THE EFFECT OF ACUTE AND CHRONIC EXERCISE ON CIRCULATING MICROPARTICLES

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June 2014

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Submitted for the award of PhD

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Submitted: June 2014

Volume 1 of 1

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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Acknowledgements

I would like to thank the following people for all they have contributed to my time and experience in DCU:

Prof. Niall Moyna for his supervision, guidance, advice and patience and for giving me the opportunity to pursue my studies.

Members of the Vascular Research Unit, Sarah, David, Bryan, Brona, Sinead, Cathal, Crionna, Mickey and Kevin for creating a special working environment. Special thanks to Dr. Sarah Kelly for her help with study three. Thanks to David, Brona and Bryan for making the last few months more bearable.

All the other postgrads I've had the pleasure of studying with, there are too many to list.

Kevin O'Brien for his assistance with study one.

Dr. Brendan Egan for his assistance with study two.

All the subjects for giving up their time.

Dr. Michael Harrison for his advice and guidance.

All the staff of the School of Health and Human Performance for creating a wonderful working environment and affording me the opportunity to study part-time.

Aisling Scally for all her help and for always being available for a chat.

Dr. Donal O'Gorman for his advice and guidance.

Dr. Noel McCaffrey for all his help with participant screening and recruitment for study three.

Dr. Ronan Murphy for his advice and guidance.

Laura Twomey and Robert Wallace for their assistance with the flow cytometry for study three.

My family and friends for their constant love and support.

My beloved wife, Claire, for her constant love, support, patience and understanding.

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Abstract

O'Connor, P.L. The Effect Of Acute And Chronic Exercise On Circulating Microparticles

Microparticles (MPs) are small membrane vesicles shed from plasma membranes following cell activation or apoptosis. They are released from a variety of cells including endothelial cells, smooth muscle cells, platelets, leukocytes, and erythrocytes. MPs are produced in a healthy state, however changes in the number and composition of MPs are associated with many disease states, including cardiovascular disease (CVD), venous thrombosis, diabetes mellitus, rheumatic disease and other inflammatory states. MPs may be used as a marker of dysfunction in many of these disease states. Exercise has been shown to have a positive effect on many of these disease states with MPs a possible biomarker of improvement.

Study 1: This study examined the effect of isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max on circulating levels of MPs in healthy young men. There was a significant increase in mean numbers of circulating endothelial MPs (EMPs) 6 h following each bout. Compared to baseline circulating platelet MPs (PMPs) did not increase during any of the acute bouts of exercise.

Study 2: This study examined the effect of 14 consecutive days of aerobic exercise on circulating MPs. There was no difference in circulating EMPs before exercise, immediately after exercise and 13 h following exercise on day 1, 3, 7, 10 and 14 of the training program. Circulating EMPs were significantly decreased 13 h following training compared to immediately post training. There were no changes in platelet MP numbers. There was no correlation between aerobic fitness and circulating MP numbers

Study 3: This study examined the effect of 4 weeks of low-volume, short duration high-intensity interval training (LS-HIIT) and cardiac rehabilitation on circulating MPs in individuals with CVD. There were no changes in MP numbers in either group following the exercise intervention. Flow-mediated dilation was significantly higher in the LS-HIIT group than the cardiac rehabilitation group following the exercise intervention

Conclusions: EMPs increase following an acute bout of isocaloric exercise at 60%, 70% and 80% $\dot{V}O_2$max in healthy young trained men. Aerobic exercise training for 14 consecutive days elicits a significant increase in aerobic fitness. Circulating EMPs decrease 13 h following training. Endothelial function is significantly improved and circulating MPs are unchanged in men and women with CVD following 8 sessions of LS-HIIT. There were no changes in circulating PMP numbers in response to acute or chronic exercise.

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Abbreviations

ACS	Acute coronary syndrome
BC	Beckman Coulter
BD	Becton Dickinson
CBC	Complete blood count
CHD	Coronary heart disease
CR	Cardiac rehabilitation
CRP	C-reactive protein
CVD	Cardiovascular disease
EC	Endothelial cell
ECG	Electrocardiograph
EDD	Endothelial dependant dilation
EE	Energy expenditure
EMP	Endothelial microparticle
ET	Endothelin

EPC	Endothelial progenitor cells
FMD	Flow-mediated dilation
FFA	Free fatty acids
GXT	Graded exercise test
HDL-C	High-density lipoprotein cholesterol
HIIE	High-intensity interval exercise
HIIT	High-intensity interval training
HMWK	High-molecular-weight kininogen
HPL	Human Performance Laboratory
IAT	Individual anaerobic threshold
ICAM	Intracellular adhesion molecule
LDL-C	Low-density lipoprotein cholesterol
LS-HIIT	Low-volume, short-duration high-intensity interval training
MCP-1	Monocyte chemotactic protein-1
MP	Microparticle

NO	Nitric oxide
PPO	Peak power output
PAD	Peripheral artery disease
PC	Phosphatidylcholine
PE	Phosphatidylethanoline
PECAM	Platelet-endothelial cell adhesion molecule
PI	Phosphatidylinositol
PK	Prekallikrein
PMN	Polymorphonuclear neutrophils
PMP	Platelet microparticle
PPP	Platelet-poor plasma
PS	Phosphatidylserine
Shh	Sonic hedgehog
T	Trained
TF	Tissue factor

TG	Triglyceride
UT	Untrained
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor

Chapter I

Introduction

Microparticles (MPs) are submicron vesicles shed from the plasma membrane of a variety of cells in response to a number of conditions including cell activation, injury, and/or apoptosis.[1] They were initially identified as membrane fragments from platelets and called 'platelet dust'. It was discovered that this 'dust' had procoagulant activity similar to the platelets from which they formed.[2] Since then it has become apparent that MPs are released from many cell types including endothelial cells, monocytes, red blood cells and granulocytes.[3] Remodelling of the plasma lipid membrane during their formation results in MPs containing glycoproteins, phospholipids and cytoplasmic components from their parental cell.[4]

Circulating MPs from a variety of cells are present in healthy individuals but their number and composition change in various disease states. Increased circulating MP numbers have been found in individuals with diabetes, kidney disease, inflammatory diseases and, in particular, cardiovascular disease (CVD).[5] Furthermore, increased endothelial MPs (EMPs) and platelets MPs (PMPs) are linked to hypertension, hypercholesterolemia, obesity, coronary artery disease, peripheral artery disease, acute coronary syndromes and CVD.[6] MPs have recently been proposed as a novel biomarker of cardiovascular disease and have been linked to

established biomarkers. A number of studies have found a significant relation between circulating MPs and IL-6, adhesion molecules and blood pressure.[7-9]

Acute and chronic exercise has many well documented positive effects on processes associated with CVD such as aerobic fitness, obesity, hypertension, inflammation, coagulation and endothelial function.[10,11] To date, relatively few studies have evaluated the effect of acute or chronic exercise on circulating MP numbers. Furthermore, no studies have evaluated the relation between MPs and endothelial function in men and women with CVD.

Purpose

The purpose of the following series of studies is to determine the acute and chronic effects of exercise on circulating MP numbers, and evaluate the relation between endothelial function and circulating MPs before and after training in men and women with CVD.

Objectives

1. To determine the acute effects of isocaloric bouts of exercise at different intensities on circulating MP formation in young healthy men
2. To determine the effects of exercise training on circulating MP numbers in young healthy men

3. To examine the relation between circulating MPs and aerobic fitness and endothelial function before and after training in men and women with CVD

Null Hypotheses

1. There will be no significant difference in circulating levels of MPs following isocaloric bouts of exercise at 60%, 70%, and 80% $\dot{V}O_2$max in healthy young physically active men
2. There will be no difference in circulating MP numbers pre-exercise, immediately following 1 h of exercise at 80% $\dot{V}O_2$peak and 13 h following exercise on days 1, 3, 7, 10 and 14 of a 14 day training program
3. Circulating levels of MPs will not change significantly following 4 weeks of LS-HIIT or CR in individuals with CVD and there will be no relation between MPs and endothelial function before or after training

Chapter II

Review of Literature

Microparticles

Microparticles (MPs) are small membrane vesicles ranging in size from 0.2 µm to 1.0 µm that are shed from plasma membranes following cell activation or apoptosis. They are released from a variety of cells including endothelial cells, smooth muscle cells, platelets, leukocytes, lymphocytes and erythrocytes.[2] Originally considered as 'cell dust' MPs are now known to play a significant role in cellular activity.[12] Although MPs are produced in a healthy state changes in their number and composition are associated with a number of disease states, including cardiovascular disease (CVD), venous thrombosis, diabetes mellitus, sickle cell disease, rheumatic disease and other inflammatory states. [1,2,13,14]

Microparticle Formation

Triggers for cell activation and apoptosis include chemical stimuli, cytokines such as TNF-α and IL-6, thrombin and endotoxin and physical stimuli such as sheer stress and hypoxia.[2] Chemical and physical stimuli lead to changes in the structure of the cell membrane cytoskeleton which ultimately results in the release of MPs (Figure 2.1). MP formation is reliant on a rise in cytosolic calcium concentration with consequent activation of calpain, protein kinases and phosphatase inhibition.[15]

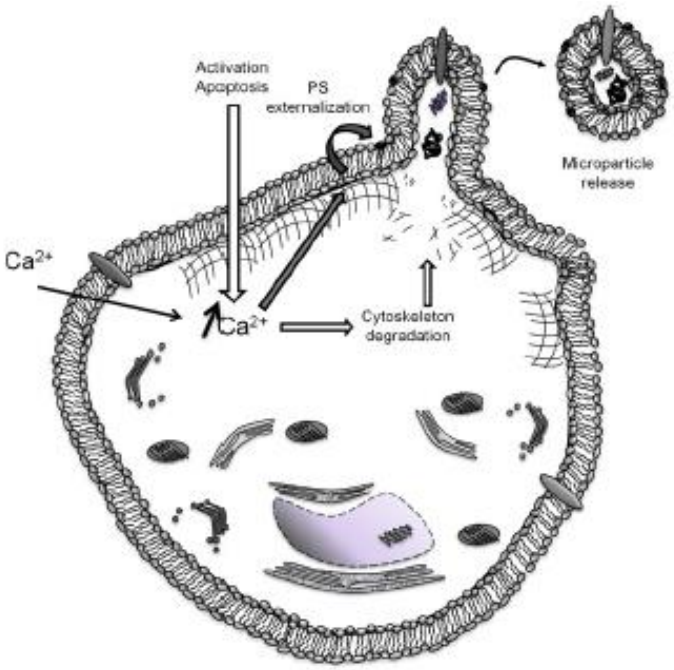


Figure 2.1: MP formation[16]

Cell Membrane Physiology

Cell membranes define the boundaries of the cells and serve as a permeability barrier. They regulate the movement of ions, gases and water, into and out of the cell and provide a surface for communication between cells and are involved in energy transduction.[17,18]

Cell Membrane Structure

Cell membranes are composed of a lipid bilayer with embedded globular proteins. On the external surface, carbohydrate groups join with lipids to form glycolipids, and with proteins to form glycoproteins.

Phospholipids are the most abundant of the membrane lipids and are normally glycerol based phosphoglycerides or sphingosine based sphingolipids. Phosphoglycerides are derived from phosphatidic acid, a molecule of diacylglycerol with a phosphate group esterified to the hydroxy group on the third carbon. The phosphate group esterified to carbon 3 is linked to small charged or polar compounds including choline (phosphatidylcholine - PC), serine (phosphatidylserine - PS), ethanolamine (phosphatidylethanolamine - PE) and inositol (phosphatidylinositol - PI). In the body, the polar head regions of the phosphoglycerides face towards the aqueous environment and the hydrophobic fatty acyl chains face inwards (Figure 2.2). In healthy humans approximately 60% of the phospholipid membrane is PC with the remainder PS, PE and sphingomyelin. Also present in the cell membrane are glycolipids and sterols. Cholesterol is the principle sterol found in animal cell membranes.

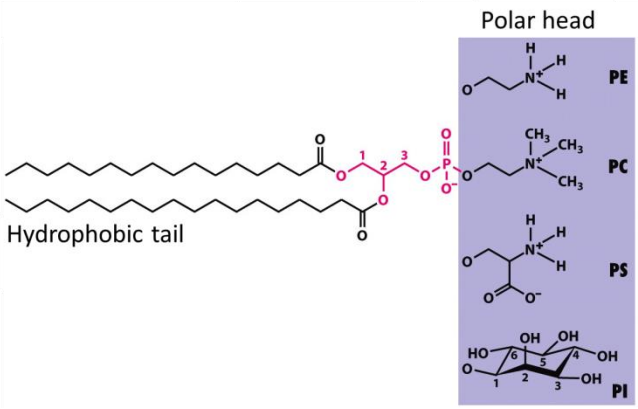


Figure 2.2: Lipid bilayer showing the hydrophilic polar heads and the hydrophobic fatty acyl chain

Membrane proteins can be grouped into i) integral proteins, ii) peripheral proteins and iii) lipid-anchored proteins. Integral proteins are transmembrane proteins and are involved in the transport of ions, solutes and electrons across the membrane. Peripheral proteins are located on the surface of the cell membrane on both the intra and extra-cellular surface. (Figure 2.3)[17] Peripheral proteins on the intracellular surface can form a fibrillar network that acts as a membrane skeleton. (Figure 2.4)[17] Common peripheral proteins in human cell membranes include spectrin, actin and tropomyosin. Lipid-anchored proteins can be found on the intra and extra-cellular surface of the cell membrane.

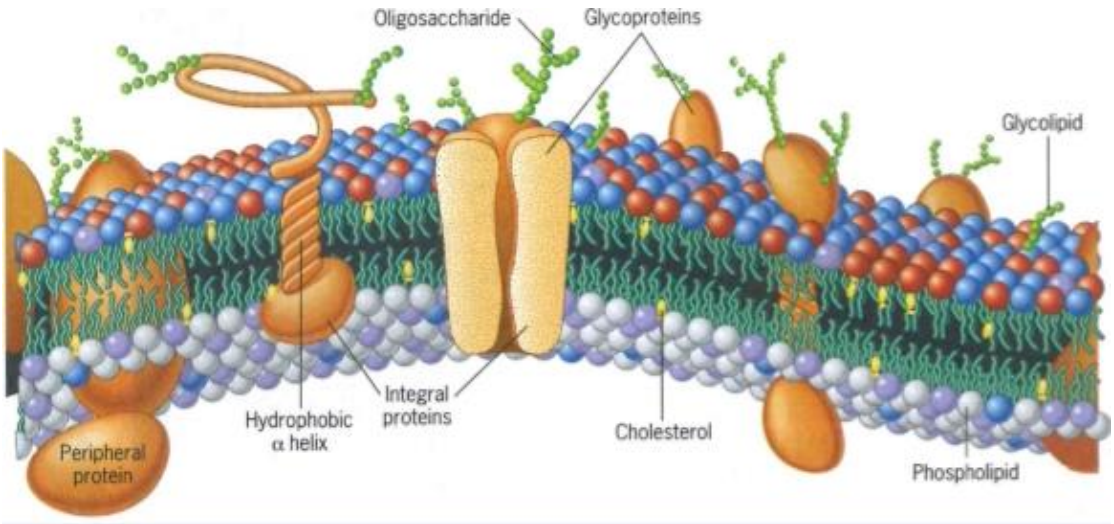


Figure 2.3: Cell membrane showing the lipids and proteins present and demonstrating the lipid bilayer.

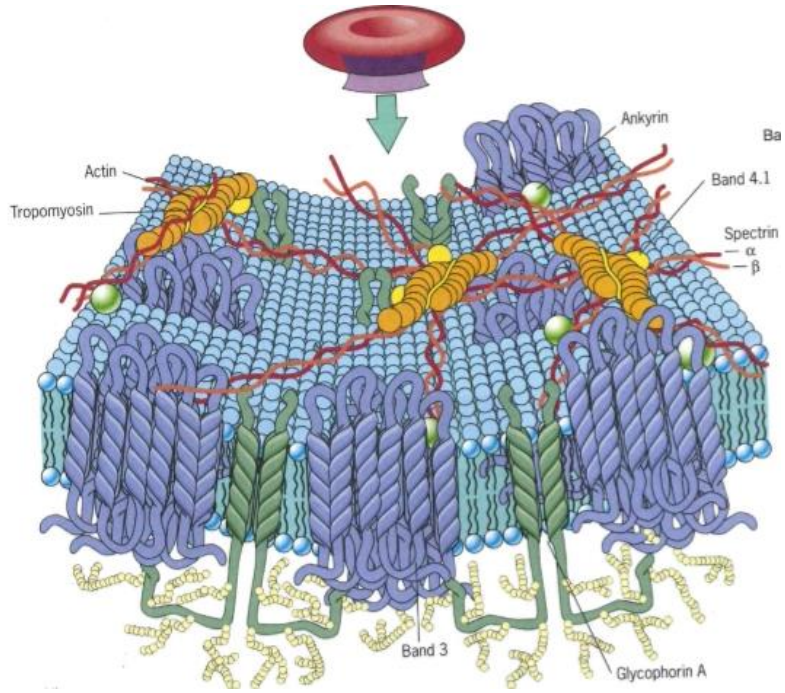


Figure 2.4: Cell membrane of a RBC showing the peripheral proteins spectrin, actin and tropomyosin forming the membrane skeleton.

Microparticle Physiology

The external membrane layer of eukaryotic cells involved in MP formation consists of PC and sphingomyelin and the internal layer consists of PS and PE. Under normal conditions the distribution of the lipids in the external and internal membrane layers is tightly controlled by ATP-dependant enzymes. [4]

An inward directed flippase, outward-directed floppase pump and a lipid scramblase control the transbilayer lipid distribution.[19] (Figure 2.5) The flippase, known as aminophospholipid translocase, is specific for PS and PE and rapidly conveys them from the outer to the inner leaflet maintaining lipid asymmetry. Floppase, a member of the ABC transporter family, reorganizes phospholipids so that PC and sphingomyelin are the dominant species in the outer lipid bilayer. Scramblase, which is ATP-independent but calcium-dependent, facilitates bidirectional movement of phospholipids between the lipid bilayers. [4]

Cell-Activated Microparticle Formation

The release of cell activation-associated MPs is time-and calcium-dependant. MP formation starts within minutes of an agonist binding to the cell. Cell activation results in an immediate increase in cytosolic calcium concentration.[2] Cell stimulation leading to substantial and continued increases in cytosolic calcium concentration may lead to the collapse of the membrane asymmetry. Cytosolic calcium influx inhibits flippase activity while stimulating scramblase and floppase activities (figure 2.5).[20] This

leads to changes in the distribution of lipids in the membrane bilayer with surface PS exposure and the formation of MPs. [19]

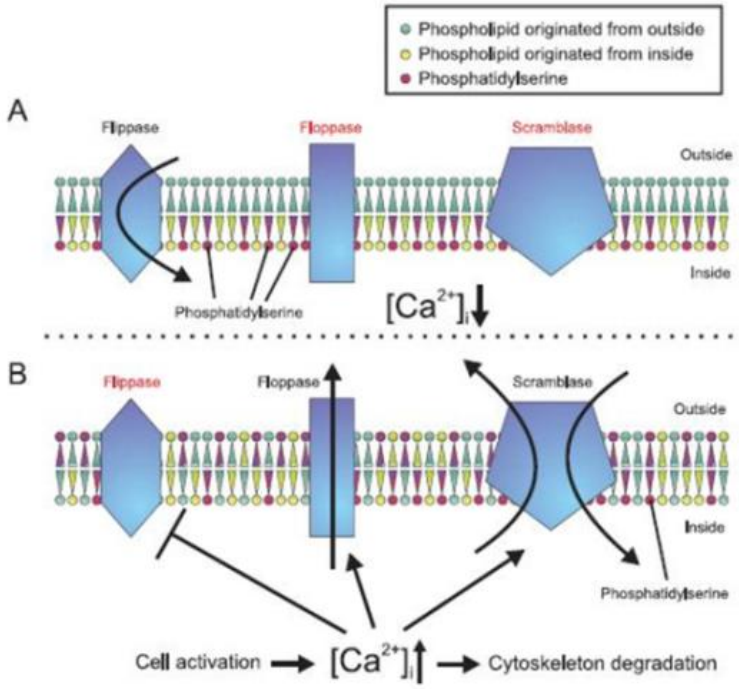


Figure 2.5. Flippase, floppase and scramblase activity

A – Resting cell membrane with low cytoplasmic concentration and active flippase. B – Cell activation leading to an increase in cytoplasmic calcium which inhibits flippase and activates floppase and scramblase. This leads to a loss of membrane asymmetry and the formation of microparticles.

Cytosolic calcium influx is also linked to cytoskeleton degradation associated with cell apoptosis.[21] The intracellular cytoskeleton is formed by a network of structural proteins which control the membrane asymmetry and cell stability via covalent protein to protein and protein to lipid interactions. Spectrin is a submembrane skeletal protein that connects lipids with actin cytoskeleton through vertical and lateral connections. During the movement of membrane phospholipids

these covalent links are disrupted leading to abolishment of the spectrin anchorage which in turns leads to membrane budding and MP formation.[4]

Cytoplasmic caspases and calpains also contribute to cytoskeleton reorganisation. These calcium dependent thiol proteases facilitate vesiculation of the cell surface by cleavage of talin, filamin and gelsolin, proteins involved in the assembly and structure of actin. (Figure 2.6)[20] Caspase-3 plays a major role in cytoskeleton reorganisation by mediating the cleavage of ROCK I phosphorylating myosin light chain resulting in cell membrane contraction, shrinkage and MP release. Independent of cell death, another rho-kinase isoform, ROCK II is activated by caspase-2 following endothelial cell stimulation by thrombin and is involved in MP release. [21]

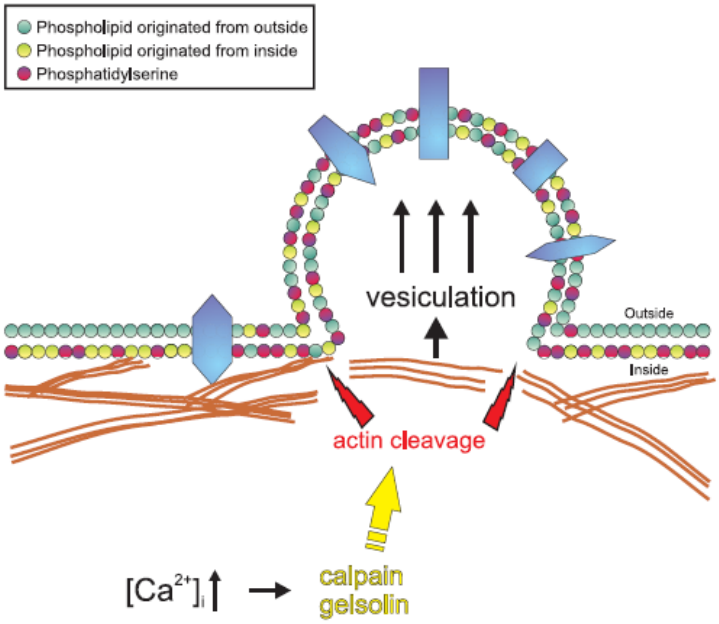


Figure 2.6: Role of calcium in microparticle formation.

An increase in cytoplasmic calcium results in actin cleavage and the rearrangement of the cell membrane resulting in the blebbing of MPs.

Microparticle Structure

MP membranes consist primarily of lipids and proteins. The number and type of lipids and proteins depends on the cellular origin of the MPs and the process which triggers their formation. Endothelial cells release phenotypically and quantitatively distinct endothelial microparticles (EMPs) depending on whether they are formed in response to cell activation or apoptosis. [22] The membranes of MPs isolated from the synovial fluid of patients with rheumatoid arthritis differ from the membrane of MPs taken from healthy individuals.[23]

MPs are surrounded by a phospholipid membrane bilayer similar to their parent cell. However, unlike their parent cell, the outer membrane contains PS and PE. In the resting cells of healthy individuals these phospholipids are contained in the inner lipid membrane.

Proteins contained in MPs originate from their cell of origin and can emanate from the cell surface or from intracellular bodies that are up-regulated or translocated by cell activation. PMPs contain P-selectin and glycoprotein 53 that originate from intracellular granule membranes. T-cell derived MPs also display glycoprotein 53 from endocytic origin.[2] PMPs also contain glycoproteins 1b (CD42b) and platelet-endothelium adhesion molecule-1 (PECAM-1) (CD31). MPs from erythrocytes stain for glycophorine A, MPs from granulocytes stain for CD66 and EMPs stain for CD31, CD34, CD51, CD62E and CD146. [24]

In addition to identifying the parent cell, the phospholipid and protein content can also distinguish between activation derived and apoptosis derived MP formation. Endothelial cells have been found *in vitro* to release phenotypically different MPs in response to activation and apoptosis. Activation of endothelial cells increases MPs expressing inducible markers, including CD54, CD62E and CD106. In contrast, endothelial cell apoptosis results in microparticles expressing CD31 and CD105. [22] Endothelial cell lines from renal microvasculature, brain microvasculature and

coronary artery microvasculature also show different responses to cell activation and apoptosis.

Microparticle Measurement

Several different methodological approaches have been used to measure MP including flow cytometry, electron-microscopy, enzyme-linked immunosorbent assay (ELISA)[24] and solid-phase capture.[25] There is currently no universally accepted standard method for the analysis of MPs although there now seems to be a concerted effort to seek agreement on a standard protocol. [26,27] Major differences currently exist in sample preparation, total MP detection and antigenic markers used. [28] The use of a wide variety of methodologies has made it difficult to directly compare results which sometimes have inconsistent or conflicting outcomes. Table 2.1 highlights six methods used to measure MPs from laboratories with established research programs on the topic.

Table 2.1: Methods of MP Measurement

					Cell-specific Identification		Cell Origin		
	Principle Technique	**Quantitation**	**Anti-coagulant**	**Prepare PPP**	**Isolation MP pellet**	**Generic MP detection**	**Platelet**	**Endothelial**	**Leukocyte**
Biro *et al.* [29]	FC	Counts	Citrate	1550 x *g* 20 min	1800 xg, 30 min	Annexin V	CD62P, CD61, CD63	CD31, CD62E or CD144	CD4, CD8
Dignat-George *et al.* [30]	FC	Counts	Citrate	1500 x *g* 15 min 13000 x *g* 2 min	-	Annexin V	CD	CD51, CD144 or CD146	CD45
Hugal *et al.* [25]	SPC	Prothromb-inase capture	Citrate	1500 x *g* 15 min 13000 x *g* 2 min	-	Annexin V, tissue factor	CD62P or GPIba	CD31 or CD62E	CD45
Jimenez *et al.* [31]	FC	Counts	Citrate	200 x *g* 10min 1500 x *g* 7 min	-	-	CD41 or CD42b & CD31	CD31+/CD42- or CD62E	CD45
Nomura [32]	ELISA	Standard PMP	EDTA	100 x *g* 20min	-	-	GP IX (capture) CD62P, CD40L	-	-

Shet *et al.* [33]	FC	Counts	Citrate	1300 x *g*, 10min	100000 x g, 60min	Annexin V	CD41a	CD144	CD14 (monocyte)

FC, flow cytometry; SPC, solid phase capture

Sample Preparation

There is currently large inter-laboratory variation (Table 2.1) in sample preparation procedures. To avoid endothelial or platelet damage and activation it is recommended that venous blood samples are collected into a tube containing anticoagulant using a needle ≥ 21-gauge and within 1 min of placing a tourniquet. Citrate is the most commonly used anticoagulant. However, EDTA and heparin have also been used in a number of studies. The first 3 ml of blood should be discarded to avoid contamination from the application of the tourniquet. The sample is gently mixed with the anticoagulant and vigorous shaking is avoided in order to avoid MP activation. The cellular elements of the blood are separated from the plasma containing MPs using centrifugation. The time between blood sampling and centrifugation has ranged from 15 min to 2 h.[26,28,34] Circulating MP levels are more than double in blood samples that are left for 2 h before centrifugation compared to samples that are immediately processed.[27]

There is also a large variation in the centrifugation protocol (Table 2.1). A two-step centrifugation is recommended but the angular velocity measured in revolutions per minute (RPM), or acceleration expressed as g speeds vary greatly. Following the first spin the supernatant is removed, leaving the bottom 200-500 µl of undisturbed supernatant. This supernatant is referred to as 'platelet-rich plasma' (PRP). The same centrifugation procedure is repeated and the final 200-500 µl of undisturbed

supernatant is referred to as 'platelet-poor plasma' (PPP). The PPP is immediately analysed or frozen. Some investigators snap freeze the PPP in liquid nitrogen and store it at -80^{o}C while others just freeze the PPP at -80^{o}C. Samples have been thawed on ice or at 37^{o}C prior to analysis.[8] Biro *et al.*, (2004) recommend snap freezing and thawing on melting ice to ensure the best possible preservation of MP structure and function. [29] Another variation involves freezing the plasma after the first spin cycle. This plasma is then thawed at 37^{o}C before analysis and centrifuged at 17000 x *g* resulting in a MP pellet. The supernatant is removed and the MP pellet is reconstituted in Annexin V buffer to be analysed. [34]

Flow cytometry uses an optical-to-electronic coupling system to simultaneously measure multiple physical characteristics of cells, as they flow in a fluid stream through a beam of light (figure 2.7). A large numbers of MPs can be analyzed in the same sample. Cell origin can also be determined by detecting multiple antigens on a single MP. For each event detected by flow cytometry the size, (forward angle scatter) and density (side scatter) are determined along with the fluorescence. The size of the MPs are defined in comparison to sizing beads (1 – 1.5 μm) used to set the upper microparticle diameter limit and to exclude larger non-MP material from the analysis. Platelets have also been used as internal sizing beads with MP identified as having a smaller forward angle light scatter than that of the smallest platelet. [35]

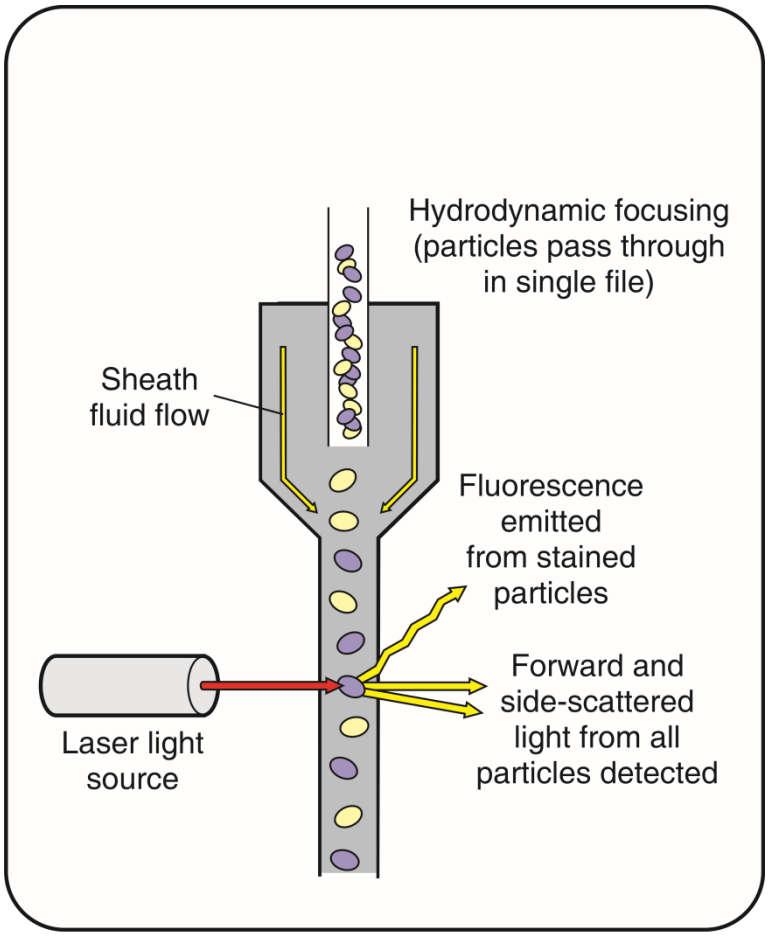


Figure 2.7: Flow cell operation

Antibodies are used to identify the cellular origin of MPs in plasma. The antibodies bind to cell-specific antigens on the surface of MPs giving information on the cell of origin. Table 2.2 adapted from Simak *et al*., (2006)[8] summarizes the major antigens that have been used to identify the cellular origin of MPs.

Single antibodies and combinations of antibodies are used to identify MP origin. For example EMPs can be identified by the expression of $CD31^{+}CD42b^{-}$.

However, the presence of an antigen does not necessarily classify the cell of origin. In blood, antigens derived from one cell type may adhere to MPs derived from another cell type. Also MPs may bind with the membrane of a different cell type and this cell may then release MPs with an adopted antigen. [8]

Table 2.2: Antigens used for MP detection

MP origin	Antigen	Alternative Name
Red Blood Cells	CD235a	Glycophorin A
Leukocytes	CD45	LCA, T200, B220
Monocytes	CD14	LPS-R
Granulocytes	CD66b	CD67, CGM6, NCA-95
TH lymphocytes	CD4	T4, L3t4 (mouse), W3/25 (rat)
TS lymphocytes	CD8	T8, Leu-2, Lyt 2,3
B lymphocytes	CD20	B1, Bp35
Platelets	CD41	GPIIb, αIIb integrin
	CD41a	GPIIbIIIa, αIIbβ_3 integrin
	CD42a	GPIX
	CD42b	GPIbα
	CD61	GPIIIa, β_3 integrin
Endothelium	CD31	PECAM-1
	CD34	gp105-120
	CD62E	E selectin
	CD51	αv integrin
	CD105	Endoglin
	CD144	VE-cadherin
	CD106	VCAM-1

The International Society on Thrombosis and Haemostasis SSC Collaborative workshop concluded that the standardisation of PMP counts by flow cytometry is feasible but is dependent on the flow cytometer and the calibration strategy used.[36] Lacroix *et al*., (2012) identified three major pre-analytical points that may impact on

MP measurement; (i) the time before the first centrifugation, (ii) agitation of the samples in transport and (iii) the centrifuge protocol. [37]

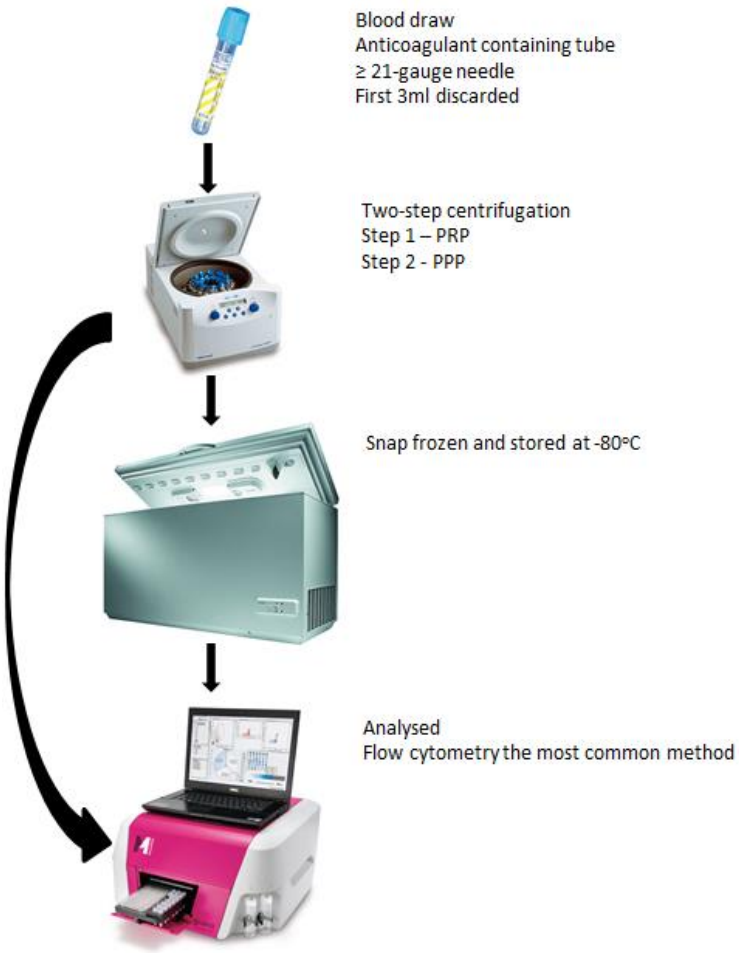


Figure 2.8: MP sample preparation overview

PRP, platelet rich plasma; PPP, platelet poor plasma

Coagulation

Coagulation is the series of highly regulated steps that results in the formation of fibrin, a non-globular protein that provides the framework for a loose platelet plug. Plasma coagulation proteins, also referred to as clotting factors, circulate in the blood

in an inactive form. Following activation of the coagulation system these clotting factors become active resulting in the formation of a blood clot composed of platelets and fibrin. The formation of fibrin involves a series of sequential reactions that commence within 15 to 20 sec following injury such as trauma to the vessel wall and surrounding tissue, trauma to the blood or contact between the blood and procoagulatory factors in the vessel wall. Two pathways are known to exist in the coagulation process, the extrinsic pathway and the intrinsic direct activation pathway. The two pathways interact extensively and converge to form a common pathway leading the formation of the enzyme thrombin, which catalyses the conversion of fibrinogen to fibrin. (Figure 2.8) [38]

The extrinsic pathway, also known as the tissue factor (TF) pathway is initiated following tissue damage. Tissue damage can lead to exposed TF on the surfaces of injured endothelial cells, leukocytes and cells of the subendothelial layers, in particular smooth muscle and fibroblasts. TF binds to Factor VIIa resulting in the formation of TF-Factor VIIa complex (figure 2.9). TF-Factor VIIa complex catalyses the conversion of Factor IX from its inactive to its active form, Factor IXa, which in turn activates Factor X, the final step of the extrinsic pathway. Factor Xa can feedback in the pathway and induce further Factor VII activation.

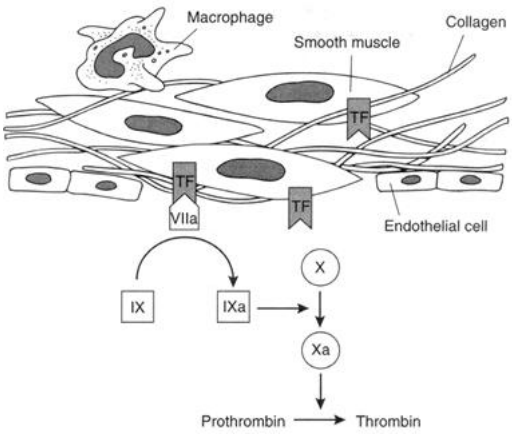


Figure 2.9: Extrinsic pathway

The intrinsic pathway, also called the direct activation pathway, begins with the auto activation of Factor XII to Factor XIIa. This auto activation occurs in the presence of high-molecular-weight kininogen (HMWK) and prekallikrein (PK). Factor XIIa acts enzymatically to convert inactive Factor XI to its activated form Factor XIa. This in turns converts Factor X to its active form, Factor Xa. Activated platelets supply the phospholipid membrane on which this reaction occurs and requires Factor VIIIa as a cofactor. Factor VIIIa is the co-factor of FIXa and together they form the tenase complex which activates Factor X.

Factor Xa, formed from either the extrinsic or the intrinsic pathway, catalyses the conversion of prothombin to thrombin. The primary role of thrombin is to convert fibrinogen to fibrin. In addition, it stimulates several of the coagulation factors and amplifies the coagulation process. Following conversion of fibrinogen to fibrin the fibrin monomers automatically polymerize with each other to form a meshwork that

holds a newly formed clot together. In the presence of Factor XIII the fibrin strands become cross-linked with covalent bonds. [38]

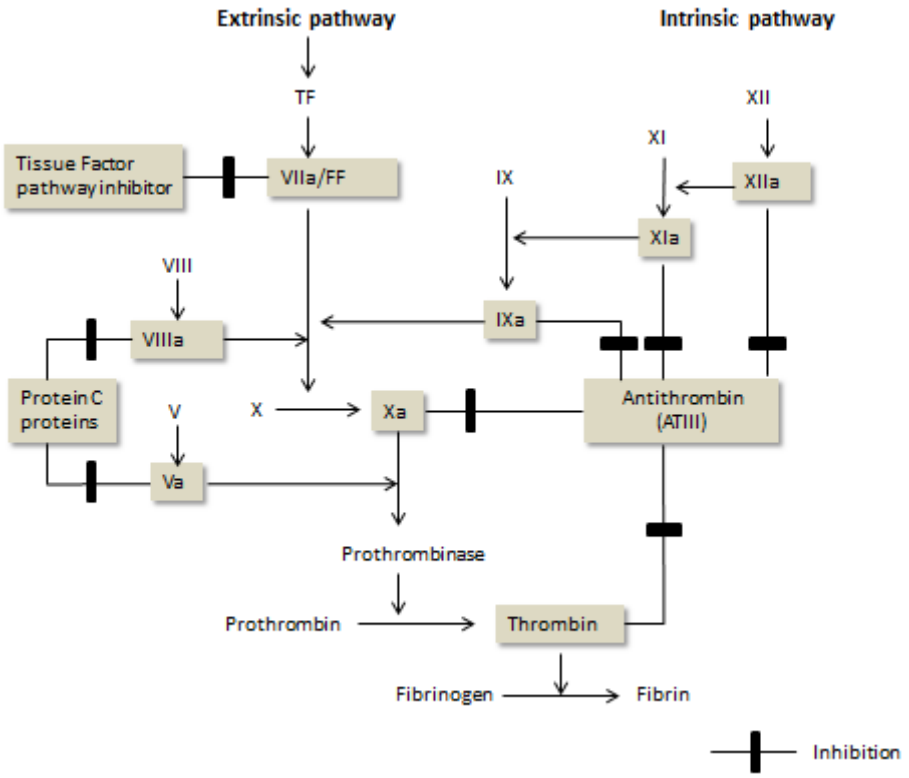


Figure 2.10: Coagulation Pathway.

Extrinsic and Intrinsic pathways interacting to form a common path for the development of fibrin.

MPs and Coagulation

MPs are involved in many of the pathways leading to the formation of fibrin. When compared to a similar surface area on an activated platelet, PMPs have at least 50- to 100-fold higher pro-coagulant properties. [39] This is due to the fact that PMPs have an increased density of procoagulant phospholipids and surface receptors for the procoagulant complexes, such as Factor X. PMPs also contain surface receptors for

both Factor VIII and Factor Va and high and low affinity binding sites for Factor IXa. [40] Phospholipids contained on the surface of MPs, particularly PS, can bind to coagulation factors and promote the formation and activity of tenase and prothrombinase complexes (figure 2.10). [2,15]

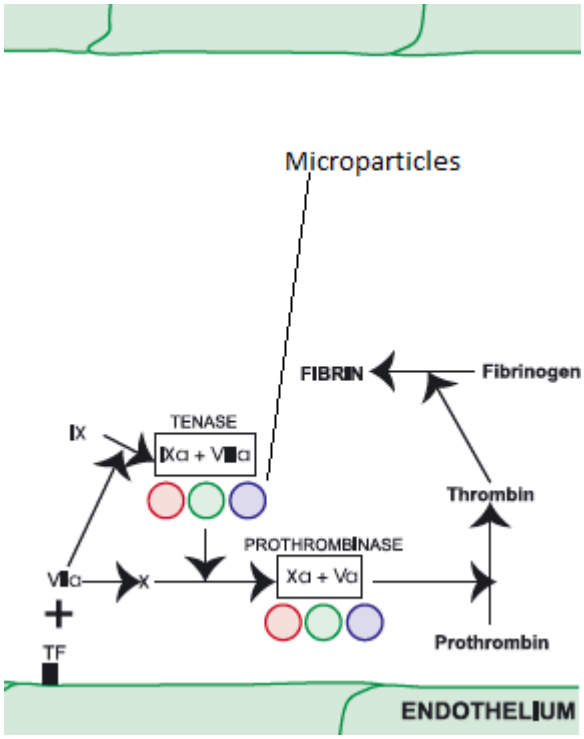


Figure 2.11: MPs in coagulation.[15]

MPs have the capacity to harbour, deliver or induce TF activity, the prime cellular initiator of coagulation.[40] The origins of these MPs include monocytes, tumor cells and endothelial cells. MPs bearing TF circulate in low numbers in healthy individuals but increase in various disease states including cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM) and with sickle cell disease. The increase in TF

bearing MPs is related to increased thrombolytic events [41] suggesting that TF present on MPs may play a role in coagulation.

Stimulation of cultured human umbilical vein endothelial cells with TNF-α increases EMPs expressing TF on their surface. Increasing the number of these EMPs in a coagulation assay shortened the plasma clotting time compared with EMPs from unstimulated cells. The EMPs induced coagulation *in vitro* via a TF/Factor VII-dependent pathway. Mallet *et al*., (2009)[42] found that atherosclerotic plaques contain procoagulant MPs that expose TF and exhibit TF procoagulant activity. The MPs were primarily of monocyte and lymphocytic origin. These MPs accounted for almost all the TF activity in the plaque indicating a direct causal relation between their presence and TF activity.

Most of the evidence for MP involvement in coagulation comes from *in vitro* studies. MP membranes exposing phospholipids facilitate the binding of coagulation factors to the membrane enabling the formation of tenase and prothrombinase complexes that in conjunction with TF can initiate blood coagulation. The negative charged phospholipid surface of MPs readily binds activated coagulation factors and exposes TF in various conditions. [2]

The presence of elevated levels of MPs in various disease conditions provides *in vivo* evidence of MP involvement in coagulation. MPs are elevated in certain diseases that involve hypercoagulation and circulating levels are reduced in several bleeding

disorders such as Scott Syndrome, Castaman's defect and Glanzmann's disease. MPs also expose TF in several clinical conditions that are associated with hypercoagulation such as the blood of patients with disseminated intravascular coagulation and the synovial fluid from inflamed arthritic joints. Hypercoagulation is a characteristic of CVD and altered numbers and procoagulant behaviour of MPs have been reported in several CVDs, such as unstable angina and atherosclerosis.[43] Agouni et al., (2008) reported an increase in procoagulant MPs in patients with the metabolic syndrome compared to healthy controls.[44] MPs are likely to play a causal role in the development of hypercoagulation in CVD. [2,24]

Anticoagulant Effects of MPs

MPs can also display anticoagulant properties. Protein C inhibitor binds preferentially to the PE on platelets and PMP membranes and inhibits phospholipid bound activated protein C.[45] MP membranes have been found to contain coagulation inhibiting proteins such as TF pathway inhibitor, thrombomodulin, endothelial protein C receptor and protein C which suggests a putative role of MPs in anticoagulation.[40,46] TF pathway inhibitor down regulates the activation of TF-factor-VIIa complex. Thrombomodulin binds to TF rendering it inactive. Endothelial protein C receptor binds protein C, which in conjunction with its cofactor, protein S, inactivates factor Va and VIIIa.[47]

MPs and Inflammation

MPs have also been found to play an important role in the inflammation process. They are a source of aminophospholipids and also provide a preferential substrate for the enzyme phospholipase A2. This enzyme releases lysophosphatidic acid from the breakdown of PC which triggers platelet aggregation and the inflammatory process.[15] PMPs and MPs of leukocyte origin stimulate the release of several endothelial cytokines including IL-6, IL-1β, IL-8 and monocyte chemotactic protein-1 (MCP-1) and monocytic cytokines including IL-1β, TNF-α and IL-8.[48] The release of IL-6 and IL-8 induce the expression of the adhesion molecules ICAM-1, VCAM-1 and E-selectin.[49]

MPs may also have a positive role in the anti-inflammatory process. The active anti-inflammatory protein annexin 1 is contained on MPs from leukocytes and these MPs inhibit the interaction between leukocytes and endothelial cells both *in vitro* and animal models.[50] Andriantsitohaina *et al*., (2012) suggest that a delicate balance between the pro- and anti-inflammatory effects of MPs should be preserved to obtain an adequately resolved inflammatory process.[16]

MPs and Endothelial Function

A healthy endothelium has anti-inflammatory and anticoagulatory properties and regulates vascular tone, vascular wall permeability and cell growth ultimately protecting the vascular system.[51] Impairment of the normal functions of the

endothelial is referred to as endothelial dysfunction and represents one the earliest events in the pathogenesis of atherosclerosis.[52] EMPs exist in low concentrations in healthy individuals but are increased in disease states linked to endothelial dysfunction.[2]

MPs from patients with acute MI are associated with severe endothelial dysfunction in healthy rat aortic rings.[53] However MPs from non-ischemic patients, at an equivalent protein concentration, did not result in endothelial dysfunction in healthy rat aortic rings.[53] An EMP concentration threshold may exist before endothelial barrier function and vasodilation become compromised.[54] Esposito *et al.,* (2006) found a higher level of circulating EMPs and PMPs in obese than lean women.[55] There was also a significant inverse relation between the number of circulating EMP and PMP and brachial artery flow mediated dilation (FMD) in the obese women. Endothelium-dependant relaxation is impaired in mice aorta injected with MPs from patients with the metabolic syndrome. The MPs reduced the ability of acetylcholine to promote endothelial-dependant relaxation.[44]

MPs play a role in the regulation of vascular tone by altering the production of nitric oxide (NO).[48] MPs from patients with the metabolic syndrome reduced NO release in endothelial cells by ~50% compared to MPs from healthy controls.[44] Brodsky *et al*., (2004) reported that EMPs in rat aortic rings diminish the production and/or availability of NO reducing endothelium-dependant relaxation.[56] In contrast,

Agouni *et al*., (2007) found that MPs containing the morphogen sonic hedgehog (Shh) on their surface induced NO production from endothelial cells and tissue.[57] When the Shh pathway was silenced there was a reduction in NO production demonstrating that the NO production is mediated directly by the Shh pathway. Shh is involved in adult and embryonic development. Vascular endothelial growth factor (VEGF) and NO-synthase are downstream targets of Shh signalling suggesting that Shh can be a modulator of VEGF regulation and NO production.[48] The study also found that MPs decrease the production of reactive oxygen species (ROS) and these two findings combined might result in an increase in NO by reducing oxidative stress and the subsequent scavenging of NO.[57]

MPs in Cardiovascular Disease

Coagulation, inflammation and endothelial dysfunction are involved in the development of cardiovascular disease (CVD). MPs from circulating blood cells or endothelial cells reflect the activation or damage to cells of the vasculature. Significant increases in MPs have been reported for a number of coronary vascular disorders including stroke, aortic aneurysm disease, peripheral vascular disease and for venous thrombo-embolism.[3] The formation and biological effects of EMPs and PMPs in CVD are summarized in figure 2.11 and figure 2.12.[6]

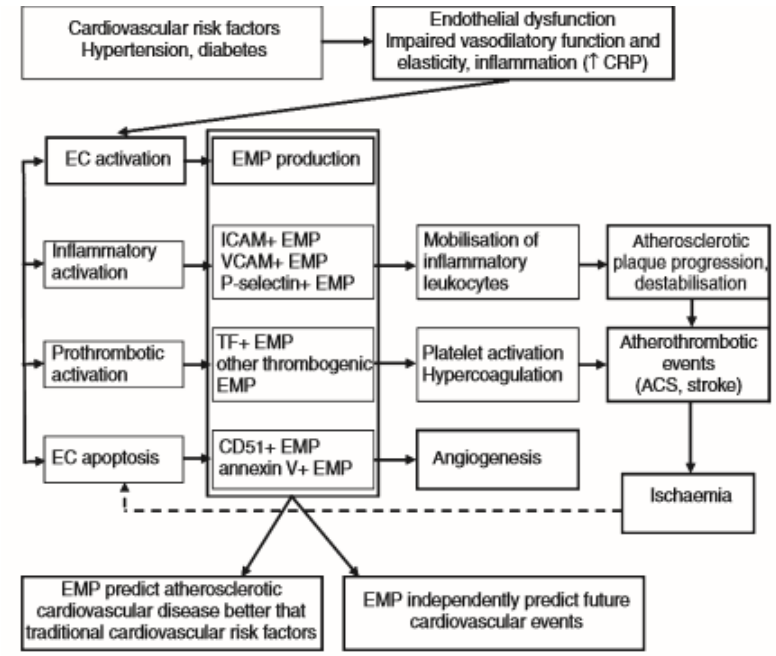


Figure 2.12: EMPs in CVD – Mechanisms for EMP production and the reported effects of the EMPs on CVD development.

CRP, C-reactive protein; EC, endothelial cell; EMP, endothelial microparticle; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; TF, tissue factor; ACS, acute coronary syndrome; CD51+, cluster of differentiation 51+

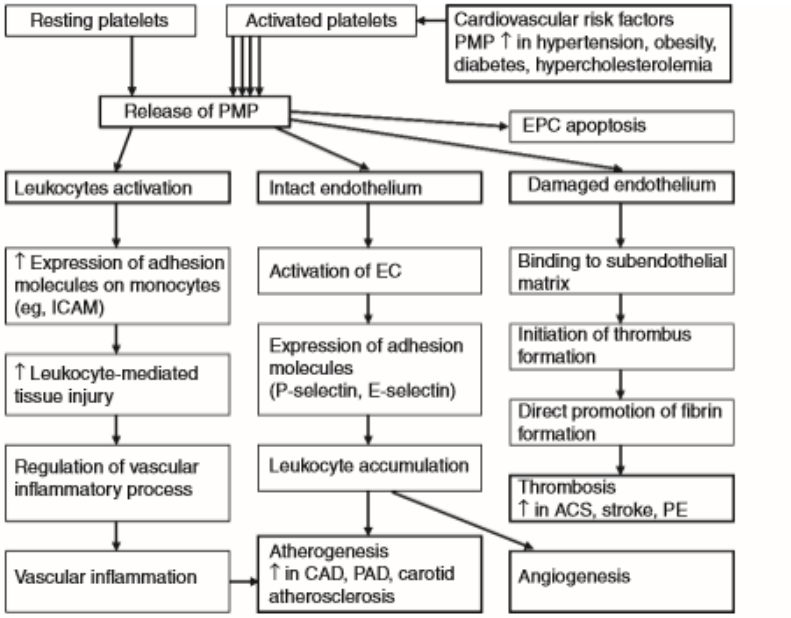


Figure 2.13: PMPs in CVD – Release of PMPs and their role in inflammation and coagulation leading the development of CVD.

PMP, platelet microparticles; ICAM, intracellular adhesion molecule; EC, endothelial cell; CAD, coronary artery disease; PAD, peripheral arterial disease; EPC, endothelial progenitor cell; ACS, acute coronary syndrome; PE, pulmonary embolism

With the proposed involvement of MPs in the development of CVD the prevailing view may be that MPs are harmful. However, it is well documented that MPs can have positive effects on coagulation, inflammation and endothelial function. Tushuizen *et al.,* (2011) have proposed a model for describing the role of MPs in CVD highlighting both the negative and positive effects of MPs (figure 2.13).[50] Under normal conditions MPs are released by cells to ensure homeostasis. The MPs assist with the regulation of physiological processes including anticoagulation, inflammation and regulation of endothelial functions. Under abnormal conditions inducing stress, cells release altered numbers of MPs that have different compositions and functions.

These stress induced MPs contribute to the development of CVD through coagulation, inflammation and endothelial dysfunction.

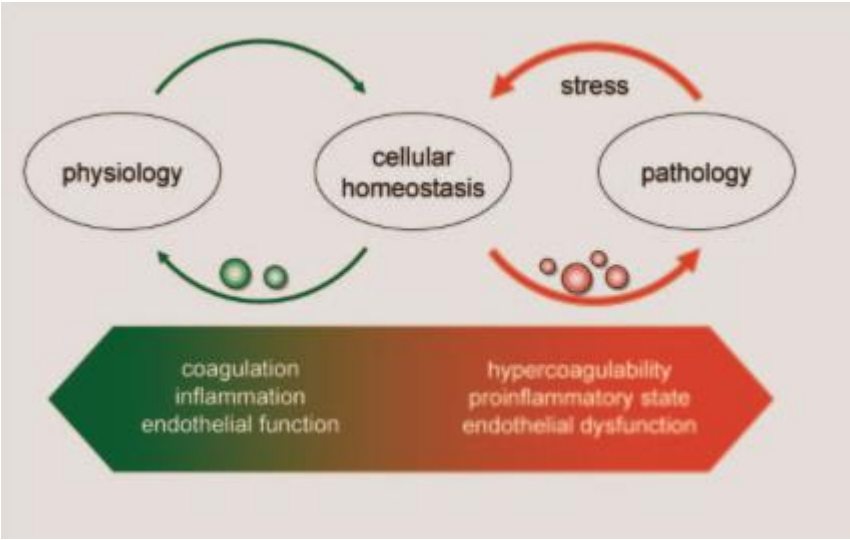


Figure 2.14: Proposed model for describing the role of MPs in normal health and in CVD.

MPs as Biomarkers for Disease

It is clear that MPs are elevated in disease states, in particular CVD, and play both positive and negative roles in coagulation, inflammation and endothelial (dys)function, depending on their origin, composition and numbers. However, the issue of whether MPs can be used as a biomarker of CVD risk or as independent predictors of CVD is still not resolved. One of the major problems is a lack of a universal method of collection, preparation and analysis. Nonetheless, there are a number of studies with large sample sizes that highlight the role of MPs as potential biomarkers of CVD. Nozaki *et al*., (2009) found that circulating levels of CD144-EMP

were independent predictors of future cardiovascular events in patients at high risk for coronary heart disease (CHD) during a 36 month follow-up.[58] Furthermore, the addition of multiple biomarkers, including CD144-EMP, high-sensitivity CRP and B-type natriuretic peptide, can identify patients vulnerable to CVD by improving the risk classification based on the Framingham risk model.[58] Esposito *et al*., (2006) found that EMPs positively correlated with endothelial dysfunction impairment, measured by endothelium-dependant flow-mediated dilation, in 41 obese women.[55] Preston *et al.,* (2003) found an increase in PMPs and EMPs in subjects with severe hypertension.[59]

MPs as Signalling Moieties

MPs have been shown to be a vehicle for the intercellular exchange of biological signals with 2 principle mechanisms for intercellular signalling proposed.[60] Firstly, MPs may activate receptors on target cells via membrane associated, bioactive molecules. Secondly, proteins, bioactive lipids, or RNA contained in MPs may be directly transferred to cells resulting in cell activation, phenotypic modification and reprogramming of cell function. Laffont *et al.,* (2013) found that platelet derived MPs release microRNA (miR-223) that regulates endothelial cells.[61] MP's ability to travel in the blood stream allows them to act as both paracrine and autocrine signalling molecules. For example, CNS-derived MPs may interact with endothelial cells in the peripheral circulation representing a novel communication method between the nervous system and the cardiovascular system.[62]

MPs and Exercise

Acute Exercise and MP

Relatively few studies have examined the relation between exercise and MPs (Table 2.3). Chaar *et al.,* (2011) examined the effects of strenuous exercise on circulating cell derived microparticles.[63] Seven young healthy men performed three $\dot{V}O_2$max tests on a cycle ergometer with a 10 min interval between the tests. Blood for haematological parameters, MPs and adhesion molecules was taken before and after the third exercise test and at 2 h post-exercise. Leukocytes and polymorphonuclear neutrophils (PMN) were significantly higher immediately after exercise and 2 h post-exercise than before exercise. Flow cytometry was used to enumerate MPs from RBC, EC, platelets and PMNs. The total number of MPs, PMPs and PMN-derived MPs were significantly higher after exercise than before exercise and remained elevated at 2 h post-exercise. Monocyte and endothelial derived MP were not detected in the blood at any of three sampling time points. This may have been due to the antibody, anti-CD106, used to measure EC derived MPs. Monocyte and EC derived MPs were measured using antibodies anti-CD14 and anti-CD106, respectively. Compared to baseline values, IL-6 was significantly elevated post-exercise and remained elevated at 2 h post-exercise. There were no significant changes in the number of circulating adhesion molecules. It was speculated that the increase in PMP and PMN derived MPs were in response to the pro- inflammatory response to exercise.[63]

Sossdorf et al., (2011) examined the effects of 90 min cycling at 80% of the individual anaerobic threshold on the concentration and procoagulant activity of MPs in eight trained (T) and eight untrained (UT) healthy men.[64] Blood samples were taken before exercise, immediately post-exercise, at 45 min post-exercise and 2 h post-exercise. Total MP counts increased significantly above baseline levels at 45 min in the T group and at 2 h in the UT group with the difference between the groups significant at 2 h. There was a significant increase in PMPs post-exercise and 45 min post-exercise in the T group with values dropping close to basal levels at 2h. PMP levels were significantly higher than baseline at 45 min post-exercise in the UT group and remained significantly higher at 2 h post-exercise. There were no significant changes in the circulating number of EMP or MMP numbers in the UT group. In the T group there was a transient significant increase in EMP and MMP numbers at 45 min. TF expression on MPs was also measured. Compared to pre-exercise values there was a significant decrease in TF-positive PMPs in both groups at 45 min post-exercise and the number remained significantly lower than baseline in the UT group at 2 h post-exercise. The number of circulating TF-positive EMPs was significantly higher than baseline in the T group at 45 min post-exercise. TF-positive MMPs were significantly higher at rest in the UT group than the T group and at 2 h post-exercise in the UT group compared to baseline. Prothrombinase activity of MPs was significantly enhanced after exercise in both groups and remained elevated 2 h post-exercise. The TF-initiated fibrin formation in MP-containing plasma was increased by approximately

15% after exercise and remained elevated compared to pre-exercise values in the T group. The increase in MPs, along with the changes in the number of TF-positive MP and the enhanced prothrombinase activity of the MPs support the premise that MPs contribute to the exercise induced changes in haemostasis. It is possible that the exercise induced sheer stress may play a role in the increase in circulating MPs[64]. This view is supported by a trend towards higher MP levels in the T group. Although both groups exercised at the same relative intensity the RPE scores and blood pressure were higher in the T group, suggesting that they were exercising at a higher relative intensity.

Kirk *et al.,* (2013) investigated whether maintaining acid-base homeostasis by ingesting sodium bicarbonate affected the release of MPs.[65] Seven young healthy men performed two bouts of 10 x 15 sec sprint cycling at 120% peak power output, following ingestion of sodium bicarbonate supplementation or placebo. Blood samples were taken pre-exercise, immediately post-exercise and at 90 min and 180 min post-exercise and circulating EMPs were measured using both CD105 and CD106 antibodies. Both CD105 and CD106 EMPs peaked at 90 min post-exercise and returned to baseline at 180 min. There was no significant difference in the circulating number of EMP's between the sodium bicarbonate and the placebo group. The increase in CD106 is in contrast to the findings of Chaar *et al.,* (2011)[63] following 3 $\dot{V}O_2$max test and may be due to differences in pre-analytical methodology and exercise protocols.

The increase in EMPs may reflect the increase in sheer stress on the endothelium in response to supra-maximal exercise. The fact that the EMPs returned to pre-exercise levels at 180 min indicates that the endothelium may have recovered from the exercise induced haemodynamic stress at this time. Sossdorf *et al.,* (2011)[64] also found that circulating MP levels returned to pre-exercise levels 2 h following 90 min of cycling at 80% IAT.

Parker *et al.,* (2012) examined the effects of a marathon race on the venous thrombotic risk markers, D-dimer, p-selectin and MPs in 23 travellers and 18 local controls. The travellers lived a minimum of a 4 h flight time from the marathon location while the controls lived within a 2 h drive of the marathon.[66] Blood samples were taken at the race location the day before the marathon and immediately after the race and the day after the marathon in the participant's home city following a flight or drive home.

MP activity increased in both groups following the marathon and the day after the marathon compared to baseline levels. There was no effect of travel, age or gender on the circulating number of MPs following the marathon. Soluble p-selectin and d-dimer also increased significantly in both groups after the race. Since soluble p-selectin, d-dimer and MP are associated with venous thrombosis and MPs and p-selectin are also linked with vascular, inflammatory and coagulation diseases the

observed increase in these biomarkers may potentially contribute to the increased risk of cardiac events linked to endurance running in vulnerable subjects.

PMP levels have been also found to increase significantly in young men and women (21 ± 0.3 years) immediately after reaching 85% of age predicted maximal heart rate following a Bruce treadmill protocol.[67] PMP remained significantly elevated 1 h following exercise. The increase in PMPs may indicate an increase in platelet activation. However, the markers of fibrinolysis and coagulation measured did not point to an activation of the coagulation system.

In contrast, Mobius-Winkler *et al.,* (2009) found no change in circulating MP ($CD42b^-/CD62E^+$) levels in 18 healthy men following 240 min of cycling at 70% IAT.[68] However, an increase in circulating endothelial progenitor cells (EPC), vascular endothelial growth factor, mature ECs and IL-6 during or after the exercise session may indicate some degree of endothelial damage. The exercise intensity may not have been high enough to induce the production of MPs.

Guiraud *et al.,* (2013) compared the effect a single bout of moderate intensity continuous exercise and high-intensity interval exercise (HIIE) matched for caloric expenditure on EMPs and PMPs in 19 men with an average age of 62 ± 12 years, and stable CHD.[69] The moderate intensity continuous bout consisted of cycling at 70% peak power output (PPO) for 28.7 min. The HIIE session consisted of a 10 min warm-up at 50% PPO followed by two 10 min bouts composed of 15 sec at 100% PPO

interspersed with 15 sec of passive recovery. The bouts were separated by 4 min of passive recovery and the session ended with a 5 min cool-down. Blood was taken 10 min before exercise and 20 min, 24 h and 72 h after exercise. MPs were enumerated using flow cytometry and EMPs were defined as $CD31^+/CD42b^-$ and $CD62E^+$ and smaller than 0.9µm. PMPs were defined as $CD42b^+$ and smaller than 0.9µm.

There were no changes in MPs after exercise in either the moderate intensity continuous exercise or the HIIE condition. There was an inverse relation between baseline EMP levels and the change in EMP levels 20 min after exercise. Individuals with the highest baseline EMPs had the greatest reduction in EMPs 20 min following the exercise. These results are similar to Chaar et al., (2011)[63] and Mobius-Winkler *et al.,* (2009)[68] who also found no increase in MP numbers after exercise. The results indicate that this type of HIIE does not induce harmful effects on the endothelial and appears safe for this population.

Exercise Training and MPs

To date, only two published studies have examined the effects of a training intervention on MP numbers[70 71]. Babbitt et al., (2013) examined the effects of 24-weeks of aerobic exercise training on EMPs and inflammation status in 42 sedentary (6 men and 36 women) African Americans with a mean age of 52.7 ± 1.0 y.[70] Participants completed 3 exercise sessions a week. The exercise sessions started with 20 min at 50% $\dot{V}O_2$max and increased to 40 min at 65% $\dot{V}O_2$max by week 8. During week 12

there was an adjustment in workload to account for improvements in aerobic fitness. Blood for EMPs, IL-6 and IL-10 was taken after a 12 hr fast before and after the training program. EMPs were identified using flow cytometry and the ability to bind CD62E+. Endothelial function assessed by flow mediated dilation (FMD) was measured before and after the exercise program. Circulating levels of CD62E+ EMPs were 47% lower than baseline at the end of the 6 month training program. There was also a significant decrease in IL-6 and an improvement in FMD but no significant change in IL-10. The changes in CD62E+ EMPS, IL-6 and IL-10 accounted for 10% of the change in FMD%.

Chen et al., (2013) examined the effects of hypoxic exercise training on CD61+ PMPs in sedentary men.[71] Although hypoxia has long been used as a training aid it may however, have a negative effect on the haemostatic system. Vigorous exercise in conjunction with hypoxia may induce a high shear stress in narrow blood vessels that increase the risk of prothrombotic events in patients with CAD.[71]

Seventy five sedentary men in their early twenties were randomly assigned to one of 5 groups and were randomly assigned to one of three experimental groups. Group 1 received 21% O_2 (normoxic) while resting; group 2 received 15% O_2 (hypoxic) while resting; group 3 and group 4 exercised on a cycle ergometer at 50% peak power output at 21% O_2 and 15% O_2 respectively and group 5 exercised at 50% of heart rate reserve at 15% O_2. Each group was administered their respective intervention for 30 $min \cdot d^{-1}$, 5 $d \cdot wk^{-1}$ for 4 weeks in a controlled chamber. Participants performed a graded

exercise test (GXT) to exhaustion before and after the intervention and blood was taken before and immediately after each GXT. Prior to the PMP measurement the blood was exposed to conditions of no stress, low-shear stress and high-shear stress using a rotational viscometer. The procoagulant activity of the PMPs was measured and expressed as FV/Va, FVIII or TF rich PMPs.

There was a shear stress induced increase in PMPs. High-shear stress enhanced the release of FV/Va, FVIII and TF rich PMPs and this was amplified following the GXT. After the training period there was a reduction in the release of PMPs and procoagulant PMPs following high-shear stress in the normoxic exercise and the hypoxic relative exercise group after the GXT. There was an increase in PMPs and procoagulant PMPs following the GXT in the hypoxic group exercising at 50% peak work rate. There were no changes in PMPs in the other two groups. These findings indicated that that it may be safer to exercise at a relative workload as there is less thrombotic risks as evidenced by a decrease in PMP or procoagulant PMPs.

Exercise and MP Response to a High Fat Meal

A number of studies have examined the prior effect of exercise on the MP response to a high fat meal. Following a high-fat meal, serum triglyceride (TG) concentrations increase. This increase in TG induces systemic oxidative stress and impairs endothelial function.[72] Ferreira *et al.,* (2004) found that postprandial hypertriglyceridemia increase EMPs.[73]

Harrison *et al.,* (2009) examined the effects of a prior bout of exercise on CD31+/42b- EMPs following a high fat meal.[74] Eight healthy, active men underwent two oral fat tolerance tests (OFTT) separated by 7 d. The study participants rested on the evening prior to one of the OFTT. On the evening prior to the other OFTT the participants cycled for 90 min at 70% $\dot{V}O_2$peak, followed by 10 x 1 min sprints interspersed with 1 min of recovery to maximise glycogen depletion. Flywheel resistance was increased by 25% for the sprints. On the morning of the oral fat tolerance test blood was taken before the meal and 30 min, 1 h, 2 h, 4 h and 6 h following the high-fat meal.

Compared to baseline values, circulating EMPs increased significantly at 2 h following the OFTT and remained elevated at 4 h and 6 h post in both trials. There was no significant difference in EMP values between the exercise and control conditions. Circulating IL-6 levels were significantly increased at 4 h and 6 h following the meal in both trials and there were no changes in sICAM-1 or sVCAM-1. There was a significant increase in the number of circulating leukocytes in both groups at 6 h following the meal compared to baseline. Fasted EMP counts were not related to any of the biomarkers in the fasted state. The increase in EMPs, IL-6 and leukocytes may indicate postprandial endothelial activation. The fact that there was an increase in EMPs with no changes in sICAM-1 or sVCAM-1 may suggest that EMPs are a more sensitive biomarker of endothelial activation than soluble adhesion molecules.

During the exercise trial there was a 40% reduction in postprandial lipemia and an 8% increase in high-density lipoprotein cholesterol (HDL-C). However, a reduction of a similar magnitude was not observed for EMPs. Considering that TG-rich lipoproteins are cytotoxic to endothelial cells *in vitro* and HDL-C has a positive effect on endothelial dependent dilation (EDD), a decrease in EMPs would be expected following the exercise trial. Moyna *et al.,* (2004) suggest that exercise may only improve endothelial function in people who have impaired endothelial function.[11] The participants in this study were young physically active men with no CVD symptoms. Furthermore, postprandial free fatty acids (FFA) were higher in the exercise trial. Increases in lipolysis at the endothelial surface, induced by exercise may decrease TG but increase FFA uptake by the endothelial cells. It is also possible that the exercise intensity may not have been high enough to induce an inflammatory response.

Strohacker *et al.,* (2012) examined the postprandial changes in circulating monocytes and biomarkers of endothelial function, including EMPs, in 4 men and 4 women following a high-fat meal with or without prior exercise.[75] Participants ingested two high-fat meal trials separated by 7 days with and without prior exercise. The exercise consisted of cycling at an intensity corresponding to 60 – 75% $\dot{V}O_2$peak for 1 h while they achieved an energy expenditure corresponding to 4 – 6 kcal·kg^{-1} body mass. The high-fat meal was taken 1 h following the exercise bout or the rest period. Blood was taken before and after the exercise/rest period, before the high-fat

meal and at 1 h intervals for 3 h. EMPs were enumerated using flow cytometry and defined as $CD31^{+}/CD42b^{-}$ and < 1.5μm.

Compared to the pre meal values circulating EMP levels at 3 h post meal were 47% higher in the meal only trial. In contrast, there was no significant increase in circulating EMP levels in the exercise condition indicating that prior exercise ameliorated the increase in EMPs 3 h following the high fat meal. Monocyte CD18 and CD11a were lower in the exercise trial and probably reflect the lower number of EMPs.

Jenkins et al., (2011) also examined the interaction between prior exercise and postprandial circulating EMPs in 10 healthy active men with a mean age of 27 yr.[76] Participants exercised at 15:00 h or rested the day before the high-fat meal. The exercise involved cycling at 70% $\dot{V}O_2$max until they expended 2.5 MJ. To ensure a fixed macronutrient composition the study participants received the same meal between 19:00 h and 20:00 h on the same evening as the exercise session. The control trial subjects rested the day before the high-fat meal. The high-fat meal was taken the following morning at 06:00 h. Blood was taken before the meal and every hour for 4 h. EMPs were enumerated using flow cytometry and defined as $CD31^{+}/CD42b^{-}$ (endothelial apoptosis) and $CD62E^{+}$ (endothelial activation) and < 1.0μm.

EMP values before and after the meal were lower with prior exercise. During the exercise trial $CD31^{+}/CD42b^{-}$ EMPs were 30% lower and $CD62E^{+}$ 55% lower than the control trial. In direct contrast to both Harrison et al., (2009)[74] and Strohacker *et al.,*

(2012)[75] the circulating levels of EMPs did not increase following the high-fat meal. Also in contrast to Harrison *et al.,* (2009)[74] there was no decrease in TG in the exercise group. Differences in exercise intensity, timing and, meal composition and timing, and methods used to quantify EMPs makes it difficult to directly compare the studies.

Table 2.3: MPs and exercise

	Participants	**Exercise Mode**	**Exercise Type**	**MP Time Points**	**MPs**	**Antigen**	**Result**	**Measurement Technique**
Charr *et al.,* (2011)[63]	7 young healthy males	Cycle ergometer	3 max test with 10min break	Pre, post, 2 h	Total MP PMP EMP MMP RBC MP PMN MP	Annexin V, CD41, CD106, CD14, CD235a, CD15	↑ post and 2h ↑ post and 2h ↔ ↔ ↔ ↑post and 2h	FC
Sossdorf *et al.,* (2011)[64]	16 young healthy males, 8 trained (T), 8 untrained (UT)	Cycle ergometer	90min at 80% IAT	Pre, post, 45 min, 2 h	Total MP PMP MMP EMP	Annexin V CD42a CD14 CD62E	↑ in both groups ↑ in T at post and 45 ↑ in UT at 45 and 2h ↑ in T at post and 45 ↑in T at 45	FC
Kirk *et al.,* (2013)[65]	7 young healthy males	Cycle ergometer	2 trials 10 x 15 sec sprints at 120% PPO with 45 sec recovery	Pre, post, 90 min, 180 min	EMP	CD105 CD106	↑ in both trials at 90 min ↑ in both trials at 90 min	FC

			1 trial with NaHCO3					
Babbitt *et al.,* (2013)[70]	42 middle aged healthy African Americans 6 men 36 women	Treadmill, stair stepping, cycling, rowing, arm ergometer, cross-trainer	6 month aerobic exercise training	Pre and post intervention	EMP	CD62E	↓ between pre and post intervention	FC
Mobius-Winkler *et al.,* (2009)[68]	18 healthy young men	Cycle ergometer	4 h at 70% IAT	16 time points during and after exercise	Total MP EMP	CD42b- CD62E+/ CD42b-	↔ ↔	
Parker *et al.,*	Marathon runners, 23 travellers, 18 local	Running	Marathon	Pre, post and next day	MP pro coagulant activity		↑ post and the next day. No travel effect	ELISA

(2012)[66]	controls							
Maruyama *et al.,* (2012)[67]	18 healthy subjects 9 men 9 women	Treadmill	Max test until 85% of APHRM	Pre, post and 1 h	PMP		↑ post and at 1 h	ELISA
Guiraud et al., (2013)[69]	19 fit men with CHD	Cycle ergometer	Moderate intensity continuous exercise or HIIE	Pre, 20 min post, 24 h and 72 h post	EMP PMP	$CD42b^-$ /$CD31^+$ $CD62E^+$ $CD42b^+$	↔ ↔ ↔	FC
Chen *et al.,* (2013)[71]	75 sedentary males 5 groups of 15	Cycle ergometer Rest	30 min, 5xweek for 4 weeks. 1) 21% O_2 rest and 2) 50% PPO 3)15% 0_2 rest and 4)50% PPO	Pre and post GXT before and after training	PMP Procoagulant PMPs	CD61+ FV, FVIII or TF rich	↓ after training following the GXT in 2) and 5) ↔ in group 1) or 3) ↑ in group 4)	FC

			5)15% 0_2 50% APHRR					

FC, flow cytometry; MP, microparticle, EMP, endothelial microparticle, PMP platelet microparticle, MMP, monocyte microparticle; RBC, red blood cell; PMN, polymorphonuclear neutrophil; T, trained; UT, untrained; IAT, individual anaerobic threshold; PPO, peak power output; HIIE, high intensity interval exercise; APHRR, age predicted heart rate reserve

The Endothelium

The vascular endothelium is a monolayer of endothelial cells that form a semi-permeable biological interface between the vascular space and the tissues.[11] Endothelial cells are highly specialized to detect diverse physical, chemical, or mechanical stimuli. In addition to their role in the regulation of vascular tone, healthy endothelial cells continuously adapt to local requirements and are essential for the maintenance of entire vascular homeostasis involving antioxidant, anti-inflammatory, profibrinolytic, and anticoagulant effects.

Vascular Endothelium – Structure

The endothelium consists of a single layer of endothelial cells (EC) providing a lining for the lumen of the entire vascular system. The cells have a squamous morphology and are aligned in the direction of laminar blood flow. ECs are between 25-50 µm in length, 10-15 µm in width and up to 5 µm in depth.[77] The human body contain up to 6 trillion ECs covering approximately 4000 to 7000 m^2, representing about 1% of body mass.[78]

Endothelial cells are separated from the underlying tissue by the basal lamina. The basal lamina connects to the ECs via integrins. The surface of the ECs exposed to blood flow is lined by a negatively charged layer called the glycocalyx. This layer allows water and small solutes to exit the blood but keeps negatively charged proteins in the

blood stream.[38] The cytoskeleton of the EC, composed mainly of actin and myosin filaments, provides the structure for the cell and attaches ECs to each other and to the basal lamina. Adjacent ECs are connected via junctional proteins which extend across the space between adjacent cells. This space between ECs is called the intracellular cleft. The junctional proteins play an important role in transport through the endothelial layer.

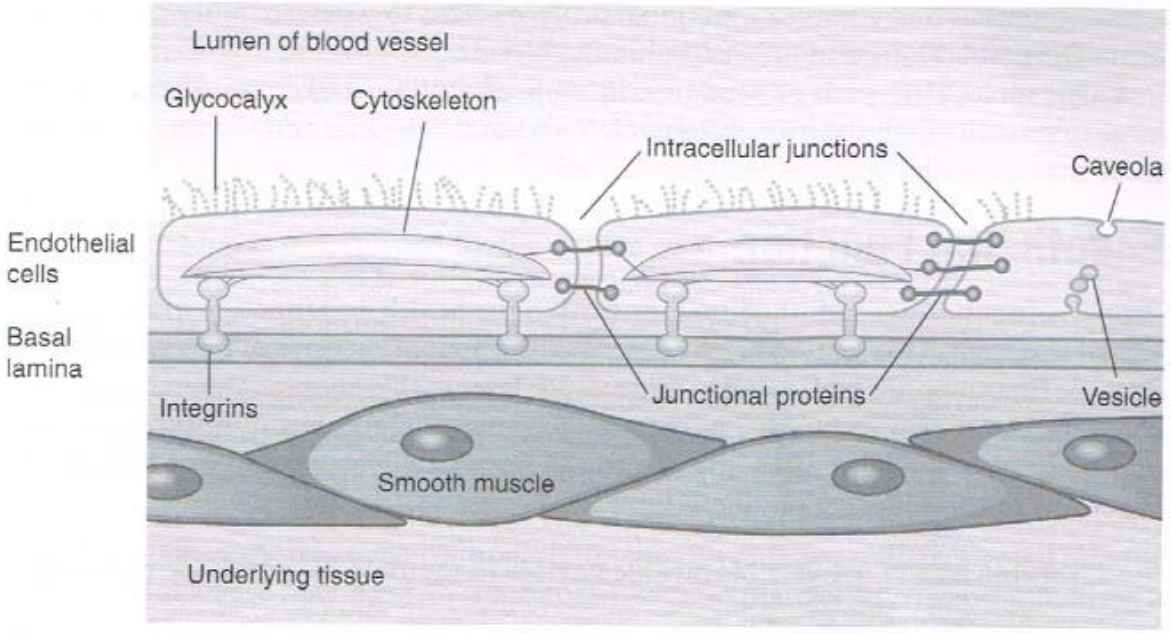


Figure 2.15: Vascular Endothelium[38]

Vascular Endothelium – Functions

The endothelium serves as a selectively permeable barrier regulating exchange of nutrients and gases between the blood and tissues. Lipid-soluble substances can

diffuse through the ECs while small, water-soluble substances diffuse through the intracellular junctions.

ECs regulate vascular tone and basal vasomotor tone by the release of vasodilators and vasoconstrictors.[77] Nitric oxide (NO) is the primary vasodilator released by EC and also plays an important role in the inhibition of thrombosis. Prostacyclins are also released by EC and play an important role in vasodilation and platelet inhibition, with PGI_2 being the active prostacyclin in ECs.[78] The primary vasoconstrictors active in ECs include endothelins (ETs) and platelet activating factor.

The endothelium has both anticoagulant and procoagulant properties. In a healthy endothelium the balance is towards anticoagulant properties, however following endothelial damage procoagulant properties become apparent.[77] A healthy endothelium has the ability to inhibit the generation and activity of thrombin which prevents fibrinogen being converted to fibrin. The endothelium expresses procoagulant properties in response to tissue factor (TF). Several agonists can stimulate TF production from the endothelium. Shear stress, thrombin, cytokines such as IL-1 and TNF-α, hypoxia and oxidised lipoproteins have all been linked to endothelium TF production.[78]

The endothelium also plays an active role in the inflammatory defence against pathogens. Following injury the endothelium produces and expresses adhesion molecules that allow circulating leukocytes to attach and transmigrate to the intima.[38]

Depending on the type of stimuli the initial adhesion molecules produced by the endothelium include P-selectin, E-selectin and L-selectin. The selectins facilitate "rolling" of leukocytes along the surface of the endothelium. Rolling facilitates the attachment of leukocytes to the endothelium by another family of adhesion molecules called intracellular adhesion molecules, ICAM-1, -2, -3, and vascular cell adhesion molecule-1 (VCAM-1). Platelet-endothelial cell adhesion molecule-1 (PECAM-1) facilitates the transmigration of leukocytes to the intima.[78]

Vascular Endothelium - Dysfunction

Endothelial dysfunction is a term used to describe a series of pathological changes in the function and structure of the endothelium. These changes include increased permeability to plasma lipoproteins, hyperadhesiveness to leukocytes and imbalances in the regulation of vascular tone and haemostasis leading to a reduced vasodilation and an increased prothrombotic and pro-inflammatory state.[38,79] Endothelial dysfunction is an early manifestation of atherosclerosis and is predictive of an increased risk for cardiovascular events.[79]

Vascular Endothelium – Exercise

Exercise training promotes an acute increase in blood flow and shear stress, improving NO availability and increasing endothelium-dependant vasodilation.[80] The improvement in NO availability may represent one of the most important mechanisms explaining the improvement in exercise-induced endothelial function. The exercise-

induced improvement in endothelial function is more obvious in individuals with impaired endothelial function.[81] Studies examining the effect of exercise on endothelial function in healthy men and women are inconsistent, while the majority of studies examining individuals with impaired endothelial function have reported improvements with exercise.

Rinder *et al.,* (2000) examined endothelial function between a group of masters athletes and sedentary men.[82] They found that endothelial-dependant dilation was greater in the athletes suggesting exercise may attenuate the decline in endothelial function associated with ageing.

Exercise intensity is an important factor when discussing improvement in the vascular endothelium in response to exercise. Moderate-intensity exercise has been shown to be effective in improving endothelial function in men and women with impaired endothelial function.[83] Recent studies have found that high-intensity interval exercise is better stimulus than moderate-intensity exercise for improving endothelial function in individuals with the metabolic syndrome[84] and in heart failure patients.[85] These findings may be due to increased bioavailability of NO following high shear stress during the high-intensity exercise.

Lipids

Lipids are a heterogeneous group of hydrophobic organic molecules and can be broadly classified as fatty acids, acylglycerols, phospholipids, eicosanoids, steroids and

lipoproteins. They have a number of important functions including the formation of membranes, energy storage, cellular signalling protection, insulation and the production of steroid hormones and bile acids.

The most commonly reported lipids in studies examining the effects of exercise on lipid profiles include total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TGs).[86] Exercise has a marked effect on atherogenic dyslipidemia, a characteristic of the metabolic syndrome. The metabolic syndrome is characterised by low HDL-C and high LDL-C and TGs.[87] HDL-C transports lipids from peripheral tissue to the liver where they are recycled or disposed and a high concentration of HDL-C is linked to a healthy cardiovascular system. In contrast, high circulating levels of LDL-C and TG are associated with an increased the risk of CVD.

Exercise Training and Lipids

Aerobic exercise, resistance training and various combinations have been successfully used to lower blood lipid levels. However, the optimal type, intensity, frequency or duration of exercise has not been established. The most commonly observed change following an exercise intervention is an increase in HDL-C, with decreases in LDL-C, total cholesterol and TGs less frequently observed.[88]

In a review covering 51 exercise interventions with 12 weeks or more of aerobic exercise Leon and Sanchez (2001) found that, on average, there was a 4.6%

increase in HDL-C, a 3.7% decrease in TGs and a 5% decrease in LDL.[88] No change in total cholesterol was reported. In a subsequent review Mann *et al.,* (2014) found that moderate-intensity exercise is effective in increasing HDL-C.[86]

O'Donovan *et al.,* (2005) compared the effect of 24 week moderate-intensity exercise program (60% $\dot{V}O_2$max) with a calorie matched high-intensity exercise program (80% $\dot{V}O_2$max) on blood lipids.[89] Improvements in lipid profiles were only found in the high-intensity group. There were significant decreases in total cholesterol, LDL-C and non-HDL-C in the high-intensity group. Dunn *et al.,* (1997) found significant reductions in total cholesterol and the total:HDL-C ratio following a 6 month aerobic exercise program which progressed to 85% of maximum aerobic power for 20 – 60 min three times a week.[90] LeMura *et al.,* (2000) investigated the effects of 16 weeks of aerobic exercise on lipid profiles in young women.[91] The exercise was progressed from three 30 min sessions weekly at 70 – 75% HR_{max} for the first 8 weeks to four 30 min sessions at 85% HR_{max} for the last 8 weeks. Significant decreases in TGs and increases in HDL-C were reported. Nybo *et al.,* (2010) compared traditional aerobic exercise with high intensity training on blood lipids.[92] The traditional aerobic exercise consisted of ~150 min of exercise a week at 65% $\dot{V}O_2$max and the high intensity exercise sessions consisted of 40 min of intense exercise a week. The high intensity session consisted of a 5 min warm-up with light jogging followed by 5 intervals of 2 min with heart rate above 95% of heart rate max at the end of 2 min period. The total:HDL-C ratio significantly improved in the traditional group. No

significant changes were found in the high intensity training group. These findings indicate that training volume is more important than training intensity in eliciting changes in lipid profiles. A review on the impact of exercise on blood lipids and lipoproteins by Trejo-Gutierrez and Fletcher (2007) recommend both high-volume and high-intensity exercise to significantly alter blood lipid profiles.[87]

Acute Exercise and Lipids

Increases in HDL-C and reductions in TGs have been reported after a single exercise session.[93] Reported increases in HDL-C vary from 4 to 43% and occur 18 – 24 h after exercise.[94] The duration and intensity of exercise needed to acutely increase HDL-C is not clearly defined but changes have been reported in moderately fit subjects after expending 350 – 400 kcal and in well-trained subjects following exercise expending 1000 kcal.[94] Reductions in TGs follow the same timeline as the increases in HDL-C and can remain low for up to 72 h. Reductions are greatest in individuals with higher pre-exercise TG levels[95] and the most consistent results have been found in trained subjects performing prolonged endurance exercise.[94]

Greene et al., (2012) investigated the effects of training on the acute effects of exercise on blood lipids and lipoproteins.[96] Overweight and obese men and women performed an acute bout of exercise on a treadmill at 70% $\dot{V}O_2$max until they expended 400kcal before and after 12 weeks of aerobic exercise training. The training consisted of land-based or aquatic-based treadmill running 3 times weekly progressing

to expending 500 kcal at 85% $\dot{V}O_2$max from week 6 - 12. The acute bout of exercise reduced the total:HDL-C ratio in men before the training intervention, and there was no difference in the effect of the acute exercise following the training intervention. Exercise training resulted in increases in HDL-C.

Study Rationale

Despite declines in prevalence in recent years, CVD remains the leading cause of morbidity and premature mortality in men and women worldwide.[132] MPs have been found to play a role in coagulation, inflammation and endothelial function/dysfunction which are all closely related to CVD. The benefits of physical activity in the primary and secondary prevention of CVD are well established. At the time of conception of study 1 and 2 there were no published research studies investigating the effects of physical activity/exercise on circulating MPs. A series of studies was undertaken to investigate the effect of acute and chronic exercise on circulating MPs.

Conventional exercise guidelines for reducing CVD risk factors and cardiovascular events have promoted continuous, moderate to vigorous intensity exercise training. Exercise performed at a vigorous intensity (≥6 METs) has been shown to induce a greater reduction in CVD risk along with greater improvements in diastolic blood pressure, glucose control and aerobic capacity than exercise performed at a moderate intensity.[10] Study 1 evaluated the dose response of moderate to high intensity exercise on circulating MPs in healthy physically active men. Participants performed an isocaloric bout of exercise at exercise intensities corresponding to 60%, 70% and 80% $\dot{V}O_2$max. Study 2 examined the effects of 14 d of exercise training on circulating MPs in healthy sedentary men. Improvements in aerobic capacity have

been linked to improvements in CVD and many of the processes leading to CVD.[81,97] Considering that an improvement of 10% in aerobic fitness has been found following 7 – 14 d of aerobic exercise[98] a 14 d training period was selected in order to provide a sufficient stimulus to improve $\dot{V}O_2$max. Participants undertook 60 min of daily exercise at 80% $\dot{V}O_2$peak to ensure a moderate to high intensity exercise session in line with previous research reporting improvements in CVD risk factors. Blood samples were taken before, immediately after and 13 h after the exercise on day 1, 3 7, 10 and 14 to investigate the possible changes in MP response to exercise during the training sessions.

Participants in study 1 and 2 were young healthy men. However, resting levels of EMPs are increased in individuals with established risk factors linked to endothelial dysfunction and CVD.[44,55] Study 3 investigated the effect of exercise training on circulating MP and endothelial function in men and women with documented CVD. Recently, low volume short-duration, high-intensity interval training (LS-HIIT) involving repeated bouts of short-duration high-intensity exercise separated by periods of active or passive recovery have been shown to be a safe and effective alternative to more traditional continuous, moderate-to-vigorous exercise programs in improving exercise capacity and endothelial function in individuals with CVD. Men and women who had been participating in a community based cardiac rehabilitation program for at least 6 months were recruited and this enabled a comparison between a traditional cardiac rehabilitation program and a LS-HIIT program. This is the first study to investigate the

relation between circulating MPs and endothelial function in response to exercise training in men and women with CVD

There is strong evidence for the link between circulating lipids and CVD.[97] Exercise training has been shown to reduce total cholesterol, LDL-C, triglycerides and increase HDL-C. However, there is a dearth of information regarding the relation between lipids and circulating MPs following acute and chronic exercise in healthy individuals and those with documented CVD. Blood lipids were measured in each of the 3 studies in order to address these issues.

Chapter III

Study I

The Dose Response of an Acute Bout of Exercise on Circulating Microparticles

Rationale

Microparticles are small membrane vesicles shed from membranes following cell activation or apoptosis. EMPs and PMPs are involved in inflammatory and coagulation processes related to the development of CVD.[6] Exercise is a commonly used non-pharmacological intervention in the prevention and treatment of CVD.[11,99,100] The acute effects of exercise on markers of coagulation, inflammation, and endothelial dysfunction have been extensively investigated. In contrast little is currently known about the acute effects of exercise on circulating MPs.

Exercise dose refers to the type, intensity, frequency and/or volume of exercise. The response refers to the physiological and psychological changes that occur when a specific dose of exercise is performed. Varying exercise intensities have been shown to elicit different responses in many of the processes linked to CVD. Studies that have controlled for accumulated energy expenditure (EE) found greater improvements in aerobic fitness at higher intensities compared to moderate intensities.[10] To date, no studies have compared the effect of exercise at different intensities on circulating levels of MPs. Since vigorous exercise elicits a greater EE than

moderate exercise of a similar duration it is important to control for EE when comparing the effect of exercise intensity on circulating levels of MPs.

The purpose of this study was to examine the effect of isocaloric bouts of exercise at different intensities on circulating MPs in healthy young men.

Specific Aims:

1. To compare the acute effects of isocaloric bouts of exercise at 60%, 70%, and 80% $\dot{V}O_2$max on circulating EMP levels in young healthy physically active men
2. To compare the acute effects of isocaloric bouts of exercise at 60%, 70%, and 80% $\dot{V}O_2$max on circulating PMP levels in young healthy physically active men

Hypothesis:

1. There will be no significant difference in circulating levels of EMPs following isocaloric bouts of exercise at 60%, 70%, and 80% $\dot{V}O_2$max in healthy young physically active men
2. There will be no significant difference in circulating levels of PMPs following isocaloric bouts of exercise at 60%, 70%, and 80% $\dot{V}O_2$max in healthy young physically active men

Methodology

Study Overview

The study design is illustrated in Figure 3.1. 10 Participants visited the Human Performance Laboratory (HPL) in DCU on 5 separate occasions. During the first visit participants underwent an initial screening consisting of a general health questionnaire (Appendix A), medical examination, body composition and an electrocardiograph (ECG) to confirm their suitability for the study. The second visit consisted of a maximal exercise test to determine maximal aerobic capacity ($\dot{V}O_2max$). During visits 3-5 participants were randomly assigned to exercise at 60%, 70% or 80% $\dot{V}O_2max$ until they expended 400 kcal. Blood samples were taken before, immediately after and at 2 h, 4 h and 6 h post-exercise.

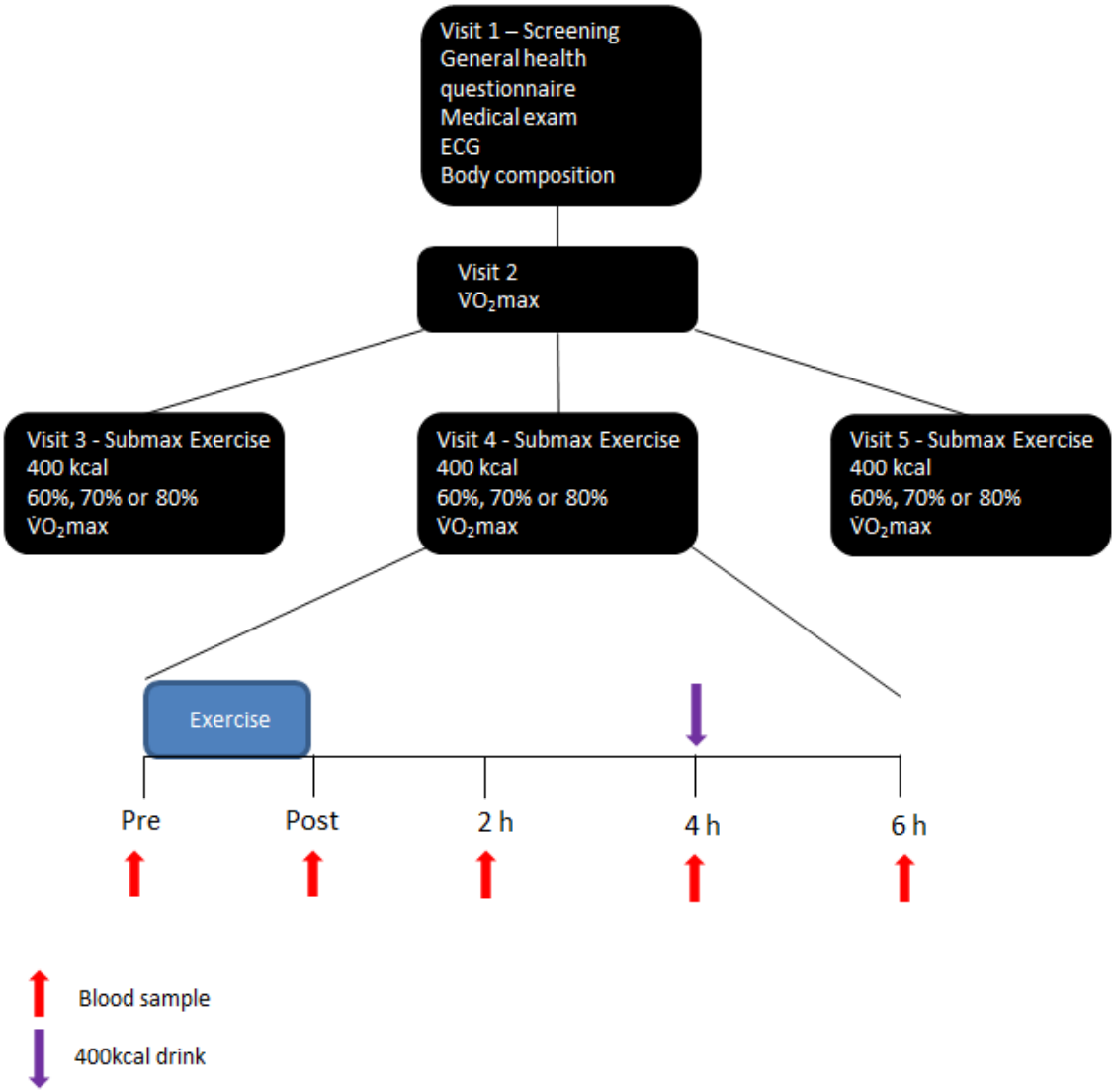


Figure 3.1: Study design

GHQ, general health questionnaire; ECG, electrocardiograph

Participant Recruitment

An email was sent to all undergraduate and post graduate students attending Dublin City University (Appendix B). Students who expressed an interest in participating in the study received a plain language statement detailing the requirements of the study (Appendix C). Written informed consent was obtained from

individuals who agreed to participate in the study (Appendix D). The study was approved by the Dublin City University Research Ethics committee (Appendix E). Participants were excluded if they were current smokers, not involved in regular physical exercise or had any other medical conditions that contraindicated exercise participation.

Maximal Oxygen Uptake

Participants performed an incremental exercise test until volitional exhaustion on a motorised treadmill (Woodway ELG 55, Waukesha, WI). The tests were conducted in a temperature controlled laboratory (19-21^{o}C, 40-55% relative humidity). The test was preceded by a 5 min warm-up at 8 km·h^{-1}. Following the warm-up participants exercised for 2 min at 10.0 km·h^{-1} followed by a further 2 min at 12.0 km·h^{-1}. The slope of the treadmill was then increased 2% a min for the first 2 min and then 1% every min until volitional exhaustion. Respiratory metabolic measures were monitored continuously throughout the test. Maximal oxygen uptake was determined by averaging the three highest consecutive 20 sec values.

Cardiorespiratory and Metabolic Measures

Respiratory metabolic responses were determined using standard open-circuit spirometry techniques (Sensormedics Vmax 29c, 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 artefact 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 $L \cdot sec^{-1}$ and integrated flow between 0-350 L min^{-1} with flow resistance <1.5 cmH_2O L^{-1} sec^{-1}. The mass flow sensor was outputted to the analyser module of the Vmax 29c 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 $L \cdot sec^{-1}$, and at least one of the four strokes had to have an average flow > 3.0 $L \cdot sec^{-1}$.

Gas Analysers

The Vmax 29c utilizes a rapid response infrared measurement technique. An O_2 and CO_2 analyser is integrated with the Vmax 29c. 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 O_2 and CO_2 Gas 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% O_2 and the balance nitrogen (N2). The second calibration gas contained 4.00 ± 0.02% CO_2, 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.

Calculation of Exercise Intensities

Oxygen uptake at 60%, 70% and 80% $\dot{V}O_2$max was estimated for each participant using the individual linear regression equation between $\dot{V}O_2$ and treadmill velocity obtained during the initial three stages of the $\dot{V}O_2$max test based on the linear

relation between oxygen uptake (y-axis) and treadmill velocity (x-axis). For a given percentage of the $\dot{V}O_2$max, the corresponding treadmill velocity was estimated by solving for x using the linear function y=mx + c, where y is $\dot{V}O_2$, x is speed, m is the slope of the relationship between $\dot{V}O_2$ and speed and c is the intercept point on the y axis.

Submaximal Exercise Session

On three separate occasions the participants reported to the Human Performance Laboratory in DCU the morning following an overnight fast. The first submaximal exercise session was performed one week after the completion of the $\dot{V}O_2$max test. A cannula was placed in a prominent forearm in order to take serial blood samples. A resting blood sample was taken following 5 min of rest in a seated position. Participants then exercised at a treadmill velocity corresponding to 60%, 70% or 80% $\dot{V}O_2$max until they expended 400 kcal. Sessions were randomised and performed at least 4 d apart. Blood samples were taken immediately after exercise while the participant was standing on the treadmill and in a seated position at 2 h, 4 h and 6 h.

The treadmill velocity was adjusted during the first 10 min of each exercise session in order to ensure that participants were exercising at the required intensity. Expired air was collected continuously throughout the exercise session and used to calculate caloric expenditure.[101] Participants were weighed to the nearest 0.1 kg in

minimal clothing immediately before and after exercise in order to monitor fluid loss during the submaximal exercise sessions. They were given a volume of plain water equivalent to 150% of weight loss to drink after the training session to ensure proper hydration levels.

Dietary Control

Participants were required to record their daily intake of food and fluids for 3 d prior to the first submaximal exercise session. They were asked to repeat this dietary pattern before the remaining two submaximal exercise sessions to avoid any possible changes from variation in day to day dietary intake. Participants consumed a 400 kcal drink immediately following the 4 h blood sample. The drink consisted of 13.9 g of protein, 55.9 g of carbohydrate and 13.4 g of fat.

Assessment of Body Composition

Body density was calculated using the sum of seven skinfolds (triceps, subscapular, pectoral, mid-axillary, suprailiac, abdomen and thigh) by the method of Jackson and Pollock (1978).[102] Skinfolds were measured using Harpenden skinfold callipers. Percentage body fat was calculated using the Siri equations.[103]

Calculations of Energy Expenditure

Values for $\dot{V}O_2$, $\dot{V}CO_2$, RER, $VE_{(STPD)}$, and FEO_2 values were averaged from expired air every 60 s and used to calculate the rate of energy expenditure using the Weir equation.[101]

Blood Sampling and Storage

Serum vacutainers for lipid analysis were allowed to stand for 30 min at room temperature before being centrifuged at 3000 rpm (1600g) for 15 min at $4^{O}C$. Blood for microparticles analysis was collected, prepared and stored according to methods described by Bernal-Mizrachi *et al*., (2004).[104] Blood was collected in pre-chilled sodium citrate vacutainers before being centrifuged twice. The first spin, 160 g for 9 min, produced platelet rich plasma (PRP). The top layer of this plasma was harvested and spun for a further 9 min at 1000g to PPP. The PPP was aliquoted and stored at -80°C. Blood for a complete blood count (CBC) was collected in EDTA vacutainers and allowed stand at room temperature before analysis.

Microparticle Analysis

Flow cytometry, using CellQuest software (FACScan, Becton Dickinson), was used to quantify microparticle numbers. Microparticles were identified in PPP based on size and fluorescence, using 1.0 µm sizing beads and anti-CD 31 FITC and anti-CD42b PE monoclonal antibodies. EMPs were defined as CD31+/42b- and PMP were

defined as CD42b+. An 85 µl volume of PPP was incubated for 20 min with 10 µl of anti-CD31 and 5 µl of anti-CD42b. Samples were then diluted with 400 µl of PBS and analysed for 60 sec at medium speed. The flow rate was calibrated before each run using 15 µl of flow count beads diluted in 485 µl of PBS. Fluorescence thresholds were set using PPP incubated with isotype-matched control antibodies. Samples were analysed in duplicate and all samples for each participant were analysed in the same run.

Lipid Analysis

Triglycerides, total cholesterol, HDL-C and LDL-C were analysed using an automated clinical chemistry analyser (Randox Daytona, Randox, NI). A standard curve was generated from supplied calibrators of zero and a known concentration and control samples were subsequently analysed to check for accuracy. Samples were run in duplicate and each participant's samples were analysed in the same run.

Leukocytes and Platelets

Leukocytes and platelet numbers were measured using a Coulter AcTdiff2 analyser (Beckman Coulter, Indianapolis, IN). Each EDTA vacutainer was mixed gently by inversion before sampling and each sample was run in duplicate.

Statistical Analysis

Prior to statistical analysis the data was checked for normality using the Shapiro-Wilk test. EMP and PMP data was log transformed to conform to normality. An exercise intensity (60%, 70% and 80% $\dot{V}O_2max$) by time (pre, post, 2 h, 4 h and 6 h post-exercise) repeated measure ANOVA was used to compare the differences in EMPs, PMP, lipids and blood counts across the 3 different exercise intensities. A one-way ANOVA was used to compare the physiological responses, speed and time across the 3 exercise intensities. Significant main effects and interactions were probed using a Bonferroni post hoc test, in the presence of significant interactions there was no analysis of main effects. 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

A total of 10 healthy, physically active young men participated in the study (age, 21.6 ± 1.0 yr; BMI, 23.2 ± 0.4 $kg \cdot m^{-2}$; % body fat, 7.7 ± 1.0; $\dot{V}O_2max$, 58.8 ± 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$).

Exercise Trials

Total exercise time, $\%\dot{V}O_2max$ and total caloric expenditure during the submaximal exercise sessions are summarised in table 3.2. There was no significant difference in caloric expenditure between the 3 exercise trials. The time required to expend 400 Kcal was significantly longer during the exercise trial at 60% $\dot{V}O_2max$ than 70% $\dot{V}O_2max$ and 80% $\dot{V}O_2max$ and during the exercise trial at 70% $\dot{V}O_2max$ than 80% $\dot{V}O_2max$. There was no significant difference between predicted $\dot{V}O_2$ and actual $\dot{V}O_2$ (Figure 3.2) or predicted treadmill velocity and actual treadmill velocity at any of the three exercise intensities. (Figure 3.3)

Table 3.1: Percent $\dot{V}O_2$, total caloric expenditure and total exercise time

	$\%\dot{V}O_2max$		
	60%	**70%**	**80%**
% $\dot{V}O_2$	60.93 ± 0.42	70.36 ± 0.44*	79.73 ± 0.45*†
Total Kcal	405.05 ± 4.45	403.90 ± 2.04	398.01 ± 2.94
Time (min)	30.88 ± 1.10	26.63 ± 0.81*	23.15 ± 0.68*†

Values are mean ± SE; *p < 0.001 vs. 60% $\dot{V}O_2max$; †p < 0.001 vs. 70% $\dot{V}O_2max$

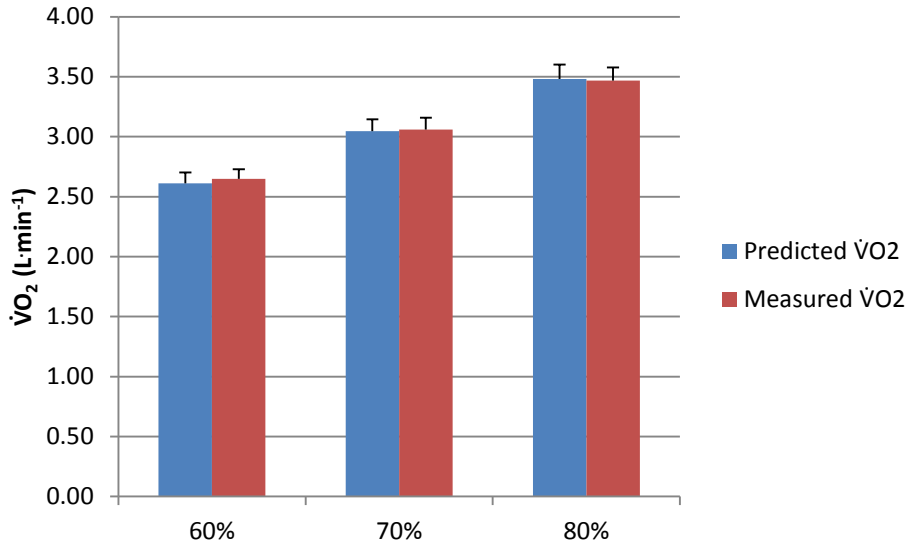


Figure 3.2: Predicted and measured $\dot{V}O_2$ ($L \cdot min^{-1}$)

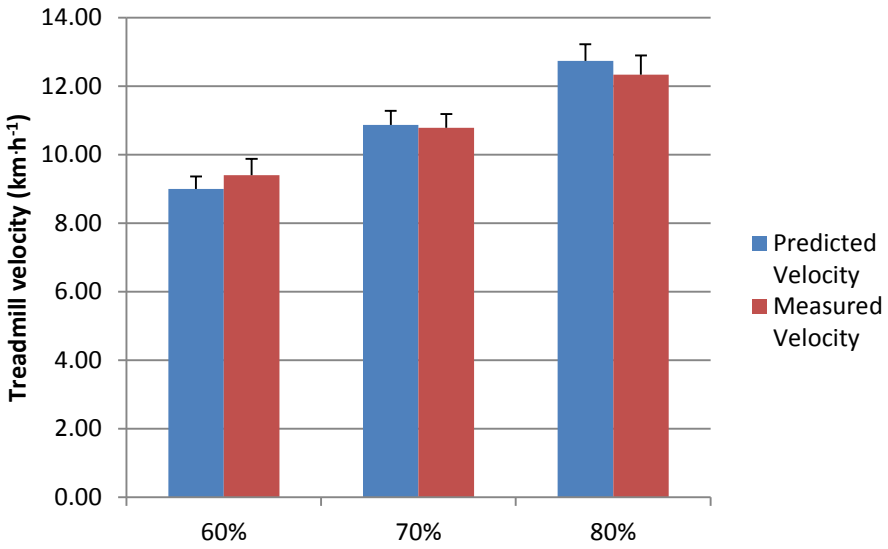


Figure 3.3: Predicted and measured treadmill velocity ($km \cdot h^{-1}$)

Endothelial Microparticles

The mean number of circulating EMPs 4 h following exercise at 70% $\dot{V}O_2$max and 6h following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max were significantly higher than pre-exercise. Compared to post-exercise there was a significant decrease in the mean number of circulating EMPs 2 h following exercise at 70% $\dot{V}O_2$max and a significant increase at 4 h following isocaloric bouts of exercise at 60% and 80% $\dot{V}O_2$max. There was a significant increase in the mean number of circulating EMPs at 4 h and 6 h following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max than 2 h post-exercise. There are no significant differences in circulating levels of EMPs between the three exercise intensities pre-exercise, post-exercise, 2 h, 4 h and 6 h post-exercise.

Table 3.2: Circulating levels of EMPs (counts·µl^{-1}) before and after isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max

	%$\dot{V}O_2$max		
	60%	**70%**	**80%**
Pre-exercise	2029 ± 333	1451 ± 116	1647 ± 201
Post-exercise	1749 ± 198	2170 ± 374	1695 ± 163
2 h post-exercise	1969 ± 230	1275 ± 225†	1448 ± 199
4 h post-exercise	2949 ± 487†‡	2740 ± 450*‡	2076 ± 165†‡
6 h post-exercise	2945 ± 328*‡	2403 ± 198*‡	2529 ± 284*‡

Values are mean ± SE. *p < 0.01 vs. pre-exercise; †p < 0.01 vs. post-exercise; ‡p < 0.05 vs. 2 h post-exercise

Platelet Microparticles

The circulating levels of PMPs before and after isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max are summarised in table 3.4. There was no significant difference in circulating PMP levels between the three different exercise intensities at pre-exercise, post-exercise, 2 h, 4 h and 6 h post-exercise.

Table 3.3: Circulating levels of PMPs (counts·µl^{-1}) before and after isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max

	%$\dot{V}O_2$max		
	60%	**70%**	**80%**
Pre-exercise	10403 ± 1402	14206 ± 2857	19651 ± 3834
Post-exercise	11595 ± 2334	17065 ± 2870	19916 ± 3790
2 h post-exercise	20218 ± 7170	29347 ± 12846	15914 ± 3095
4 h post-exercise	18664 ± 3123	17357 ± 4235	20929 ± 3803
6 h post-exercise	15891 ± 2661	27468 ± 7099	17975 ± 4371

Values are mean ± SE

Total Cholesterol

Total serum cholesterol is summarized in table 3.5. Total cholesterol was significantly higher ($p<0.05$) 2 h following exercise at 60% than 70% $\dot{V}O_2$max ($p<0.05$). There were no other significant differences in serum cholesterol levels between any of the three different exercise intensities at pre-exercise, post-exercise, 2 h, 4 h and 6 h post-exercise. Within a given exercise intensity there was no significant difference in total serum cholesterol at any time point.

Table 3.4: Total cholesterol ($mmol \cdot L^{-1}$) before and after isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max

	%$\dot{V}O_2$max		
	60%	**70%**	**80%**
Pre-exercise	4.20 ± 0.22	4.04 ± 0.16	4.15 ± 0.19
Post-exercise	4.51 ± 0.24	4.17 ± 0.14	4.43 ± 0.20
2 h post-exercise	4.77 ± 0.28*	3.98 ± 0.13	4.28 ± 0.16
4 h post-exercise	4.34 ± 0.21	3.95 ± 0.14	4.23 ± 0.18
6 h post-exercise	4.33 ± 0.19	4.16 ± 0.14	4.31 ± 0.20

Values are mean ± SE; *$p<0.05$ vs. 70% $\dot{V}O_2$max

Triglycerides

Serum triglyceride concentrations at rest and at four time points following an isocaloric bout of exercise at 60%, 70% and 80% $\dot{V}O_2$max are summarised in table 3.6. Compared to pre-exercise there was a significant increase ($p<0.01$) in the mean serum triglyceride concentrations immediately following exercise at 80% $\dot{V}O_2$max. Mean serum triglyceride concentrations were significantly lower than baseline 2 h following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max ($p<0.01$). Compared to 2 h

post-exercise there was a significant increase in the mean serum triglyceride concentrations 6 h following isocaloric bouts of exercise at 60% and 70% $\dot{V}O_2$max ($p<0.01$). There was no significant difference in triglyceride concentrations between any of the three different exercise intensities at pre-exercise, post-exercise, 2 h, 4 h and 6 h post-exercise.

Table 3.5: Triglycerides ($mmol \cdot L^{-1}$) before and after isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max

	%$\dot{V}O_2$max		
	60%	**70%**	**80%**
Pre-exercise	0.94 ± 0.08	0.95 ± 0.06	0.90 ± 0.08
Post-exercise	1.01 ± 0.10	1.06 ± 0.05	1.03 ± 0.08¥
2 h post-exercise	0.86 ± 0.10†	0.84 ± 0.07†	0.90 ± 0.08†
4 h post-exercise	0.97 ± 0.15	0.90 ± 0.09	0.98 ± 0.12
6 h post-exercise	1.17 ± 0.15*	1.10 ± 0.10*	1.04 ± 0.10

Values are mean ± SE. †$p < 0.01$ vs post-exercise; ‡$p < 0.01$ vs 2h; *$p < 0.01$ vs 2 h; ¥$p < 0.01$ vs pre-exercise

HDL-C

Circulating levels of HDL-C at rest and at 4 time points following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max are summarized in table 3.7. Compared to pre-exercise Serum HDL-C levels were significantly higher ($p<0.01$) immediately following exercise at 80% $\dot{V}O_2$max than pre-exercise and significantly lower ($p<0.01$) following exercise at 70% $\dot{V}O_2$max than immediately post-exercise. There was no significant difference in serum HDL-C concentrations between the three exercise intensities at pre-exercise, post-exercise, 2 h, 4 h and 6 h post-exercise.

Table 3.6: HDL-C ($mmol \cdot L^{-1}$) before and after isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2max$

	%$\dot{V}O_2max$		
	60%	**70%**	**80%**
Pre-exercise	1.29 ± 0.09	1.24 ± 0.05	1.29 ± 0.08
Post-exercise	1.31 ± 0.09	1.29 ± 0.07	1.38 ± 0.09†
2 h post-exercise	1.32 ± 0.09	1.21 ± 0.06*	1.33 ± 0.09
4 h post-exercise	1.29 ± 0.08	1.24 ± 0.07	1.32 ± 0.10
6 h post-exercise	1.30 ± 0.09	1.27 ± 0.07	1.32 ± 0.08

Values are mean ± SE. *$p < 0.01$ vs. post-exercise; †$p < 0.01$ vs. pre-exercise

LDL-C

Serum LDL-C concentrations at rest and following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2max$ are summarized in table 3.8. There were no significant difference in the serum [LDL-C] at any time within a given exercise intensity or between the three exercise intensity levels at pre-exercise, post-exercise, 2 h , 4 h and 6 h post-exercise.

Table 3.7: LDL-C ($mmol \cdot L^{-1}$) before and after isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2max$

	%$\dot{V}O_2max$		
	60%	**70%**	**80%**
Pre-exercise	2.49 ± 0.16	2.37 ± 0.13	2.45 ± 0.13
Post-exercise	2.75 ± 0.23	2.40 ± 0.10	2.58 ± 0.13
2 h post-exercise	3.05 ± 0.30	2.39 ± 0.11	2.54 ± 0.11
4 h post-exercise	2.61 ± 0.16	2.31 ± 0.11	2.46 ± 0.12
6 h post-exercise	2.50 ± 0.15	2.39 ± 0.12	2.52 ± 0.15

Values are mean ± SE

Leukocytes

The number of circulating leukocytes at rest and following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max are summarized in table 3.9. At each of the three exercise intensities the number of circulating leukocytes was significantly higher immediately post-exercise and at 4 h post-exercise than pre-exercise. Compared to pre-exercise, there was a significant increase in the mean number of circulating leukocytes 6 h following an isocaloric bout of exercise at 60% and 80% $\dot{V}O_2$max and at 2 h following exercise at 80% $\dot{V}O_2$max. There was no significant difference the number of circulating leukocyte between the three different exercise intensities at pre-exercise, post-exercise, 2 h, 4 h and 6 h post-exercise.

Table 3.8: Leukocytes ($x10^{3}\cdot\mu l^{-1}$) before and after isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max

	%$\dot{V}O_2$max		
	60%	**70%**	**80%**
Pre-exercise	6.21 ± 0.29	6.58 ± 0.54	6.39 ± 0.61
Post-exercise	7.49 ± 0.50†	8.55 ± 0.61†	9.48 ± 0.81†
2 h post-exercise	7.11 ± 0.59	7.55 ± 0.79	8.02 ± 0.68†
4 h post-exercise	7.46 ± 0.41‡	7.99 ± 0.61‡	8.41 ± 0.69†
6 h post-exercise	7.69 ± 0.41†	7.47 ± 0.63	8.07 ± 0.64†

Values are mean ± SE. †$p < 0.01$ vs. pre-exercise; ‡$p < 0.05$ vs. pre-exercise.

Platelets

Platelet numbers at rest and following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max are summarised in table 3.10. Compared to pre-exercise there was a

significant increase in the number of circulating platelets immediately following isocaloric bouts of exercise at 70% and 80% $\dot{V}O_2$max. Circulating levels of platelets were significantly higher immediately post-exercise than 2 h and 4 h post-exercise following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max and at 6 h following exercise at 80% $\dot{V}O_2$max. There was no significant difference in platelet numbers between the three different exercise intensities at pre-exercise, post-exercise, 2 h, 4 h and 6 h post-exercise.

Table 3.9: Platelets ($x10^{3} \cdot \mu l^{-1}$) before and after 3 isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max

	%$\dot{V}O_2$max		
	60%	**70%**	**80%**
Pre-exercise	276.00 ± 22.24	260.50 ± 16.93	267.40 ± 20.52
Post-exercise	311.60 ± 22.06†	326.10 ± 23.65*†	343.30 ± 30.12*†‡
2 h post-exercise	277.30 ± 20.18	263.80 ± 17.19	269.00 ± 20.72
4 h post-exercise	279.70 ± 20.74	275.30 ± 20.07	281.40 ± 21.61
6 h post-exercise	290.80 ± 15.24	291.60 ± 23.47	284.50 ± 2.93

Values are mean ± SE. *p < 0.01 vs pre-exercise; †p < 0.05 vs. 2 h post-exercise and 4 h post-exercise; ‡p < 0.01 vs 6 h post-exercise

Summary

Isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max resulted in significantly higher circulating levels of EMPs at 6 h post-exercise than pre-exercise. Compared to pre-exercise values there was no significant change in circulating levels of PMPs at any time up to 6 h following isocaloric bouts of exercise at 60%, 70% and 80%

$\dot{V}O_2$max. An exercise induced leucocytosis was evident immediately post-exercise and 4 h post-exercise following isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max and at 6 h following isocaloric bouts of exercise at 60% and 70% $\dot{V}O_2$max. Platelet levels immediately post-exercise were significantly higher than baseline following exercise at the two highest intensities.

Discussion

The first study examined the acute effects of an isocaloric bout of exercise (400 kcal) at 60%, 70% and 80% $\dot{V}O_2$max on circulating EMP and PMP numbers. Circulating EMP numbers were significantly higher than pre-exercise values at 6 h following exercise at 60%, 70% and 80% $\dot{V}O_2$max. The findings indicate that the exercise induced alterations in circulating EMPs are possibly related to caloric expenditure and independent of exercise intensity. The minimum caloric expenditure required to increase circulating EMPs is currently unknown and should be addressed in future studies.

The time course of the acute exercise-induced changes in EMP numbers in the present study is in contrast to previously findings. Kirk *et al.,* (2013) found an increase in EMPs (CD105 and CD106) 90 min following a bout of repeated supra-maximal sprint cycling. The values returned to baseline at 180 min post-exercise.[65] Sossdorf *et al.,* (2011) found an increase in EMPs (CD62E) in trained individuals 45 min following 1.5 h of cycling at 80% IAT and values returned to basal levels 2 h post-exercise.[64] In the present study there was an increase in EMPs immediately following the exercise bout at 70% $\dot{V}O_2$max with values returning to baseline at 2 h post-exercise. Mobius-Winkler et al., (2009) [68] found an increase in markers of endothelial damage but no change in EMPs (CD42b-/CD62E+) following 240 min of cycling at 70% IAT. Methodological differences across studies make it difficult to compare results. Participant fitness

levels, relative workload, blood sampling time points, exercise duration and modality may affect the release of MPs and should be controlled in future studies. MP origin also makes it difficult to compare studies. CD62E EMPs are associated with cell activation, while the EMPs in the present study are associated with cell apoptosis.

Similar to previous studies circulating PMPs numbers were significantly higher than EMP numbers at all time points in the three exercise trials. Compared to baseline values, there was no change in circulating PMP numbers following an isocaloric bout of exercise at 60%, 70% or 80% $\dot{V}O_2$max. In contrast, Sossdorf *et al.,* (2011) reported an increase in PMP numbers following 90 min of cycling at 80% IAT.[64] The fact that the bout of exercise was considerably longer than the present study may help to explain the different findings. However, Maruyama *et al.,* (2012) using ELISA, found an increase in PMPs following an incremental exercise test to only 85% of age predicted heart rate maximum.[67] It is possible that the ELISA may be a more sensitive method of measuring of PMP numbers or that it could be less sensitive and misclassified the number of circulating PMPs.

The number of circulating leukocytes increased significantly immediately following exercise at 60%, 70% and 80% $\dot{V}O_2$max and remained elevated at all time points up to 6 h following exercise at 80% $\dot{V}O_2$max. Platelet numbers follow a similar trend with circulating numbers significantly higher immediately post-exercise and remaining slightly elevated for up to 6 h of recovery.

Exercise induced leucocytosis is a common response to acute exercise and is due primarily to an increase in circulating numbers of leukocytes and lymphocytes.[105] The degree of leucocytosis is dependent on the intensity, duration and type of exercise. A major source of leukocytes appears to be the marginated pool. Leukocytes adhere to areas of the vascular endothelium outside the main axial flow where blood flow in much slower. An increase in shear stress appears to be responsible for the majority of leukocyte demargination in response to exercise. Other factors that may influence demargination include opening of capillaries and the direct effect of catecholamine's on β1 adrenergic receptors on cell surface adhesion molecules, in multiple vascular beds.[106]

The increase in platelet numbers was not related to an increase in PMPs. PMPs have been linked to increases in inflammation in previous studies.[15,107] It is possible that the inflammatory response to the acute exercise bouts may not have reached a threshold for the release of PMP. The longest trial, (60% $\dot{V}O_2$max) was 31 min in duration and this may not be long enough to activate PMPs. The large inter-individual variation in PMP numbers makes it difficult to detect significant changes. Differences in blood processing and antibodies used in PMP analyses may also help to explain the different findings.

At all exercise intensities circulating triglyceride levels were significantly lower 2 h post-exercise than immediately post-exercise and in the 60% and 70% $\dot{V}O_2$max trials

values are significantly higher 6 h post-exercise than 2 h post-exercise. Acute exercise transiently reduces serum triglycerides with the effect not always immediately apparent.[93,108] Decreased levels of post-exercise triglycerides have been linked to increased levels of muscle lipoprotein lipase activity. Lipoprotein lipase (LPL), located on the capillary endothelium of extrahepatic tissues, catalyzes the rate limiting step in the hydrolysis of triglycerides from circulating chylomicrons following a meal and endogenously produced very low density lipoprotein (VLDL). Quantitatively, the majority of LPL is found in muscle and adipose tissue, where the liberated free fatty acids are taken up and either stored or oxidized, respectively.[109] The significant increase in triglycerides immediately following exercise at 80% $\dot{V}O_2$max was an unexpected finding and may have been due to plasma fluid shifts. [96]

This study aimed to compare the acute effects of isocaloric bouts of exercise at moderate to vigorous intensities on circulating EMP and PMP levels in young healthy physically active men. EMP levels increase following acute exercise at all intensities. There are no changes in PMP levels.

Chapter IV

Study II

The Effect of Exercise Training on Microparticle Formation in Men

Rationale

Isolated exercise sessions elicit acute responses that when repeated produce more permanent adaptations, referred to as the exercise training response. Exercise training has been reported to have numerous beneficial effects on cardiovascular and metabolic health. MPs have been linked to a number of the processes linked to these beneficial effects. To date the effect of exercise training on MP formation is not currently known. The purpose of this study was to examine the effects of exercise training on circulating levels of EMPs and PMPS in young healthy men.

Specific Aims:

1. To examine the time-course changes in circulating EMP numbers pre-exercise, immediately following 1 h of exercise at 80% $\dot{V}O_2$peak and 13 h following exercise on days 1, 3, 7, 10 and 14 of a 14 day training program
2. To examine the time-course changes in circulating PMP numbers pre-exercise, immediately following 1 h of exercise at 80% $\dot{V}O_2$peak and 13 h following exercise on days 1, 3, 7, 10 and 14 of a 14 day training program

3. To examine the changes in total cholesterol, LDL-C, HDL-C and triglycerides following 14 consecutive days of training at 80% $\dot{V}O_2$peak

Hypothesis:

1. There will be no significant difference in circulating EMP numbers pre-exercise, immediately following 1 h of exercise at 80% $\dot{V}O_2$peak and 13 h following exercise on days 1, 3, 7, 10 and 14 of a 14 day training program
2. There will be no difference in circulating PMP numbers pre-exercise, immediately following 1 h of exercise at 80% $\dot{V}O_2$peak and 13 h following exercise on days 1, 3, 7, 10 and 14 of a 14 day training program
3. There will be no significant difference in total cholesterol, LDL-C, HDL-C and triglycerides following 14 consecutive days of training at 80% $\dot{V}O_2$peak

Methodology

Study Overview

An overview of the study design is illustrated in figure 4.1. Eight healthy, sedentary men performed 60 min of submaximal exercise training once a day for 14 consecutive days. Prior to commencing the training, participants visited the Human Performance Laboratory (HPL) in DCU on two separate occasions. During the first visit they underwent a brief medical screen consisting of a general health questionnaire (Appendix A), medical examination, body composition assessment, ECG and an exercise test on a cycle ergometer to measure peak oxygen uptake ($\dot{V}O_2$peak). Participants returned to the HPL within a week and performed a test to verify the work rate corresponding to 80% $\dot{V}O_2$peak. Exercise commenced 7 d after the verification trial. Peak oxygen uptake was re-assessed 48 to 72 h following the final exercise session.

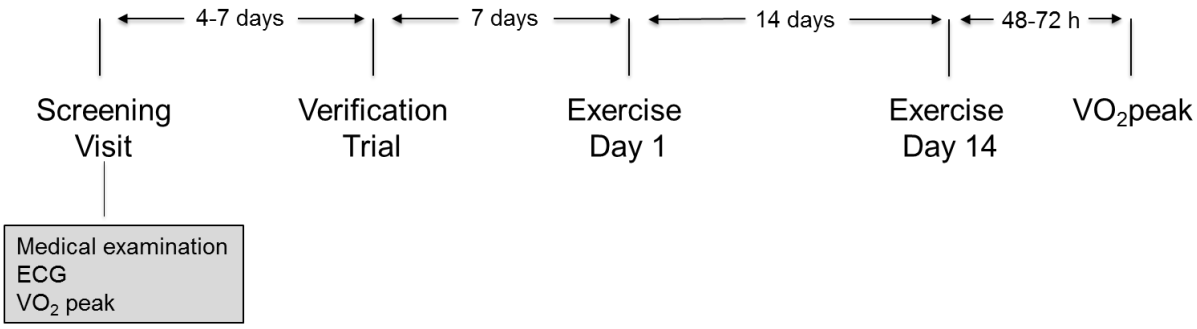


Figure 4.1: Study overview

Participants

Eight healthy, sedentary men volunteered to participate in the study. Participants were excluded if they were current smokers, involved in regular physical exercise or had any other medical conditions that contraindicated exercise participation.

Participant Recruitment

An email was sent to all registered undergraduate students, graduate students and staff in Dublin City University (Appendix F). Individuals expressing interest received a plain language statement detailing the requirements of the study (Appendix G). Written informed consent was obtained from individuals who met the inclusion and exclusion criteria and agreed to take part in the study. (Appendix H). The study was approved by the Dublin City University Research Ethics committee. (Appendix I)

Training program

Training consisted of 14 x 1 h supervised exercise training sessions on 14 consecutive days. (Figure 4.2) Each training session was 60 min in duration and performed at an intensity corresponding to 80% of pre-training $\dot{V}O_2$peak. To avoid any influence from changes in circadian variation on exercise performance participants performed their session at the same time ± 1 h each day. Participants trained between

17:00 and 20:00. Plain water was allowed to be consumed ad libitum throughout the training sessions.

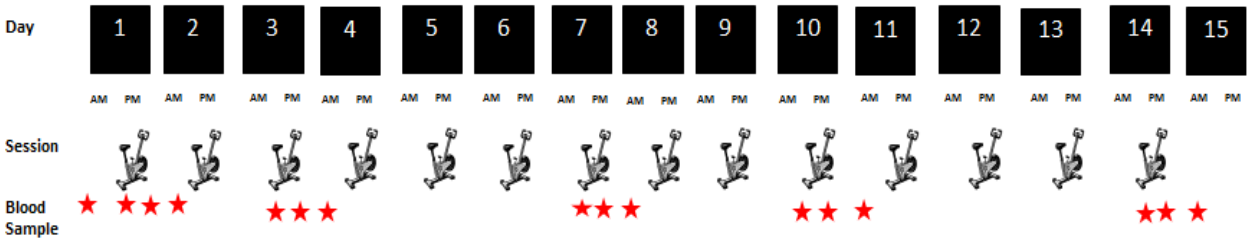


Figure 4.2: Exercise overview

Exercise intensity was monitored by measuring oxygen uptake between 15-20 min, 35-40 min and 55-60 min of each exercise session and workload was adjusted when appropriate. Heart rate and RPE were recorded at 5 min intervals throughout each training session. To ensure that exercise intensity matched the expected increase in $\dot{V}O_2$peak during the training session, target $\dot{V}O_2$ was increased by 10% after day 7 of training. Using similar training protocols, Gulve and Spina (1995)[98] and Spina *et al.,* (1996)[110] have shown a 9-10% increase in $\dot{V}O_2$peak after 7-10 days of cycle ergometer training.

A total of 16 blood samples were taken during the period of exercise training. On the morning of day 1, participants reported to the HPL after an overnight fast and the first blood sample was taken (#1). Participants returned later on day one for their first training session. Blood was taken before (#2 pre-exercise) and after the training session (#3 post-exercise). Participants were asked not to eat or consume caffeine for

2 h before the start of the training session. The following morning, day 2, participants returned to the HPL after an overnight fast and a blood sample was taken (#4). Blood was taken before and after the third training session (#5, #6) and again the following morning, day 4, after an overnight fast (#7). This procedure was repeated for training session 7 (#8, #9, #10), session 10 (#11, #12, #13) and session 14 (#14, #15, #16).

Workload Determination

The $\dot{V}O_2$ relating to a given power output was calculated based on the linear relation between oxygen uptake (y-axis) and power output (x-axis). For a given percentage of $\dot{V}O_2$peak, the corresponding power output was estimated by solving for x using the linear function y=mx + c, where y is $\dot{V}O_2$, x is power output, m is the slope of the relation between $\dot{V}O_2$ and power and c is the intercept point on the y axis.

Verification of Exercise Intensity

Each participant performed three 10 min verification trials involving exercising at 15 W below the predicted power output, 15 W above the predicted power output and at the predicted power output. They were encouraged to keep the pedal cadence between 75 and 80 rpm for all exercise sessions.

Peak Oxygen Uptake

Participants performed an incremental exercise test to volitional exhaustion on an electronically braked cycle ergometer (Ergoline 900, SensorMedics, Yorba Linda, CA)

to determine $\dot{V}O_2$peak. Following a 5 min warm-up at 80 W, the workload was increased by 40 W every 2 min until volitional exhaustion. Respiratory metabolic measures were monitored continuously throughout the test. Peak oxygen uptake was determined by averaging the three highest consecutive 20 sec values.

Assessment of Body Composition

Body density was calculated using the sum of seven skinfolds (triceps, subscapular, pectoral, mid-axillary, suprailiac, abdomen and thigh) by the method of Jackson and Pollock (1978).[102] Skinfolds were measured using Harpenden skinfold callipers. Percentage body fat was calculated using the Siri equation.[103]

Calculations of Energy Expenditure

Values for $\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{V}E_{(STPD)}$, and FEO_2 were recorded from expired air using 60 s averages and used to calculate the rate of energy expenditure using equations from Kuo *et al.,* (2005).[111]

Cardiorespiratory and Metabolic Measures

As described in the methods section of Chapter 3

Mass Flow Sensor Heated Wire Anemometer-Mode of Operation

As described in the methods section of Chapter 3.

Mass Flow Sensor Calibration

As described in the methods section of Chapter 3.

Gas Analysers

As described in the methods section of Chapter 3.

Calibration of O_2 and CO_2 Gas Analysers

As described in the methods section of Chapter 3.

Blood Sampling and Storage

Morning blood samples were taken from a prominent forearm vein using standard venipuncture. Prior to the blood draw participants rested in a seated position for 5 min with legs uncrossed to avoid plasma fluid shift changes. Pre and post-exercise samples were taken using a cannula placed in a prominent forearm vein. Pre-exercise samples were collected following 5 min rest in a seated position. Post-exercise samples were taken at the end of the training session with the participant sitting on the cycle ergometer.

Serum vacutainers were allowed stand for 30 min at room temperature before being centrifuged at 3000 rpm (1600 g) for 15 min at $4^{O}C$. Blood for microparticles analysis was collected, prepared and stored according to methods described by Bernal-Mizrachi *et al*., (2004).[104] Blood was collected in pre-chilled sodium citrate vacutainers

before being centrifuged twice. The first spin, 160 g for 9 min, produced platelet rich (PRP) plasma. The top layer of this plasma was harvested and spun for a further 9 min at 1000 g to produce platelet poor plasma (PPP). The PPP was aliquoted and stored at -80^{O}C.

Microparticle Analysis

As described in the methods section of Chapter 3.

Lipid analysis

As described in the methods section of Chapter 3.

Dietary Control

Participants were required to record their daily intake of food and fluids for 3 d before the start of the training sessions. They repeated this dietary pattern throughout the training phase to avoid any possible changes from variation in day to day dietary intake. Participants were weighed to the nearest 0.1 kg in minimal clothing immediately before and after each training session in order to monitor fluid loss during the training sessions. To ensure proper hydration levels they were given a volume of plain water equivalent to 150% of weight loss to drink after the training session. After each training session, participants were given two cereal bars (Nutrigrain, Kellog's, UK) to consume with the water. The cereal bars contained 50 g of carbohydrates, 3 g of protein and 7 g of fat.

Statistical analysis

Prior to statistical analysis the data was checked for normality using the Shapiro-Wilk test. EMP and PMP data were log transformed to conform to normality. An independent t-test was used to compare anthropometric data, $\dot{V}O_2$peak, lipids, MPs and estimated EE before and after the 14 days training and to compare O_2 consumption between week 1 and week 2 of training. A repeated measure ANOVA was used to compare MP formation during the 14 d of training. A one way ANOVA was used to compare circulating MP levels within training days. A one way ANOVA was used to compare morning samples, pre-exercise samples and post-exercise samples. Significant main effects were probed using a Bonferroni post hoc test. The relation between changes in MP numbers and changes in aerobic fitness after the intervention was established using Pearson's product moment correlation. 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

After completion of the experiment one of the participants was diagnosed with a chronic inflammatory disease. As this condition may affect MP formation his data was excluded and data from the remaining 7 participants was analysed. They were (mean ± SE) 20.7 ± 0.9 yr, had a body mass of 75.2 ± 3.4 kg and had an estimated % body fat of 12.2 ± 2.2. Physical characteristics and aerobic endurance before and after the 14 d training program are summarised in Table 4.1. There were no significant changes in body mass or BMI following the 14 d training period. There was an 18.3% ($p<0.01$) and 17.9% ($p<0.01$) increase in relative and absolute $\dot{V}O_2$peak respectively, following the 14 d of training.

Table 4.1: Anthropometrics

	Time	
	Pre Training	**Post Training**
BMI ($kg \cdot m^{-2}$)	23.3 ± 1.0	23.2 ± 1.0
$\dot{V}O_2$max ($L \cdot min^{-1}$)	2.88 ± 0.43	3.36 ± 0.25*
$\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$)	39.4 ± 3.9	45.8 ± 3.1*

Values are mean ± SE; *$p < 0.01$ vs. pre training

Oxygen consumption

The absolute O_2 consumption and percentage of $\dot{V}O_2$peak averaged across the entire 14 d training period are summarised in table 4.2. The average absolute $\dot{V}O_2$ was

significantly higher in week 2 than week 1. During the 14 d training period participants exercised at 80.7 ± 2.0% and 68.9 ± 2.5% of the pre training V̇O2peak of the post training $\dot{V}O_2$peak, respectively.

Table 4.2: Average metabolic data

	Metabolic Data		
	$\dot{V}O_2$ (L min^{-1})	**% $\dot{V}O_2$peak Pre**	**% $\dot{V}O_2$peak Post**
Day 1 to 7	2.24 ± 0.10	78.0 ± 2.0	66.6 ± 2.2
Day 8 to 14	2.39 ± 0.11*	83.4 ± 2.1*	71.2 ± 2.7*
Day 1 to 14	2.32 ± 0.11	80.7 ± 2.0	68.9 ± 2.5

Values are mean ± SE. *p < 0.01 vs. day 1 - 7

Energy Expenditure

Average EE for the first 7 days, the second 7 days and the 14 d training period (mean ± SE) are summarised in figure 4.3. The average training EE over the 14 day period was 695 ± 30 kcal. The average training EE was significantly higher in week 2 than week 1 (672 ± 30 vs. 718 ± 34 kcal).

Relative and absolute O_2 uptake, EE and % contribution of CHO and fats during exercise on day 1, 7 and 14 are summarised in table 4.3. Oxygen consumption was 8.6% higher on day 14 than day 1, although the difference did not reach statistical significance (p = 0.07). Relative $\dot{V}O_2$ was significantly higher on day 14 than day 1. There was a non-significant (p = 0.07) increase in EE over the 14 days. The percentage contribution from CHOs was significantly higher at day 7 compared to day 1. The % contribution from fat was significantly lower at day 7 compared to day 1.

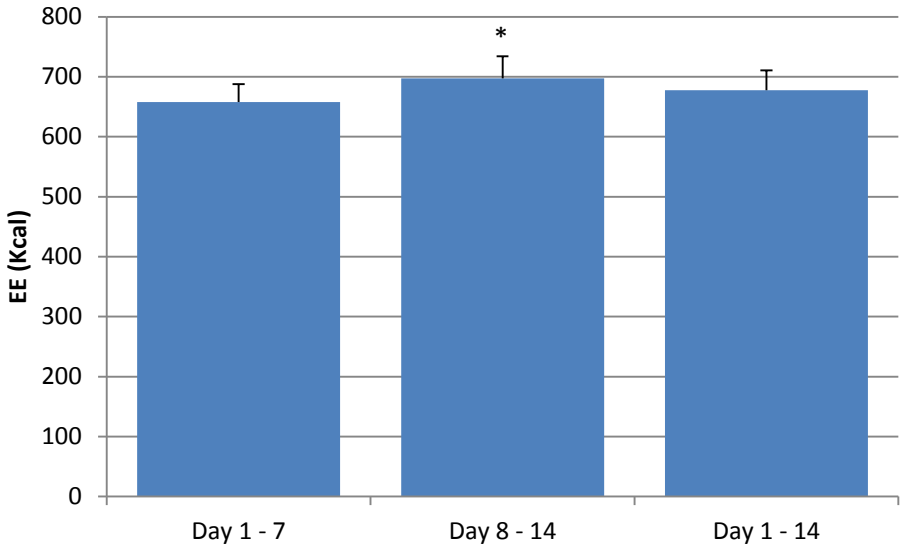


Figure 4.3: Estimated EE during the training period; *p<0.05 vs day 1 - 7

Table 4.3: Oxygen uptake, energy expenditure and substrate utilisation during exercise on day 1, 7 and 14

	Metabolic Data					
	$\dot{V}O_2$ (L/min)	**% $\dot{V}O_2$peak Pre**	**% $\dot{V}O_2$peak Post**	**Estimated EE (kcal)**	**% CHO oxidation**	**% Fat oxidation**
Day 1	2.22 ± 0.1	77.5 ± 2.1	65.9 ± 1.5	665 ± 22	86.0 ± 2.0	14.0 ± 2.0
Day 7	2.26 ± 0.1	78.7 ± 1.4	67.2 ± 1.9	680 ± 27	89.6 ± 2.1*	10.4 ± 2.1*
Day 14	2.41 ± 0.1	83.8 ± 2.2*	71.5 ± 2.5	724 ± 39	89.5 ± 1.0	10.5 ± 1.0

Values are mean ± SE. *p < 0.05 vs. day 1

Endothelial Microparticles

Circulating levels of EMPs before training and 13 h following the last training session are illustrated in figure 4.4. There was a significant decrease in EMPs 13 h

following the last exercise bout than baseline. Circulating levels of EMPs at pre-exercise, immediately post-exercise and 13 h post-exercise following 5 of the exercise bouts (day 1, 3, 7, 10, 14) are illustrated in figure 4.5. There were no differences between morning EMP numbers on day 2, 4, 8, 11, or 15. There was no significant difference between the pre-exercise circulating EMP numbers on day 1, 3, 7, 10 and 14. There was no difference between the post-exercise EMP numbers on day 1, 3, 7, 10 and 14. On training days 3, 7, 10 and 14 EMP values increased (non-significantly) immediately after exercise compared to pre-exercise values. With the exception of the day 10 exercise bout, circulating EMPs were significantly lower than immediately post-exercise 13 h following each of the exercise bouts. On training days 1, 7 and 14 EMP values were significantly lower 13 h post-exercise than the pre-exercise values (figure 4.5).

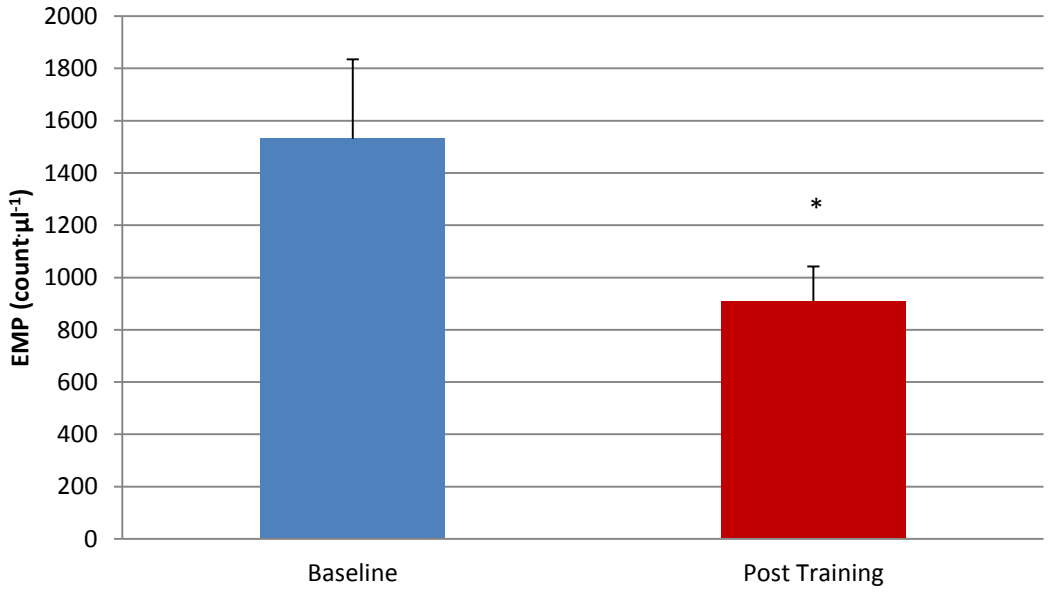


Figure 4.4: EMP values before training and 13 h after the last bout. *p < 0.05 vs. pre training

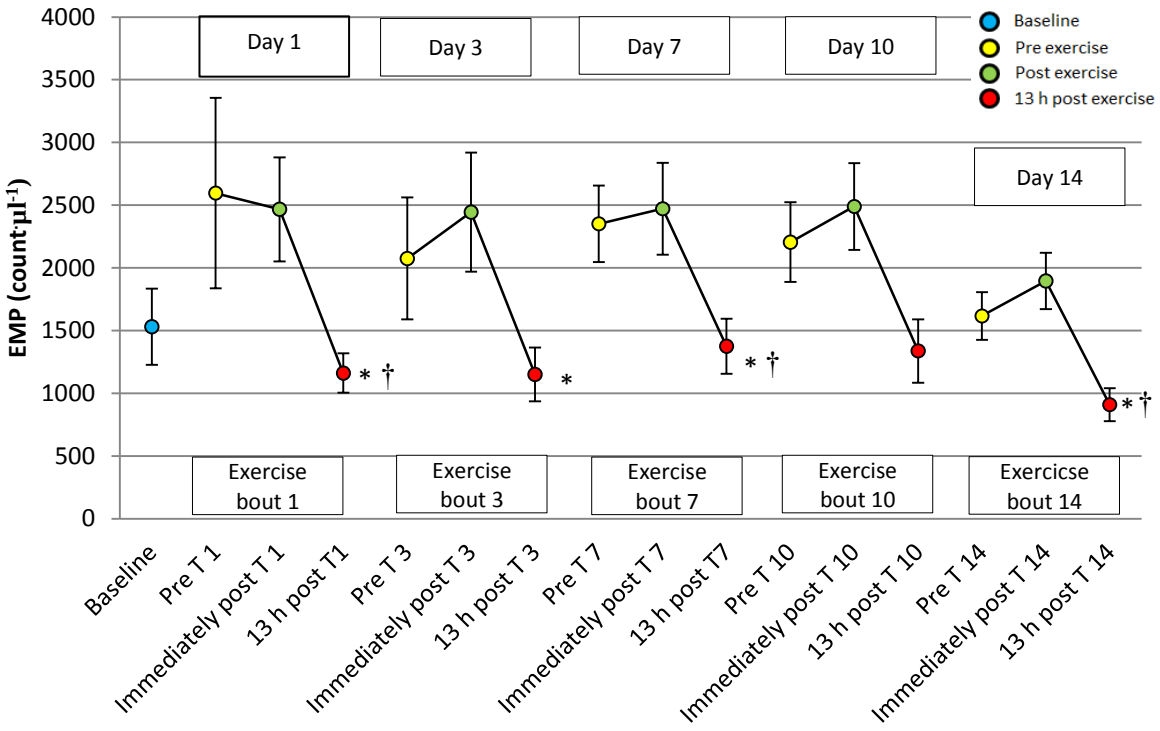


Figure 4.5: EMP values around training bouts 1, 3, 7, 10 and 14; *p < 0.05 vs. post-exercise, †p < 0.05 vs. pre-exercise

Platelet Microparticles

PMP values during the 14 d training period are illustrated in figure 4.6. There were no significant differences in the number of circulating PMP at any time point during the 14 d of training. The only pattern apparent during the training sessions is a decrease immediately post-exercise compared to pre-exercise on training days 1, 3, 7, 10 and 14. However none of the values reached statistical significance.

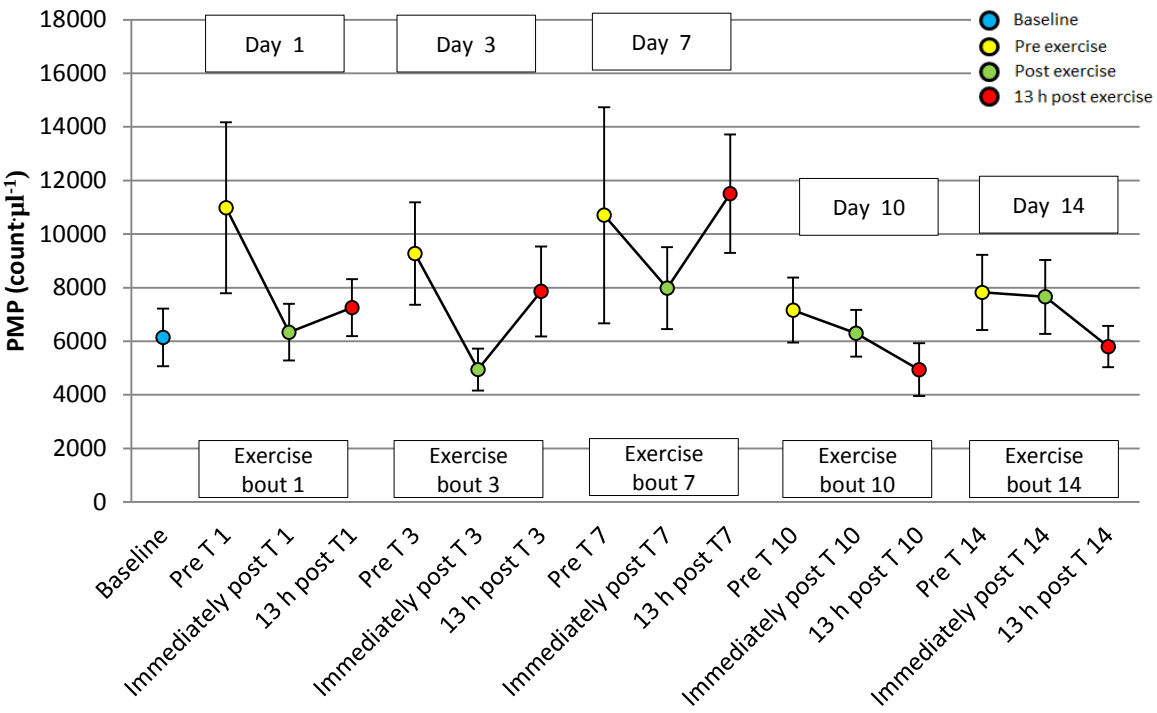


Figure 4.6: PMP values around training bouts 1, 3, 7, 10 and 14

Endothelial Microparticles and Aerobic Fitness

There was no relation between changes in circulating EMP values between pre training and values on day 15 and changes in aerobic fitness following exercise training in young healthy men ($r=0.453$, $p=0.307$).

Lipids

Serum triglycerides, total cholesterol, LDL-C and HDL-C were measured before training, at day 8 and 13 h after the last training bout and the results are illustrated in figure 4.7. Serum cholesterol and LDL-C concentrations were significantly lower on day 8 than baseline. Post training serum cholesterol and LDL-C concentrations remained low compared to baseline. However, the difference did not reach significance ($p = 0.057$). There were no significant changes in triglyceride or HDL-C concentrations during or following the 14 d training protocol.

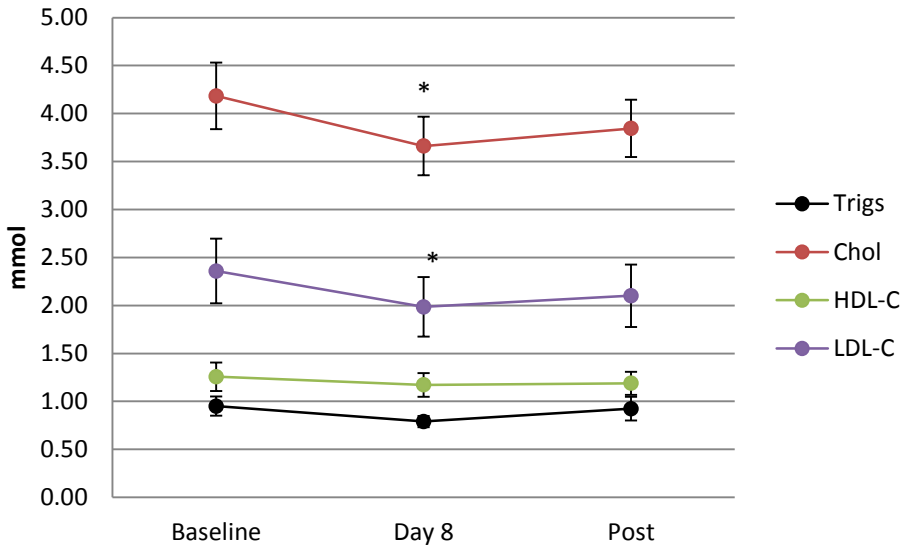


Figure 4.7: Lipid values at baseline, day 8, post 14 d training. *p < 0.05 vs. pre training

Summary

Following 14 consecutive days of exercise training there was a significant increase in aerobic fitness. There was a significant decrease in circulating EMPs 13 h following the last exercise bout compared to baseline. Circulating EMPs did not change significantly immediately following any if the five exercise bouts. In contrast circulating EMPs 13 h following exercise were significantly lower than pre-exercise on days 1, 7 and 14 and significantly lower than post-exercise on days 1, 3, 7 and 14. There was no relation between changes in circulating EMP numbers and changes in aerobic fitness. Total serum cholesterol and LDL-C were significantly lower than pre training values after 7 days of training. They remained lower than baseline after 14 days but were not significantly lower.

Discussion

Study two examined the effect of 14 consecutive days of exercise on MP numbers in sedentary men. Participants cycled at 80% $\dot{V}O_2$peak for 1 h each day and blood was taken before exercise, immediately after exercise and 13-14 h following exercise on 5 separate days during the 2 weeks of training. The high volume and intensity training protocol was employed in an attempt to elicit a significant change in aerobic fitness in a short time period. The 14 d training was a sufficient stimulus to improve aerobic fitness as indicated by a significant increase in both absolute and relative $\dot{V}O_2$peak.

EMP numbers followed a similar trend throughout the duration of the study. Compared to pre-exercise values there was on average a small non-significant 10% increase in EMPs immediately post-exercise. Circulating EMP levels were on average 50% below pre-exercise values and 45% below immediately post-exercise the following morning (+13 h). The morning EMP values were significantly lower than pre-exercise on day 1, 7 and 14 and significantly lower than post-exercise values on day 1, 3, 7 and 14. Increased EMP numbers have been associated with CVD risk factors so it is possible the lower EMP numbers point to cardio protective benefits of the exercise. The circulating levels of PMPs did not change at any time during the 15 days.

It is difficult to draw any definite conclusion from the fact that circulating EMP numbers were significantly lower than pre training values 13 h following the 14 d

training period. This is due to the fact that no exercise was performed the day prior to the pre training blood sample whereas participants exercise for 1 h at 80% $\dot{V}O_2$peak 13 h prior to the blood draw. In hindsight, a blood sample for MP quantification post training should have been taken 2 – 3 days following the cessation of the final exercise session to avoid possible interference from the exercise.

It is evident from the pre-exercise EMP numbers that time of day and possibly daily activities have an effect on EMP numbers. Pre-exercise circulating EMP numbers, taken in the evening between 17:00 and 19:00 h following a 2 h fast, were consistently higher than morning samples, taken 13 h after the training session and following an overnight fast. Although blood samples were not taken on the morning of the training sessions it is unlikely, based on the measurements taken on 5 of the 14 days, that there would be a significant difference in circulating EMPs from the values measured on the morning after each training session. Future studies need to more rigorously assess diurnal variation in circulation EMP levels. Future research will also need to take time of day into account when designing their research.

This is the first short term training study to assess the changes in EMP numbers in young healthy men. Babbitt *et al.,* (2013) found a decrease in EMPs and IL-6 and an improvement in FMD following 6 months of aerobic exercise training in middle aged, healthy African American men and women.[70] Participant and methodological differences makes it difficult to compare the results between the two training studies.

During the 14 days of training there was no change in MP numbers pre-exercise, immediately post-exercise or 13 h post-exercise across the 5 training days in which MPs were measured. Average caloric expenditure equated to 695 kcal per day, or approximately 10000 kcal over the 14 day period. There was a significant increase in both relative and absolute $\dot{V}O_2$peak and a decrease in total cholesterol and LDL-C but no change in MP numbers. This consistent response at each time point may indicate that MPs in young healthy men are resistant to change despite the high workload during the training protocol.

An increase in HDL-C is the most commonly reported change in lipid profiles following exercise training with decreases in LDL-C, total cholesterol and TGs less frequently reported.[112] In study 2 there were no changes in HDL-C or TGs. Total cholesterol and LDL-C and were significantly lower on morning 8 compared to baseline values. Although the LDL-C values remained lower on morning 15 the decrease did not reach significance ($p = 0.057$). Changes in LDL-C and total cholesterol are associated with exercise training programs in which participants expend more than 1200 kcal·wk^{-1}.[113] Changes are also more likely to be found in previously sedentary individuals. Participants in study 2 had an average $\dot{V}O_2$peak of 39.4 ± 3.9 ml·kg·min^{-1} at pre training, which places them in the bottom 25% of aerobic fitness for their age.[114] The average estimated EE for each session over the 14 days was 695 ± 32 kcal which is considerably higher than the 1200 kcal/wk reported to be associated with decreases in LDL-C and

total cholesterol. The training protocol may however, not have been of sufficient length to elicit changes in HDL-C.

There was no relation between changes in aerobic fitness and changes in MP numbers. The large individual variations between the changes in MP numbers and the small sample size make correlations difficult to examine.

The aim of study 2 was to compare circulating EMP and PMP numbers pre-exercise, immediately following 1 h of exercise at 80% $\dot{V}O_2$peak and 13 h following exercise on days 1, 3, 7, 10 and 14 of a 14 day training program. EMP numbers were significantly lower 13 h after exercise possibly due to a cardio protective role of the exercise. There were no significant changes in PMP numbers. Triglycerides and LDL-C improved during the course of the training and could also be linked to improvements in CVD risk factors.

Chapter V

Study III

Microparticles and Endothelial Function Response to Exercise Training in Individuals with Cardiovascular Disease

Rationale

The vascular endothelium is a complex dynamic barrier that plays a central role in maintaining vascular integrity. Established CVD risk factors promote the development of atherosclerosis through their deleterious effects on endothelial structure and function. 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 and is evident prior to angiographic detection of the disease.

Circulating levels of EMPs are increased in individuals with established cardiovascular disease risk factors linked to endothelial dysfunction.[44,55] Furthermore, MPs from patients following a myocardial infarction cause severe endothelial dysfunction in healthy rat aortic rings.[115] Impairment in endothelial function may be related to a reduced production and/or availability of NO. To date, no *in vivo* studies have examined the relation between circulating MPs and endothelial function in men and women with CVD.

An increase in cardiorespiratory fitness in response to traditional continuous, moderate-to-vigorous exercise training programs can reverse endothelial dysfunction in individuals with atherosclerotic CVD. Relatively few studies have examined the effects of exercise training on MP formation in this clinical population. More recently, low volume short duration, high-intensity interval training (LS-HIIT) involving repeated bouts of short-duration high intensity exercise separated by periods of active or passive recovery have been shown to be a safe and effective alternative to more traditional continuous, moderate-to-vigorous exercise programs in improving exercise capacity and endothelial function in individuals with CVD. Studies in healthy individuals have found physiological and metabolic improvements following 4 - 8 sessions of LS-HIIT involving 30 sec exercise bouts.

The purpose of this study is to compare the effects of 4 weeks of LS-HIIT and cardiac rehabilitation (CR) on endothelial function and circulating microparticles in individuals with CVD.

Specific Aims:

1. To compare the number of circulating EMPs before and after 4 weeks of LS-HIIT or CR in men and women with cardiovascular disease
2. To compare the number of circulating PMPs before and after 4 weeks of LS-HIIT or CR in men and women with cardiovascular disease

3. To compare endothelial function before and after 4 weeks of LS-HIIT or CR in men and women with cardiovascular disease
4. To examine the relation between circulating MPs and endothelial function in men and women with CVD before and after exercise training.

Hypothesis:

1. Circulating levels of EMPs will not significantly change following 4 weeks of LS-HIIT or CR in individuals with cardiovascular disease
2. Circulating levels of PMPs will not significantly change following 4 weeks of LS-HIIT or CR in individuals with cardiovascular disease
3. Endothelial function will significantly improve following 4 weeks of LS-HIIT or CR in individuals with cardiovascular disease
4. Improvements in endothelial function will not correlated with MPs in individuals with cardiovascular disease

Methodology

Participants

Twenty-two men and four women with documented CVD, who had been participating in a community-based phase IV cardiac rehabilitation programme for a minimum of 6 months and received medical clearance were recruited for the study. Exclusion criteria were smoking, unstable angina, uncontrolled hypertension (systolic blood pressure (BP) >180 mmHg, diastolic BP >100 mmHg), resting tachycardia or unstable/acute heart failure, or any other medical condition that contraindicated exercise participation. Participants were randomly assigned to LS-HIIT (n=15, 66.6 ± 1.73 yr; mean ± SE) or to continued participation in CR (n=11, 68.6 ± 1.71 yr; mean ± SE).

Study Overview

An overview of the study design is illustrated in figure 5.1. Following an initial screening visit the participants visited the Health and Human Performance Laboratories on two occasions before and after the 4 weeks of LS-HIIT or CR.

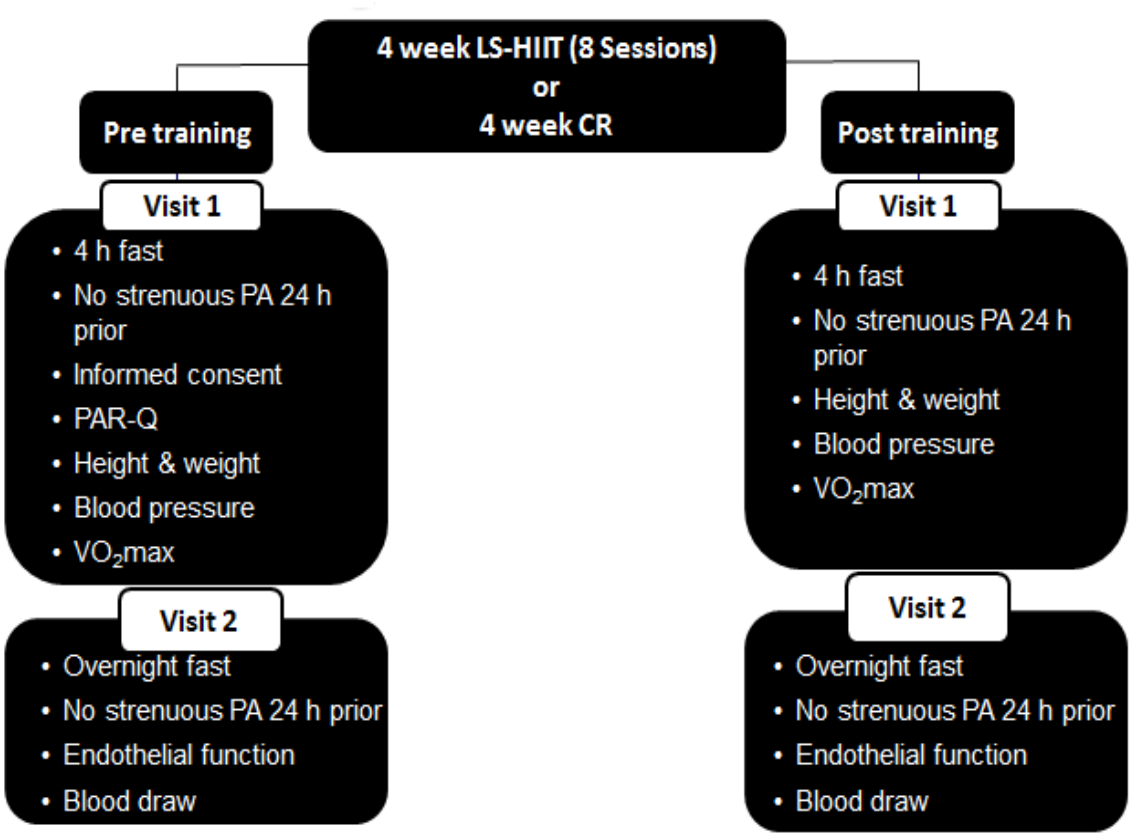


Figure 5.1: Overview of the study design

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

Visit 1: Participants abstained from strenuous physical activity for 24 h, and abstained from food, alcohol and caffeine for at least 4 h before the visit. During this visit, height, weight, and blood pressure were measured and maximal aerobic capacity ($\dot{V}O_2max$) was assessed.

Visit 2: Participants abstained from strenuous physical activity for 24 h and arrived at the Vascular Research Unit at DCU following an overnight fast. Resting blood pressure was obtained, blood sample was drawn and endothelial function was assessed.

Training Protocols

High Intensity Interval Training

Participants assigned to LS-HIIT, undertook two supervised high intensity interval sessions per week for 4 weeks, and did not attend the 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 – 90 %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. Angina score was obtained after each 45 sec exercise bout. The electrical activity of the heart and O_2 saturation were continuously monitored with a 12 lead ECG and pulse oximetry, respectively. Blood pressure was measured and RPE was obtained at the end of each exercise block.

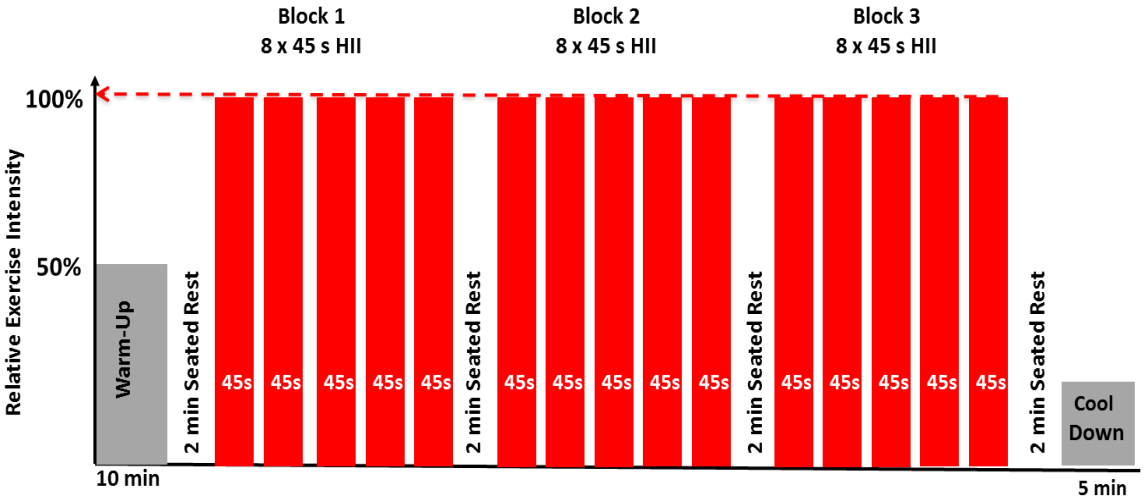


Figure 5.2: Overview of a LS-HIIT session

Cardiac Rehabilitation

Participants in the CR group continued to attend a community-based CR program, two days per week. The cardiac rehabilitation program was multi-faceted but predominantly focused on exercise. Exercise classes were 60 min in duration and involved aerobic exercise and some form of resistance training. Sessions commenced with a 10 min warm up at a low exercise intensity and were followed by 40 min at moderate-intensity. The participants exercised in small groups and rotated between the various exercise stations during each class. The class concluded with a 10 min cool-down.

Anthropometrics

Height and body 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.

Determination of Maximal Oxygen Uptake

Maximal aerobic capacity ($\dot{V}O_2max$) was assessed on a treadmill (Woodway ELG 55, Waukesha, WI) using a Balke protocol that was modified to allow participants reach volitional fatigue in 8 – 12 min. (Appendix L) A 2 min warm up preceded each test and the gradient was increased 2.5% every 2 min until the participant reached volitional fatigue or presented with contraindications to exercise. Participants were verbally encouraged to give their best effort. Respiratory metabolic measures and ECG were continuously monitored throughout the test. RPE was recorded every 2 min. Maximal oxygen uptake was determined by averaging the three highest consecutive 20 second values.

Cardiorespiratory and Metabolic Measures

As described in the methods section of Chapter 3

Mass Flow Sensor Heated Wire Anemometer-Mode of Operation

As described in the methods section of Chapter 3

Mass Flow Sensor Calibration

As described in the methods section of Chapter 3

Gas Analysers

As described in the methods section of Chapter 3

Calibration of O_2 and CO_2 Gas Analysers

As described in the methods section of Chapter 3

Ratings of Perceived Exertion

RPE was obtained using the 16-point Borg category RPE scale.[116] 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.[117] 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. During the HIIT sessions the participants were instructed to make their subjective assessments of perceived exertion relative to these minimum and maximum standards (perceptual anchors).

Electrocardiographic Monitoring (ECG)

The electrical activity of the heart was monitored 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 function was assessed by measuring brachial artery dilatory response to reactive hyperemia and glyceryl-trinitrate.[118] Participants arrived to the Vascular Research Unit, DCU at 07:00 following an overnight fast. They were not allowed to exercise, ingest substances, such as caffeine or vitamin C, which may affect flow mediated dilation (FMD), for at least 6 h before the study. Water consumption was permitted and where possible, vasoactive medications were withheld for at least 4 half-lives. Since the 4 women participants were post-menopausal menstrual cycle phase was not controlled.

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 5.3 illustrates the FMD protocol. 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 30 sec post-deflation for 5 min. FMD was normalised for the shear stimulus. Shear rate was calculated using the formula, 4 x peak velocity/peak diameter.[119]

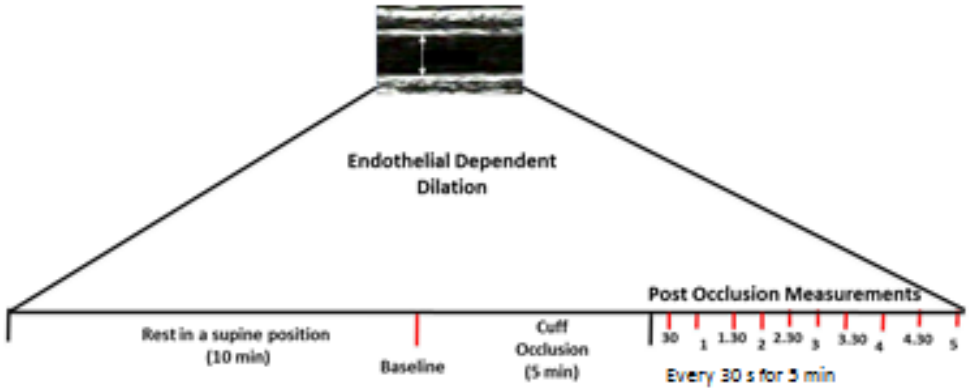


Figure 5.3: Overview of endothelial-dependent dilation

Endothelial-Independent Dilation

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 post GTN administration for 5 min (Figure 5.4). Brachial artery diameter was

analyzed off-line, using a semi-automated custom designed edge-detection software program.

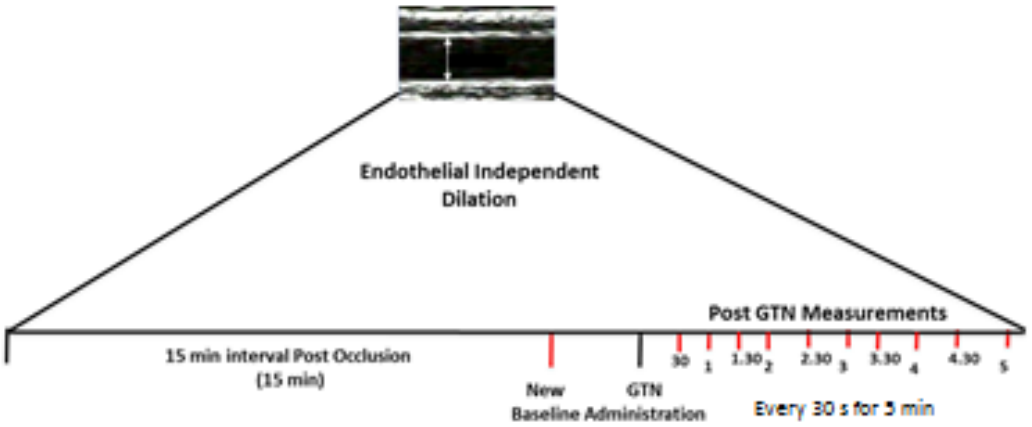


Figure 5.4: Overview of endothelial-independent dilation

All images were selected for analysis using a standard dialog box (Figure 5.5). 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. 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.

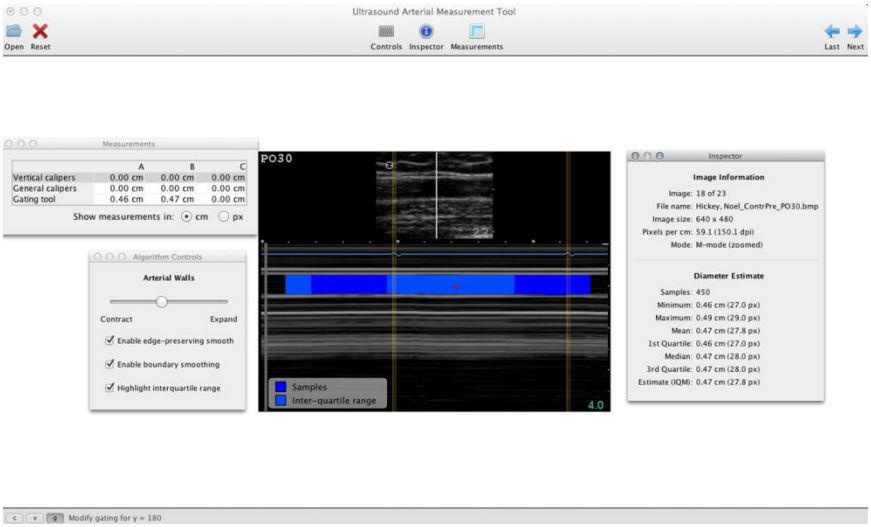


Figure 5.5: Standard dialog box

Blood sampling and storage

Blood samples were taken from a prominent forearm vein using standard venipuncture. Serum vacutainers were allowed stand for 30 min at room temperature before being centrifuged at 3000 rpm (1600 g) for 15 min at 4^{o}C. Blood for microparticles analysis was collected, prepared and stored according to methods described by Lacroix *et al.,* (2012).[37] Whole blood was gently mixed with the coagulant by inversion. Centrifugation was completed within 15 min at 3730 rpm at 20^{o}C to pellet the cells. The plasma containing the MPs was carefully aspirated, leaving a 1 cm layer undisturbed above the buffy layer. The cell-free plasma was aliquotted (250 µL portions) into eppendorf tubes. The platelet rich plasma (PRP) was then centrifuged for a second time at 5160 rpm for 15 min at 20^{o}C. The platelet poor plasma (PPP) was

aliquotted (250 µL portions) into fresh eppendorf tubes, snap-frozen in liquid nitrogen, and stored at 80^{o}C until analysis.

Microparticle Analysis

Flow cytometry, using GUAVA easyCYTE8 HT Flow Cytometry System (Millipore Corporation, Billerica, MA) was used to quantify microparticle numbers. Microparticles were identified in PPP based on size and fluorescence, using 1.0 µm sizing beads and anti-CD 31 FITC, anti-CD 41a PE and anti-CD 62E monoclonal antibodies. EMPs were defined as CD31+/CD41a- and CD62E+ and PMPs were defined as CD41a+ and CD31+/CD41a+.

Prior to use, antibodies were diluted and centrifuged to remove aggregates. Antibodies CD31 and CD41a were diluted 1:2 in filtered (0.2 µm) PBS. Antibody CD62E was diluted 1:50 in filtered PBS. The diluted antibodies were then centrifuged at 19000 g for 5 min at 4^{o}C in order to remove aggregates and the supernatant pipetted into fresh Eppendorf tubes.

Samples were removed from the freezer and allowed to thaw slowly on ice. Samples were doubled stained with CD31/CD41a and single stained with CD62E. For the doubled stained samples 15 µl of both CD31 and CD41a were added to 30 µl of PPP. For the single stained sample 15 µl of CD62E was added to 30 µl of PPP. Samples were then incubated in the dark for 20 min at room temperature. Following incubation 500 µl of PBS was added to each sample and mixed well. A 200 µl volume

of each sample was loaded into one well on the plate and analysed. Fluorescence thresholds were set using PPP incubated with isotype-matched control antibodies. All samples were analysed in duplicate and samples for each participant were analysed in the same run. Samples were counted for 5000 counts at a low flow rate.

Lipid analysis

As described in the methods section of Chapter 3.

Statistical analysis

Prior to statistical analysis the data was checked for normality using the Shapiro-Wilk test. A group (HIIT and CR) x time (pre training and post training) repeated measures ANOVA was used to compare the mean differences within and between groups. A one way repeated measure ANOVA was used to compare physiological responses averaged across the 3 sets during the HIIT training sessions. Significant main effects were probed using a Bonferroni post hoc test. The relation between MP numbers and FMD before and after the intervention was established using Pearson's product moment correlation. 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

Physical characteristics and blood lipid levels before and after LS-HIIT and CR are presented in table 5.1. There was no significant difference in height, body mass, BMI or blood lipids between the two experimental groups at baseline or after 4 weeks of training. Compared to pre training serum triglycerides were significantly higher ($p=0.02$) in the LV-HIIT and HDL-C significantly lower ($p=0.02$) post training in CR. Participant medications are summarised in table 5.2

Maximal Exercise Tests

The responses at maximal exercise are summarised in table 5.3. Treadmill time to exhaustion was significantly greater in the LS-HIIT at post training than pre training. There was no significant difference in treadmill velocity, treadmill grade, $\dot{V}O_2max$, heart rate, ventilation and RER between the two experimental groups before and after 4 weeks of training. There was no significant difference in treadmill velocity, treadmill grade, $\dot{V}O_2max$, heart rate, ventilation and RER in LS-HIIT or CR between pre training and post training.

High Intensity Interval Training

ST-segment changes ranged from 1 – 3 mm, and returned to normal during the 15 sec passive recovery. None of the participants reported angina symptoms during or following the 4 weeks of LS-HIIT.

The average physiological and perceptual responses for each LS-HIIT exercise block are summarized in table 5.4. Treadmill velocity was significantly greater during block 2 and 3 than block 1. Heart rate, %HRmax and RPE were significantly higher during block 2 than block 1 and during block 3 than block 1 and 2. SPO_2 was significantly lower during block 3 than block 1. There are no differences in treadmill grade or blood pressure between the 3 training blocks.

Table 5.1: Physical characteristics and blood lipids of the participants before and after training

	Group			
	LS-HIIT (n=15)		CR (n=11)	
	Pre-Training	Post-Training	Pre-Training	Post-Training
Height (cm)	170.63 ± 1.77	170.64 ± 1.78	167.61 ± 2.49	167.64 ± 2.51
Body mass (kg)	80.80 ± 2.65	80.41 ± 2.58	77.83 ± 3.62	79.28 ± 4.06
BMI ($kg \cdot m^{-2}$)	27.70 ± 0.67	27.31 ± 0.72	27.75 ± 1.31	28.17 ± 1.70
Total cholesterol ($mmol \cdot L^{-1}$)	3.40 ± 0.21	4.48 ± 0.55	3.63 ± 0.30	3.40 ± 0.17
HDL-cholesterol ($mmol \cdot L^{-1}$	1.21 ± 0.05	1.26 ± 0.08	1.26 ± 0.10	1.03 ± 0.10*
LDL-cholesterol ($mmol \cdot L^{-1}$)	1.42 ± 0.09	1.80 ± 0.19	1.79 ± 0.38	1.51 ± 0.14
Triglycerides ($mmol \cdot L^{-1}$)	0.96 ± 0.10	1.28 ± 0.12*	1.08 ± 0.18	1.12 ± 0.07

Values are means ± SE; *$p < 0.05$ vs. pre-training

Table 5.2: Participant medications

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

Table 5.3: Responses at maximal exercise before and after training

	Group			
	LS-HIIT (n=15)		CR (n=11)	
	Pre-Training	Post-Training	Pre-Training	Post-Training
Treadmill velocity ($km \cdot h^{1}$)	5.50 ± 0.30	5.38 ± 0.36	5.30 ± 0.26	5.91 ± 0.77
Treadmill grade (%)	12.00 ± 0.82	12.31 ± 0.72	9.50 ± 0.92	10.54 ± 1.26
Time to exhaustion (s)	524.54 ± 30.77	591.77 ± 27.13*	584.86 ± 64.82	550.00 ± 57.47
$\dot{V}O_2max$ ($ml \cdot kg^{1} \cdot min^{1}$)	30.48 ± 2.05	32.23 ± 2.15	28.52 ± 1.85	29.20 ± 2.38
$\dot{V}O_2max$ ($L \cdot min^{1}$)	2.37 ± 0.21	2.40 ± 0.16	2.03 ± 0.16	2.10 ± 0.23
Heart rate (bpm)	138.20 ± 3.63	135.87 ± 3.55	126.90 ± 4.57	129.38 ± 7.96
Ventilation ($L \cdot min^{1}$)	63.95 ± 5.43	66.20 ± 4.37	56.02 ± 4.87	59.63 ± 6.57
RER	1.13 ± 0.02	1.14 ± 0.02	1.09 ± 0.03	1.15 ± 0.03

Values are means ± SE; *$p < 0.05$ vs. week 1

Table 5.4: Average responses during each exercise block of LS-HIIT

	Exercise Blocks			
	1	**2**	**3**	**Mean**
Treadmill velocity ($km \cdot h^{-1}$)	6.83 ± 0.39	7.06 ± 0.46*	7.17 ± 0.50*	7.02 ± 0.46
Grade (%)	4.13 ± 0.43	4.15 ± 0.43	4.10 ± 0.44	4.13 ± 0.48
Heart rate (bpm)	108.51 ± 4.02	114.53 ± 4.67†	122.03 ± 5.10†‡	115.00 ± 4.64
%HRmax	77.50 ± 3.58	82.25 ± 4.17†	86.02 ± 4.4†‡	81.60 ± 4.19
Systolic blood pressure (mmHg)	163.22 ± 5.89	160.55 ± 6.17	156.23 ± 6.65	160.03 ± 7.50
Diastolic blood pressure (mmHg)	74.09 ± 1.16	73.43 ± 1.42	71.36 ± 1.43	73.10 ± 2.12
RPE	12.92 ± 0.49	13.83 ± 0.45¥	14.63 ± 0.40¥‡	13.88 ± 0.56
SPO_2	95.47 ± 0.57	95.10 ± 0.56	95.06 ± 0.54*	95.10 ± 0.65

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

Endothelial Microparticles

Circulating levels of CD31+ and CD62E+ EMPs are summarised in table 5.5. Before training, the number of circulating CD31+ and CD62E+ EMPs was similar in LS-HIIT and CR. Compared to pre training, there was no change in the number of circulating CD31+ EMPs (Figure 5.6) or CD62E+ EMPs (Figure 5.7) in either LS-HIIT or CR after 4 weeks of training.

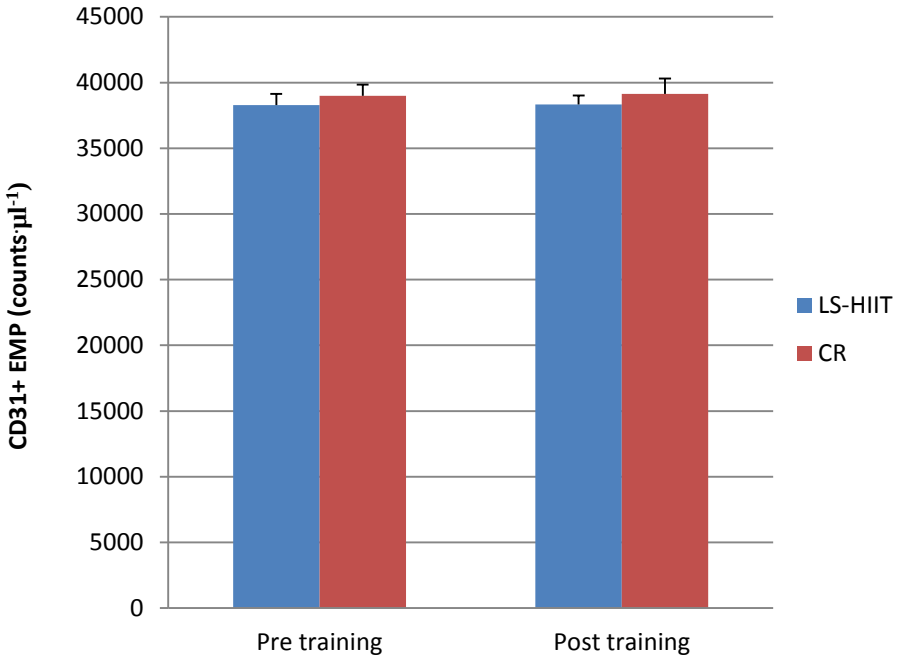


Figure 5.6: Circulating CD31+ EMPs pre and post training

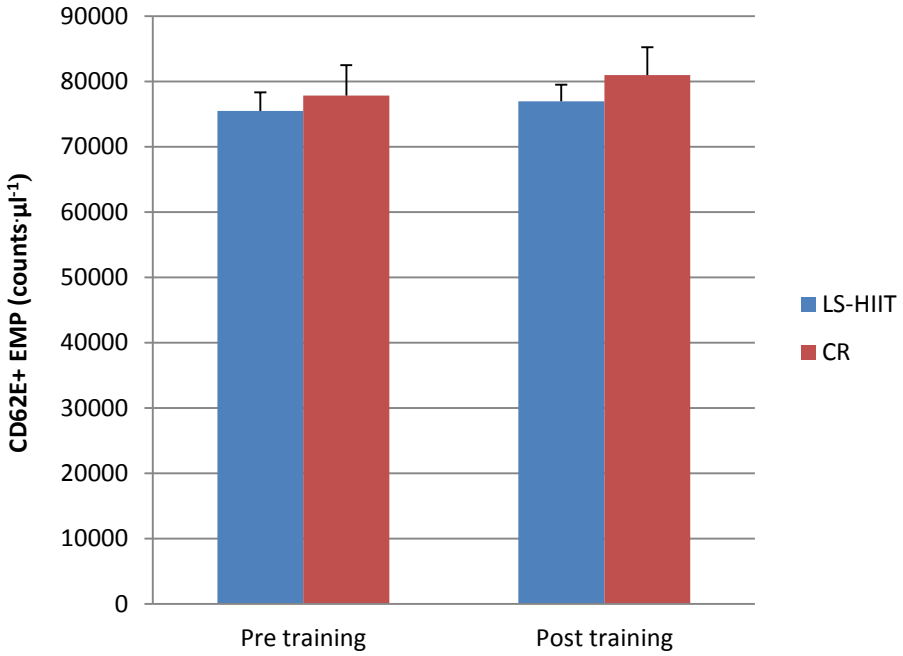


Figure 5.7: Circulating CD62E+ EMPs before and after training

Platelet Microparticles

Circulating levels of CD41a+ and CD41a+/CD31+ PMPs are summarised in table 5.5. Before training, the number of circulating CD41a+ and CD41a+/CD31+ PMPs was similar in LS-HIIT and CR. Compared to pre-training, there was no change in the number of circulating CD41a+ PMPs (Figure 5.8) or CD41a+/CD31+ PMPs (Figure 5.9) in either LS-HIIT or CR after 4 weeks of training.

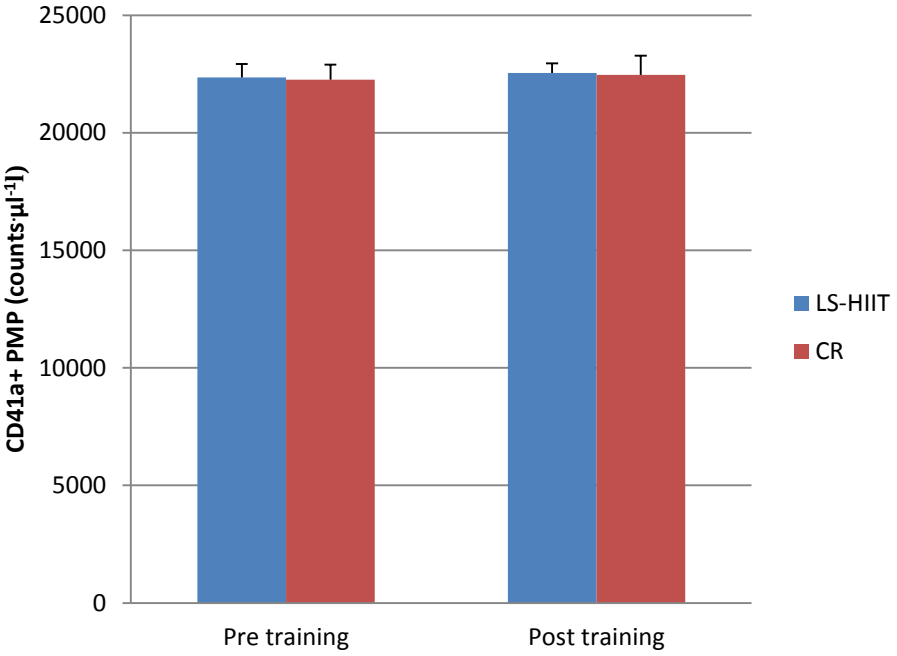


Figure 5.8: CD41a+ PMPs before and after 4 weeks

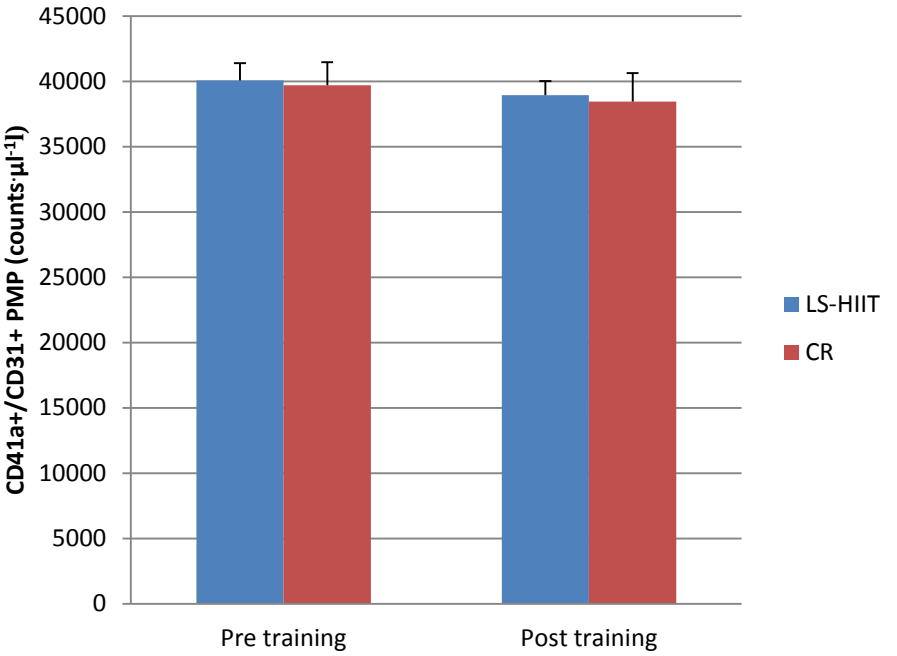


Figure 5.9: CD41a+/CD31+ PMP before and after 4 weeks

Table 5.5: Circulating microparticles pre training and post training

	Group			
	LS-HIIT (n=15)		CPCR (n=11)	
	Pre-Training	Post-Training	Pre-Training	Post-Training
CD31+ EMP (counts·µl^{-1})	38258 ± 857	38337 ± 673	39003 ± 847	39128 ± 1168
CD62E+ EMP (counts·µl^{-1})	75488 ± 2806	76951 ± 2518	77825 ± 4670	80947 ± 4275
CD41a+ PMP (counts·µl^{-1})	22355 ± 573	22544 ± 412	22253 ± 639	22461 ± 814
CD31+/CD41a+ PMP (counts·µl^{-1})	40062 ± 1328	38927 ± 1091	39690 ± 1759	38453 ± 2162

Values are means ± SE

Endothelial Function

Pre-training flow mediated endothelial dependent dilation was similar in LS-HIIT and CR. Post training flow mediated endothelial dependent dilation was significantly higher than pre training in LS-HIIT. Brachial artery FMD was also significantly higher LS-HIIT than CR group training (Figure 5.10).

There was no significant difference in endothelial-independent dilation between the two experimental groups before and after training. Compared to pre training there was no significant difference in endothelial-independent dilation in the LS-HIIT or CR after 4 weeks of training. (Figure 5.11) Compared to pre-training, there was no change in shear rate in LS-HIIT (951.2 ± 294.1 s^{-1} vs. 1020.5 ± 245.1 s^{-1}) or CR (1009.5 ± 179.9 s^{-1} vs. 932.5 ± 146.2 s^{-1}) post training. There was no relation between changes in endothelial function and changes in aerobic fitness (r=0.351, p=0.108)

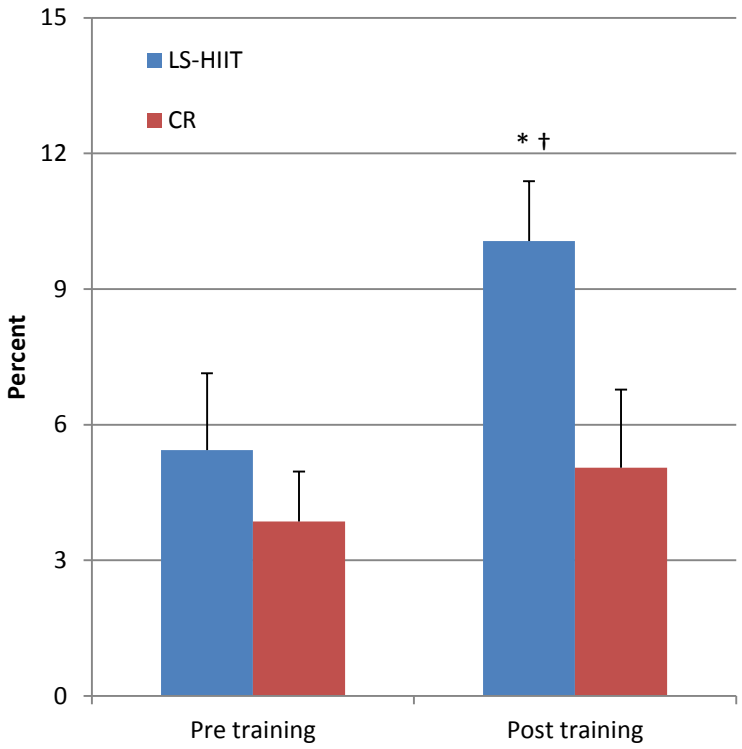


Figure 5.10: Percent change in FMD in LS-HIIT and CR group pre training and post training. *p < 0.05 vs pre training; †p < 0.05 vs CR

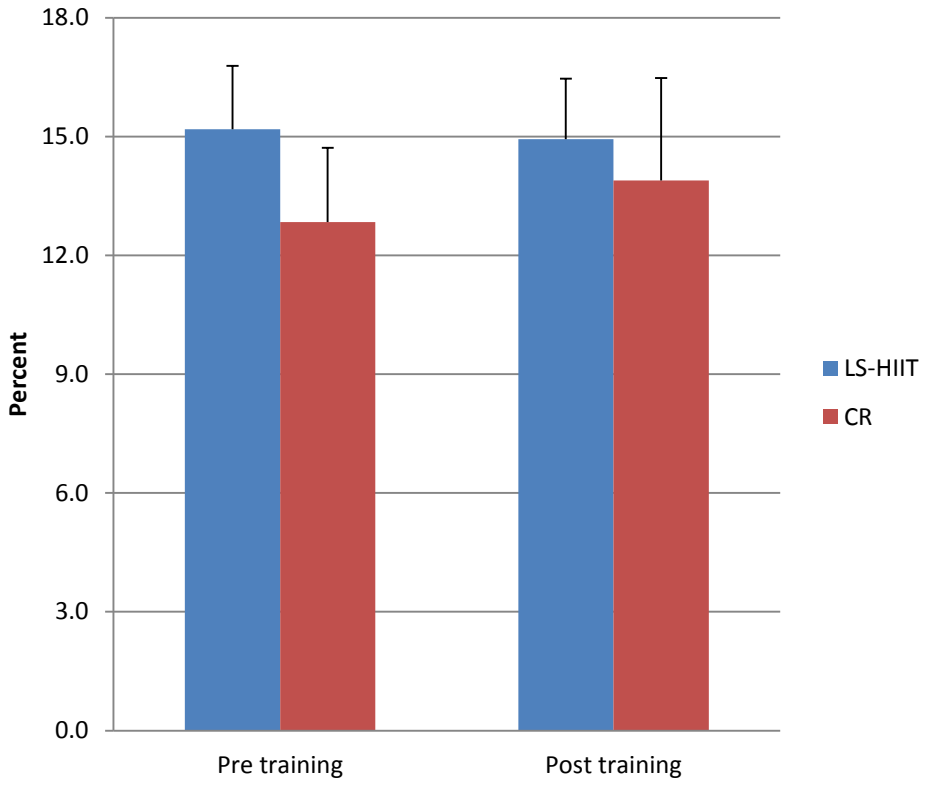


Figure 5.11: Percent change in brachial artery diameter (%) following GTN administration pre training and post training

Microparticles and Endothelial Function Correlations

Pre training there was a significant relation between circulating CD31+ EMP numbers and absolute and percentage change in FMD. (Figure 5.11) Post training CD41+ PMP numbers in the CR group were significantly related to the post training absolute and percentage change in FMD (Figure 5.12). After the 4 weeks of training there was a significant inverse relation between CD31+/41a+ PMP numbers and the absolute and relative change in FMD in the LS-HIIT group. (Figure 5.13) There was a

significant relation between CD31+/41a+ PMP numbers and the absolute and relative change in FMD in the CR group. (Figure 5.14)

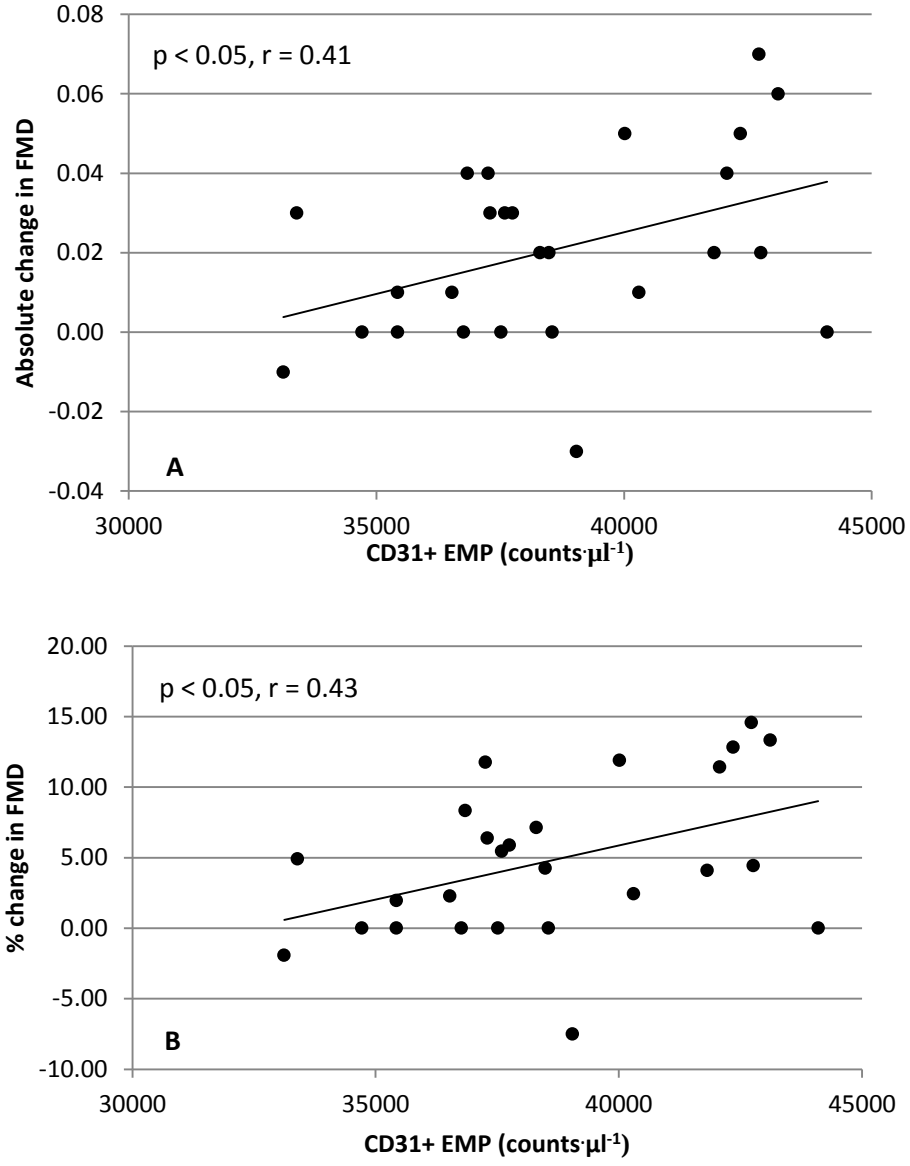


Figure 5.12: Relation between CD31+ EMP numbers and absolute (A) and percentage change (B) in FMD pre training

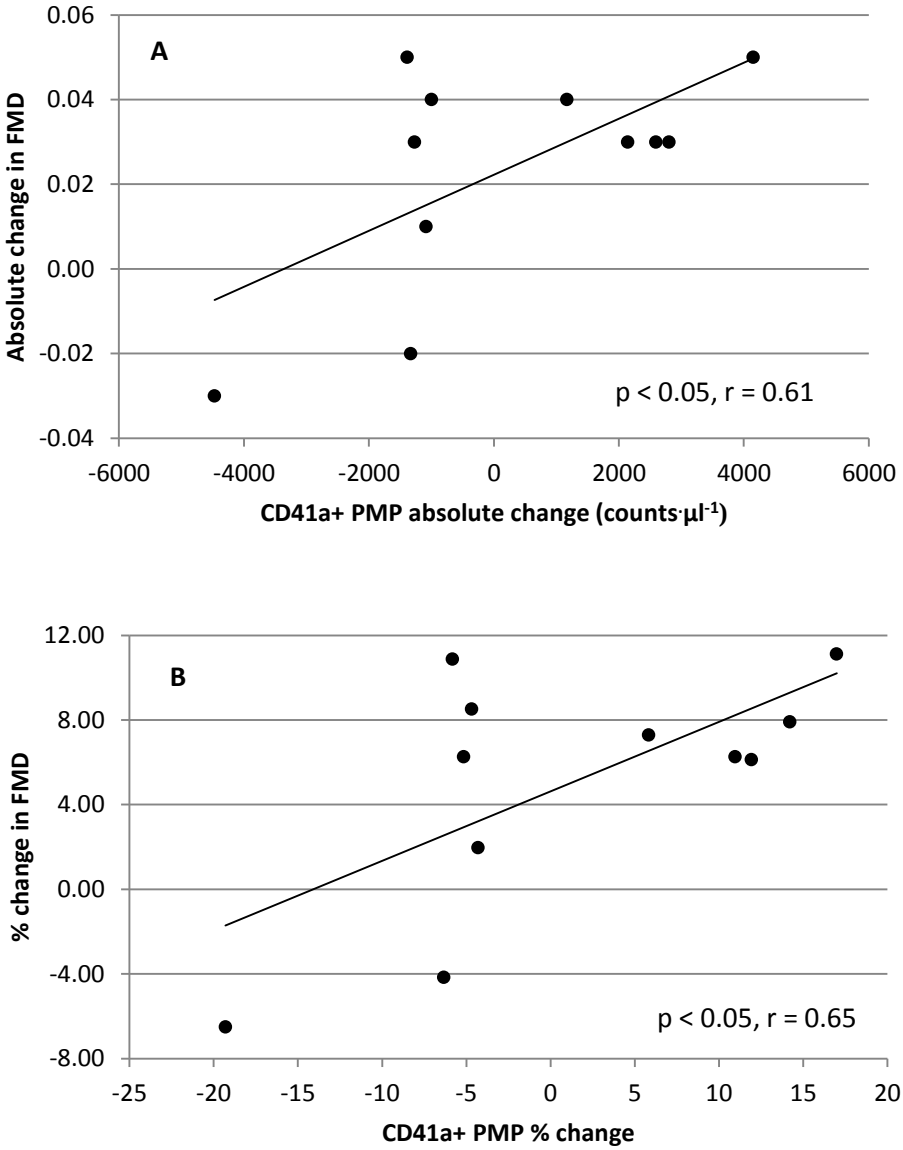


Figure 5.13: Relation between absolute change in FMD and CD41a+ PMP (A) and % change in FMD and CD41a+ PMP (B) following the intervention in the CR group.

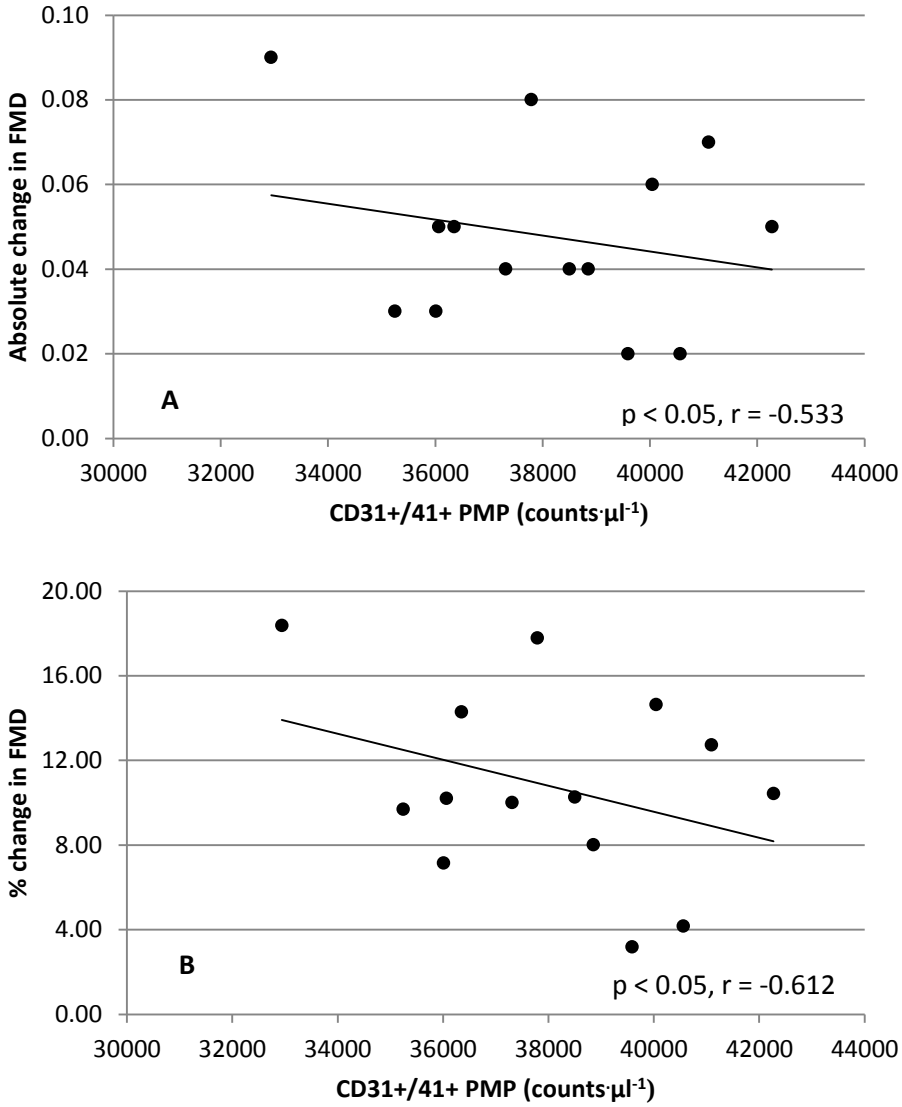
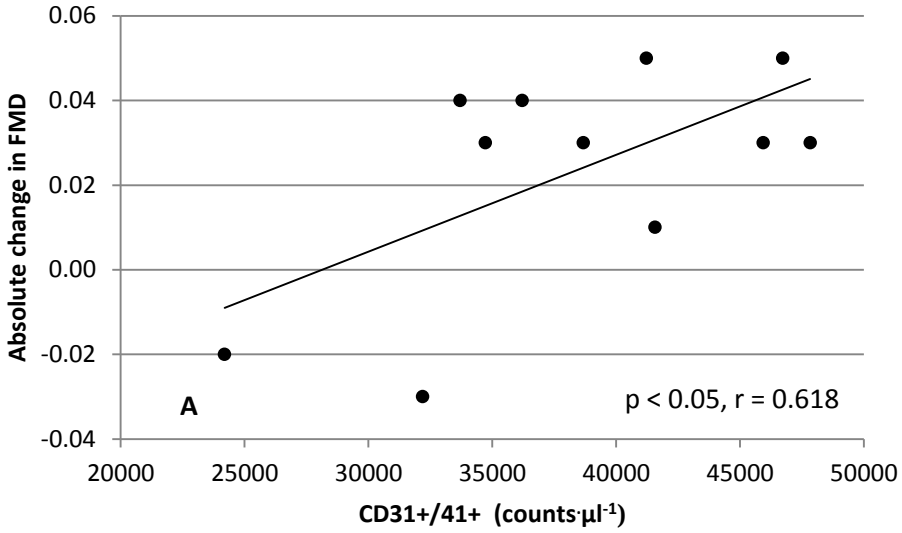


Figure 5.14: Relation between CD31+/41+ PMP numbers and the absolute (A) and percentage (B) change in FMD following the intervention in the LS-HIIT group.



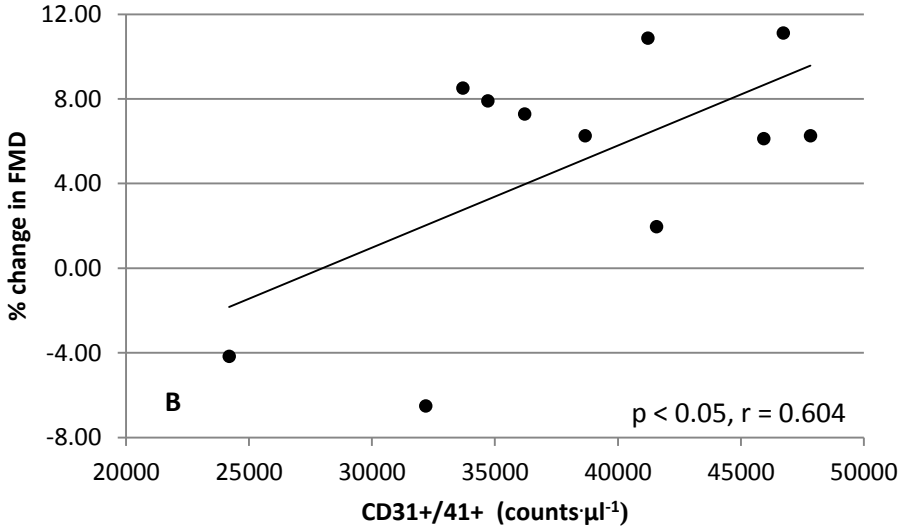


Figure 5.15: Relation between CD31+/41+ PMP numbers and the absolute (A) and percentage (B) change in FMD following the intervention in the CR group.

Summary

There was no increase in aerobic fitness following 4 weeks of LS-HIIT or CR. There was however, a statistically significant improvement in treadmill time to exhaustion in the LS-HIIT group only. There were no changes in circulating MP numbers between or within groups before or after the exercise intervention. There was no difference in FMD between groups at baseline. There was an improvement in FMD in the LS-HIIT group only following the exercise intervention and at post training the FMD was significantly higher in the LS-HIIT group. There was a positive relation between CD31+ EMP numbers and absolute and percentage change in FMD pre training.

Discussion

Study 3 evaluated the effect of 4 weeks of LS-HIIT and CR on circulating MPs, aerobic fitness and endothelial function in men and women with CVD and evaluated the relation between MPs and endothelial function. Conventional exercise guidelines for reducing CVD risk factors and cardiovascular events have promoted continuous, moderate to vigorous intensity exercise training. Exercise performed at a vigorous intensity (≥6 METs) has been shown to induce a greater reduction in CVD risk along with greater improvements in diastolic blood pressure, glucose control and aerobic capacity than exercise performed at a moderate intensity.[10] In recent years LS-HIIT has been proposed as a safe and effective method for improving both CVD risk factors and aerobic fitness in patients with CVD.[120] A number of studies have found an increase in aerobic fitness and endothelial function in patients with CVD following a HIIT exercise program.[85,121,122] In the present study there was a significant improvement in endothelial function following 4 weeks of HIIT but no change in $\dot{V}O_2$max. There was however, an increase in the time to exhaustion during the incremental treadmill exercise test.

The improvement in endothelial function may be due in part to the positive effect of exercise on the regulation of endothelial NO bioavailability. Exercise induced increases in shear-stress augments the expression of nitric oxide synthase in endothelial cells and the repeated sheer stress during HIIT may partly explain the

improvements in flow mediated dilation. Shear stress can also increase the endothelium's antioxidative defence mechanisms by increasing the activity of superoxide dismutase.[123]

Differences in exercise prescription and study duration may help to explain the relatively small improvement in $\dot{V}O_2$max. Previous studies that have found a significant improvement in aerobic fitness following short-term HIIT have primarily involved healthy young college age students.[124,125] Although the exercise prescription in the present study involved LS-HIIT it is unlikely that the older patients with CVD will be able to exercise at the same intensity as healthy college age students. Previous HIIT studies involving college age students involved 2 sessions.wk^{-1} for 2 – 4 weeks whereas HIIT studies involving individuals with CVD have been greater than 8 weeks in duration. It is possible that 2 session of HIIT per week for 4 weeks may not have been of sufficient duration to increase aerobic fitness.

Recent epidemiological research found that a 1 MET increase in aerobic fitness is associated with a 13% decrease in all-cause mortality and a 15% decrease in CHD/CVD.[126] Although not statistically significant, $\dot{V}O_2$max increased 5.7% and by 0.5 METS in the LS-HIIT group. It is feasible that this increase in aerobic fitness has important positive influences on the cardiovascular health in men and women with CVD.

There were no changes in EMP or PMP numbers following the LS-HIIT or the CR. A recent study that used similar laboratory protocols and antibodies to the present study found no change in circulating EMP or PMP numbers up to 72 h following an acute bout of HIIT in men with CHD.[69] The fact that there was no change in serum troponin would indicate that HIIT does not induce myocardial injury in the short-term and is safe in this population. There is some evidence to indicate that circulating EMPs may also be used to indicate myocardial injury.[23] Troponin was not measured in the present study but the fact that circulating CD62E+ or CD31+ did not change in response to LS-HIIT may indicate that the training regime employed in the present study is safe and does not induce myocardial injury or endothelial activation. Clinical trials will need to be undertaken to further investigate the efficacy of EMP's as a putative biomarker for myocardial injury. The fact that there was no significant change in circulating MPs despite an improvement in endothelial function indicates that MPs may not be a suitable marker of endothelial function.

CD31+ EMPS are associated with endothelial cell apoptosis and are elevated in disease states associated with vascular dysfunction such as the metabolic syndrome and acute coronary syndromes (ACS).[104,127] In contrast, CD62E+ EMPs are associated with an increase in activation of endothelial cells. Circulating levels of CD31+ MPs are significantly higher in individuals with the metabolic syndrome and ACS than healthy controls. In contrast, circulating levels of CD62E+ EMPs were similar in both groups

suggesting that there may be increased levels of endothelial cell apoptosis in patients with the metabolic syndrome and ACS.

Feng *et al.,* (2010) also found significantly higher circulating CD31+ EMPs in diabetic patients than healthy controls.[128] There was no difference in CD62E+ EMPs between the LS-HIIT and the CR groups. The absence of a healthy control group in the present study makes it difficult to determine if circulating EMP numbers are elevated. There was however, no significant change in CD31+ or CD62E+ following the exercise intervention suggesting that the LS-HIIT does not induce greater endothelial cell apoptosis or activation than CR.

The fact that there was a significant positive relation between circulating CD31+ and changes in endothelial-dependent FMD before training indicates that increased circulating CD31+ EMP numbers may be associated with improved vascular health in the present study. This is in contrast to a previous study showing a significant inverse relation between CD31+ EMP numbers and changes in FMD in diabetic patients but not in healthy controls.[128] The participants in the current study were participating in a structured CR program for at least 6 months. Previous exercise may have had an effect on EMP phenotype. It is also possible that CD31+ EMPs are related to alterations due to the diabetic condition as Tramontano *et al.,* (2010) also found a significant increase in CD31+ EMPs in diabetic patients compared to non-diabetic controls.

The significant positive relation between the absolute and percentage change in FMD and the absolute and percentage change in circulating CD41+ in the CR group is a unique finding and suggests that this MP may have cardio protective properties. Although the increase in the circulating number of CD41+ PMPs was related to an increase in endothelial function the relatively small sample size and two obvious outliers (figure 5.12) makes it difficult to draw any definite conclusions on the clinical relevance of this finding.

Following the training study there was a significant inverse relation between circulating CD31+/41+ PMP numbers and changes in FMD in the LS-HIIT group indicating that lower numbers of CD31+/41+ PMPs are associated with improved endothelial function. It is possible that PMPs positive for both CD31+ and CD41+ have a more deleterious effect on the endothelium than CD41+ alone. In contrast, circulating CD31+/41+ PMP numbers were positively related to changes in FMD in the CR group. The correlation coefficient may however have been significantly influenced by two outliers. The relation between CD31+/41+ PMP numbers and FMD was no longer significant when the outliers were removed from the statistical analysis. Future studies involving men and women with CVD should further investigate the acute and chronic effects of exercise on MPs and investigate if there is a minimum threshold of exercise needed to induce changes in MPs.

The aim of study 3 was to i) compare the number of circulating EMPs and PMPs before and after 4 weeks of LS-HIIT or CR in men and women with CVD and ii) to examine the relation between MP numbers and FMD, a measure of endothelial function associated with CVD. There were no changes in MP numbers in either group despite an improvement in FMD in the LS-HIIT group.

Chapter VI

Synthesis of Findings

MPs are small membrane vesicles shed from cells following activation or apoptosis. Small numbers of MPs are constantly released from a variety of cells under normal physiological conditions. The effect of exercise on circulating MP numbers is relatively unknown. To date no studies have quantified the dose-response of exercise intensity on MP production. With the exception of one study that evaluated the effects of a 6 month exercise training program on MP production in overweight and obese African Americans there is currently no information on the effects of short or long-term training on MP production in healthy or disease populations. Although there is evidence that circulating MP numbers increase in men and women with CVD, no published studies have examined the relation between exercise training, MPs and endothelial function in this population. A series of studies were undertaken to assess the effects of acute and chronic exercise on the number and composition of MPs and to examine the relation between MPs and endothelial function before and after exercise training.

Study one examined the effects of isocaloric bouts of exercise at 60%, 70% and 80% $\dot{V}O_2$max on circulating EMP and PMP numbers. For the most part, circulating EMPs did not change significantly immediately after exercise and at 2 h post exercise. However, circulating EMPs were elevated at 4 h and 6 h post exercise compared to 2 h

post exercise at all 3 exercise intensities. It may be that following the exercise bouts there is increased gene expression resulting in cell activation and MP production. There may be a cellular response to the over production of proteins leading to MPs containing these proteins being released from cells. Unpublished work by Fitzpatrick and Murphy (DCU) has described an increase in the protein Lasp-1 in sheer induced MPs. Lasp-1 is an adhesion protein essential for cell migration and survival in reply to growth factors and extra cellular matrix proteins.[129]

Study two examined the effect of 14 consecutive days of exercise on circulating MP numbers in sedentary men. Changes in aerobic fitness have been linked to improvements in the processes associated with the development of CVD and the present study succeeded in significantly improving both absolute and relative aerobic fitness. Despite the fact that circulating MPs have been associated with CVD, the study did not find a relation between changes in aerobic fitness and changes in circulating MPs. There was a significant decrease in EMP numbers compared to pre-training levels. However, it is possible that EMP numbers may have been influenced by the acute bout of exercise the previous evening.

Although not statistically significant, circulating EMP values were lower at all time points on training day 14 compared to day 1, 3, 7 and 10. PMP values on day 10 and day 14 were different to values on days 1, 3 and 7 with less variation within samples and very similar responses. It is possible that there is an adaptive response

affecting both the release of EMPs and PMPs following 10 to 14 days of exercise training. The lower PMP values may be a result of improvements in platelet function. It is possible that lower numbers of MPs are released from platelets after the first week of training due to alteration in megakaryocytopoiesis.

Study 3 evaluated the effects of exercise training on circulating MPs in men and women with documented CVD. Endothelial dysfunction is one of the earliest events in the development of atherosclerosis and is predictive of an increased risk for cardiovascular events.[79] Brachial artery flow mediated dilation was assessed to provide a measure of endothelial function and to assess the relation between endothelial function and circulating MPs before and after 4 weeks of exercise training.

In contrast to the first two studies, there was no change in EMPs in men and women with CVD despite an improvement in the FMD in the LS-HIIT group. This may be due to the fact that the i) laboratory procedures used to measure MPs were different in study 3 compared to the first two studies, ii) circulating EMPs are not sensitive to changes in endothelial function and iii) a higher training volume may be required to increase EMPs in men and women with CVD.

The participants in study 3 had been participating in a community based CR program for at least 6 months prior to the start of the study. It is unclear if this exercise period affected circulating MP numbers. The lack of an age matched, non-exercising control group makes it difficult to evaluate the independent effect of

exercise training on circulating MPs. Different analytical methods make it difficult to compare the results of study 3 to study 1 and 2. Efforts are currently being made to standardise MP analysis.

In conclusion, independent of exercise intensity circulating levels MPs increase in healthy physically active young men at 6 h following an acute bout of exercise involving a caloric expenditure of 400 kcal. Circulating EMPs decrease significantly in healthy sedentary young men 13 h following a vigorous exercise session lasting 1 h with a possible training effect following 10 – 14 days. In men and women with CVD eight sessions of LS-HIIT significantly improves brachial artery and does not significantly change circulating MP numbers. Future studies should i) establish the putative role of MPs as markers of CVD, ii) describe the *in vivo* mechanisms involved in MP release and iii) establish disease specific MP biomarkers.

Study Limitations

At the present time there are many limitations associated with the measurement of microparticles. These include blood sampling techniques, anti-coagulants, the time prior to blood processing, number and speed of centrifugations, freezing and storage time, and antibodies used for detection. Enumeration of MPs in study 3 involved different preparation protocols and a different flow cytometer than used for study 1 and study 2. The change in the preparation protocol reflected a change in the guidelines over the time period of the study.

A major limitation to the work is that post exercise data points were not subject to correction for a possible exercise induced haemoconcentration or haemodilution. Bouts of acute exercise can produce a transient haemoconcentration immediately after exercise, while exercise training can cause long term expansion of the plasma volume.[130] Both of which may affect lipid and MP results.

The sample size in study 2 was small and there was no control group. In addition, participants in study 2 were asked to consume a similar diet for the duration of the exercise training intervention. This was monitored verbally but changes in daily dietary intake may have influenced MP numbers. The final post training blood sample was taken 13 h following the last training session. In order to make a valid comparison with baseline, a 48 h time period should be allowed between the final training session and the blood draw.

More than 50% of the participants in study 3 were taking HMG CoA reductase inhibitors. This class of drug has been shown to effect circulating MPs.[131]

Future Direction

This series of experiments aimed to assess the acute and chronic effects of exercise on MP production in young healthy men and the effects of chronic LS-HIIT and CR on circulating MPs in men and women with CVD. Future research should determine the appropriate volume of exercise needed to induce changes in MP numbers in different populations and examine the relation between exercise induced changes in MPs and established markers of cardiovascular health. If flow cytometry is to be used as the gold standard method of MP analyses more work needs to be done on establishing standard protocols for sample collection, preparation and analysis.

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Appendices

Appendix A

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 which you suffer from and any medication which you may be taking by prescription or otherwise:

……

……

……

Date: Signature:

Authorising Signature:

Appendix B

E-mail to be sent to staff and students

The School of Health and Human Performance are conducting research looking at how different exercise intensity effect levels of microparticles in the blood. Microparticles are tiny fragments of cells that may be linked with disease such as heart disease.

The study would involve having your fitness assessed on a treadmill and on three other days jogging at different intensities on the treadmill until 400 kcal are burned. To look at the microparticles we need to take some blood samples.

We are looking for men and women between 18-30 years, who have no history of cardiovascular disease, and do not smoke.

Following the research you would get information on your fitness levels and receive fitness and nutritional advice if required.

Appendix C

Plain Language Statement

Title: Effect of an acute bout of exercise on circulating microparticles.

Principal Investigators

Prof. Niall Moyna	niall.moyna@dcu.ie	(Phone No. 7008802)
Paul O'Connor	paul.l.oconnor@dcu.ie	(Phone No. 7008474)
Dr. Noel McCaffrey	noel.mccaffery@dcu.ie	(Phone No. 7008187)

School of Health and Human Performance, Dublin City University

What is the study about?

Microparticles are tiny fragments of cells that can be found in the blood. During some diseases, the level of microparticles circulating in the blood rises. There is now evidence that elevated levels of these microparticles can lead to cardiovascular disease. Regular physical activity has been shown to reduce the risk for cardiovascular disease. The purpose of this study is to determine the effects of exercise intensity on the number of microparticles in healthy asymptomatic individuals.

You will be asked to make 5 visits to the School of Health and Human Performance in DCU. Each visit will last approximately 1 hour. You will arrive for each visit after an overnight fast. During the first visit you will have a blood sample taken and undergo a brief examination by a medical doctor to make sure you are suitable for the study. About 10 days after this visit you will be contacted by phone or email to let you know if you are eligible to participate in the study. During the second visit you will exercise on a treadmill for approximately 15-mins to determine your fitness level. The exercise will get progressively harder, but you should try to keep going as long as you can. During visits 3-5, you will exercise at an intensity equal to 60, 70 and 80%, of your maximal capacity until you burn 400 calories. The exercise intensity of each visit will be determined randomly. A blood sample will be taken immediately before and after exercise, and again at 2 hours, 4 hours and 6 hours after the exercise. You will be asked to drink a nutritious shake 2 hours after the exercise.

Exercise does carry a risk of injury, such as a pulled muscle, muscle soreness or in extreme cases abnormal heart rhythm, heart attack or death. The benefits of being involved in this study include a detailed assessment of your fitness, your response to exercise and your cholesterol levels. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate thesis and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. 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. All data will be destroyed after 5 years. Your participation in this research project is voluntary and you may withdraw your consent at any time.

If participants 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

Appendix D

Informed Consent

INFORMED CONSENT FORM

Title: Dose-response of circulating microparticles to acute bouts of exercise

Principal Investigators: Prof. Niall Moyna, Dr. Noel McCaffrey and Kevin O'Brien

Purpose

To determine the effects of a different intensities of exercise on the number of endothelial and platelet derived microparticles in healthy asymptomatic individuals..

This is what will happen during the research study

1. I will have the purpose of the study, each of the steps involved and the risks of participating in the study explained to me. I will have the opportunity to ask any questions and if I am happy with the answers I will:
   i). Provide written informed consent for participation in the research project.
   ii). I will have a small blood sample taken from a vein in my arm, undergo a brief physical examination by a medical doctor, and complete a medical history form, which will ask questions about my general health, personal and family health history, smoking, exercise, and dietary habits.
   iii). I will be contacted by phone or email to let me know if I am eligible to participate in the study.
   iv) I am eligible to participate in the study I will visit the laboratory in the School of Health and Human on 2 occasions separated by at least one week. Each visit will involve exercising on a treadmill.
2. **Visit 1: Screening**

   I will have a small blood sample taken from a vein in my arm, undergo a brief physical examination by a medical doctor, and complete a medical history form, which will ask questions about my general health, personal and family health history, smoking, exercise, and dietary habits. I will have a number of measurements of my body size and shape. My percentage body fat will be measured by the thickness of skin and fat on 7 sites around the body including the chest, leg, arm, back and side of the body. I will practice walking and running on a treadmill while wearing a special mouthpiece like a snorkel in my mouth to measure the amount of air I breathe in and out.

   I understand that any of these procedures or tests may be waived at the discretion of the doctor for the following reasons: (i) I have completed the same or similar step in the past 6 months as part of another research protocol at the School of Health and Human Performance; (ii) It has been determined that I am not eligible to participate in this research project; and thus completion of the entire screening process will not be necessary.

   Visit 2: Fitness Evaluation

   I will undergo an exercise test designed to measure my fitness. I understand that I will walk or run on a treadmill, with the slope gradually increasing until fatigue, breathlessness, chest pain and/or symptoms that indicate to the doctor or myself that I should stop exercise. I will wear a mouthpiece to measure the amount of air I breathe in and out. My heart will be monitored by an electrocardiogram (ECG) throughout the test.

   Visits 3-5: Experimental Trials

   I will walk or run on a treadmill at 60, 70 and 80% of my maximal capacity until I use 400 calories. During this time I will also wear the mouthpiece, to determine oxygen use. I will have a plastic tube called a cannula, inserted into a vein in my arm to allow blood

samples to be taken. This plastic tubing is introduced into my vein with a needle, which is then withdrawn. The plastic cannula will remain in my arm, and will be used to take blood samples before and during exercise. A 10.0 ml blood sample will be taken at the beginning and end of each experimental trial, and at 6 h and 24 h after exercise. The 24 h blood sample will be taken using standard venipuncture. About two and half tablespoons of blood will be taken all together. After exercise I will eat a balanced breakfast and 2 other meals throughout the day. This is to control my caloric intake, as high-fat meals can increase microparticle levels.

Sometimes there are side effects from performing exercise tests. These side effects are often called risks, and for this project, the risks are:

1. Exercise testing carries with it a very small risk of exercise induced asthma, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. The risk of sudden death during exercise for healthy men is 1:15000-18000. Because I will be asked to give a maximum effort, I may experience some muscle soreness in my arms and legs or nausea following the maximal exercise test. It should be noted that if the experimental protocol is adhered to, the likelihood of these risks occurring is minimal.
2. I understand that the insertion and placement of a cannula (to take blood samples) should be minimally painful but a slight ache may be felt and a small bruise may appear on my arm. There is also a small risk of infection, but by using the appropriate techniques this risk is minimal.

There may be benefits from my participation in this study. These are:

1. I will receive a copy of my personal results, body fat and fitness measurements and cholesterol levels
2. I understand that no other benefits have been promised me.

Expenses incurred during the study

I understand that I will not be reimbursed for my participation in this study

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

I have read the Plain Language Statement	Yes ☐ No ☐
I understand the information provided	Yes ☐ No ☐
I have had an opportunity to ask questions and discuss this study	Yes ☐ No ☐
I have received satisfactory answers to all my questions	Yes ☐ No ☐

My confidentiality will be guarded:

Dublin City University will protect all the information about me, and my part in this study, within the limitations of the law. My identity or personal information will not be revealed or published. All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records. In addition, the study findings may be presented at scientific meetings and published in a scientific journal and/or as part of a postgraduate thesis, but my identity will not be divulged and only presented as part of a group.

If I have questions about the research project, I am free to call Niall M. Moyna at 01-7008060.

Taking part in this study is my decision.

I understand that my participation in this study is voluntary and that I may withdraw my consent at any time by notifying any of the investigators. I may also request that my data and samples be removed from the database or storage and destroyed. My withdrawal from this study, or my refusal to participate, will in no way affect my relationship with Dublin City University or my entitlements as a student or staff member. I understand that my participation in this research

may be terminated by the investigator without regard to my consent if I am unable or unwilling to comply with the guidelines and procedures explained to me.

Signature:

I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to take part in this research project

Participants Signature: _____

Name in Block Capitals: _____

Witness: _____

Date: _____

This Informed Consent form was officially approved by the DCU Research Ethics Committee on:

____/____/____

Official DCU Stamp:

If you have concerns about this study and wish to contact someone independent, you may contact:

The Chairperson of the Dublin City University Research Ethics Committee c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000

Appendix E

Dublin City University
RESEARCH ETHICS COMMITTEE

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING **HUMAN PARTICIPANTS**

Application No. *(office use only)* DCUREC/2006/ ☐☐☐

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. *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 Dose response of circulating microparticles to acute bouts of exercise

PRINCIPAL INVESTIGATOR (S) Prof. Niall M. Moyna, Dr. Noel McCaffrey, Paul O'Connor

1. ADMINISTRATIVE DETAILS

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

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

Project Start Date: **Oct 2006** **Project End date:** **Oct 2008**

1.1 INVESTIGATOR CONTACT DETAILS

Supervisors and co-supervisors of student projects are Principal Investigators. PhD and Doctoral students can be listed as Investigators

PRINCIPAL INVESTIGATORS:

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*

Prof	Moyna	Niall	7008802	7008888	niall.moyna@dcu.e
Dr	Mc Caffrey	Noel	7008187	7008888	noel.mccaffery@dcu.ie
Mr	O'Connor	Paul	7008474	7008888	paul.l.oconnor@dcu.ie

OTHER INVESTIGATORS:

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*

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 *(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): _____ _____ _____

Print name(s)	Niall M. Moyna	Noel McCaffrey	Paul O'Connor
Date:	*22 August 2006*	*22 August 2006*	*22 August 2006*

7 PROJECT OUTLINE

2.1 LAY DESCRIPTION

Microparticles are tiny fragments of cells that can be found in the blood. Under normal physiological conditions, low levels of microparticles are continually being shed into the blood from cells that line the inside of blood vessels, and appear to cause no problems. However, during some diseases, the level of endothelium-derived microparticles circulating in the blood rises. There is now evidence that elevated levels of these microparticles can lead to cardiovascular disease. Regular physical activity has been shown to reduce the risk for cardiovascular disease. The purpose of this study is to determine the effects of different bouts of exercise intensity on circulating microparticles in healthy asymptomatic people. The study will involve 5 separate exercise trials of approximately 1 hour in duration. During the first visit subjects will read and sign an informed consent, undergo a medical examination questionnaire, have a blood sample taken, and familiarise themselves with walking on a treadmill. The second visit will be used to assess their cardiovascular fitness level. During the experimental trials (visits 3-5), they will exercise at an intensity equal to 60, 70 and 80% of their maximal capacity until they burn 400 calories. The exercise intensity of each visit will be randomly determined. A blood sample will be taken immediately before and after exercise, and again at 6 and 24 hours after the exercise.

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH

Aims of the Research

1. To determine the effects of a different intensities of exercise on the number of endothelial and platelet derived microparticles in healthy asymptomatic individuals
2. To use an in-vivo cell culture to examine the cellular/molecular mechanism(s) responsible for the effect of fitness on EMP.

Microparticles (MP) are cell fragments shed from formed elements such as circulating platelets, leukocytes, and vascular endothelial cells. During their formation, EMP are packaged and exocytosed in a portion of the original plasma membrane. Depending on the dynamic morphological state of the parent cell, phenotypically varied EMP may be secreted. Specific phenotypic subsets carry identifiable surface markers that allow them to be detected. Microparticles of different origin have been shown to play an important role in atherosclerotic and inflammatory vascular disease. For example, high levels of endothelial microparticles (EMP) are associated with endothelial dysfunction by altering the production and/or bioavailability of nitric oxide (NO). EMP's also inhibit mitral valve growth. Significant differences exist between microparticle fractions found in the circulation of healthy subjects and those found in patients with atherosclerotic cardiovascular disease, diabetes, hypertension and end-stage renal failure.

.3 PROPOSED METHOD

Study Overview: Subjects will visit the Cardiovascular Research Unit in the School of Health and Human Performance on 5 separate occasions. Each visit will last approximately 1 h. During the first visit the nature and risks of the study will be explained, and written informed consent will be obtained. Subjects will undergo a medical examination, have a 10 ml blood sample taken, and familiarize themselves with exercising on a treadmill. Subjects who meet the entry criteria will return to the

laboratory for a second visit to assess their maximal aerobic capacity (VO_2max). During the final visit subjects will exercise on a treadmill at 60, 70 and 80% of their maximal capacity until they use 400 kcal. Blood sample will be taken immediately before and after exercise, and again at 6 and 24 hours after the exercise.

Maximal Exercise Test: VO_2max will be assessed using a ramped treadmill protocol. The test will begin with an initial speed between 5.0 - 7.0 mph and 0% gradient, and will increase in 0.5% incline every 2 min, until volitional exhaustion. A 12 lead ECG, blood pressure, rating of perceived exertion, and expired gases will be monitored throughout the test.

Experimental Trials (Visits 3-5): Subjects will arrive at 9.00 am following an overnight fast. An indwelling catheter will be inserted in a prominent forearm vein in order to take blood samples. Subjects will exercise on a treadmill at 60, 70 and 80% VO_2max until they burn 400 kcal. The exercise intensity of each visit will be determined randomly. Metabolic measurements and heart rate will be recorded continuously throughout each trial. A 10 ml blood sample will be taken at the beginning and end of each experimental trial, and at 6 h and24 h after exercise. The 24 h blood sample will be taken using standard venipuncture. The experimental trials (visits 3-5) will be undertaken within 5 d of the beginning of menses in menstruating women to reduce the confounding effects of menstrual cycle mediated alterations in estrogen and progesterone. During the 18 h following exercise, subjects will be required to eat 3 balanced meals provided by the researcher. Research has shown that high-fat meals can elevate EMP levels. There is a need to standardise the food intake throughout the 24 h of the experimental trial.

Respiratory Metabolic Measures: Breath-by-breath expired O_2, CO_2 and ventilatory volume will be determined using open circuit spirometry (Vmax 29, Sensormedics Corp., Yorba Linda CA).

Blood Sample Analysis: Blood samples will be processed and stored at -80°C until analysis.

2.4 PARTICIPANT PROFILE

Ten apparently healthy men and ten apparently healthy women (18-30 yr) will be recruited.

Exclusion criteria: Smoking, anaemia, metabolic syndrome, history of heart disease or uncontrolled thyroid dysfunction, liver or kidney dysfunction, pancreatitis, pregnancy and other major signs or symptoms suggestive of cardiovascular and pulmonary disease (angina, dizziness or syncope, orthopnea or paroxysmal dyspnea, ankle oedema, palpitations or tachycardia, intermittent claudication known heart murmur or unusual fatigued or shortness of breath with usual exercise).

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED

A recruitment advertisement will be emailed to DCU staff and students. In addition, a recruitment advertisement will be posted on the DCU campus. Permission will be sought from the relevant authorities prior to posting the advertisement. Contact details for the research team will be included on the advertisement. Potential subjects will undergo a brief telephone interview to assess their suitability for inclusion in the study. Suitable candidates will be asked to attend a screening session in the School of Health and Human Performance. They will be told by agreeing to attend the screening session they are not obligated to participate in the study. A brief 10 min presentation will be given to each potential subject to explain the nature, benefits, risks and discomforts of the study. The informed consent will be explained. Any individual with doubts about participating in the study will be encouraged to take time before making a final decision.

E-mail to be sent to staff and students

The School of Health and Human Performance are conducting research looking at how different exercise intensity effect levels of microparticles in the blood. Microparticles are tiny fragments of cells that may be linked with disease such as heart disease.

The study would involve having your fitness assessed on a treadmill and on three other days jogging at different intensities on the treadmill until 400 kcal are burned. To look at the microparticles we need to take some blood samples.

We are looking for men and women between 18-30 years, who have no history of cardiovascular disease, and do not smoke.

Following the research you would get information on your fitness levels and receive fitness and nutritional advice if required.

.

If you would like to hear more about this study or would consider participating, please contact Paul O'Connor (Tel: 7008474; e-mail: paul.l.oconnor@dcu.ie)

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 study findings may be presented at scientific meetings and published in scientific journals. Participants will receive a written report outlining the principle findings of the study. Study participants will receive a one-page report summarizing the results from the tests that were undertaken during the screening visit. The identity of individual subjects will not be divulged, and will only be presented as part of a group.

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

3.0 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

The nature and risks involved in the study will be explained prior to starting the study and a contact number will be provided.

Exercise. There is a risk of delayed muscle soreness following an exercise session in sedentary individuals. 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.

Blood draws. There may be discomfort during the insertion of a cannula in a vein and the development of a small bruise at the site of puncture. An individual trained in phlebotomy will insert the IV catheter. The total amount of blood taken during the entire study will be approximately 50 ml. This is much less than the 570 ml of blood that is usually donated at blood banks. Individuals who are anaemic will be excluded form participation in this study.

The laboratory is equipped with an emergency crash cart and defibrillator. A medical doctor will supervise the maximal exercise test. Standard sterile techniques will be used for blood sampling.

Alternatives to the risks

All of the methods employed for the study are standard procedures for exercise testing and blood collection. These procedures are currently the best methods available for the questions being addressed. The investigators are very experienced in the implementation of these techniques from previously published studies.

Confidentiality is an important issue during data collection. Participants' identity or 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 file and saved in a password protected file in a computer in DCU. The principle investigator, graduate students and medical director will have access to the data. The study results may be used as part of a series of studies being conducted by the Cardiovascular Research Unit.

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

☒ YES ☐ NO Subjects will receive a summary of the

results of the screening visit. This will include their fitness levels (VO_2max) ECG, body composition, complete blood count and 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 The exposure to blood and muscle tissue and needles are minimal but 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 deal with all aspects of this study and an emergency plan for adverse events. The laboratory is equipped with an emergency crash cart and defibrillator. An individual trained in Advanced Cardiac Life Support (ACLS) will be present during each test. In the unlikely event of an adverse outcome, an ambulance will be called and the subject will immediately be sent to Beaumont Hospital.

3.7 MONITORING

The principal investigator will be involved in all aspects of the research, including subject recruitment and data collection. The research team have weekly meeting 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.)*

4.0 INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS

Niall Moyna is an exercise physiologist. He is a professor and head of the School of Health and Human Performance in the Faculty of Health and Human Performance at DCU. He was Director of the Applied Physiology Laboratory in the Division of Cardiology in the University of Pittsburgh Medical Centre and a Senior Research Scientist and Director of the Clinical Research Laboratory in the Division of Cardiology at Hartford Hospital, Connecticut, USA. Prof. Moyna is currently the associate Director of the Vascular Health Research Centre at DCU. He is certified in Advanced Cardiac Life Support.

Dr. Noel Mc Caffrey will act as the study physician. He is a sports medicine physician with an adjunct position in the Faculty of Science and Health.

Paul O'Connor is a laboratory technician in the School of Health and Human Performance. Kevin graduated from the Sport Science and Health programme at DCU with 1st class honours in 2003. He has extensive knowledge of all the equipment and procedures in the School of Health and Human Performance. Paul is certified in Occupational First Aid and Advanced Cardiac Life Support.

5.0 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 ENSURED?

Confidentiality is an important issue during data collection. Participant's identity, or other personal information, will not be revealed or published. Subjects will be assigned an ID number under which all personal information will be stored in a secure file and saved in password protected file in a computer at DCU. The investigators alone will have access to the data.

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY:

☒ YES ☐ NO

6.0 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL

For the purpose of this section, "Data" includes that in a raw or processed state (e.g. interview audiotape, transcript or analysis). "Samples" include body fluids or tissue samples.

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

Stored at DCU ☒
Stored at another site ☐

6.2 WHO WILL HAVE ACCESS TO DATA/SAMPLES?

Access by named researchers only ☒
Access by people other than named researcher(s) ☐
Other: ☐

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

The data will be shredded after five years. Prof. Niall Moyna will carry this out.

7.0 FUNDING

7.4 HOW IS THIS WORK BEING FUNDED?

The study is being funding from resources within the School of Health and Human Performance

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

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

☐ YES ☒ NO

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

8.0 PLAIN LANGUAGE STATEMENT

Title: Effect of an acute bout of exercise on circulating microparticles.

Principal Investigators

Prof. Niall Moyna	niall.moyna@dcu.ie	(Phone No. 7008802)
Kevin O' Brien	kevin.obrien7@mail.dcu.ie	(Phone No. 7008470)
Dr. Noel McCaffrey	noel.mccaffery@dcu.ie	(Phone No. 7008187)

School of Health and Human Performance, Dublin City University

What is the study about?

Microparticles are tiny fragments of cells that can be found in the blood. During some diseases, the level of microparticles circulating in the blood rises. There is now evidence that elevated levels of these microparticles can lead to cardiovascular disease. Regular physical activity has been shown to reduce the risk for cardiovascular disease. The purpose of this study is to determine the effects of exercise intensity on the number of microparticles in healthy asymptomatic individuals.

You will be asked to make 5 visits to the School of Health and Human Performance in DCU. Each visit will last approximately 1 hour. You will arrive for each visit after an overnight fast. During the first visit you will have a blood sample taken and undergo a brief examination by a medical doctor to make sure you are suitable for the study. About 10 days after this visit you will be contacted by phone or email to let you know if you are eligible to participate in the study. During the second visit you will exercise on a treadmill for approximately 15-mins to determine your fitness level. The exercise will get progressively harder, but you should try to keep going as long as you can. During visits 3-5, you will exercise at an intensity equal to 60, 70 and 80%, of your maximal capacity until you burn 400 calories. The exercise intensity of each visit will be determined randomly. A blood sample will be taken immediately before and after exercise, and again at 6 hours and 24 hours after the exercise. You will be asked to eat 3 balanced meals throughout the 24 h of the experimental trials.

Exercise does carry a risk of injury, such as a pulled muscle, muscle soreness or in extreme cases abnormal heart rhythm, heart attack or death. The benefits of being involved in this study include a detailed assessment of your fitness, your response to exercise and your cholesterol levels. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate thesis and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. 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. All data will be destroyed after 5 years. Your participation in this research project is voluntary and you may withdraw your consent at any time.

If participants 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

Appendix F

E-mail to be sent to staff and students

The School of Health and Human Performance at DCU are conducting a research study to investigate the influence of the intensity of exercise training on changes in skeletal muscle that occur with training. The purpose of this project is to look at the impact of training at two different exercise intensities for 14 days on muscle function. One group will exercise at a low intensity for 60 minutes each day and another group will exercise at a high intensity for 30 minutes each day.

We are looking for healthy males between 18-35 years, who have not been involved in regular exercise for the last 6 months and do not smoke. The study would involve a fitness test on a bicycle followed by two weeks of exercise training. To determine the impact of exercise on the muscle cells, we would take muscle biopsies from the leg at different points during the training programme.

If you would like to hear more about this study or would consider participating, please contact Brendan Egan (Tel: 7008472; e-mail: brendan.egan6@mail.dcu.ie)

Appendix G

Plain Language statement

Title: The influence of training intensity on adaptations to exercise training in human skeletal muscle

Principal investigators:
Dr. Donal O'Gorman - donal.ogorman@dcu.ie 01-7008060
Mr. Brendan Egan - brendan.egan6@mail.dcu.ie 01-7008472
Metabolic Physiology Research Unit, School of Health and Human Performance, Dublin City University

It is well known that exercise training has many health benefits, including weight loss and the prevention of diabetes and cardiovascular disease. Despite the known benefits, we don't fully understanding what changes occur in the body, especially skeletal muscle. Learning more about these changes would have important implications for (i) future treatments of these diseases and (ii) more specific exercise recommendations.

The purpose of this project is to look at how the intensity/effort of exercise influences the adaptations to training. You will be asked to make several visits to the lab.

- On the first visit you will have a medical examination from the doctor, to make sure you are suitable for the study. You will then exercise on a bicycle for approximately 15 minutes to determine your fitness level.
- On the second visit to the lab you will cycle at either 40% or 80% of your maximal ability to verify the intensity that you will train at. This will take approximately 30 minutes.
- After a week, you will begin an exercise training program lasting 14 days. You will be required to train every day at either 40% of your maximal ability for approximately 60-mins or 80% for approximately 30-mins. Your training will be supervised at all times.
- A muscle biopsy will be taken from your leg before you start the training programme and following 1-, 3-, 7-, 10- and 14-days of training. A blood sample will also be taken at this time, and at the exercise session the previous evening.
- For three days prior to the commencement of training you will be asked to record your daily food intake. You will be advised to continue this pattern of intake for the duration of the program.

Exercise does carry a risk of injury, such as a pulled muscle, muscle soreness or in extreme cases abnormal heart rhythm, heart attack or death. The benefits of being involved in this study include a detailed assessment of your fitness, your response to exercise and your energy use. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate thesis and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. Confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be participant to subpoena, freedom of information claim or mandated reporting by some professions. All data will be destroyed after 5 years. Your participation in this research project is voluntary and you may withdraw your consent at any time. There will not be any penalty nor will your rights as a student or staff member of DCU be affected in any way.

This research project is being funded from a grant awarded within DCU and the funding of a postgraduate student by the Irish Research Council for Science, Engineering and Technology (IRSCET).

If participants 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

Appendix H

Informed Consent

Title: The influence of training intensity on adaptations to exercise training in human skeletal muscle

Principal investigators: Dr. Donal O'Gorman, and Mr. Brendan Egan: School of Health and Human Performance

Other investigators: Brian Carson, David Ashley, Dr. Gavin McHugh, Dr. Ray Walls, Prof. Niall Moyna, Paul O'Connor

Purpose
The purpose of this project is to look at the impact of changing the intensity of exercise training on changes that occur in muscle proteins in response to 14 days of exercise training.

This is what will happen during the research study

1. I will have the purpose of the study, each of the steps involved and the risks of participating in the study explained to me. I will have the opportunity to ask any questions and if I am happy with the answers I will:
    a. Provide written informed consent for participation in the research project.
    b. I will then complete a medical history form, which will ask questions about my general health, personal and family health history, smoking, exercise, and dietary habits.
    c. I will talk with a medical doctor about the information I have provided and I understand, based on the information provided, the medical doctor may exclude me from participating in the research project.
    d. I will be provided with dietary advice, which I will follow for three days prior to each visit to the laboratory. I will not consume caffeine or alcohol during this period.
2. Pre-test evaluation
    a. I will have a number of measurements of my body size and shape. Firstly, my blood pressure will be measured. I will have an electrocardiogram (ECG) done to evaluate my heart. This is a painless, 5 minute procedure that involves lying down and having adhesive pads attached to my arms, legs and chest. My height and weight will be measured in light clothing, and without shoes. My percentage body fat will be measured by the thickness of skin and fat on 7 sites around the body including the chest, leg, arm, back and side of the body. I will provide a blood sample to check some health indicators.
    b. I understand that any of these procedures or tests may be waived at the discretion of the doctor for the following reasons: (i) I have completed the same or similar step in the past 6 months as part of another research protocol at the School of Health and Human Performance; (ii) It has been determined that I am not eligible to participate in this research project; and thus completion of the entire screening process will not be necessary.
3. Exercise capacity and determination of exercise intensity
    a. I will undergo an exercise test designed to measure my fitness, and to evaluate my current physical condition. I understand that I will pedal on a stationary bicycle, with the pedal resistance getting more difficult every 2 minutes until, fatigue, breathlessness, chest pain and/or symptoms that indicate to the doctor or myself that I should stop exercise. To assess my fitness I will have a mouthpiece similar to a snorkel in my mouth to measure the amount of air I breathe in and out.
    b. At least 4 days later, I will cycle on a stationary bike for 30 minutes to determine the resistance that corresponds to either 40% or 80% of my exercise capacity (depending on the training group that I will be assigned to). During this time I will also wear the mouthpiece to determine oxygen use
4. Impact of training on muscle cell function
    a. Approximately one week later I will begin the exercise training phase of the study. I will exercise on consecutive days for 14 days.

b. I will be assigned to one of two training groups and as such will train at an intensity corresponding to either 40% of my maximum ability for approximately 60 minutes or at an intensity corresponding to 80% of my maximum ability for approximately 30 minutes depending on my group. I understand that I will be breathing more often, and will sweat, but that I will be able to maintain a conversation.
c. I understand that all my exercise training sessions will take place in the MPRU on a stationary bicycle under supervision by one of the study's investigators and that training sessions will take place in the late afternoon to early evening.
d. On the morning of the first day of training, I will come to the MPRU between 0800h and 0900h after an overnight fast, with only water taken for the previous 10 hours. After resting for 15 minutes, I will have a blood sample taken from my arm and a muscle biopsy taken from my thigh. For the biopsy I will have the area anaesthetised with local anaesthetic, then a small 0.5 cm incision will be made in the skin and a needle inserted briefly into the muscle. A small piece of muscle, less that 0.15 of a gram, will be taken from my leg. The incision is pulled close with sterile strips and my leg will be wrapped snugly with an elastic bandage to maintain pressure. Before I leave I will be given contact information and supplies to change the dressing around the biopsy sites.
e. I will then leave the lab before returning that afternoon to complete the first exercise training session. On the morning following the 1st, 3rd, 7th, 10th, and 14th training sessions, I will report to the MPRU under the same fasting conditions for subsequent blood and muscle sample collection. I will have additional blood samples taken immediately prior to and immediately after the 1st, 3rd, 7th, 10th, and 14th training sessions. This will complete my participation in the study.
f. In preparation for the training phase I will be required to record my daily food intake in the three days leading up to the commencement of training. I will maintain a similar level of dietary intake throughout the two week training period.
g. I will be weighed immediately prior to and immediately after each training session to determine the volume of fluid loss through sweating that has occurred. I will be advised to replace this loss with the appropriate amount of fluid based on established recommendations. However, I understand that water will be available ad libitum throughout the training sessions.

Sometimes there are side effects from performing exercise tests. These side effects are often called risks, and for this project, the risks are:

1. Exercise testing carries with it a very small risk of exercise induced asthma, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. The risk of sudden death during exercise for healthy men is 1:15000-18000. Because I will be asked to give a maximum effort, I may experience some muscle soreness in my arms and legs or nausea following the maximal exercise test. It should be noted that if the experimental protocol is adhered to that the likelihood of these risks occurring is minimal.
2. I understand that the insertion of a needle into a superficial arm vein (to take blood samples) should be minimally painful but a slight ache may be felt and a small bruise may appear on my arm. There is also a small risk of infection, but by using the appropriate techniques this risk is minimal.
3. I understand that there may be discomfort during the muscle biopsy with some pain and delayed soreness afterward. There is a risk of bleeding, bruising, infection and scaring of the skin. After the biopsy, my leg may feel stiff and sore. Temporary numbness of the skin near the biopsy may also occur.

There may be benefits from my participation in this study. These are:

1. I will receive a copy of my personal results, body fat and fitness measurements and energy use during exercise when the study is finished.
2. I will be given guidelines to maintain or increase my physical activity/training program if I feel I would like to continue to exercise after the study.
3. I understand that no other benefits have been promised me.

Costs associated with the study

I understand that there will not be any payment for participation in this study but legitimate expenses to a maximum value of €150 will be recompensed.

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

I have read the Plain Language Statement Yes/No

I understand the information provided	Yes/No
I have had an opportunity to ask questions and discuss this study	Yes/No
I have received satisfactory answers to all my questions	Yes/No

My confidentiality will be guarded:

Dublin City University will protect all the information about me, and my part in this study, within the limitations of the law. My identity or personal information will not be revealed or published. All records associated with my participation in the study will be participant to the usual confidentiality standards applicable to medical records. In addition, the study findings may be presented at scientific meetings and published in a scientific journal and/or as part of a postgraduate thesis, but my identity will not be divulged and only presented as part of a group.

If I have questions about the research project, I am free to call Donal O'Gorman at 01-7008060.

Taking part in this study is my decision.

I understand that my participation in this study is voluntary and that I may withdraw my consent at any time by notifying any of the investigators. I may also ask that the information gathered about me be removed from the database, or storage, and destroyed. My withdrawal from this study, or my refusal to participate, will in no way affect my relationship with Dublin City University or my entitlements as a student or staff member. I understand that my participation in this research may be terminated by the investigator without regard to my consent if I am unable or unwilling to comply with the guidelines and procedures explained to me.

Project Funding

This research project is being funded from an award to Dr. Donal O'Gorman from the Faculty of Science and Health at Dublin City University and funding for a postgraduate student from the Irish Research Council on Science, Engineering and Technology.

Signature:

I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to take part in this research project

Participants Signature: _____

Name in Block Capitals: _____

Witness: _____

Date: _____

Appendix I

Dublin City University
RESEARCH ETHICS COMMITTEE

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING **HUMAN PARTICIPANTS**

Application No. *(office use only)* DCUREC/2007/ ☐☐☐

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. *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	**Adaptations in human skeletal muscle following short term exercise training at two different intensities**
PRINCIPAL INVESTIGATOR(S)	**Donal O'Gorman, PhD** **Brendan Egan**

Guidelines to Applicants

1.1 PRINCIPAL INVESTIGATOR(S): *Supervisors and co-supervisors of student projects are Principal Investigators. PhD and Doctoral students can be listed as Investigators.*

2.0 PROJECT OUTLINE: *Provide a brief outline of the project, aims, methods, duration, funding, profile of participants and proposed interaction with them. This description must be in everyday language that is free from jargon. Please explain any technical terms or discipline-specific phrases.*

2.1 LAY DESCRIPTION: *Provide a brief outline of the project, including what participants will be required to do. This description must be in everyday language which is free from jargon. Please explain any technical terms or discipline-specific phrases. (No more than 300 words).*

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH: *State the aims and significance of the project (approx. 400 words). Where relevant, state the specific hypothesis to be tested. Also please provide a brief description of current research, a justification as to why this research should proceed and an explanation of any expected benefits to the community.* ***NB – all references cited should be listed in an attached bibiliography.***

2.3 PROPOSED METHOD: *Provide an outline of the proposed method, including details of data collection techniques, tasks participants will be asked to do, the estimated time commitment involved, and how data will be analysed. If the project includes any procedure which is beyond already established and accepted techniques please include a description of it. (No more than 400 words.)*

2.4 PARTICIPANT PROFILE: *Provide number, age range and source of participants. Please provide a justification of your proposed sample size. Please provide a justification for selecting a specific gender.*

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED: *Please provide specific details as to how you will be recruiting participants. How will people be told you are doing this research? How will they be approached and asked if they are willing to participate? If you are mailing to or phoning people, please explain how you have obtained their names and contact details. This information will need to be included in the plain language statement. If a recruitment advertisement is to be used, please ensure you attach a copy to this application.*

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES*: Identify, as far as possible, all potential risks to participants (physical, psychological, social, legal or economic etc.), associated with the proposed research. Please explain what risk management procedures will be put in place.*

3.6 ADVERSE/UNEXPECTED OUTCOMES: *Please describe what measures you have in place in the event that there are any unexpected outcomes or adverse effects to participants arising from involvement in the project.*

3.7 MONITORING: *Please explain how you propose to monitor the conduct of the project (especially where several people are involved in recruiting or interviewing, administering procedures) to ensure that it conforms with the procedures set out in this application. In the case of student projects please give details of how the supervisor(s) will monitor the conduct of the project.*

3.8 SUPPORT FOR PARTICIPANTS: *Depending on risks to participants you may need to consider having additional support for participants during/after the study. Consider whether your project would require additional support, e.g., external counseling available to participants. Please advise what support will be available.*

4.0 INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS: *List the academic qualifications and outline the experience and skills relevant to this project that the researchers and any supporting staff have in carrying out the research and in dealing with any emergencies, unexpected outcomes, or contingencies that may arise.*

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED? *Please bear in mind that where the sample size is very small, it may be impossible to guarantee anonymity/confidentiality of participant identity. Participants involved in such projects need to be advised of this limitation.*

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY: *Participants need to be aware that confidentiality of information provided can only be protected within the limitations of the law - i.e., it is possible for data to be participant to subpoena, freedom of information claim or mandated reporting by some professions. Depending on the research proposal you may need to specifically state these limitations.*

6.0 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL: *For the purpose of this section, "Data" includes that in a raw or processed state (e.g. interview audiotape, transcript or analysis). "Samples" include body fluids or tissue samples.*

8.0 PLAIN LANGUAGE STATEMENT: *Written information in plain language that you will be providing to participants, outlining the phases and nature of their involvement in the project and inviting their participation. Please note that the language used must reflect the participant age group and corresponding comprehension level.*

9.0 INFORMED CONSENT FORM: *This is a very important document that should be addressed by participants to researchers, requiring participants to indicate their consent to specific statements, and give their signature.*

FOR FURTHER INFORMATION AND NOTES ON THE DEVELOPMENT OF PLAIN LANGUAGE STATEMENTS AND INFORMED CONSENT FORMS, PLEASE CONSULT THE DCU REC WEBSITE: WWW.DC.IE/RESEARCH/ETHICS

1. ADMINISTRATIVE DETAILS

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

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

Project Start Date: May 2007 **Project End date: April 2008**

1.1 INVESTIGATOR CONTACT DETAILS *(see Guidelines)*

PRINCIPAL INVESTIGATOR(S):

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Dr.	O'Gorman	Donal	7008060	7008888	donal.ogorman@dcu.ie
	Egan	Brendan	7008472		brendan.egan6@mail.dcu.ie

OTHER INVESTIGATORS:

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
	O'Connor	Paul	7008474		paul.l.oconnor@.dcu.ie
Prof.	Moyna	Niall	7008802	7008888	niall.moyna@dcu.ie
	Carson	Brian	7008472		brian.carson2@mail.dcu.ie
	Ashley	David	7008472		david.ashley2@mail.dcu.ie
Dr.	McCaffrey	Noel	7008187		mccaffs@eircom.net
Dr.	McHugh	Gavin	7008472		mchughgavin@yahoo.co.uk
Dr.	Walls	Ray	7008472		raywalls1@hotmail.com

FACULTY/DEPARTMENT/SCHOOL/ CENTRE: Metabolic Research Unit, School of Health and Human Performance

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): _____

_____

Print name(s) in block letters: _____

_____

__

Date: _____ _____

2. PROJECT OUTLINE

2.1 LAY DESCRIPTION *(see Guidelines)*

Exercise training has many important health-related benefits. In addition to helping with weight loss, it can decrease the risk of developing many chronic diseases. Despite the many positive outcomes, we know relatively little about how exercise contributes to these changes. It is known that exercise training improves the function of muscle cells. Recent research has shown that many of the changes that occur in response to one exercise session have been shown to depend on the intensity/effort of the exercise. How this occurs is poorly understood at present. The purpose of this study is to determine the impact of exercise training at two different intensities on the changes in muscle tissue.

Participants will undertake a medical screening and have their fitness measured on the first visit. On a separate day the intensity corresponding to 40% and 80% of their maximal fitness will be determined during a short exercise session.

Participants will be divided into two groups, one training at 40% of their maximal ability for approximately 60-mins and the other at 80% for approximately 30-mins. Training will take place each day for 14 days. In order to measure the changes in the function of muscle cells, muscle biopsies will be taken on selected days during the training period.

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH *(see Guidelines)*

Aims of Research
To determine the impact of the intensity/effort of exercise on gene expression (the initial step in making new proteins) and protein content in response to short-term exercise training in human skeletal muscle.

Significance
There is very little known about the impact of exercise training on gene expression in muscle cells. Determining the influence of intensity/effort of exercise training on the regulation of gene expression would help further our understanding of the benefits of exercise and the mechanism of its effects.

Brief description of current research
Research studies have shown that 16 hours following an exercise session there is an increase in the expression of GLUT-4 (Neufer & Dohm, 1993), a key protein in glucose transport into muscle cells. It has also been shown that exercise training will lead to an increase in the amount of this protein in muscle cells (Kraniou et al., 2004), and helps to reduce blood glucose levels in patients with type 2 diabetes and those who are overweight and insulin resistant (common before diabetes). We have previously shown an increase in GLUT4 protein following short-term exercise training in type 2 diabetics (O'Gorman et al., 2006). However, it is not known how exercise results in this increase in expression. We have also shown that key cellular proteins are regulated

to a greater or lesser extent depending on the intensity of exercise (Egan et al., 2007). A transcriptional co-activator, PGC-1α, is involved in the regulation of a number of key proteins in glucose and fat metabolism (Puigserver & Spiegelman, 2003). Acute exercise and exercise training both lead to an increase in PGC-1α and is thought to play a role in the exercise-related training adaptation (Pilegaard et al., 2003). Important enzymes activated by exercise (i.e. AMPK and CaMK) are also activated in an intensity-dependent manner (Rose et al., 2006; Sriwijitkamol et al., 2007, Egan et al., 2007), with a greater degree of activation evident at higher exercise intensities even when the energy cost of exercise is the same. We want to further our research findings from one exercise session, to determine the impact on changes in muscle proteins following short term exercise training.

Hypothesis to be tested
Stated as a null hypothesis, no difference will exist in the time-course or magnitude of the exercise training-induced response to exercise at two different intensities when energy expenditure is the same.

Implications of this Research
While there may not be any direct benefits to society from this study, the results will contribute to our knowledge of the regulation of gene expression following exercise. This has implications for the prescription of exercise training, particularly whether the intensity of each exercise bout is an important determinant in the positive outcomes associated with such training programmes. It is possible that this research will identify novel mechanisms for understanding energy regulation in response to exercise training.

- Egan B, Carson BP, Garcia-Roves PM, Sarsfield FM, Chibalin AV, Monedero J, O'Connor P, Moyna NM, McCaffrey N, Zierath JR, O'Gorman DJ (2007) Intensity-dependent effect of exercise on contraction-mediated signalling and metabolic gene expression in human skeletal muscle. Nuclear Receptor Pathways to Metabolic Regulation. Keystone Symposia, Steamboat Springs, CO. (Abstract)

- Kraniou GN, Cameron-Smith D, Hargreaves M (2004) Effect of short-term training on GLUT-4 mRNA and protein expression in human skeletal muscle. Experimental Physiology. 89:559-63

- Neufer PD, Dohm GL (1993) Exercise induces a transient increase in transcription of the GLUT-4 gene in skeletal muscle. American Journal of Physiology (Cell Physiology). 265:C1597-603

- O'Gorman DJ, Karlsson HK, McQuaid S, Yousif O, Rahman Y, Gasparro D, Glund S, Chibalin AV, Zierath JR, Nolan JJ (2006) Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes. Diabetologia. 49:2983-2992

- Pilegaard H, Saltin B, Neufer PD (2003) Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. Journal of Physiology. 546:851-8

- Puigserver P, Spiegelman BM (2003) Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocrine Reviews. 24:78-90

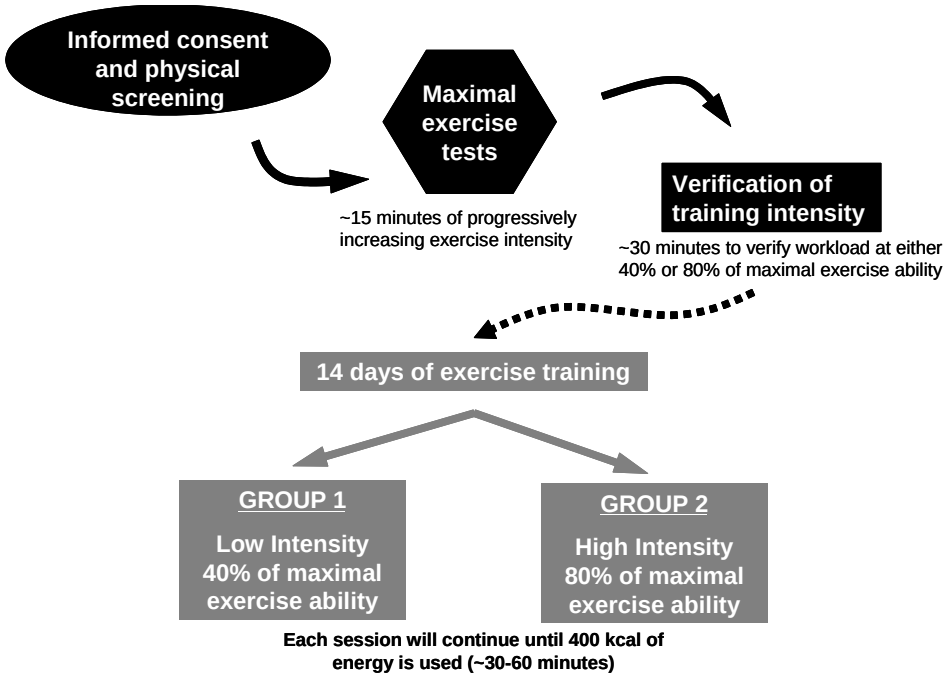
- Rose AJ, Kiens B, Richter EA (2006) Ca2+-calmodulin-dependent protein kinase expression and signalling in skeletal muscle during exercise. Journal of Physiology. 574:889-903.

- Sriwijitkamol A, Coletta DK, Wajcberg E, Balbontin GB, Reyna SM, Barrientes J, Eagan PA, Jenkinson CP, Cersosimo E, Defronzo RA, Sakamoto K, Musi N (2007) Effect of acute exercise on AMPK signaling in skeletal muscle of participants with type 2 diabetes: a time-course and dose-response study. Diabetes. 56:836-48

2.3 PROPOSED METHOD *(see Guidelines)*

Participants

The study will take place in the Metabolic Physiology Research Unit (MPRU) in the School of Health and Human Performance. The nature and risks of the study will be explained, and any questions will be answered to the satisfaction of the participants before obtaining written informed consent. Participants will complete a medical history questionnaire and be interviewed by the study physician to determine eligibility. Participant height, weight blood pressure and body composition, as well as resting 12 lead ECG and a blood sample will be evaluated as part of the physical examination and medical clearance. The blood sample (3 ml) will be used to ensure that the immune system is not fighting infection and that the proportion of red blood cells is normal. Participants will have a normal body mass index and will be excluded if they exercise regularly, smoke, have diabetes, suffer from other acute or chronic diseases or use drugs that the physician and investigators decide would interfere with the normal adaptation to the proposed intervention. Participants will be asked to keep a food diary for 3 days prior to starting exercise and then to maintain a similar diet throughout the study. Participants will abstain from caffeine for 24 hours prior to each biopsy (see below) and from alcohol for the duration of the training programme.



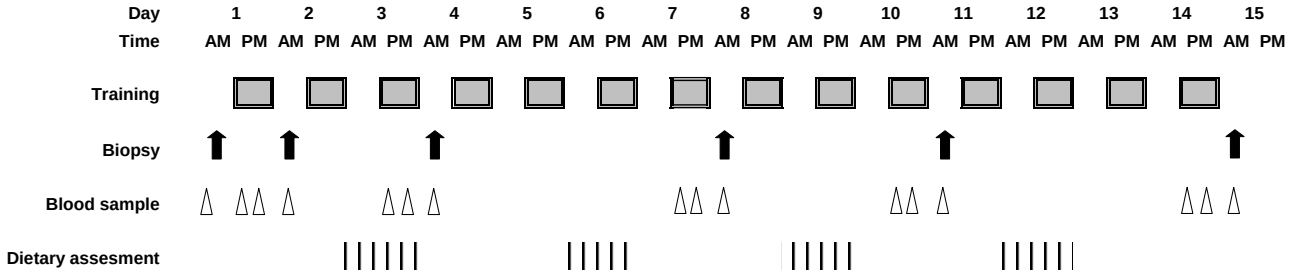
Research procedures

Stage 1 - Maximal oxygen consumption: This test follows the standard operating procedure for all similar trials submitted to the Research Ethics Committee. Participants will exercise on a stationary cycle ergometer to volitional fatigue. After 5 minutes at an easy pace the resistance to pedalling will increase every 2 minutes until it is not possible for the participant to maintain cycling cadence. During the test the participant will have a mouthpiece, similar to a snorkel in their mouth. This is connected to an oxygen analyser that will determine the amount of oxygen consumed by the body. Generally the more oxygen someone is capable of consuming the higher the fitness level. The participant will have their heart rate and rate of perceived exertion (a subjective measure of the degree of effort involved) monitored throughout the test. This test takes approximately 15 minutes.

Stage 2 – Verification of exercise intensity: On a separate day, participants will exercise to determine the workload that corresponds to either 40% or 80% of their maximal oxygen uptake (determined from the maximal test) depending on which training group each participant is randomly assigned to. The exercise intensity/effort will be verified by oxygen consumption, heart rate and perceived exertion and will take approximately 30 minutes.

Stage 3 – Exercise training phase: Five to seven days after the verification test, participants will begin the exercise training phase of the study. Participants will be required to exercise on 14 consecutive days. One group will train at an intensity corresponding to 40% of their maximum ability for approximately 60 minutes and the other group will train at an intensity corresponding to 80% of their maximum ability for approximately 30 minutes. They will use the exact same amount of energy (400 kcal) in each training session. This magnitude of training load is consistent with that employed in similar designs and is well tolerated by sedentary individuals (Spina et al., 1996; Gibala et al., 2006). All exercise training sessions will take place in the MPRU on a stationary bicycle under supervision by one of the study's investigators. Training sessions will take place in the late afternoon to early evening depending on the participants' availability.

On the morning of the first day of training, participants will report to the MPRU between 0800h and 0900h after an overnight fast. They will rest for 15 minutes and then have a blood sample (6 ml) using venepuncture and a muscle biopsy taken from *m. vastus lateralis*. They will then be free to leave the lab before returning that afternoon to complete the first exercise training session. On the morning following the 1st, 3rd, 7th, 10th, and 14th training sessions, participants will report to the MPRU under the same conditions for subsequent blood and muscle sample collection. Participants will have additional blood samples taken immediately prior to and immediately after the exercise sessions prior to a muscle biopsy.



Meals

Participants will be required to record their daily food intake in the three days leading up to the beginning of the exercise training phase. They will be advised to maintain a similar level of dietary intake throughout the two week training period. Each participant will be weighed immediately prior to and immediately after each training session to determine the volume of fluid loss through sweating that has occurred. They will be advised to replace this loss with the appropriate amount of fluid based on established recommendations. However, water will be available ad libitum throughout the training sessions. Body mass will be monitored on a daily basis as a surrogate measure of both hydration and energy balance. At 3 day intervals, dietary intake will be assessed as per the days prior to beginning of the exercise programme to ensure an appropriate number of calories is being taken and to monitor any dietary changes that may have inadvertently occurred since the beginning of training.

Rationale for muscle biopsy samples

A total of six biopsies will be taken from each participant. This number is not uncommon in this type of research (Pilegaard et al., 2003). A biopsy is needed prior to the start of the training programme in order to establish a baseline value to which subsequent changes can be compared. We intend to track the rate of change in muscle function in response to training per se and measure the effect of exercise intensity on this rate of change. In order to complete a time-course study we will require muscle samples at regular intervals during the 14 day training programme.

- Gibala MJ, Little JP, van Essen M, Wilkin GP, Burgomaster KA, Safdar A, Raha S, Tarnopolsky MA. Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. Journal of Physiology 15: 901-11, 2006.
- Pilegaard H, Saltin B, Neufer PD (2003) Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. Journal of Physiology. 546:851-8
- Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, Holloszy JO. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. Journal of Applied Physiology 80: 2250-2254, 1996.

2.4 PARTICIPANT PROFILE *(see Guidelines)*

Sixteen healthy (no current illness or history of clinical conditions that may preclude them from exercise) but untrained males, aged 18-35, will volunteer to participate in the study.

Sample size calculations from citrate synthase activity in two studies with a similar design indicate that 7 (Spina et al. 1996) to 13 (Scarritt et al. 1999) subjects are required to significantly increase mitochondrial enzyme activity (a key outcome for this study). These calculations are based on an alpha level of 0.05 and a beta level of 0.2. We propose to recruit 8 subjects in each group.

- Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, Holloszy JO. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. Journal of Applied Physiology 80: 2250-2254, 1996.
- Starritt EC, Angus D, Hargreaves M. Effect of short-term training on mitochondrial ATP production rate in human skeletal muscle. Journal of Applied Physiology 86: 450-454, 1999.

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED *(see Guidelines)*

Volunteers will be recruited from the general population, including students and staff at Dublin City University. The study will be advertised by e-mail (below) to provide individuals with information about the study and contact details. Potential participants will be asked to come to the School of Health and Human Performance where they will have the study explained to them. They will have an opportunity to ask questions and, before leaving, will be provided with a blank medical history form, an explanation of the study and a blank informed consent for them to review. If they wish to participate in the study they will have to provide written informed consent, which will be witnessed, on their next visit to the School of Health and Human Performance. Contact details will be provided to ensure all queries or concerns of the participant can be dealt with immediately.

E-mail to be sent to staff and students
The School of Health and Human Performance at DCU are conducting a research study to investigate the influence of the intensity of exercise training on changes in skeletal muscle that occur with training. The purpose of this project is to look at the impact of training at two different exercise intensities for 14 days on muscle function. One group will exercise at a low intensity for 60 minutes each day and another group will exercise at a high intensity for 30 minutes each day.

We are looking for healthy males between 18-35 years, who have not been involved in regular exercise for the last 6 months and do not smoke. The study would involve a fitness test on a bicycle followed by two weeks of exercise training. To determine the impact of exercise on the muscle cells, we would take muscle biopsies from the leg at different points during the training programme.

If you would like to hear more about this study or would consider participating, please contact Brendan Egan (Tel: 7008472; e-mail: brendan.egan6@mail.dcu.ie)

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*?*

Participants will be provided with a report, which will summarise their fitness characteristics on the bicycle ergometer and all other relevant information such as percentage body fat and blood profile. The results will form the basis for a postgraduate thesis and will be presented at scientific meetings or published in a scientific journal. The identity of individual participants will not be divulged and will only be presented as part of a group.

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)

1. The influence of exercise intensity on skeletal muscle gene expression. REC

2. The influence of contraction frequency on skeletal muscle gene expression. REC/2006/20

3. RISK AND RISK MANAGEMENT

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

☒ YES ☐ NO *If YES, this proposal will be participant 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 *(see Guidelines)*

Muscle biopsy. There may be discomfort during the muscle biopsy with some pain and delayed soreness afterward. There is a risk of bleeding, bruising, infection and scaring of the skin. Temporary numbness of the skin near the biopsy site may occur.
Blood draws. There may be discomfort during the insertion of a needle in a vein and the development of a small bruise at the site of puncture.
Exercise. There is a risk of delayed muscle soreness following an exercise session in a group of untrained individuals. The risk of sudden death during exercise for healthy men is 1:15000-18000.

We will take all possible precautions to avoid infection during these procedures. Blood samples will be taken with sterile disposable needles, drapes, and gauze. Reusable biopsy needles are cleaned and sterilized prior to use. Sterile techniques are used during blood sampling and muscle biopsy procedures.

Alternatives to the risks
All of the procedures described in the methodology section of the study are standard procedures for the evaluation of blood and muscle tissue. These procedures are currently the best methods for the questions being addressed. The investigators are very experienced in the implementation of these techniques from previously published studies

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

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

Participants will receive a copy of their personal results, body fat and fitness measurements after the study. The will also be provided with feedback on their responses to the exercise sessions and be provided with recommendations for exercise training. As such they will already have begun a regular exercise training programme, which if continued will result in health benefits in both the present and future.

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 *(If YES, please describe.)*

The exposure to blood and muscle tissue and needles are minimal but the School of Health and Human Performance has standard operating procedures for the handling of biological products.

3.6 ADVERSE/UNEXPECTED OUTCOMES *(see Guidelines)*

The School of Health and Human Performance has the facilities to deal with all aspects of this study and an emergency plan for adverse events. The laboratory is equipped with an emergency crash cart and defibrillator. An individual trained in Advanced Cardiac Life Support (ACLS) will be present during each test. 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 *(see Guidelines)*

The principal investigator will be involved in all aspects of the research, including participant recruitment and data collection. The research team have weekly meeting 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 *(see Guidelines)*

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.)*

3.10 EXPENSE ALLOWANCE

Participants will not be paid to participate in this study but we will cover any expenses associated with participation in this study to a maximum value of €150

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

Donal O'Gorman is a Lecturer in Exercise Physiology at the School of Health and Human Performance at Dublin City University. Donal has been involved in the publication of numerous papers investigating the role of exercise in the regulation of metabolism for general health and in Type 2 diabetes.
Brendan Egan is a PhD student at the School of Health and Human Performance. Brendan completed an MSc in Sport Nutrition and has a wide range of experience in exercise metabolism. He recently completed a similar project looking at the effect of acute exercise on skeletal muscle gene expression.
Paul O'Connor is a technical officer at the School of Health and Human Performance who is pursuing a Masters degree part-time. He has several years of experience in all aspects of exercise testing and research implementation
Professor Niall Moyna is Head of Department at the School of Health and Human Performance. Niall has over 10 years of research experience, both in Ireland and abroad, in many aspects of exercise training, exercise metabolism, cardiovascular metabolism and has had over 20 research communications published in peer-reviewed journals.
Brian Carson is a PhD student at the School of Health and Human Performance. Brian graduated from the Sport Science and Health programme at DCU with 1st class honours in 2005. He is currently active in similar project looking at the effect of altering the contraction frequency of acute exercise on skeletal muscle gene expression.
David Ashley is a PhD student at the School of Health and Human Performance. David graduated from the Sport Science and Health programme at DCU with 2nd class honours in 2005.
Dr. Noel McCaffrey will act as the study physician. He is a sports medicine physician with an adjunct position in the Faculty of Science and Health.
Dr. Gavin McHugh will act as study physician. He is a specialist registrar in orthopaedics and has been taking muscle biopsies as part of his own research.
Dr. Ray Walls will also act as the study physician. He is a specialist registrar in orthopaedics and has been taking muscle biopsies as part of his own research

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? *(see Guidelines)*

Confidentiality is an important issue during data collection. Participant's identity, or other personal information, will not be revealed or published. Participants will be assigned an ID number under which all personal information will be stored in a secure file and saved in password protected file in a computer at DCU. The principal investigator, graduate student and study physicians 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 <u>HOW</u>, <u>WHEN</u> AND <u>BY WHOM</u> THIS WILL BE DONE?

Data will be shredded after 5 years by Dr. Donal O'Gorman. At the same time biological samples will be disposed of by a technician at the School of Health and Human Performance consistent with standard protocols for the treatment of biohazard materials.

7. FUNDING

7.1 HOW IS THIS WORK BEING FUNDED?
This work is being funded by a grant from the Targeted Research Initiative Fund from the Faculty of Science & Health and postgraduate IRSCET funding.

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

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

☐ YES ☐ NO

7.4HOW WILL PARTICIPANTS BE INFORMED OF THE SOURCE OF THE FUNDING?
The source of funding will be included in the plain language statement and the informed consent document.

8. PLAIN LANGUAGE STATEMENT *(Approx. 400 words – see Guidelines)*

Title: The influence of training intensity on adaptations to exercise training in human skeletal muscle

Principal investigators:
Dr. Donal O'Gorman - donal.ogorman@dcu.ie 01-7008060
Mr. Brendan Egan - brendan.egan6@mail.dcu.ie 01-7008472
Metabolic Physiology Research Unit, School of Health and Human Performance, Dublin City University

It is well known that exercise training has many health benefits, including weight loss and the prevention of diabetes and cardiovascular disease. Despite the known benefits, we don't fully understanding what changes occur in the body, especially skeletal muscle. Learning more about these changes would have important implications for (i) future treatments of these diseases and (ii) more specific exercise recommendations.

The purpose of this project is to look at how the intensity/effort of exercise influences the adaptations to training. You will be asked to make several visits to the lab.

- On the first visit you will have a medical examination from the doctor, to make sure you are suitable for the study. You will then exercise on a bicycle for approximately 15 minutes to determine your fitness level.
- On the second visit to the lab you will cycle at either 40% or 80% of your maximal ability to verify the intensity that you will train at. This will take approximately 30 minutes.
- After a week, you will begin an exercise training program lasting 14 days. You will be required to train every day at either 40% of your maximal ability for approximately 60-mins or 80% for approximately 30-mins. Your training will be supervised at all times.
- A muscle biopsy will be taken from your leg before you start the training programme and following 1-, 3-, 7-, 10- and 14-days of training. A blood sample will also be taken at this time, and at the exercise session the previous evening.
- For three days prior to the commencement of training you will be asked to record your daily food intake. You will be advised to continue this pattern of intake for the duration of the program.

Exercise does carry a risk of injury, such as a pulled muscle, muscle soreness or in extreme cases abnormal heart rhythm, heart attack or death. The benefits of being involved in this study include a detailed assessment of your fitness, your response to exercise and your energy use. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate thesis and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. Confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be participant to subpoena, freedom

of information claim or mandated reporting by some professions. All data will be destroyed after 5 years. Your participation in this research project is voluntary and you may withdraw your consent at any time. There will not be any penalty nor will your rights as a student or staff member of DCU be affected in any way.

This research project is being funded from a grant awarded within DCU and the funding of a postgraduate student by the Irish Research Council for Science, Engineering and Technology (IRSCET).

If participants 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

9. INFORMED CONSENT FORM *(Approx. 300 words – see Guidelines)*

Title: The influence of training intensity on adaptations to exercise training in human skeletal muscle

Principal investigators: Dr. Donal O'Gorman, and Mr. Brendan Egan: School of Health and Human Performance

Other investigators: Brian Carson, David Ashley, Dr. Gavin McHugh, Dr. Ray Walls, Prof. Niall Moyna, Paul O'Connor

Purpose
The purpose of this project is to look at the impact of changing the intensity of exercise training on changes that occur in muscle proteins in response to 14 days of exercise training.

This is what will happen during the research study

5. I will have the purpose of the study, each of the steps involved and the risks of participating in the study explained to me. I will have the opportunity to ask any questions and if I am happy with the answers I will:
    a. Provide written informed consent for participation in the research project.
    b. I will then complete a medical history form, which will ask questions about my general health, personal and family health history, smoking, exercise, and dietary habits.
    c. I will talk with a medical doctor about the information I have provided and I understand, based on the information provided, the medical doctor may exclude me from participating in the research project.
    d. I will be provided with dietary advice, which I will follow for three days prior to each visit to the laboratory. I will not consume caffeine or alcohol during this period.
6. Pre-test evaluation
    a. I will have a number of measurements of my body size and shape. Firstly, my blood pressure will be measured. I will have an electrocardiogram (ECG) done to evaluate my heart. This is a painless, 5 minute procedure that involves lying down and having adhesive pads attached to my arms, legs and chest. My height and weight will be measured in light clothing, and without shoes. My percentage body fat will be measured by the thickness of skin and fat on 7 sites around the body including the chest, leg, arm, back and side of the body. I will provide a blood sample to check some health indicators.
    b. I understand that any of these procedures or tests may be waived at the discretion of the doctor for the following reasons: (i) I have completed the same or similar step in the past 6 months as part of another research protocol at the School of Health and Human Performance; (ii) It has been determined that I am not eligible to participate in this research project; and thus completion of the entire screening process will not be necessary.
7. Exercise capacity and determination of exercise intensity
    a. I will undergo an exercise test designed to measure my fitness, and to evaluate my current physical condition. I understand that I will pedal on a stationary bicycle, with the pedal resistance getting more difficult every 2 minutes until, fatigue, breathlessness, chest pain and/or symptoms that indicate to the doctor or myself that I should stop exercise. To assess my fitness I will have a mouthpiece similar to a snorkel in my mouth to measure the amount of air I breathe in and out.

b. At least 4 days later, I will cycle on a stationary bike for 30 minutes to determine the resistance that corresponds to either 40% or 80% of my exercise capacity (depending on the training group that I will be assigned to). During this time I will also wear the mouthpiece to determine oxygen use

8. Impact of training on muscle cell function
    a. Approximately one week later I will begin the exercise training phase of the study. I will exercise on consecutive days for 14 days.
    b. I will be assigned to one of two training groups and as such will train at an intensity corresponding to either 40% of my maximum ability for approximately 60 minutes or at an intensity corresponding to 80% of my maximum ability for approximately 30 minutes depending on my group. I understand that I will be breathing more often, and will sweat, but that I will be able to maintain a conversation.
    c. I understand that all my exercise training sessions will take place in the MPRU on a stationary bicycle under supervision by one of the study's investigators and that training sessions will take place in the late afternoon to early evening.
    d. On the morning of the first day of training, I will come to the MPRU between 0800h and 0900h after an overnight fast, with only water taken for the previous 10 hours. After resting for 15 minutes, I will have a blood sample taken from my arm and a muscle biopsy taken from my thigh. For the biopsy I will have the area anaesthetised with local anaesthetic, then a small 0.5 cm incision will be made in the skin and a needle inserted briefly into the muscle. A small piece of muscle, less that 0.15 of a gram, will be taken from my leg. The incision is pulled close with sterile strips and my leg will be wrapped snugly with an elastic bandage to maintain pressure. Before I leave I will be given contact information and supplies to change the dressing around the biopsy sites.
    e. I will then leave the lab before returning that afternoon to complete the first exercise training session. On the morning following the 1st, 3rd, 7th, 10th, and 14th training sessions, I will report to the MPRU under the same fasting conditions for subsequent blood and muscle sample collection. I will have additional blood samples taken immediately prior to and immediately after the 1st, 3rd, 7th, 10th, and 14th training sessions. This will complete my participation in the study.
    f. In preparation for the training phase I will be required to record my daily food intake in the three days leading up to the commencement of training. I will maintain a similar level of dietary intake throughout the two week training period.
    g. I will be weighed immediately prior to and immediately after each training session to determine the volume of fluid loss through sweating that has occurred. I will be advised to replace this loss with the appropriate amount of fluid based on established recommendations. However, I understand that water will be available ad libitum throughout the training sessions.

Sometimes there are side effects from performing exercise tests. These side effects are often called risks, and for this project, the risks are:

4. Exercise testing carries with it a very small risk of exercise induced asthma, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. The risk of sudden death during exercise for healthy men is 1:15000-18000. Because I will be asked to give a maximum effort, I may experience some muscle soreness in my arms and legs or nausea following the maximal exercise test. It should be noted that if the experimental protocol is adhered to that the likelihood of these risks occurring is minimal.
5. I understand that the insertion of a needle into a superficial arm vein (to take blood samples) should be minimally painful but a slight ache may be felt and a small bruise may appear on my arm. There is also a small risk of infection, but by using the appropriate techniques this risk is minimal.
6. I understand that there may be discomfort during the muscle biopsy with some pain and delayed soreness afterward. There is a risk of bleeding, bruising, infection and scaring of the skin. After the biopsy, my leg may feel stiff and sore. Temporary numbness of the skin near the biopsy may also occur.

There may be benefits from my participation in this study. These are:

4. I will receive a copy of my personal results, body fat and fitness measurements and energy use during exercise when the study is finished.
5. I will be given guidelines to maintain or increase my physical activity/training program if I feel I would like to continue to exercise after the study.
6. I understand that no other benefits have been promised me.

Costs associated with the study
I understand that there will not be any payment for participation in this study but legitimate expenses to a maximum value of €150 will be recompensed.

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

I have read the Plain Language Statement	Yes/No
I understand the information provided	Yes/No
I have had an opportunity to ask questions and discuss this study	Yes/No
I have received satisfactory answers to all my questions	Yes/No

My confidentiality will be guarded:
Dublin City University will protect all the information about me, and my part in this study, within the limitations of the law. My identity or personal information will not be revealed or published. All records associated with my participation in the study will be participant to the usual confidentiality standards applicable to medical records. In addition, the study findings may be presented at scientific meetings and published in a scientific journal and/or as part of a postgraduate thesis, but my identity will not be divulged and only presented as part of a group.

If I have questions about the research project, I am free to call Donal O'Gorman at 01-7008060.

Taking part in this study is my decision.
I understand that my participation in this study is voluntary and that I may withdraw my consent at any time by notifying any of the investigators. I may also ask that the information gathered about me be removed from the database, or storage, and destroyed. My withdrawal from this study, or my refusal to participate, will in no way affect my relationship with Dublin City University or my entitlements as a student or staff member. I understand that my participation in this research may be terminated by the investigator without regard to my consent if I am unable or unwilling to comply with the guidelines and procedures explained to me.

Project Funding
This research project is being funded from an award to Dr. Donal O'Gorman from the Faculty of Science and Health at Dublin City University and funding for a postgraduate student from the Irish Research Council on Science, Engineering and Technology.

Signature:
I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to take part in this research project

Participants Signature: _____

Name in Block Capitals: _____

Witness: _____

Date: _____

10. CHECKLIST

Please check that all supplementary information is attached to your application (in both hard and soft 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.

	ATTACHED	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:

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 an 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)

Appendix J

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

PRINCIPAL INVESTIGATOR(S) 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 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)

Guidelines to Applicants

1.1 PRINCIPAL INVESTIGATOR(S): *The named Principal Investigator is the person with primary responsibility for the research project. Doctoral researchers and Research Masters or their supervisors may be listed as Principal Investigators, depending on the conventions of the discipline and on the individual case. It should be made clear, in subsequent sections of this application, who is carrying out the research procedures. In the case of Taught Masters and undergraduate student projects the supervisors are Principal Investigators.*

2.0 PROJECT OUTLINE: *Provide a brief outline of the project, aims, methods, duration, funding, profile of participants and proposed interaction with them. This description must be in everyday language that is free from jargon. Please explain any technical terms or discipline-specific phrases.*

2.1 LAY DESCRIPTION: *Provide a brief outline of the project, including what participants will be required to do. This description must be in everyday language which is free from jargon. Please explain any technical terms or discipline-specific phrases. (No more than 300 words).*

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH: *State the aims and significance of the project (approx. 400 words). Where relevant, state the specific hypothesis to be tested. Also please provide a brief description of current research, a justification as to why this research should proceed and an explanation of any expected benefits to the community.* ***NB – all references cited should be listed in an attached bibliography.***

2.3 PROPOSED METHOD: *Provide an outline of the proposed method, including details of data collection techniques, tasks participants will be asked to do, the estimated time commitment involved, and how data will be analysed. If the project includes any procedure which is beyond already established and accepted techniques please include a description of it. (No more than 400 words.)*

2.4 PARTICIPANT PROFILE: *Provide number, age range and source of participants. Please provide a justification of your proposed sample size. Please provide a justification for selecting a specific gender.*

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED: *Please provide specific details as to how you will be recruiting participants. How will people be told you are doing this research? How will they be approached and asked if they are willing to participate? If you are mailing to or phoning people, please explain how you have obtained their names and contact details. This information will need to be included in the plain language statement. If a recruitment advertisement is to be used, please ensure you attach a copy to this application.*

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES*:* *Identify, as far as possible, all potential risks to participants (physical, psychological, social, legal or economic etc.), associated with the proposed research. Please explain what risk management procedures will be put in place.*

3.6 ADVERSE/UNEXPECTED OUTCOMES: *Please describe what measures you have in place in the event that there are any unexpected outcomes or adverse effects to participants arising from involvement in the project.*

3.7 MONITORING: *Please explain how you propose to monitor the conduct of the project (especially where several people are involved in recruiting or interviewing, administering procedures) to ensure that it conforms with the procedures set out in this application. In the case of student projects please give details of how the supervisor(s) will monitor the conduct of the project.*

3.8 SUPPORT FOR PARTICIPANTS: *Depending on risks to participants you may need to consider having additional support for participants during/after the study. Consider whether your project would require additional support, e.g., external counselling available to participants. Please advise what support will be available.*

4.0 INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS: *List the academic qualifications and outline the experience and skills relevant to this project that the researchers and any supporting staff have in carrying out the research and in dealing with any emergencies, unexpected outcomes, or contingencies that may arise.*

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED? *Please bear in mind that where the sample size is very small, it may be impossible to guarantee anonymity/confidentiality of participant identity. Participants involved in such projects need to be advised of this limitation.*

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY: *Participants need to be aware that confidentiality of information provided can only be protected within the limitations of the law - i.e., it is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions. Depending on the research proposal you may need to specifically state these limitations.*

6.0 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL: *For the purpose of this section, "Data" includes that in a raw or processed state (e.g. interview audiotape, transcript or analysis). "Samples" include body fluids or tissue samples.*

8.0 PLAIN LANGUAGE STATEMENT: *Written information in plain language that you will be providing to participants, outlining the phases and nature of their involvement in the project and inviting their participation. Please note that the language used must reflect the participant age group and corresponding comprehension level.*

9.0 INFORMED CONSENT FORM: *This is a very important document that should be addressed by participants to researchers, requiring participants to indicate their consent to specific statements, and give their signature.*

FOR FURTHER INFORMATION AND NOTES ON THE DEVELOPMENT OF PLAIN LANGUAGE STATEMENTS AND INFORMED CONSENT FORMS, PLEASE CONSULT THE DCU REC WEBSITE: WWW.DCU.IE/RESEARCH/ETHICS

. ADMINISTRATIVE DETAILS

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

- ☒ Research Project
- ☐ Practical Class
- ☐ Student Research Project
  - *(please give details)*
  - ☐ ResearchMasters
  - ☐ 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
Dr.	Murphy	Ronan	01 7008824	01 7008888	ronan.murphy@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

5. 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.

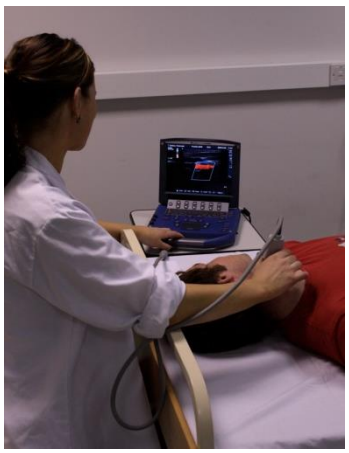


Figure 1: Carotid artery ultrasound reactivity

Figure 2: Brachial artery

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 (VO_{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 (VO_{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 (VO_{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)

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 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 Ronan Murphy has 12 years of post PhD experience and training in cell and molecular biology, vascular biology, and thrombosis & haemostasis. He received his undergraduate degree and Ph.D. with NUI Galway. Following this he worked for two years as a Clinical Research Scientist in the field of Pharmacogenomics. He was awarded a Fellowship from the HRB to work on bleeding disorders. Thereafter, he went to work for Prof. S.J. Shattil, at The Scripps Research Institute, San Diego (2000-2003). He has also been a visiting scientist to the Blood Research Institute, Milwaukee, USA.

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 <u>HOW</u>, <u>WHEN</u> AND <u>BY WHOM</u> 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?

☐ YES ☒ NO *(If Yes, please specify how this conflict of interest will be addressed.)*

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)

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. 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.

II. 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.

III. 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.

VI. 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.

VII. 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

1. 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.

2. 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.

3. 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.

4. 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.

5. Testing Session 4 will be a repeat of the test carried out during during Testing Session 2.

6. 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).

7. 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 K

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

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP | SCPE

Appendix L

VO_2max 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

VO_2max 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

VO_2max 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

VO_2max 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

VO_2max 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