accurate method for normalizing FMD responses is believed to be the area under the curve shear response from post-occlusion to the time at which peak diameter occurs [71]. Presently, the Vascular Research Laboratory in DCU is not equipped to measure viscosity and the ultrasound system does not have the functionality for simultaneous acquisition of M mode diameter and pulsed-wave Doppler velocity signals, or the continuous measurement of blood flow velocity. Duplex ultrasound for simultaneous capture is recommended where available. For these reasons shear rate was calculated as an accepted estimate of shear stress, using peak blood flow velocity (equation: shear rate = 4*peak velocity/peak diameter) [69].
- When assessing peak aerobic capacity in individuals with CVD, it may be difficult to attain true peak heart rate values due to medication affecting HR. This can have a deleterious effect when using heart rate to establish training intensities. Use of effort perception may be useful to help ensure participants reach desired intensities during exercise training.

Conclusions

The developed ADM software system is a valid and reliable tool for brachial artery diameter assessment. Self-regulated or high-intensity interval exercise does not provide the required stimulus to elicit acute improvements in endothelial function in

men and women with CVD. In contrast, 8 sessions of high-intensity interval training significantly improve endothelial function and exercise capacity in patients with at least 6 months participation in a community-based cardiac rehabilitation programme. Anecdotal evidence would indicate that upon completion of a 4-week HIIT programme, participants experienced increased self-efficacy and confidence in exercising alone and at higher intensities.

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Appendices

Appendix A

School Of Health and Human Performance
Dublin City University

General Health Questionnaire

Name:…….. Occupation:……

Address:…….

Telephone: (Home)…….. (Work):……..

_____

Do you have, or have you ever suffered from: -Diabetes? Yes / No

-Asthma? Yes / No

Epilepsy? Yes / No

Have you ever had pains in your chest or heart? Yes / No

Do you ever feel faint or have spells of dizziness? Yes / No

Do you have or have you ever had high blood pressure? Yes / No

Do you have a muscle, back or joint problem that could be aggravated by physical activity or made worse with exercise? Yes / No

Do you have any current injuries? Yes / No

In the past week, have you suffered from any illness which required you to be in bed or off work for one day or more? Yes / No

Do you smoke? If yes, how many per day? Yes / No

Do you drink? If yes, how many units per week? Yes / No

Is there a good physical reason not mentioned here why you should not carry out laboratory testing? Yes / No

Please provide any further information concerning any condition/complaints that you suffer from and any medication which you may be taking by prescription or otherwise:

Date: Signature:_____ Authorising Signature:_____

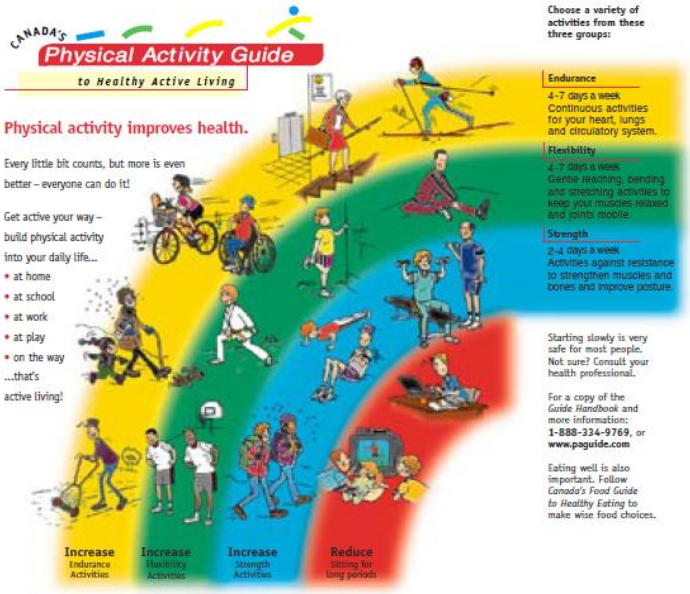
Appendix B

PAR-Q

...continued from other side

PAR-Q & YOU

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)



Choose a variety of activities from these three groups:

Endurance
4-7 days a week
Continuous activities for your heart, lungs and circulatory system.

Flexibility
4-7 days a week
Gentle reaching, bending and stretching activities to keep your muscles relaxed and joints mobile.

Strength
2-4 days a week
Activities against resistance to strengthen muscles and bones and improve posture.

Starting slowly is very safe for most people. Not sure? Consult your health professional.

For a copy of the *Guide Handbook* and more information: **1-888-334-9769**, or **www.paguide.com**

Eating well is also important. Follow *Canada's Food Guide to Healthy Eating* to make wise food choices.

Get Active Your Way, Every Day – For Life!

Scientists say accumulate 60 minutes of physical activity every day to stay healthy or improve your health. As you progress to moderate activities you can cut down to 30 minutes, 4 days a week. Add-up your activities in periods of at least 10 minutes each. Start slowly... and build up.

Time needed depends on effort

Very Light Effort	Light Effort *60 minutes*	Moderate Effort *30-60 minutes*	Vigorous Effort *20-30 minutes*	Maximum Effort
• Strolling • Dusting	• Light walking • Volleyball • Easy gardening • Stretching	• Brisk walking • Biking • Raking leaves • Swimming • Dancing • Water aerobics	• Aerobics • Jogging • Hockey • Basketball • Fast swimming • Fast dancing	• Sprinting • Racing

Range needed to stay healthy

You Can Do It – Getting started is easier than you think

Physical activity doesn t have to be very hard. Build physical activities into your daily routine.

- Walk whenever you can – get off the bus early, use the stairs instead of the elevator.
- Reduce inactivity for long periods, like watching TV.
- Get up from the couch and stretch and bend for a few minutes every hour.
- Play actively with your kids.
- Choose to walk, wheel or cycle for short trips.
- Start with a 10 minute walk – gradually increase the time.
- Find out about walking and cycling paths nearby and use them.
- Observe a physical activity class to see if you want to try it.
- Try one class to start – you don t have to make a long-term commitment.
- Do the activities you are doing now, more often.

Benefits of regular activity:	Health risks of inactivity:
- better health - improved fitness - better posture and balance - better self-esteem - weight control - stronger muscles and bones - feeling more energetic - relaxation and reduced stress - continued independent living in later life	- premature death - heart disease - obesity - high blood pressure - adult-onset diabetes - osteoporosis - stroke - depression - colon cancer

Health Canada Santé Canada — Canadian Society for Exercise Physiology

Source: Canada's Physical Activity Guide to Healthy Active Living, Health Canada, 1998 http://www.hc-sc.gc.ca/hppb/paguide/pdf/guideEng.pdf

FITNESS AND HEALTH PROFESSIONALS MAY BE INTERESTED IN THE INFORMATION BELOW:

The following companion forms are available for doctors' use by contacting the Canadian Society for Exercise Physiology (address below):

The **Physical Activity Readiness Medical Examination (PARmed-X)** – to be used by doctors with people who answer YES to one or more questions on the PAR-Q.

The **Physical Activity Readiness Medical Examination for Pregnancy (PARmed-X for Pregnancy)** – to be used by doctors with pregnant patients who wish to become more active.

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Mottola, M., Wolfe, L.A. (1994). Active Living and Pregnancy. In: A. Quinney, L. Gauvin, T. Wall (eds.), **Toward Active Living: Proceedings of the International Conference on Physical Activity, Fitness and Health.** Champaign, IL: Human Kinetics.

PAR-Q Validation Report, British Columbia Ministry of Health, 1978.

Thomas, S., Reading, J., Shephard, R.J. (1992). Revision of the Physical Activity Readiness Questionnaire (PAR-Q). **Can. J. Spt. Sci.** 17:4 338-345.

For more information, please contact the:

Canadian Society for Exercise Physiology
202-185 Somerset Street West
Ottawa, ON K2P 0J2
Tel. 1-877-651-3755 • FAX (613) 234-3565
Online: www.csep.ca

The original PAR-Q was developed by the British Columbia Ministry of Health. It has been revised by an Expert Advisory Committee of the Canadian Society for Exercise Physiology chaired by Dr. N. Gledhill (2002).

Disponible en français sous le titre «Questionnaire sur l'aptitude à l'activité physique - Q-AAP (revisé 2002)».

Supported by: Health Canada Santé Canada

Appendix C

Submission to Ethics Committee

Dublin City University

RESEARCH ETHICS COMMITTEE

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING HUMAN PARTICIPANTS

Application No. *(office use only)* DCUREC/2009/ ☐☐☐

Period of Approval *(office use only)* ……/……/…… to ……/……/….

This application form is to be used by researchers seeking ethics approval for individual projects and studies. The **signed original and an electronic copy** of your completed application must be submitted to the DCU Research Ethics Committee.

NB - The hard copy must be signed by the PI. The electronic copy should consist of one file only, which incorporates all supplementary documentation. The completed application must be proofread and spellchecked before submission to the REC. All sections of the application form should be completed. Applications which do not adhere to these requirements will not be accepted for review and will be returned directly to the applicant.

Applications must be completed on the form; answers in the form of attachments will not be accepted, except where indicated. No handwritten applications will be accepted. **Research must not commence until written approval has been received from the Research Ethics Committee.**

PROJECT TITLE	Effect of Exercise Duration on Vascular Health in Patients with CVD
PRINCIPAL INVESTIGATOR	Prof. Niall M. Moyna

Please confirm that all supplementary information is included in your application (in both signed original and electronic copy). If questionnaire or	**INCLUDED**	**NOT APPLICABLE**

interview questions are submitted in draft form, a copy of the final documentation must be submitted for final approval when available.			
Bibliography	☐		☒
Recruitment advertisement	☐		☒
Plain language statement/Information Statement	☒		☐
Informed Consent form	☒		☐
Evidence of external approvals related to the research	☐		☒
Questionnaire	☐ draft	☐ final	☒
Interview Schedule	☐ draft	☐ final	☒
Debriefing material	☐		☒
Other	☐		☒

Please note:

1. Any amendments to the original approved proposal must receive prior REC approval.
2. As a condition of approval investigators are required to document and report immediately to the Secretary of the Research Ethics Committee any adverse events, any issues which might negatively impact on the conduct of the research and/or any complaint from a participant relating to their participation in the study

Please submit the **signed original, plus the electronic copy** of your completed application to: Ms. Fiona Brennan, Research Officer, Office of the Vice-President for Research (fiona.brennan@dcu.ie, Ph. 01-7007816)

1. ADMINISTRATIVE DETAILS

THIS PROJECT IS:

(tick as many as apply)

☒ Research Project

☐ Practical Class

☐ Student Research Project

☐ Funded Consultancy

☐ Clinical Trial

☐ Other - *Please Describe:*

Final Year Research Project

☐ Research Masters ☐ Taught Masters

☒ PhD ☐ Undergraduate

Project Start Start: 01/01/10 **Project End End: 31/12/11**

Date: **Date:**

1.1 INVESTIGATOR CONTACT DETAILS

PRINCIPAL INVESTIGATOR(S):

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Prof	Moyna	Niall	01 7008802	01 7008888	niall.moyna@dcu.ie

OTHER INVESTIGATORS:

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Dr.	Mc Caffrey	Noel	087 2797597	01 7008888	noel.mccaffrey@dcu.ie
Dr.	Woods	Catherine	01 7008008	01 7008888	Catherine.woods@dcu.ie
Dr.	Susta	Davide	085 7781748	01 7008888	davide.susta@dcu.ie
Ms.	Hughes	Sarah	086 8673608	01 7008888	sarah.hughes3@mail.dcu.ie
Ms.	Furlong	Bronagh	086 3687961	01 7008888	brona.furlong2@mail.dcu.ie
Ms.	Gray	Cleona	01 8034478	01 8034252	cgray@mater.ie

FACULTY/DEPARTMENT/SCHOOL/ CENTRE: School of Health and Human Performance

1.2 WILL THE RESEARCH BE UNDERTAKEN ON-SITE AT DUBLIN CITY UNIVERSITY?

☒ YES ☐ NO

1.3 IS THIS PROTOCOL BEING SUBMITTED TO ANOTHER ETHICS COMMITTEE, OR HAS IT BEEN PREVIOUSLY SUBMITTED TO AN ETHICS COMMITTEE?*)*

☐ YES ☒ NO

DECLARATION BY INVESTIGATORS

The information contained herein is, to the best of my knowledge and belief, accurate. I have read the University's current research ethics guidelines, and accept responsibility for the conduct of the procedures set out in the attached application in accordance with the guidelines, the University's policy on Conflict of Interest and any other condition laid

down by the Dublin City University Research Ethics Committee or its Sub-Committees. I have attempted to identify all risks related to the research that may arise in conducting this research and acknowledge my obligations and the rights of the participants.

If there any affiliation or financial interest for researcher(s) in this research or its outcomes or any other circumstances which might represent a perceived, potential or actual conflict of interest this should be declared in accordance with Dublin City University policy on Conflicts of Interest.

I and my co-investigators or supporting staff have the appropriate qualifications, experience and facilities to conduct the research set out in the attached application and to deal with any emergencies and contingencies related to the research that may arise.

Signature(s):

***Principal investigation**:* Niall Moyna

Print name(s) in block letters: *Niall M. Moyna*

Date: *12 November 2009*

2. PROJECT OUTLINE

2.1 LAY DESCRIPTION

The purpose of this study is to assess the effect of exercise duration on vascular health in patients with cardiovascular disease (CVD).

Cardiovascular disease is the primary cause of death in Ireland. Cardiovascular disease results in damage to blood vessels due to the build up of plaque. Plaque can begin to accumulate early in life, long before CVD has been diagnosed. Rupture of these plaques can lead to a blockage of the artery resulting in a heart attack or stroke. The build up of plaque can be measured in the arteries of the neck using a simple ultrasound procedure. This involves measuring the thickness of the wall of the carotid artery (carotid intima media thickness). We will measure the carotid intima media thickness (CIMT) in patients who have known CVD. Participants will lie on a table and have an ultrasound probe placed on their neck to get a clear image of their carotid arteries to allow the vessel wall to be assessed (Figure 1).

The inner lining of blood vessels is called the endothelium. Damage to the endothelium is an early indicator of CVD. The ability of the brachial artery in the upper arm to increase in diameter following 5 min of blood flow restriction is called brachial artery reactivity (BAR) and is a measure of the health of blood vessels. We will measure BAR in patients with CVD before and 1 hour and 24 hours after 20 min and 40 min of exercise. BAR will be measured using ultrasound while the subject is lying on a bed (Figure 2).

The damaged endothelium also releases cells into the blood stream. We will take blood samples before and 1 hour and 24 hours after the exercise to measure how many of these cells are in the blood.

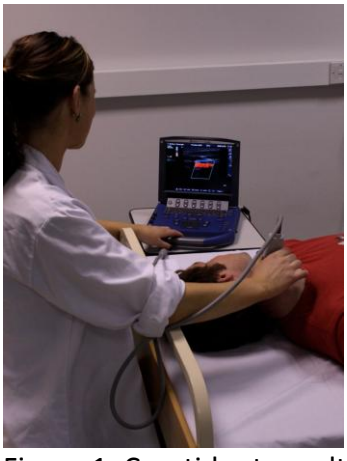


Figure 1: Carotid artery ultrasound

Figure 2: Brachial artery reactivity

AIMS OF AND JUSTIFICATION FOR THE RESEARCH

Cardiovascular disease (CVD) collectively refers to diseases of the heart and circulatory system, and typically includes coronary heart disease (CHD), stroke, and peripheral vascular diseases (e.g. atherosclerosis, deep vein thrombosis, hypertension). Ireland has the highest mortality rate from CVD in the EU (40%). The socio-economic impact of CVD to Ireland and the EU is €169 billion/year. Physical activity plays a critical role in both the primary and secondary prevention of CVD. In individuals with documented CVD, physical activity is an established, inexpensive, and generally safe secondary intervention that is associated with a 31% reduction in cardiovascular-related mortality.

The beneficial effects of physical activity are multifactorial and are related to direct and indirect protective mechanisms that impact blood vessel health (endothelium function, inflammation, vessel remodeling), blood clotting processes (platelet activation and fibrinolysis), and cardiac performance. This study will examine the effect of exercise duration on circulating endothelial cells (CECs), endothelial progenitor cells (EPCs) and microparticles (MPs) in individuals with CVD. These cells are released by the damaged endothelium, and currently little is known about the effect of acute exercise on their number and function. Carotid intima media thickness (CIMT) will be used to estimate CVD. Endothelial function will be assessed using endothelial dependent dilation (reactive hyperemia) and endothelial independent dilation (nitroglycerin).

The aim of this study is to compare the effect of exercise duration on vascular health in patients with CVD.

The purpose of this study is to

1. Examine the relation between CIMT and the number of CECs, EPCs and MPs at rest in individuals with documented CVD.
2. Examine the effect of exercise duration on blood levels of CECs, EPCs and MPs in individuals with documented CVD.
3. Examine the relation between BAR and the number of CECs, EPCs and MPs at rest and 1 hour and 24 hours after 20 min and 40 min of acute exercise in individuals with documented CVD.

2.3 PROPOSED METHOD

Overview

The study will take place in the Cardiovascular Research Unit in the School of Health and Human Performance. Subjects will visit the laboratory on 3 separate occasions. The first visit will be used to take a blood sample and measure CIMT. During the second and third visit the subjects will exercise on a treadmill for 20 min or 40 min. They will have a blood sample taken and endothelial dependent

and independent dilation assessed before, and 1 hour and 24 hours after the treadmill exercise.

Carotid Intima Media Thickness: Thickness of the carotid intima-media will be assessed using a 12.0 MHz linear-array transducer (SonoSite, MicroMaxx). Recordings will be obtained with the subject resting in a supine position, with the head turned slightly to the contralateral side. The common carotid artery, including the carotid bulb, will be visualized, and 2 longitudinal B-mode images of the left and the right common carotid arteries at end diastole will be recorded and electronically stored. Measurements of CIMT will be conducted in the 10-mm linear segment proximal to the carotid bulb at 2 plaque-free sites twice in the near wall and twice in the far wall on both sides and combined as mean CIMT. The combination of readings from the near and far walls yields the strongest association with cardiovascular disease. The artery will be scanned longitudinally without colour flow to assess the grey scale image, and with colour flow to identify difficult anatomy and delineate irregularities in plaque.

Brachial Artery Protocol: Endothelial dependent dilation will be determined in response to reactive hyperemia following 5 min of lower arm occlusion. A blood pressure cuff will be placed on the left arm for blood pressure monitoring and another on the right lower arm for occlusion. ECG leads will be attached to monitor heart rate. Subjects will rest for 10 min in a supine position. Blood pressure will be determined during the final 2 minutes of the rest period. Baseline blood flow and brachial artery diameter (SonoSite, MicroMaxx) will be recorded. The right arm blood pressure cuff will then be inflated to approximately 220-230 mmHg and maintained at that pressure for 5 minutes. The cuff will then be rapidly deflated after 5 min of occlusion. Doppler blood flow measurement will be obtained during the first minute following cuff deflation. Brachial artery diameter will be assessed at one and three minutes post occlusion. Subjects will then rest for 15 minutes to eliminate endothelium dependent effects on brachial artery diameter. After this period, endothelial independent dilation will be assessed. Baseline blood flow and brachial artery diameter will be recorded and used as a baseline prior to sublingual nitroglycerine administration. Nitroglycerin (0.4mg) will be placed under the subjects tongue. Doppler blood flow measurement will be obtained three minutes following the sublingual nitroglycerin administration and brachial artery diameter measurements will be assessed 3 and 5 minutes post nitroglycerin administration. Endothelial dependent and independent dilation assessment will be repeated at 1 hour and 24 hours after exercise. If the subject is taking Viagra they will notify Sarah Hughes. They will not be permitted to take Viagra for at least 24 hours before the administration of nitroglycerin.

Acute Exercise: Subjects will exercise for 20 min or 40 min at their preferred intensity (self regulated). The exercise order will be randomized. A 12 lead ECG

will be used to continuously monitor the electrical activity of the heart. Rating of perceived exertion will be measured every 5 min.

2.4 PARTICIPANT PROFILE

Men and women aged 40-65 yr currently enrolled in HeartSmart Phase IV community based cardiac rehabilitation programme in DCU Sport's complex will be recruited. Phase IV cardiac rehabilitation aims to assist patients with the long-term maintenance of lifestyle changes. In 2006, DCU, Beaumont Hospital, The Mater Hospital and Connolly Memorial Hospital established HeartSmart, a community-based phase IV physical activity programme. This programme is located at DCU, and is specifically designed for patients who have successfully completed hospital based phase III cardiac rehabilitation programme. Patients, who meet the inclusion criteria, are referred to the programme by the cardiac rehabilitation teams at the three partner hospitals. To date, approximately 200 individuals have enrolled in the DCU HeartSmart programme; with on average 80 people exercising in the classes each week. These individuals have diagnosed CVD and have passed a medical and physical examination that permits them to exercise. Subjects recruited will be participating in HeartSmart for a minimum of 4 wk.

Inclusion Criteria:

- Stable angina
- Prior myocardial infarction
- Undergone revascularisation procedure.
- Participants must be able to achieve 30 min of continuous walking without symptoms (cardiac chest pain/discomfort, severe breathlessness, dizziness or palpitations) or be able to undertake activities of at least 5 METS (manually washing a car, digging/turning over soil, walking/jogging a mile in less than 15 minutes) without symptoms.
- Participants must be clinically stable and in good health for a minimum of two weeks prior to beginning the study
- Participants must be attending HeartSmart for a minimum of 4 wk

Exclusion Criteria:

Potential subjects will be excluded if

- They smoke,
- Have unstable angina
- Systolic blood pressure >180 mmHg and/or diastolic blood pressure > 100 mmHg,

- Resting tachycardia
- Unstable or acute heart failure.

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED

Men and women participating in the HeartSmart cardiac rehabilitation programme in DCU Sport for a minimum of 4 wk will be informed of the research study. The study will be announced at the end of a HeartSmart class and a brief summary of the study will be provided to explain the study to the individuals and provide contact details. Following an expression of interest, potential subjects will be asked to attend a screening session in the DCU Sports Centre. They will be told by agreeing to attend the screening session they are not obligated to participate in the study. A brief presentation will be given to each potential subject to explain the nature, benefits, risks and discomforts of the study. The will be provided with a plain language statement, and the informed consent will be explained. They will be encouraged to ask questions, and any individual with doubts about participating in the study will have an opportunity to ask questions. Individuals who wish to participate in the study will have to provide written informed consent. Contact details will be provided to ensure all queries or concerns of the participant can be dealt with immediately.

2.6 PLEASE EXPLAIN WHEN, HOW, WHERE, AND TO WHOM RESULTS WILL BE DISSEMINATED, INCLUDING WHETHER PARTICIPANTS WILL BE PROVIDED WITH ANY INFORMATION AS TO THE FINDINGS OR OUTCOMES OF THE PROJECT*?*

The results will form the basis for a postgraduate thesis and will be presented at scientific meetings and published in scientific journals. The identity of individual participants will not be divulged. Group information will only be presented. Participants will be provided with a copy of their results, summarising information such as body mass index, blood pressure and cholesterol levels.

2.7 OTHER APPROVALS REQUIRED *Has permission to gain access to another location, organisation etc. been obtained? Copies of letters of approval to be provided when available.*

☐ YES ☐ NO ☒ NOT APPLICABLE

(If YES, please specify from whom and attach a copy. If NO, please explain when this will be obtained.)

2.8 HAS A SIMILAR PROPOSAL BEEN PREVIOUSLY APPROVED BY THE REC?

☐ YES ☒ NO

3. RISK AND RISK MANAGEMENT

3.1 ARE THE RISKS TO SUBJECTS AND/OR RESEARCHERS ASSOCIATED WITH YOUR PROJECT GREATER THAN THOSE ENCOUNTERED IN EVERYDAY LIFE?

☒ YES ☐ NO If YES, this proposal will be subject to full REC review

If NO, this proposal may be processed by expedited administrative review

3.2 DOES THE RESEARCH INVOLVE?

	YES	NO
• use of a questionnaire? (attach copy)?	☐	☒
• interviews (attach interview questions)?	☐	☒
• observation of participants without their knowledge?	☐	☒
• participant observation (provide details in section 2)?	☐	☒
• audio- or video-taping interviewees or events?	☐	☒
• access to personal and/or confidential data (including student, patient or client data) without the participant's specific consent?	☐	☒
• administration of any stimuli, tasks, investigations or procedures which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process?	☐	☒
• performance of any acts which might diminish the self-esteem of participants or cause them to experience embarrassment, regret or depression?	☐	☒
• investigation of participants involved in illegal activities?	☐	☒
• procedures that involve deception of participants?	☐	☒
• administration of any substance or agent?	☒	☐
• use of non-treatment of placebo control conditions?	☐	☒
• collection of body tissues or fluid samples?	☒	☐
• collection and/or testing of DNA samples?	☐	☒
• participation in a clinical trial?	☐	☒
• administration of ionising radiation to participants?	☐	☒

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES

1. Exercise carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Subjects will exercise at a submaximal intensity and will be continuously monitored using a 12 lead ECG.

2. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks. The amount of blood drawn is not harmful.

3. Assessment of endothelial dependent and independent dilation will require restriction of blood flow for 5 minutes. This may cause slight discomfort in the arm, which will go away after the blood pressure cuff in deflated. The nitroglycerin used may induce a headache that may last 5 - 10 minutes.

Alternatives to the risks: It is not possible to assess endothelial dependent and independent dilation without the use of brachial artery reactivity and the administration of nitroglycerin. It is not possible to analyse cardiovascular biomarkers without taking a sample of blood. The investigators are certified and experienced in these techniques.

3.4 ARE THERE LIKELY TO BE ANY BENEFITS (DIRECT OR INDIRECT) TO PARTICIPANTS FROM THIS RESEARCH?

☒ YES ☐ NO Participants will be provided with a copy of their results, summarising information such as body mass index, blood pressure and their cholesterol levels

3.5 ARE THERE ANY SPECIFIC RISKS TO RESEARCHERS? *(e.g. risk of infection or where research is undertaken at an off-campus location)*

☒ YES ☐ NO Working with blood and needles carries risks, however the exposure to blood and needles is minimal and the School of Health and Human Performance has standard operating procedures for the handling of biological products.

3.6 ADVERSE/UNEXPECTED OUTCOMES

The School of Health and Human Performance has the facilities to implement all aspects of this study and has an emergency plan for adverse events. In the unlikely event of a major adverse outcome, an ambulance will be called and the participant will immediately be sent to Beaumont Hospital. In the unlikely event of a minor adverse outcome, the situation will be dealt with by the attending study physician with subsequent attention at the on-campus VHI SwiftCare clinic if required.

3.7 MONITORING

The principal investigator will be involved in all aspects of the research, including participant recruitment and data collection. The research team will have weekly meetings to update on all aspects of the study. The School of Health and Human Performance has a detailed list of Standard Operating Procedures for each of the protocols in this study. All researchers, including students, must be familiar with the procedures and the Safety Statement before beginning data collection.

3.8 SUPPORT FOR PARTICIPANTS

This project does not require additional support for participants

3.9 DO YOU PROPOSE TO OFFER PAYMENTS OR INCENTIVES TO PARTICIPANTS?

☐ YES ☒ NO *(If YES, please provide further details.)*

4. INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS *(Approx. 200 words – see Guidelines)*

Prof. Moyna is an exercise physiologist and has extensive experience in cardiovascular research. Prior to joining the staff in DCU Niall was Director of the Applied Exercise physiology laboratory in the Division of Cardiology at the University of Pittsburgh Medical Centre, and Director of Clinical Research in the Division of Cardiology, Hartford Hospital, Connecticut, USA.

Ms. Sarah Hughes is a graduate student in the School of Health and Human Performance, DCU. She has extensive experience in studies involving human experimentation, and has undertaken extensive training in ultrasonography under the guidance of Cleona Gray, Chief Vascular Technologist in the Department of Vascular Surgery in the Mater Hospital, Dublin. She is a certified phlebotomist.

Dr. Noel Mc Caffrey is a physician and member of staff in the School of Health and Human Performance. He was instrumental in establishing the community based HeartSmart cardiac rehabilitation program in DCU. Dr McCaffrey is actively involved in the supervision of the HeartSmart classes. His role in the study is to screen potential subjects, supervise laboratory based exercise tests and assist with manuscript preparation.

Dr. Davide Susta is a physician and member of staff in the School of Health and Human Performance. Davide has extensive experience in clinical exercise

testing. His role in the study is to supervise laboratory based exercise tests and assist with manuscript preparation.

Dr. Catherine Woods is Head of the School of Health and Human Performance, Catherine and Dr. Noel McCaffrey established the community based HeartSmart cardiac rehabilitation program in DCU. Dr Woods is actively involved in the supervision of the HeartSmart classes. He role in the study is to assist with the recruitment of subjects and manuscript preparation.

Cleona Gray is Senior Vascular Technologist in the Vascular Lab in the Mater Hospital. Cleona has trained Sarah Hughes in ultrasonography and is currently training Brona Furlong (see below) in this technique. Cleona's involvement in the research project will continue in the form of a supervisory and troubleshooting role.

Brona Furlong has recently graduated first in her class with a 1st class honours degree in Sports Science and Health. She is currently enrolled as a PhD student in the School of Health and Human Performance. She has extensive experience in blood sampling and research involving human subjects.

5. CONFIDENTIALITY/ANONYMITY

5.1 WILL THE IDENTITY OF THE PARTICIPANTS BE PROTECTED?

☒ YES ☐ NO

IF YOU ANSWERED YES TO 5.1, PLEASE ANSWER THE FOLLOWING QUESTIONS:

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED?

Confidentiality is an important issue during data collection. Participant's identity and other personal information will not be revealed, published or used in further studies. Subjects will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password-protected file in a computer at DCU. The principal investigator, and collaborators listed on this ethics application will have access to the data.

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY: *(Have you included appropriate information in the plain language statement and consent form? See Guidelines)*

☒ YES ☐ NO *(If NO, please advise how participants will be advised.)*

6 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL *(see Guidelines)*

6.1 HOW WILL THE DATA/SAMPLES BE STORED? *(The REC recommends that all data be stored on campus)*

Stored at DCU ☒
Stored at another site ☐ **(***Please explain where and for what purpose)*

6.2 WHO WILL HAVE ACCESS TO DATA/SAMPLES?

Access by named researchers only ☒
Access by people other than named researcher(s) ☐ **(***Please explain who and for what purpose)*
Other*:* ☐ **(***Please explain**)***

6.3 IF DATA/SAMPLES ARE TO BE DISPOSED OF, PLEASE EXPLAIN HOW, WHEN AND BY WHOM THIS WILL BE DONE?

The principal investigator will be responsible for security of the data. The data will be kept in locked cabinet in the Cardiovascular Research Unit in the School of Health and Human Performance in DCU. Access to the data will only be attainable by the named researchers. Data will be kept for a minimum of five years from the date of publication of the research. Aside from the named researchers, no others will have access to the raw data. Data will be shredded by Prof. Moyna after 5 years.

.7. FUNDING

7.1 HOW IS THIS WORK BEING FUNDED?

School of Health and Human Performance/CLARITY

7.2 PROJECT GRANT NUMBER (If relevant and/or known)
NA

7.3 DOES THE PROJECT REQUIRE APPROVAL BEFORE CONSIDERATION FOR FUNDING BY A GRANTING BODY?

☐ YES ☐ NO ☒ Not Applicable

7.4 HOW WILL PARTICIPANTS BE INFORMED OF THE SOURCE OF THE FUNDING?

☐ YES ☐ NO ☒ Not Applicable

Plain Language Statement

Dublin City University

Project Title: Effect of Exercise Duration on Vascular Health in individuals with cardiovascular disease

The Research Study will take place in the School of Health and Human Performance, DCU.

The principle investigator is: Prof. Niall M. Moyna, (Tel: 7008802 Fax: 7008888) EMAIL niall.moyna@dcu.ie

I. Disease of the heart and blood vessels is called cardiovascular disease (CVD), and is the main cause of death in Ireland. CVD increases the thickness of blood vessels, and also reduces the ability of blood vessel to dilate (get bigger). We can use a simple ultrasound procedure to measure the health of your blood vessel. Tiny pieces of the damaged blood vessel wall break off into the blood and these can be measured by taking a blood sample. People who exercise regularly have less chance of getting CVD. The purpose of this study is to 1) measure the health of your blood vessels and 2) to evaluate the effect of 20 min or 40 min of exercise on your blood vessels. You will be allowed to take part in the study if you meet the entry criteria and sign the informed consent.

II. If you agree to take part in the study you will be asked to make 3 visits to the Cardiovascular Research Unit in the School of Health and Human Performance in DCU. On your first visit you, that will last approximately 1 hour, you will have a blood sample taken and an ultrasound picture will be taken of a blood vessel in your neck. During the second and third visit you will walk on a treadmill for 20 min or 40 min. You will be allowed to select the treadmill speed that you prefer. About 2 tablespoons of blood will be taken before you exercise and 1 hour and 24 hours after you exercise. The health of a blood vessel in your arm will be measured at the same times that blood samples are taken. This will be done by using an ultrasound to take an image of your blood vessel and involves blocking the blood flow to your arm for 5 minutes using a blood pressure cuff and taking a nitroglycerin tablet under your tongue. The second and third visit will last approximately 2 hours. You will fast for at least 12 hours and will not be allowed to exercise for at least 24 hours before these visits.

III. 1. Exercise carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients.

2. Drawing blood may cause a slight pain where the needle is inserted and may leave a bruise. A person trained to take blood will be used to decrease these risks.

3. Taking an ultrasound image of your arm requires blocking the blood flow to your arm for 5 minutes using a blood pressure cuff. This may cause slight discomfort in your arm, which will go away after the blood pressure cuff in deflated. The nitroglycerin used in this study may cause a headache that could last 5 to 10 min.

IV. Your confidentiality will be guarded. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate project and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. You will be assigned an ID number under which all personal information will be stored in the secure locked filing cabinet and saved in a password protected file in a computer at DCU. You need to be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

VI. Involvement in this study is completely voluntary. You may withdraw from the Research Study at any point.

VIII. If you have concerns about this study and wish to contact an independent person, please contact: The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000

Informed Consent

Dublin City University

Project Title **Effect of Exercise Duration on Vascular Health in Patients with cardiovascular disease**

Principle Investigator Prof. Niall M. Moyna

Introduction to this study

Disease of the heart and blood vessels is called cardiovascular disease (CVD), and is the main cause of death in Ireland. CVD can be indentified by measuring the thickness of the carotid artery in your neck using ultrasound. CVD damages blood vessels and reduces their ability to dilate (get bigger). We can also use a simple ultrasound procedure to measure how much the blood vessels can dilate. Damaged blood vessels release cells into the blood which can be measured by taking a blood sample. Regular physical activity improves the health of blood vessels, and also has a beneficial effect on many of the risk factors for CVD. This study will measure the health of your blood vessels. In addition we will evaluate how exercise of different durations affects your blood vessels.

Participants Requirements

1. I will visit the Cardiovascular Research Unit in the School of Health and Human Performance DCU on 3 separate days. During my first visit (which lasts approximately I hour), I will have a blood sample taken and complete a medical history questionnaire that will include a list of all the medications I am currently taking. An ultrasound measurement will be taken of my neck to measure my carotid artery (See Figure 1). This test is painless and lasts about 15 min.

2 During my second and third visit I will walk/jog on a treadmill for 20 min or 40 min. I will be allowed to select the treadmill speed. A researcher will toss a coin to determine which test I do first. A blood sample will be taken and the health of the arteries in my arm will be measured before and 1 hour and 24 hours after I finish the exercise. The total amount of blood drawn will be 2 tablespoons (30 cc). I will have electrodes placed on my chest to allow the researchers observe the electrical activity of my heart during exercise. The second and third visit will last approximately 2 hours. I will fast for at least 12 hours and will not exercise for at least 24 hours before my second and third visit. If I am taking Viagra I will notify Sarah Hughes. I will not take Viagra for at least 24 hours before these two visits.

3. The test the health of the arteries in my arms I will lie on my back, and an ultrasound will be placed on my upper arm to create an image of my artery. After the first image is recorded, a blood pressure cuff will be inflated on my forearm to block blood flow for five minutes. This may be uncomfortable. The

cuff will be released and the images of my arteries repeated. I will rest for 15 minutes and then have a nitroglycerin pill placed under my tongue. The nitroglycerin will cause my arm arteries to enlarge and how much they enlarge will again be documented by taking a third set of pictures.

Potential risks to participants from involvement in the Research Study

1. Exercise carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients.

2. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks.

3. The amount of blood drawn is not harmful, however, if I have a history of anemia, I should inform the investigator.

4. The pictures of my arm arteries require blocking the blood flow to my arm for 5 minutes. This may cause slight discomfort in the arm, which will go away after the blood pressure cuff in deflated. The nitroglycerin used in this study may induce a headache that could last 5 to 10 min.

Benefits (direct or indirect) to participants from involvement in the Research Study

After completing the study I will be provided with a copy of my results, summarising information such as my body mass index, blood pressure and cholesterol levels. There are no other direct benefits to me.

Participant – please complete the following (circle Yes or No for each question)

Have you read or had read to you the Plain Language Statement?	Yes ☐ No ☐
Do you understand the information provided?	Yes ☐ No ☐
Have you had an opportunity to ask questions and discuss this study?	Yes ☐ No ☐
Have you received satisfactory answers to all your questions?	Yes ☐ No ☐

Advice as to arrangements to be made to protect confidentiality of data, including that confidentiality of information provided is subject to legal limitations.

Your identity and other personal information will not be revealed, published or used in further studies. You will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password protected file in a computer at DCU. The named investigators will have access to the data. Data will be shredded after 5 years by Prof. Moyna.

Confidentiality is insured, but you must be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

If you are in a dependent relationship with any of the researchers their involvement in the project will not affect ongoing assessment/grades/management or treatment of health at DCU.

Signature:

I have read and understood the information in this form. The researchers have answered my questions and concerns, and I have a copy of this consent form. Therefore, I (print name) _____ consent to take part in this research project entitled Effect of Exercise Duration on Vascular Health in Patients with cardiovascular disease.

Participants Signature: _____

Name in Block Capitals _____

Witness: _____

Date: _____

Appendix D

Submission to Ethics Committee

Dublin City University

RESEARCH ETHICS COMMITTEE

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING HUMAN PARTICIPANTS

Application No. *(office use only)* DCUREC/2010/ ☐☐☐

Period of Approval *(office use only)* ...../...../.....to...../...../....

This application form is to be used by researchers seeking ethics approval for individual projects and studies. The **signed original and an electronic copy** of your completed application must be submitted to the DCU Research Ethics Committee.

NB - The hard copy must be signed by the PI. The electronic copy should consist of <u>one file only</u>, which incorporates all supplementary documentation. The completed application must be proofread and spellchecked before submission to the REC. All sections of the application form should be completed. Applications which do not adhere to these requirements will not be accepted for review and will be returned directly to the applicant.

Applications must be completed on the form; answers in the form of attachments will not be accepted, except where indicated. No handwritten applications will be accepted. **Research must <u>not</u> commence until written approval has been received from the Research Ethics Committee.**

PROJECT TITLE	Comparisons of two Exercise Training Programmes on Vascular Health in Patients with Cardiovascular Disease
PRINCIPAL INVESTIGATOR(S)	Prof. Niall M. Moyna

Please confirm that **<u>all</u>** supplementary information is included in your application (in both signed original and electronic copy). If questionnaire or interview questions are

submitted in draft form, a copy of the final documentation must be submitted for final approval when available.

	INCLUDED		NOT APPLICABLE
Bibliography	☐		☒
Recruitment advertisement	☐		☐
Plain language statement/Information Statement	☒		☐
Informed Consent form	☒		☐
Evidence of external approvals related to the research	☐		☒
Questionnaire	☐ draft	☐ final	☒
Interview Schedule	☐ draft	☐ final	☒
Debriefing material	☐		☒
Other	☐		☒

Please note:

3. Any amendments to the original approved proposal must receive prior REC approval.
4. As a condition of approval investigators are required to document and report immediately to the Secretary of the Research Ethics Committee any adverse events, any issues which might negatively impact on the conduct of the research and/or any complaint from a participant relating to their participation in the study

Please submit the **signed original, plus the electronic copy** of your completed application to: Ms. Fiona Brennan, Research Officer, Office of the Vice-President for Research (fiona.brennan@dcu.ie, Ph. 01-7007816)

. ADMINISTRATIVE DETAILS

THIS PROJECT IS: *(tick as many as apply)*

☒ Research Project
☐ Practical Class
☐ Student Research Project *(please give details)*
☐ Resear chMast ers
☐ Taught Masters
☒ PhD
☐ Undergraduate
☐ Funded Consultancy
☐ Clinical Trial
☐ Other - *Please Describe:*

Project Start Date: 01/02/11 **Project End Date** **01/06/11**

1.1 INVESTIGATOR CONTACT DETAILS *(see Guidelines)*

PRINCIPAL INVESTIGATOR(S):

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Prof	Moyna	Niall	01 7008802	01 7008888	niall.moyna@dcu.ie

OTHER INVESTIGATORS:

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Dr.	Mc Caffrey	Noel	087 2797597	01 7008888	noel.mccaffrey@dcu.ie
Dr.	Woods	Catherine	01 7008008	01 7008888	catherine.woods@dcu.ie
Ms.	Hughes	Sarah	086 8673608	01 7008888	sarah.hughes3@mail.dcu.ie
Ms.	Furlong	Bróna	086 3687961	01 7008888	brona.furlong2@mail.dcu.ie
Ms.	Gray	Cleona	01 8034478	01 8034252	cgray@mater.ie

FACULTY/DEPARTMENT/SCHOOL/ CENTRE:

(NB – if Nursing, please note all students including PhD's must attach the letter from the Nursing Ethics Advisory Committee to this application)

1.2 WILL THE RESEARCH BE UNDERTAKEN ON-SITE AT DUBLIN CITY UNIVERSITY?

☒ YES ☐ NO *(If NO, give details of off-campus location.)*

1.3 IS THIS PROTOCOL BEING SUBMITTED TO ANOTHER ETHICS COMMITTEE, OR HAS IT BEEN PREVIOUSLY SUBMITTED TO AN ETHICS COMMITTEE?*)*

☐ YES ☒ NO *(If YES, please provide details and copies of approval(s) received etc.)*

DECLARATION BY INVESTIGATORS

The information contained herein is, to the best of my knowledge and belief, accurate. I have read the University's current research ethics guidelines, and accept responsibility for the conduct of the procedures set out in the attached application in accordance with the guidelines, the University's policy on Conflict of Interest and any other condition laid down by the Dublin City University Research Ethics Committee or its Sub-Committees. I have attempted to identify all risks related to the research that may arise in conducting this research and acknowledge my obligations and the rights of the participants.

If there any affiliation or financial interest for researcher(s) in this research or its outcomes or any other circumstances which might represent a perceived, potential or actual conflict of interest this should be declared in accordance with Dublin City University policy on Conflicts of Interest.

I and my co-investigators or supporting staff have the appropriate qualifications, experience and facilities to conduct the research set out in the attached application and to deal with any emergencies and contingencies related to the research that may arise.

Signature(s):

Principal investigator(s): Niall Moyna

Print name(s) in block letters: *Niall M. Moyna*

Date: 2 December 2010

2. PROJECT OUTLINE

4.2 LAY DESCRIPTION

Cardiac Rehabilitation is a structured exercise and education programme designed to help patients recover from their heart event. It is a multi-disciplinary approach to improve short-term recovery and to promote long-term changes in lifestyle. Patients in Ireland currently enter an 8-10 week hospital-based cardiac rehabilitation programme (Phase III) after discharge from hospital. This involves a gradual increase in physical activity, continuation of risk-factor modifications and development of maintenance programs. This is followed by a community based Phase IV Cardiac Rehabilitation that aims to assist patients who have successfully completed a hospital based phase III programme.

In 2006, DCU established a community-based phase IV Cardiac Rehabilitation programme (HeartSmart) in collaboration with Beaumont Hospital, The Mater Misericordiae University Hospital and Connolly Memorial Hospital. The

HeartSmart programme is located in the DCU Sport Centre. Patients, who meet the inclusion criteria, are referred to the programme by the cardiac rehabilitation teams at the three partner hospitals. Individuals normally attend 2 classes per week. The classes are normally 60 minutes in duration and involve primarily aerobic exercise to stress the cardiovascular system and some form of resistance training. Participants exercise in small groups and rotate between the various exercises during a class. Substantial improvements in physical fitness, psychological well being and quality of life are evident after 3-6 months. Further improvements in fitness levels and cardiovascular health may require participants to exercise at higher intensities under supervision.

The purpose of this study is to compare the effect of a traditional 4 week community based cardiac rehabilitation exercise programme (HeartSmart) and an individualized 4 week high intensity intermittent exercise programme on vascular health in individuals who have participated in HeartSmart for at least 6 months. Aerobic fitness and the health of the arteries (Figure 1 and 2) will be assessed before and at the end of the 4 weeks. In addition blood samples will be taken at the same time points. The electrical activity of the heart will be continuously monitored during each class.

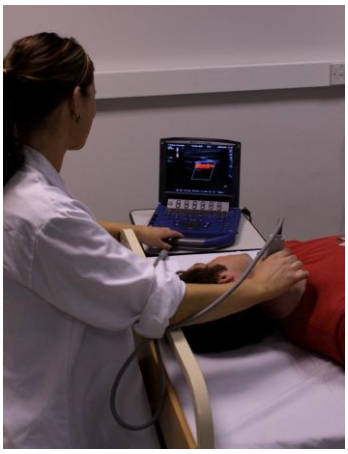


Figure 1: Carotid artery ultrasound

Figure 2: Brachial artery reactivity

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH

Cardiovascular disease (CVD) collectively refers to diseases of the heart and circulatory system, and typically includes coronary heart disease (CHD), stroke, and peripheral vascular diseases (e.g. atherosclerosis, deep vein thrombosis, hypertension). Ireland has the highest mortality rate from CVD in the EU (40%). The socio-economic impact of CVD to Ireland and the EU is €169 billion/year. Physical activity plays a critical role in both the primary and secondary prevention of CVD. In individuals with documented CVD, physical activity is an established, inexpensive, and generally safe secondary intervention that is associated with a 31% reduction in cardiovascular-related mortality. The

beneficial effects of physical activity are multifactorial and are related to direct and indirect protective mechanisms that impact blood vessel health (endothelium function, inflammation, vessel remodeling), blood clotting processes (platelet activation and fibrinolysis), and cardiac performance. Substantial improvements in physical fitness, psychological well being and quality of life are evident after 3-6 months. Further improvements in fitness levels and cardiovascular health may require participants to exercise at higher intensities under supervision.

This study will compare the effect of a 4 week community-based exercise programme (HeartSmart) and an individualized 4 week high intensity intermittent exercise programme on aerobic fitness and circulating endothelial cells (CEC), endothelial progenitor cells (EPC) and microparticles (MP) in individuals with CVD. CEC, EPC and MP are released by a damaged endothelium (innermost layer of blood vessel walls), and currently little is known about the effect of exercise on their number and function. Carotid intima media thickness (CIMT) will be used to estimate CVD. Endothelial function will be assessed using flow mediated dilation (endothelial dependent) and endothelial independent dilation (this involves the administration of vasodilator, glyceryl trinitrate).

The aim of this study is to compare the effect of a traditional 4 week community based exercise programme (HeartSmart) and an individualized 4 week high intensity intermittent exercise programme on vascular health in patients with CVD who have participated in a community-based phase IV Cardiac Rehabilitation programme for at least 6 months.

The purpose of this study is to

1. Compare the effect of a traditional 4 week community-based cardiac rehabilitation exercise programme and an individualized 4 week high intensity intermittent exercise programme on vascular health in patients with CVD.

2. Compare the effect of a traditional 4 week community based cardiac rehabilitation exercise programme and an individualized 4 week high intensity intermittent exercise programme on aerobic fitness in patients with CVD.

3. Compare the effect of a traditional 4 week community based cardiac rehabilitation exercise programme and an individualized 4 week high intensity intermittent exercise programme on CECs, EPCs and MPs in patients with CVD.

2.3 PROPOSED METHOD

Overview

Subjects participating in HeartSmart for more than 6 months will be recruited. The study will take place in the Vascular Research Unit in the School of Health and Human Performance. Subjects will be randomly assigned to a traditional cardiac rehabilitation (TCR) group or to an individually tailored high intensity intermittent exercise (HIT) training group. Subjects in the TCR will continue to attend HeartSmart twice a week for the duration of the 4 week study. Subjects in training group will attend 2 supervised exercise sessions per week in the Vascular Research Unit. Subjects in the training group will wear electrodes on their chest to monitor the electrical activity of their heart throughout the exercise sessions.

Subjects will visit the laboratory for testing sessions on 2 occasions before and 2 occasions after the 4 week exercise programme. The first testing session will be used to further explain the requirements of the study, obtain informed consent, take a blood sample, measure CIMT and to assess endothelial function. During the second testing session aerobic fitness ($\dot{V}O_{2peak}$) will be measured. At the end of the 4 week programme the third testing session will be a repeat of the tests carried out during testing session 1, and similarly testing session 4 will consist of the same tests as carried out during testing session 2. .

Traditional Cardiac Rehab Group: Subjects will attend HeartSmart twice per week. They will wear a heart rate monitor during these classes each week.

Training Group: Subjects will attend a high intensity intermittent exercise programme in the Vascular Research Unit twice a week. They will wear an ECG during every class. These exercise sessions will be supervised and include a 15 min warm-up. The sessions will involve individually prescribed programmes based on each subjects symptom-limited graded exercise test. Subjects will perform bouts of high intensity exercise at 60-100% HR_{max} interspersed with periods of recovery. The total duration of the high intensity intermittent exercise will increase from 5 min at week 1 up to 15 min at week 4. Each session will take place on a treadmill. Subjects will finish with a 10 min cool down and stretch. Before and after the final interval exercise session (Session 8) a blood sample will be drawn and endothelial function will be assessed.

Testing Session 1: Approximately 1.5 hour in duration. Subjects will read and sign the informed consent, have their CIMT measured and their endothelial function assessed. Approximately 25 ml of blood will be taken from a vein in the arm.

Testing Session 2: Approximately 1 hour in duration. Aerobic fitness level ($\dot{V}O_{2peak}$) will be assessed and 10 μL of blood will be taken using a lancing device to prick the earlobe.

Testing Session 3: Approximately 1.5 hour in duration. The testing procedures that were carried out during testing session 1 will be repeated.

Testing Session 4: Approximately 1 hour in duration. The testing procedures that were carried out during testing session 2 will be repeated.

Carotid Intima Media Thickness (CIMT): Thickness of the carotid intima-media will be assessed using a 12.0 MHz linear-array transducer (SonoSite, MicroMaxx). Recordings will be obtained with the subject resting in a supine position, with the head turned slightly to the contralateral side. The common carotid artery, including the carotid bulb, will be visualized, and 2 longitudinal B-mode images of the left and the right common carotid arteries at end diastole will be recorded and electronically stored. Measurements of CIMT will be conducted in the 10-mm linear segment proximal to the carotid bulb at 2 plaque-free sites twice in the near wall and twice in the far wall on both sides and combined as mean CIMT. The combination of readings from the near and far walls yields the strongest association with cardiovascular disease. The artery will be scanned longitudinally without colour flow to assess the grey scale image, and with colour flow to identify difficult anatomy and delineate irregularities in plaque.

Brachial Artery Reactivity (BAR): Endothelial dependent dilation will be determined in response to reactive hyperemia following 5 min of lower arm occlusion. A blood pressure cuff will be placed on the left arm for blood pressure monitoring and another on the right lower arm for occlusion. ECG leads will be attached to monitor heart rate. Subjects will rest for 10 min in a supine position. Blood pressure will be determined during the final 2 minutes of the rest period. Baseline blood flow and brachial artery diameter (SonoSite, MicroMaxx) will be recorded. The right arm blood pressure cuff will then be inflated to approximately 220-230 mmHg and maintained at that pressure for 5 minutes. The cuff will then be rapidly deflated after 5 min of occlusion. Doppler blood flow measurement will be obtained during the first minute following cuff deflation. Brachial artery diameter will be assessed at one and three minutes post occlusion. Subjects will then rest for 15 minutes to eliminate endothelium dependent effects on brachial artery diameter. After this period, endothelial independent dilation will be assessed. Baseline blood flow and brachial artery diameter will be recorded and used as a baseline prior to sublingual glyceryl trinitrate administration. Glyceryl trinitrate (0.4mg) will be placed under the subjects tongue. Doppler blood flow measurement will be obtained three minutes following the sublingual glyceryl trinitrate administration and brachial artery diameter measurements will be assessed 3 and 5 minutes post glyceryl trinitrate administration.

Peak Aerobic Capacity ($\dot{V}O_{2peak}$) Assessment: Peak aerobic capacity will be determined on a treadmill using open circuit spirometry. During this assessment subjects will be fitted with a mouthpiece or facemask. ECG will be continuously monitored using a 12 lead ECG, and a physician will be present.

2.4 PARTICIPANT PROFILE

A total of 20 men aged 40-65 yr, enrolled in HeartSmart in DCU Sport's complex for at least 6 months, will be recruited. The subjects will be referred from Beaumont Hospital, The Mater Hospital and Connolly Memorial Hospital phase III cardiac rehabilitation programme. Subjects will have documented CVD, will have passed a medical and physical examination that permits them to exercise. Each participant's cardiologist will be informed of his/her participation in the study

Inclusion Criteria:

- Male
- Involved in HeartSmart programme for more than 6 months
- Referred from Beaumont Hospital, The Mater Hospital and Connolly Memorial Hospital phase III cardiac rehabilitation programme
- Stable angina
- Able to achieve 30 min of continuous walking without symptoms (cardiac chest pain/discomfort, severe breathlessness, dizziness or palpitations) or be able to undertake activities of at least 5 METS (manually washing a car, digging/turning over soil, walking/jogging a mile in less than 15 minutes) without symptoms
- Clinically stable and in good health for a minimum of 2 weeks prior to beginning the study

Exclusion Criteria:

Potential subjects will be excluded if

- Current smoker
- Unstable angina
- Systolic blood pressure >180 mmHg and/or diastolic blood pressure > 100 mmHg
- Resting tachycardia
- Unstable or acute heart failure
- Ventricular arrhythmias during maximal exercise test
- Prolonged ST-segment depression (> 2mm) during maximal exercise test
- Severe angina during maximal exercise test

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED

Men enrolled in the HeartSmart cardiac rehabilitation programme in DCU Sport for at least 6 months will be informed of the research study. A brief summary of

the study and contact details will be provided to patients enrolling in HeartSmart. Following an expression of interest, potential subjects will be asked to visit the Vascular Research Laboratory in the School of Health and Human Performance. They will be told that by agreeing to visit the laboratory they are not obligated to participate in the study. The nature, benefits, risks and discomforts of the study will be explained. In addition, they will be provided with a plain language statement, and the informed consent will be explained. They will be encouraged to ask questions. Individuals who wish to participate in the study will have to provide written informed consent.

2.6 PLEASE EXPLAIN WHEN, HOW, WHERE, AND TO WHOM RESULTS WILL BE DISSEMINATED, INCLUDING WHETHER PARTICIPANTS WILL BE PROVIDED WITH ANY INFORMATION AS TO THE FINDINGS OR OUTCOMES OF THE PROJECT*?*

The results will form the basis for a postgraduate thesis and will be presented at scientific meetings and published in scientific journals. The identity of individual participants will not be divulged. Group information will only be presented. Participants will be provided with a copy of their results, summarising information such as body mass index, blood pressure and cholesterol levels.

2.7 OTHER APPROVALS REQUIRED *Has permission to gain access to another location, organisation etc. been obtained? Copies of letters of approval to be provided when available.*

YES ☐ NO ☒ NOT APPLICABLE ☐

(If YES, please specify from whom and attach a copy. If NO, please explain when this will be obtained.)

2.8 HAS A SIMILAR PROPOSAL BEEN PREVIOUSLY APPROVED BY THE REC?

☒ YES ☐ NO

(If YES, please state both the REC Application Number and Project Title)

REC/2010/49 **EFFECT OF AN 8 WEEK COMMUNITY BASED CARDIAC EXERCISE REHABILIATION PROGRAMME ON VASCULAR HEALTH IN PATIENTS WITH CARDIOVASCULAR DISEASE**

3. RISK AND RISK MANAGEMENT

3.1 ARE THE RISKS TO SUBJECTS AND/OR RESEARCHERS ASSOCIATED WITH YOUR PROJECT GREATER THAN THOSE ENCOUNTERED IN EVERYDAY LIFE?

☒ YES ☐ NO If YES, this proposal will be subject to full REC review

If NO, this proposal may be processed by expedited administrative review

---

3.2 DOES THE RESEARCH INVOLVE:

	YES	N
• use of a questionnaire? (attach copy)?	☐	☒
• interviews (attach interview questions)?	☐	☒
• observation of participants without their knowledge?	☐	☒
• participant observation (provide details in section 2)?	☐	☒
• audio- or video-taping interviewees or events?	☐	☒
• access to personal and/or confidential data (including student, patient or client data) without the participant's specific consent?	☐	☒
• administration of any stimuli, tasks, investigations or procedures which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process?	☐	☒
• performance of any acts which might diminish the self-esteem of participants or cause them to experience embarrassment, regret or depression?	☐	☒
• investigation of participants involved in illegal activities?	☐	☒
• procedures that involve deception of participants?	☐	☒
• administration of any substance or agent?	☒	☐
• use of non-treatment of placebo control conditions?	☐	☒
• collection of body tissues or fluid samples?	☒	☐
• collection and/or testing of DNA samples?	☐	☒
• participation in a clinical trial?	☐	☒
• administration of ionising radiation to participants?	☐	☒

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES

1. Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Subjects will be continuously monitored using a 12 lead ECG and a physician will be present.

2. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks. The amount of blood drawn is not harmful.

3. Assessment of endothelial dependent and independent dilation will require restriction of blood flow for 5 minutes. This may cause slight discomfort in the arm, which will go away after the blood pressure cuff in deflated. The glyceryl trinitrate used may induce a headache that may last 5 - 10 minutes.

Alternatives to the risks

It is not possible to assess endothelial dependent and independent dilation without the use of brachial artery reactivity and the administration of glyceryl trinitrate. Subjects will be informed that they must refrain from Viagra, PDE5 inhibitors and all other sexual enhancers (herbal or otherwise) 48 hours prior to participation in the study.

Analysis of cardiovascular biomarkers cannot be undertaken without a sample of blood. The investigators are certified and experienced in phlebotomy and ultrasonography.

3.4 ARE THERE LIKELY TO BE ANY BENEFITS (DIRECT OR INDIRECT) TO PARTICIPANTS FROM THIS RESEARCH?

☒ YES ☐ NO Participants will be provided with a copy of their results, summarising information such as blood pressure and fitness levels

3.5 ARE THERE ANY SPECIFIC RISKS TO RESEARCHERS? *(e.g. risk of infection or where research is undertaken at an off-campus location)*

☒ YES ☐ NO Working with blood and needles carries risks, however the exposure to blood and needles is minimal and the School of Health and Human Performance has standard operating procedures for the handling of biological products.

3.6 ADVERSE/UNEXPECTED OUTCOMES

The School of Health and Human Performance has the facilities to implement all aspects of this study and has an emergency plan for adverse events. In the unlikely event of a major adverse outcome, an ambulance will be called and the participant will immediately be sent to Beaumont Hospital. In the unlikely event of a minor adverse outcome, the situation will be dealt with by the attending study physician with subsequent attention at the Mater Smithfield Rapid Injury Clinic if required.

3.7 MONITORING

The principal investigator will be involved in all aspects of the research, including participant recruitment and data collection. The research team will have weekly meetings to update on all aspects of the study. The School of Health and Human Performance has a detailed list of Standard Operating Procedures for each of the protocols in this study. All researchers, including students, must be familiar with the procedures and the Safety Statement before beginning data collection.

3.8 SUPPORT FOR PARTICIPANTS

This project does not require additional support for participants

3.9 DO YOU PROPOSE TO OFFER PAYMENTS OR INCENTIVES TO PARTICIPANTS?

☐ YES ☒ NO *(If YES, please provide further details.)*

4. INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS *(Approx. 200 words – see Guidelines)*

Prof. Moyna is an exercise physiologist and has extensive experience in cardiovascular research.

Dr. Noel McCaffrey is a physician with extensive experience in exercise related research

Ms. Sarah Hughes is a graduate student in the School of Health and Human Performance, DCU. She has extensive experience in studies involving human experimentation, and has undertaken extensive training in ultrasonography under the guidance of Cleona Gray, Chief Vascular Technologist in the Department of Vascular Surgery in the Mater Hospital, Dublin. Sarah is also a certified phlebotomist.

Dr. Catherine Woods is Head of the School of Health and Human Performance, Catherine and Dr. Noel McCaffrey established the community based HeartSmart cardiac rehabilitation program in DCU. Dr Woods is actively involved in the co-ordination of the HeartSmart classes.

Brona Furlong has recently graduated first in her class with a 1st class honours degree in Sports Science and Health. She is currently enrolled as a PhD student in the School of Health and Human Performance. She has extensive experience

in blood sampling and research involving human subjects. Brona will assist with data collection and supervising classes.

Cleona Gray is Senior Vascular Technologist in the Vascular Lab in the Mater Hospital. Cleona has trained Sarah Hughes and Brona Furlong in Ultrasonography. Cleona's involvement in the research project will continue in the form of a supervisory and troubleshooting role. She will provide technical support in performing carotid and brachial ultrasound scanning

5. CONFIDENTIALITY/ANONYMITY

5.1 WILL THE IDENTITY OF THE PARTICIPANTS BE PROTECTED?

☒ YES ☐ NO *(If NO, please explain)*

IF YOU ANSWERED YES TO 5.1, PLEASE ANSWER THE FOLLOWING QUESTIONS:

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED?

Confidentiality is an important issue during data collection. Participant's identity and other personal information will not be revealed, published or used in further studies. Subjects will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password-protected file in a computer at DCU. The principal investigator, and collaborators listed on this ethics application will have access to the data.

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY: *(Have you included appropriate information in the plain language statement and consent form? See Guidelines)*

☒ YES ☐ NO *(If NO, please advise how participants will be advised.)*

6 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL *(see Guidelines)*

6.1 HOW WILL THE DATA/SAMPLES BE STORED? *(The REC recommends that all data be stored on campus)*

Stored at DCU ☒
Stored at another site ☐ **(***Please explain where and for what purpose)*

6.2 WHO WILL HAVE ACCESS TO DATA/SAMPLES?

Access by named researchers only ☒

Access by people other than named researcher(s) ☐ **(***Please explain who and for what purpose)*

Other *:* ☐ **(***Please explain)*

6.3 IF DATA/SAMPLES ARE TO BE DISPOSED OF, PLEASE EXPLAIN HOW, WHEN AND BY WHOM THIS WILL BE DONE?

The principal investigator will be responsible for security of the data. The data will be kept in locked cabinet in the Cardiovascular Research Unit in the School of Health and Human Performance in DCU. Access to the data will only be attainable by the named researchers. Data will be kept for a minimum of five years from the date of publication of the research. Aside from the named researchers, no others will have access to the raw data. Data will be shredded by Prof. Moyna after 5 years.

7. FUNDING

7.1 HOW IS THIS WORK BEING FUNDED?

School of Health and Human Performance/CLARITY – SFI

7.2 PROJECT GRANT NUMBER (If relevant and/or known)

P07625 - SFI 07/CE/I1147

7.3 DOES THE PROJECT REQUIRE APPROVAL BEFORE CONSIDERATION FOR FUNDING BY A GRANTING BODY?

☐ YES ☒ NO

7.5 HOW WILL PARTICIPANTS BE INFORMED OF THE SOURCE OF THE FUNDING?

Participants will be informed of the source of funding in the Plain Language Statement.

7.5 DO ANY OF THE RESEARCHERS, SUPERVISORS OR FUNDERS OF THIS PROJECT HAVE A PERSONAL, FINANCIAL OR COMMERCIAL INTEREST IN ITS OUTCOME THAT MIGHT COMPROMISE THE INDEPENDENCE AND INTEGRITY OF THE RESEARCH, OR BIAS THE CONDUCT OR RESULTS OF THE RESEARCH, OR UNDULY DELAY OR OTHERWISE AFFECT THEIR PUBLICATION?

Plain Language Statement

Dublin City University

Project Title: Comparisons of two Exercise Training Programmes on Vascular Health in Patients with Cardiovascular Disease

The Research Study will take place in the School of Health and Human Performance, DCU.

The principal investigator is: Prof. Niall M. Moyna, (Tel: 7008802, Fax: 7008888, Email: niall.moyna@dcu.ie)

III. Disease of the heart and blood vessels is called cardiovascular disease (CVD), and is the main cause of death in Ireland. CVD increases the thickness of blood vessels, and also reduces the ability of blood vessel to dilate (get bigger). We can use a simple ultrasound procedure to measure the health of your blood vessel. Tiny pieces of the damaged blood vessel wall break off into the blood and these can be measured by taking a blood sample. People who exercise regularly have less chance of getting CVD. People who have CVD also can reduce their risk of having a heart attack or stroke by exercising regularly). The purpose of this study is to compare the effect of a traditional cardiac rehabilitation exercise programme and the effect of a high intensity exercise programme on your fitness level and the health of your blood vessels over the course of 4 weeks. You will be allowed to take part in the study if you meet the entry criteria and sign the informed consent.

IV. If you agree to take part in the study you will attend exercise sessions in the Vascular Research Unit in the School of Health and Human Performance in DCU twice a week for 4 weeks. In addition to this you will have 2 testing sessions carried out before you begin this training programme and 2 at the end (Testing Session 1, 2, 3 and 4). You will not be allowed to exercise for at least 24 hours before each of these Testing Sessions.

V. Before the study begins you will be assigned by chance to either a traditional cardiac rehabilitation exercise group, which is called the Control Group or an Interval Training Group.

Control Group: If you are assigned to the traditional cardiac rehabilitation group, you will be asked to visit the School of Health and Human Performance in DCU on 2 occasions before the 4 weeks begin and on 2 occasions at the end of the 4 weeks. You will continue participating

in the HeartSmart classes two days per week. During each class you will wear a strap around your chest to monitor your heart rate.

Intermittent Exercise Group: If you are assigned to the interval training group, you will visit to the Vascular Research Unit in the School of Health and Human Performance in DCU for the first 2 Testing Sessions before the 4 week programme begins and for the second 2 Testing Sessions at the end of the 8 weeks. You will attend exercise sessions in the Vascular Research Unit, DCU 2s days per week for 4 weeks. During each exercise session you will wear small pads (ECG) and a strap around your chest to monitor your heart rate.

Testing Session 1:

- The first Testing Session will take place in the Vascular Research Unit in the School of Health and Human Performance in DCU and will last approximately 1.5 hours
- You will sign an informed consent and an ultrasound picture will be taken of a blood vessel in your neck
- You will have a blood sample taken (about 2 tablespoons)
- The health of a blood vessel in your arm will be measured using an ultrasound to take an image of your blood vessel. This will involve blocking the blood flow to your arm for 5 minutes using a blood pressure cuff and taking one spray of glyceryl trinitrate under your tongue. You will be fasting before this test
- If you are taking Viagra, other PDE5 inhibitors or any herbal sexual enhancers you will not take any of these for at least 48 hours before this testing visit

Testing Session 2

- This session will last about 1 hour
- Your fitness level will be measured
- During your fitness assessment you will wear a mouthpiece and exercise on a treadmill
- You will wear small pads on your chest (ECG) to monitor your heart throughout exercise
- A small droplet of blood will be taken from the earlobe before and after the exercise test

Testing Session 3

- The third Testing Session will take place at the end of the 4 weeks again in the Vascular Research Unit in DCU and will last approximately 1.5 hour
- You will undergo the same tests as you did during Testing Session 1
- If you are taking Viagra, other PDE5 inhibitors or any herbal sexual enhancers you will not take any of these for at least 48 hours before this testing visit

Testing Session 4

- The fourth testing session will also take place at the end of the 4 weeks again in the Vascular Research Unit in DCU and will last approximately 1 hour
- Your fitness will be assessed in the same way as Testing Session 2

Interval Exercise sessions

- The Interval Exercise Sessions will be monitored in the Vascular Research Unit
- You will wear small pads (ECG) and a strap around on your chest to monitor your heart throughout exercise
- During the second and eighth interval sessions you will wear a mouthpiece or a facemask during the exercise
- You will have the health of your arteries assessed and blood samples drawn before and after the final (eighth) session
- If you are taking Viagra, other PDE5 inhibitors or any herbal sexual enhancers you will not take any of these for at least 48 hours before interval session 8.
- You will fast for at least 10 hours prior to interval session 8.

III. 1. Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Your heart will be continuously monitored using a 12 lead electrocardiogram (ECG) and a physician will be present. A 12 lead ECG is a special machine that takes 12 different views of your heart (like photographs) while you are exercising. You will have electrodes placed on your chest to allow the researchers observe the electrical activity of your heart during exercise.

2. Drawing blood may cause a slight pain where the needle is inserted and may leave a bruise. A person trained to take blood will be used to decrease these risks.

3. Taking an ultrasound image of your arm requires blocking the blood flow to your arm for 5 minutes using a blood pressure cuff. This may cause slight discomfort in your arm, which will go away after the blood pressure cuff in deflated. The glyceryl trinitrate spray used in this study may cause a headache that could last 5 to 10 min.

IV. Your confidentiality will be guarded. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate project and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. You will be assigned an ID number under which all personal information will be stored in the secure locked filing cabinet and saved in a password protected file in a computer at DCU. You need to be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

VII. Involvement in this study is completely voluntary. You may withdraw from the Research Study at any point. Withdrawal from this study will not affect your participation in the HeartSmart programme or the medical treatment of your condition.

VIII. This research is funded by Science Foundation Ireland.

VIII. If you have concerns about this study and wish to contact an independent person, please contact: The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000

Informed Consent

Dublin City University

Project Title Comparisons of two Exercise Training Programmes on Vascular Health in Patients with Cardiovascular Disease

Principal Investigator Prof. Niall M. Moyna

Introduction to this study

Disease of the heart and blood vessels is called cardiovascular disease (CVD), and is the main cause of death in Ireland. Measuring the thickness of the carotid artery in your neck using ultrasound can indentify CVD. CVD damages blood vessels and reduces their ability to dilate (get bigger). We can also use a simple ultrasound procedure to measure how much the blood vessels can dilate. Damaged blood vessels release cells into the blood, which can be measured by taking a blood sample. Regular physical activity improves the health of blood vessels, and also has a beneficial effect on many of the risk factors for CVD. This study will measure the health of your blood vessels. In addition, we will compare the effect of a traditional 4 week cardiac rehabilitation programme and the effect of a high intensity intermittent exercise programme on your fitness level and the health of your blood vessels.

Participants Requirements

2. I will take part in a 4 week exercise programme in the Vascular Research Unit in the School of Health and Human Performance in DCU. I will visit DCU for a 2 Testing Sessions before and 2 at the end of the 4 weeks.

   Traditional Cardiac Rehab Group: If I am assigned to this group, after Testing Sessions 1 and 2, I will continue participation in the HeartSmart classes twice per week. During each class I will wear a strap around my chest to monitor my heart rate. After 4 weeks of HeartSmart I will return to the Vascular Research Unit for my 3rd and 4th exercise testing visit.

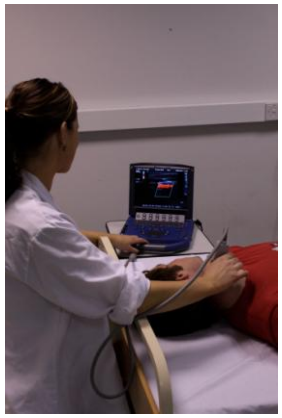
   Intermittent Training Group: If I am assigned to the Intermittent training group I will attend 2 supervised exercise sessions per week in the Vascular Research Unit, DCU. During every session I will wear small pads on my chest and a strap around my chest to monitor my heart. After 4 weeks of intermittent training I will return to the Vascular Research Unit for Testing Session 3 and 4.

3. During my first Testing Session (which lasts approximately 1.5 hour), I will have a blood sample taken and an ultrasound measurement will be taken of my neck to measure my carotid artery (See Figure 1). This test is painless and lasts about 15 min. I will also have a blood sample taken from a vein in my arm. The total amount of blood drawn will be **2 tablespoons**. The health of an artery in my

arm will also be measured. If I am taking Viagra, other PDE5 inhibitors or any herbal sexual enhancers I will not take any of these for at least 48 hours before this testing visit.

4. My second Testing Session will last about 1 hour and I will have my fitness assessed. During this test the electrical activity of my heart will be assessed by a 12 lead electrocardiogram (ECG). This is a special machine that takes 12 different views of my heart (like photographs). I will have electrodes (small pads) placed on my chest and a heart rate monitor to allow the researchers observe the electrical activity of my heart during exercise. I will be fitted with a mouthpiece and will undergo a treadmill exercise test to assess my fitness level.
5. At the end of the 4 week programme**,** I will return to the Vascular Research Unit in the School of Health and Human Performance DCU for my third Testing Session. This will be a repeat of the tests carried out during Testing Session 1. If I am taking Viagra, other PDE5 inhibitors or any herbal sexual enhancers I will not take any of these for at least 48 hours before this testing visit.
6. Testing Session 4 will be a repeat of the test carried out during during Testing Session 2.
7. During the 4 week exercise programme, all training sessions will take place in the Vascular Research Unit in DCU.
    - During these sessions I will wear a mouthpiece and walk on a treadmill
    - I will have a small droplet of blood taken from my earlobe at the end of each session
    - I will wear electrodes and a strap on my chest to monitor my heart throughout the exercise
    - Before and after the last exercise session I will have a blood sample taken and the health of a blood vessel in my arm will be measured. I will fast for 10 hour before this session. If I am taking Viagra, other PDE5 inhibitors or any herbal sexual enhancers I will not take any of these for at least 48 hours before the last exercies sessions (Session 8).
8. I will not exercise for at least 24 hours before each visit to the Vascular Research Unit.
6. To test the health of the arteries in my neck (testing session 1) I will lie on my back and an ultrasound will be placed on my neck and a number of photographs of the artery will be taken.
7. To test the health of the arteries in my arm (exercise session 2 and 3, and testing session 2) I will lie on my back, and an ultrasound will be placed on my upper arm to create an image of my artery. After the first image is recorded, a blood pressure cuff will be inflated on my forearm to block blood flow for five minutes. This may be uncomfortable. The cuff will be released and the images of my arteries repeated. I will rest for 15 minutes and then have one spray of glyceryl

trinitrate under my tongue. The glyceryl trinitrate will cause my arm arteries to enlarge and how much they enlarge will again be documented by taking a third set of pictures.



Potential risks to participants from involvement in the Research Study

1. Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Your heart will be continuously monitored using a 12 lead ECG and a physician will be present.

2. Drawing blood may cause a slight pain where the needle is inserted and can leave a bruise. A person trained to take blood will be used to decrease these risks.

3. The amount of blood drawn is not harmful, however, if I have a history of anaemia, I should inform the investigator.

4. The pictures of my arm arteries require blocking the blood flow to my arm for 5 minutes. This may cause slight discomfort in the arm, which will go away after the blood pressure cuff in deflated. The glyceryl trinitrate used in this study may induce a headache that could last 5 to 10 min.

Benefits (direct or indirect) to participants from involvement in the Research Study

After completing the study I will be provided with a copy of my results, summarising information such as my body mass index, blood pressure and cholesterol levels. There are no other direct benefits to me.

Participant – please complete the following (circle Yes or No for each question)

Have you read or had read to you the Plain Language Statement? Yes ☐ No ☐

Do you understand the information provided? Yes ☐ No ☐

Have you had an opportunity to ask questions and discuss this study? Yes ☐ No ☐

Have you received satisfactory answers to all your questions? Yes ☐ No ☐

Advice as to arrangements to be made to protect confidentiality of data, including that confidentiality of information provided is subject to legal limitations.

Your identity and other personal information will not be revealed, published or used in further studies. You will be assigned an ID number under which all personal information will be stored in a secure locked cabinet and saved in a password protected file in a computer at DCU. The named investigators will have access to the data. Data will be shredded after 5 years by Prof. Moyna.

Confidentiality is insured, but you must be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

If you are in a dependent relationship with any of the researchers their involvement in the project will not affect ongoing assessment/grades/management or treatment of health at DCU. Withdrawal from this study will not affect your participation in the HeartSmart programme or the medical treatment of your condition.

Signature:

I have read and understood the information in this form. The researchers have answered my questions and concerns, and I have a copy of this consent form. Therefore, I (print name) _____ consent to take part in this research project entitled Comparisons of two Exercise Training Programmes on Vascular Health in Patients with Cardiovascular Disease.

Participants Signature: _____

Name in Block Capitals _____

Witness: _____

Date: _____

Appendix E

$\dot{V}O_2$peak Protocol 1

Stage	Time	Speed	Slope
Warm Up	2:00	3.4	0.0
1	2:00	4.0	2.5
2	2:00	4.0	5.0
3	2:00	4.0	7.5
4	2:00	4.0	10.0
5	2:00	4.0	12.5
6	2:00	4.0	15.0
7	2:00	4.0	17.5
8	2:00	4.0	20.0
9	2:00	4.0	22.5
10	2:00	4.0	22.5
11	99:00	4.0	22.5
Recovery	5:00	3.2	0.0

$\dot{V}O_2$peak Protocol 2

Stage	Time	Speed	Slope
Warm Up	2:00	4.0	0.0
1	2:00	4.8	2.5
2	2:00	4.8	5.0
3	2:00	4.8	7.5
4	2:00	4.8	10.0
5	2:00	4.8	12.5
6	2:00	4.8	15.0
7	2:00	4.8	17.5
8	2:00	4.8	20.0
9	2:00	4.8	22.5
10	2:00	4.8	22.5
11	99:00	4.8	22.5
Recovery	5:00	3.2	0.0

$\dot{V}O_2$peak Protocol 3

Stage	Time	Speed	Slope
Warm Up	2:00	3.4	0.0
1	2:00	5.5	2.5
2	2:00	5.5	5.0
3	2:00	5.5	7.5
4	2:00	5.5	10.0
5	2:00	5.5	12.5
6	2:00	5.5	15.0
7	2:00	5.5	17.5
8	2:00	5.5	20.0
9	2:00	5.5	22.5
10	2:00	5.5	22.5
11	99:00	5.5	22.5
Recovery	5:00	3.2	0.0

$\dot{V}O_2$peak Protocol 4

Stage	Time	Speed	Slope
Warm Up	2:00	6.0	0.0
1	2:00	6.0	2.5
2	2:00	6.0	5.0
3	2:00	6.0	7.5
4	2:00	6.0	10.0
5	2:00	6.0	12.5
6	2:00	6.0	15.0
7	2:00	6.0	17.5
8	2:00	6.0	20.0
9	2:00	6.0	22.5
10	2:00	6.0	22.5
11	99:00	6.0	22.5
Recovery	5:00	3.2	0.0

$\dot{V}O_2$peak Protocol 5

Stage	Time	Speed	Slope
Warm Up	2:00	7.0	0.0
1	2:00	9.0	2.5
2	2:00	9.0	5.0
3	2:00	9.0	7.5
4	2:00	9.0	10.0
5	2:00	9.0	12.5
6	2:00	9.0	15.0
7	2:00	9.0	17.5
8	2:00	9.0	20.0
9	2:00	9.0	22.5
10	2:00	9.0	22.5
11	99:00	9.0	22.5
Recovery	5:00	3.2	0.0