Effect of Exercise Training on Vascular Function in Active and Inactive Vascular Beds

Deirdre Upton, BA (Hons)

A Dissertation Submitted in Partial Fulfillment of the Requirement for the
Degree of Master of Science by Research

Department of Health, Sport and Exercise Science
School of Health Science
Waterford Institute of Technology

Research Supervisor: Dr. Michael Harrison

July 2009

Declaration

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgement of collaborative research and discussions.

Signed: . . . .

Abstract

Title: Effect of exercise training on vascular function in active and inactive vascular beds.

Author: Deirdre Upton

Introduction: Evidence exists that regular aerobic exercise benefits vascular health. This beneficial effect is potentially related to repeated increases in the flow of blood across endothelial cells during exercise. We hypothesised that any exercise effect on endothelial dependent dilation is localised to vascular beds in the exercised limb. An additional objective was to determine the effects of exercise training on circulating biomarkers of endothelial activation, including soluble adhesion molecules and cell-derived microparticles.

Methods: Nine overweight, sedentary men (age 34 ± 4 y (mean $\pm$ SD) BMI 29.8 ± 4.3 kg·m^{-2}, $\dot{V}O_{2peak}$ 33.2 ± 4.6 ml·min^{-1}·kg^{-1}, systolic blood pressure 131.8 ± 8.6 mmHg, diastolic blood pressure 82.6 ± 4.6 mmHg) participated in the study. All volunteers underwent a two-week exercise intervention, which comprised of two-legged cycle-ergometry exercise (30-45 min at 70% $\dot{V}O_{2peak}$ for 5 d of each week) combined with one arm handgrip exercise (one contraction every 2 s at 40% maximum voluntary contraction for half of each exercise session). Allocation of dominant and non-dominant arm to handgrip training or control condition was counterbalanced. The control arm remained perfectly still during exercise.

Results: Preliminary work indicated the medium term coefficient of variation for various indices of reactive hyperaemia to be between 10 and 15%. Peak reactive hyperaemia increased in both the handgrip and control arms following training ($p<0.05$) but the increase in the handgrip-trained arm was considerably greater ($48 \pm 12\%$ vs. $16 \pm 8\%$, $p<0.05$ for interaction). The duration of reactive hyperaemia and the total reactive hyperaemia increased in the handgrip-trained arm ($p<0.05$) but was unchanged in the control arm. With the exception of an increase in soluble vascular cell adhesion molecule, training did not influence soluble adhesion molecules or cell derived microparticle counts.

Conclusions: Short-term exercise training increases vascular function in active vascular beds with little or no change in inactive beds, in sedentary overweight men. Biomarkers of endothelial activation are largely unaffected by short-term exercise training.

Acknowledgements

I would like to thank the following people for their assistance, help and encouragement in the completion of this work:

May I offer my sincere thanks and gratitude to Dr. Michael Harrison for all his assistance, guidance and supervision during the last two years.

Many thanks must be given to Dr. Kieran O' Connor for the collection of blood samples.

To the volunteers who gave up their time, without whom this project would not of been possible.

Finally, very special thanks to all my family and friends, for their love and support during my time as a student. To my nieces and nephews, for all the noise and distractions that made me feel human.

Dedication

I dedicate all of my work to Aoife. She feels as though she has taken every exam with me and her confidence in me never wavered. Her motivation made me a better person and she continues to show me what service to others really is. Aoife thank you for the endless and exceptional support, sacrifices, inspiration and love.

Table of Contents

4   Presentation of Results

5   Discussion of Results

List of Figures

List of Tables

List of Appendices

Chapter 1

Introduction

1.0   Introduction

Cardiovascular disease (CVD) is the leading cause of mortality in Ireland accounting for 36% of all deaths (Irish Heart Foundation, 2009). Atherosclerosis constitutes the single most important contributor to this growing burden of disease. The innermost layer of the blood vessel is made up of endothelial cells, which play a crucial role in preventing the development of atherosclerotic vascular disease. The endothelium produces a number of vasodilator and vasoconstrictor substances that maintain vascular homeostasis, and regulate vascular tone (Verma and Anderson, 2002; Vane et al., 1990). Endothelial dependent dilation (EDD) describes the dilation of vessels that results from the secretion of vasodilators from the endothelium. The impairment of EDD may be the earliest event in the development of atherosclerosis (Tousoulis et al., 2008). Closely related to EDD is endothelial activation. This involves changes in the endothelium toward a pro-inflammatory state, in response to particular stimuli, including cytokines and oxidants. Endothelial activation induces the expression of adhesion molecules (Libby, 2002) and shedding of endothelial microparticles (vanWijk et al., 2003).

Substantial evidence exists that regular aerobic exercise prevents the age related decline and can restore the loss in EDD (DeSouza, 2000; Rinder et al., 2000). The beneficial exercise effects appear greatest, and may be confined to individuals whose endothelial function is impaired at baseline. Exercise training has been reported to improve EDD in heart transplant patients (Pierce et al., 2008) and in young sedentary men (Murray et al., 2006; O'Sullivan, 2003). It is unclear however if exercise training involving large muscle groups benefits endothelial function in all vascular beds (generalised effect) or only those that experience increases in blood flow during exercise (local effect). This is an important question as vascular beds such as those in the carotid artery, which are prone to atherosclerosis, (Talusan et al., 2005) may not experience increases in blood flow from exercise.

Studies (Higashi et al., 1999; Goto et al., 2003; O'Sullivan, 2003; Maiorana et al., 2000; Murray et al., 2006) have reported improvements in upper limb EDD as a result of lower limb training, raising the possibility of a generalised exercise effect. An increase in forearm reactive hyperaemia was reported after 12 weeks of moderate intensity cycle ergometry training (Goto et al., 2003) and brisk walking (Higashi et al., 1999). One study reported a 60% increase in forearm reactive hyperaemia after one week of cycle training (Murray et al., 2006). Other studies point towards the primacy of a local effect

(Pierce et al., 2008; Motohiro et al., 2005; Demopoulos et al., 1997; Kobayashi et al., 2003). Improvements in EDD were seen in the calf but not in the forearm after 12 weeks of walking in heart transplant patients (Pierce et al., 2008) and cycling in patients with severe congestive heart failure (Demopoulos et al., 1997). Kobayashi et al. (2003) reported a significant increase in FMD in the tibial artery with no change in the brachial artery in patients with chronic heart failure after three months cycle ergometry exercise training (Kobayashi et al., 2003). Thijssen and Hopman (2008) note that studies that show an improvement in forearm endothelial function after lower limb exercise mainly involve walking and running exercise, which does involve some level of upper body movement (Thijssen & Hopman, 2008).

To date, no study has compared the changes in forearm EDD that result from a combined generalised and local exercise stimulus to changes resulting from a generalised stimulus only, while eliminating all movement in the unexercised limb.

Direct assessment of endothelial function is complex; it involves specialised expensive equipment, is time consuming, and requires suitably trained personnel and a high level of patient co-operation. Hence, there is growing interest in the use of biomarkers of endothelial activation to reflect vascular health status. Elevated levels of cellular adhesion molecules represent an early marker of endothelial activation and the potential development of cardiovascular disease (Meigs et al., 2004). Levels of adhesion molecules in plasma have been shown to correlate to its membrane-bound expression (Leeuwenberg et al., 1992; Schram and Stehouwer, 2005). Cell derived microparticles are a novel biomarker of vascular health status (Curtis et al., 2008). Endothelial cells shed these membrane vesicles during cell activation or from apoptosis (Jimenez et al., 2003). Augmented release of microparticles by endothelial cells has been documented in patients with many vascular diseases including angina, hypertension, pre-eclampsia and atherosclerosis (Van Wijk et al., 2003; Jimenez et al., 2003). Currently, there is considerable research interest in microparticle function and in their putative role as sensitive markers of disease activity (Malat et al., 2000; Bernal-Mizrachi et al., 2003). To date few studies (Jilma et al., 1997; Pierce et al., 2008; Smith et al., 2000) have examined the influence of exercise training on adhesion molecules. Furthermore, no study has examined the influence of exercise training on microparticles.

The primary purpose of the present study is to examine the hypothesis that any exercise effect in endothelial dependent dilation is localised to vascular beds in the exercised limb. This was determined using a novel research design involving two legged cycle-ergometry exercise training combined with one arm handgrip exercise. Two legged cycle ergometry was undertaken to provoke substantial hemodynamic increases in heart rate, stroke volume and systolic blood pressure throughout the vasculature, including in the control arm which was kept perfectly still in order to eliminate any local exercise effect. Changes in endothelial dependent dilation were assessed in the handgrip trained and the control arms pre- and post-training. A second objective is to determine the effect of the exercise training on novel biomarkers of endothelial activation including endothelial microparticles and platelet microparticles.

Chapter 2
Literature Review

2.0   Cardiovascular System

The cardiovascular system consists of the heart, blood vessels, and the cells and plasma that make up the blood. The blood vessels of the body represent a closed delivery system, which functions to transport blood around the body. The primary function of the blood is to supply substances such as oxygen, nutrients and hormones to tissues and to remove waste products. The key role of the cardiovascular system in maintaining homeostasis is dependant upon the movement of blood throughout the body (Waugh and Grant, 2006). There needs to be a continuous movement of blood through the vessels that permeate every tissue and every cell in the body. This section will focus on the general structure of the peripheral vascular system, including arteries, capillaries, and veins.

2.1   Arterial System

There are two arteries that emerge from the heart, the aorta from the left ventricle and the pulmonary artery from the right ventricle. Arteries are described as efferent vessels that carry blood away from the heart. They are responsible for ensuring that a blood supply, rich in nutrients, is able to reach the peripheral vascular beds. All arteries in the body transport oxygenated blood from the heart to body tissues except the pulmonary vascular tree that carries deoxygenated blood from the heart to the lungs (Waugh and Grant, 2006). Every artery that enters an organ and branches into 6-8 smaller vessels to become arterioles while arterioles branch 2-5 times to eventually become capillaries.

2.1.1   Arteries

Arteries vary considerably in size and their walls consist of three layers of tissue or tunicae [Figure 2.1]. These are tunica adventitia or externa, tunica media and tunica intima (Waugh and Grant, 2006). The outermost layer of the vessel, the tunica adventitia, is composed of loose fibrous connective tissue, as well as collagen and elastic fibres with some smooth muscle fibres. This layer contains nerves and lymphatic vessels in addition to a capillary network to nourish the walls of large arteries (Marieb and Hoehn, 2007). The middle layer, tunica media is the thickest layer [Figure 2.1]. The tunica media is made up of smooth muscle cells, connective tissues and elastic fibres. Smaller arteries consist principally of plain muscle fibres, arranged in lamellae. These lamella vary in number according to the size of the artery, it is to this

that the thickness of the wall of the artery is mainly due (Moore, Agur and Dalley, 2006). In the larger arteries there is more elastic tissue than smooth muscle. The greater prevalence of elastic tissue helps larger arteries to stretch in order to adjust for the change in pressure as the ventricles in the heart contract forcefully and pump blood into these types of vessels (Marieb and Hoehn, 2007). The main examples are the aorta and the pulmonary arteries.

The innermost layer of the vessel, the tunica intima, is made up of one layer of endothelial cells, a thin subendothelial layer of connective tissue, and an internal elastic layer called the internal elastic lamina (Moore, Agur and Dalley, 2006). The endothelial cells are in direct contact with the blood flow.

Figure 2.1   Artery and Vein
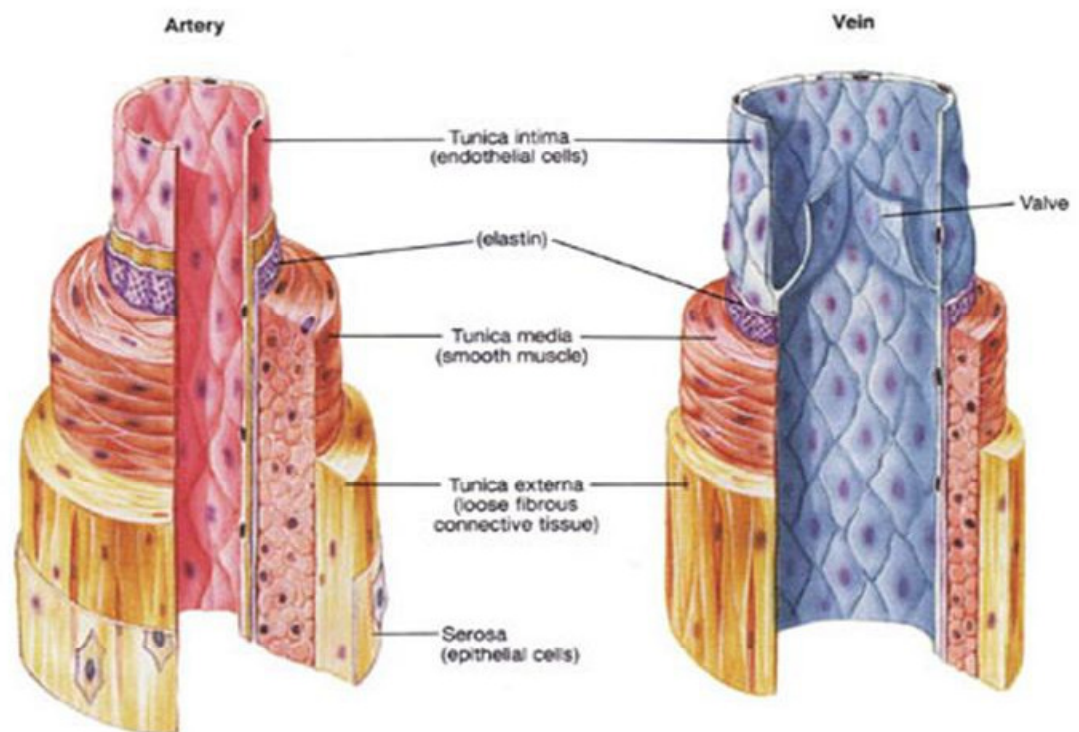
Source: Fox, S. (1993)

The walls of arteries and veins consist of three layers of tunicae, tunica externa, media and intima. The innermost layer is made up of endothelial cells.

Arteries serve as passageways for blood from the heart to the organs, because of their large radius they offer little resistance to blood flow (Waugh and Grant, 2006) and thus are termed conduit vessels. As the heart pumps blood, a greater volume enters the

arteries. The arteries elasticity enables them to expand to hold the larger volume.
When the heart relaxes, the stretched arterial walls passively recoil, pushing the blood
further along the vessels (Sherwood, 2005).

2.1.2   Arterioles

Arteries located further away from the heart branch out into smaller and smaller vessels,
then into arterioles shortly before they subdivide to form capillaries.   The intima of an
arteriole is also composed of endothelial cells. Unlike arteries, arteriolar walls contain
very little elastic connective tissue (Waugh and Grant, 2006). However, the media is
made up of two layers of smooth muscle cells that are innervated by sympathetic nerve
fibres (Marieb and Hoehn, 2007). Increased sympathetic activity leads to arteriolar
vasoconstriction, whereas decreased sympathetic activity results in arteriolar
vasodilation (Sherwood, 2005).

Arterioles are the major resistance vessels in the vascular tree because their radius is
small enough to offer considerable resistance to flow.   Thus the total peripheral
resistance, that is the total resistance to blood flow, is mainly determined by the radius
of the arterioles (Marieb and Hoehn, 2007; Sherwood, 2005; Moore, Agur and Dalley,
2006). Arterioles are very important because they are the most highly regulated blood
vessels in the body, and contribute the most to overall blood pressure. In contrast to the
low resistance of the arteries, the high degree of arteriolar resistance causes a noticeable
drop in mean arterial pressure as blood flows through these vessels (Marieb and Hoehn,
2007; Sherwood, 2005; Moore, Agur and Dalley, 2006).

2.2   Capillaries

Capillaries are the smallest blood vessels; they enable the interchange of nutrients,
oxygen and many other substances between blood and surrounding tissue (Waugh and
Grant, 2006). No exchange occurs in the larger blood vessels, arteries and veins serve
only as conduits (Darovic, 2002). Capillary walls are mainly composed of only a tunica
intima layer, that is, a one-layer endothelium so thin that gases and molecules such as
oxygen and proteins can pass through them driven by osmotic and hydrostatic gradients.
There are three types of capillaries, continuous, fenestrated and sinusoidal or
discontinuous (Marieb and Hoehn, 2007).   Continuous capillaries have a sealed

endothelium and only allow small molecules to diffuse. Fenestrated capillaries have pores in the endothelial cells to increase the capillary permeability for small molecules (Marieb and Hoehn, 2007). They are found in areas where large quantities of fluids and nutrients are exchanged with blood. The third type, sinusoidal, are a type of fenestrated capillary that have larger openings in the endothelium (Smith and Kampine, 1990). These types of capillaries allow red blood cells and serum proteins to enter. These are mainly found in the liver, bone marrow, spleen, lymph nodes and adrenal cortex (Marieb and Hoehn, 2007).

2.3 Venous System

As blood passes through capillary beds, it gets depleted of oxygen and nutritional molecules and loaded with by-products. Venous circulation is responsible for returning the blood to the heart after exchanges of gases, nutrients and wastes have occurred between the blood and body cells (Marieb and Hoehn, 2007). Furthermore, blood returns back to the heart for reoxygenation through the venules then through the small veins until it reaches the superior and inferior vena cava, which are the largest veins in the body.

2.3.1 Veins

Veins are blood vessels that return blood at low pressure to the heart. Because veins are subjected to lower pressures than arteries, vein walls are thinner and more distensible (Waugh and Grant, 2006). These characteristics allow them to expand and accommodate large volumes of fluids with small changes in intravascular pressure. At any moment, approximately 65% to 70% of circulating blood volume is in the veins (Waugh and Grant, 2006).

The walls of the veins are thinner than those of arteries but they have the same three layers of tissue [Figure 2.1]. They are thinner because there is less muscle and elastic tissue in the tunica media, again because veins carry blood at a lower pressure than arteries. Another difference between arteries and veins is that many veins possess valves, which prevent backflow of blood, ensuring that it flows towards the heart. The valves are formed by in-foldings of tunica intima, where each fold forms a bicuspid valve, which is strengthened by connective tissue (Marieb and Hoehn, 2007). Valves

are abundant in the limbs, especially the lower limbs where the blood must travel a considerable distance and where blood is moved against gravity.

2.4 Hemodynamics

Hemodynamics, meaning blood movement, is the study of blood flow. It is an important part of cardiovascular physiology dealing with the forces the heart has to develop to circulate blood and the physical factors that govern blood flow through the cardiovascular system (Klabunde, 2004).

These are the same physical factors that govern the flow of any fluid, and are based on a fundamental law of physics, namely Ohm's Law, which states that current (I) equals the voltage difference (ΔV) divided by resistance (R). In relation to blood, flow is equal to the pressure difference (ΔP) divided by the resistance (R) to flow offered by the blood vessel (Marieb and Hoehn, 2007) [Equation 2.1].

Equation 2.1

$$F = \frac{\Delta P}{R}$$

2.4.1 Blood Flow

Blood flow is volume or quantity of blood passing through a certain point in the circulation in a given time (ml/min) (Marieb and Hoehn, 2007). Total blood flow or cardiac output at rest is approximately 5,000 ml/min^{-1} and increases to 17,500 ml/min^{-1} with a rise in tissue activities, such as exercise (Fuster et al., 2004). It is called cardiac output because it is the quantity of blood pumped by the heart in volume unit per time (Rowell, 1986). Blood flow can be categorised into two types laminar [Figure 2.2a] and turbulent [Figure2.2b].

Figure 2.2a Laminar Blood Flow
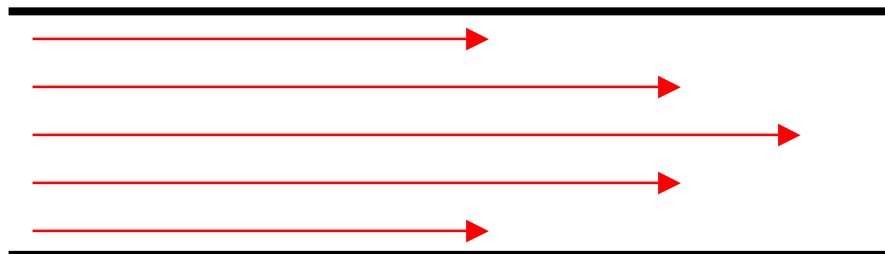
Figure 2.2b   Turbulent Blood Flow
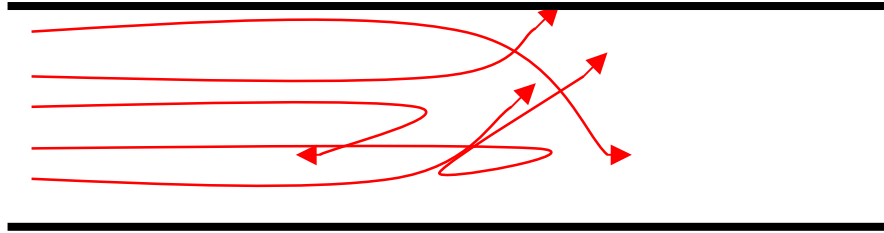
With laminar blood flow the flow is parallel to the vessel wall. In turbulent flow the direction of the flow is not parallel to the vessel wall and blood flows in different directions.

Under normal conditions, the flow of fluids is laminar, the direction of the flow is parallel to the vessel wall and is basically straight [Figure 2.2a]. The thin fluid layer adjacent to the cylinder wall is extremely slow or even motionless (Noble et al., 2005). Hence, the lowest velocity is found along the vessel wall. However, the fluid layers interior to the external slow layer must shear against it and therefore move a bit faster. The highest velocity is found in the centre of the vessel (Noble et al., 2005).

Generally in the body, blood flow is laminar (Beck, 2008). However, under conditions of high flow, or in vessels with considerable plaque development, laminar flow can be disrupted and become turbulent (Beck, 2008). In turbulent flow, the direction of the flow is not parallel to the vessel wall and blood flows in different directions [Figure 2.2b] (Davidovits, 2007). Blood flow can also be seen as oscillatory flow, that is, antegrade and retrograde flow (back-and-forth). Turbulent and oscillatory flows require considerably greater pressures than laminar to drive fluid through the same tube (Guyton, 1991). This in turn requires the heart to work harder to generate a given flow. Flow which is turbulent or oscillatory causes much more resistance in the blood vessel than laminar flow therefore laminar flow allows for better and easier delivery of blood. There is a considerable turbulence of flow at the branches of large arteries. Additionally, during ventricular contraction turbulent flow occurs at the proximal portions of the aorta and pulmonary artery, however in small vessels blood flow is predominantly laminar (Noble et al., 2005).

2.4.2   Blood Pressure

Blood pressure is the force per unit area (measured in mmHg) exerted by the blood against the walls of the blood vessels (Plowman & Smith, 2007). All levels of arterial

pressure put mechanical stress on the arterial walls. The rhythmic contraction of the heart generates this pressure during the left ventricular contraction and relaxation phases of the cardiac cycle (Klabunde, 2004). A pressure of 50 mmHg means that the force exerted is sufficient to push a column of mercury up to a level 50 mm high. It is the differences in blood pressure, that is, the pressure gradient that provides the force that keeps the blood moving, which is always from an area of high pressure to an area of low pressure, throughout the body (Marieb and Hoehn, 2007). During the contraction (systole) phase of the cardiac cycle pressure generated by the heart at rest is about 120 mmHg. This measurement is usually referred to as pressure in the brachial artery. It is specifically the maximum arterial pressure during ventricular contraction (Marieb and Hoehn, 2007).

Following contraction the ventricle relaxes, the elasticity of the arterial system maintains a driving pressure for steady flow of blood to the periphery, until the next surge of blood (Guyton, 1991). During this relaxation (diastole) phase of the cardiac cycle, blood pressure decreases to about 80 mmHg. Diastolic blood pressure is indicative of peripheral vascular resistance especially at the level of arterioles and capillaries. At rest the length of time the heart is in diastole is approximately twice as long as as it is in systole, hence the mean pressure across the circulation is not simply an average of systolic and diastolic pressures. Mean arterial pressure is the average pressure within an artery over a complete cycle of one heartbeat. It is estimated from Equation 2.2 below (Guyton, 1991):

Equation 2.2

$$MAP = DBP + [(SBP-DBP)/3]$$

2.4.3   Resistance

Resistance is opposition to flow and is a measure of the amount of friction blood encounters as it passes through the vessels. As the majority of the friction encountered is in the peripheral circulation, peripheral resistance is the term generally used. The reciprocal of resistance is conductance. Conductance reflects the magnitude of active change in blood flow and vascular tone (O'Leary, 1991). Changes in resistance have been noted as being small as baseline blood flow is high, whereas changes in vascular conductance are large (O' Leary, 1991). Vascular conductance is calculated as blood

flow divided by mean arterial pressure and vascular resistance as mean arterial pressure divided by blood flow (Dinenno et al., 2001).

Resistance to blood flow is determined by the length and diameter of individual vessels, the organisation of the vascular network and the physical characteristics of the blood (Klabunde, 2004). The three important sources of resistance are blood viscosity, vessel length, and vessel diameter. Resistance to flow is directly related to the viscosity of the blood. The viscosity of blood is three to four times the viscosity of water due to the presence of red cells and proteins (Klabunde, 2004). The greater the viscosity, the less easily molecules slide past one another and the more difficult it is to get and keep the fluid moving. The viscosity of blood is strongly influenced by three factors hematocrit, temperature and flow. At very low flow states blood viscosity can increase quite significantly and a decrease in blood temperature causes an increase in viscosity by approximately 2% per °C (Klabunde, 2004). Hematocrit is the proportion of blood volume that is occupied by red blood cells, normally expressed as a percentage of a given volume of whole blood. As hematocrit increases viscosity increases (Klabunde, 2004). Resistance is directly proportional to vessel length (Marieb and Hoehn, 2007). At a constant radius, the longer the vessel the greater the surface area in contact with the blood, the greater the resistance to flow (Klabunde, 2004).

As blood viscosity and vessel length are normally unchanging, they are not the most influential factors in the control of vascular resistance (Klabunde, 2004). Changes in blood vessel radius is the underlying factor that control blood flow in the arterial and venous systems throughout the circulation. The resistance to blood flow differs at each level in the vasculature. Fluid passes more readily through a large vessel than through a smaller vessel (Klabunde, 2004). Resistance is quite high in the arteriolar network because of the small radius of each vessel. Fluid close to the wall of a vessel is slowed by friction as it passes along the wall, whereas fluid in the centre of the vessel flows more freely and faster (Noble et al., 2005). Changes in blood vessel radius are frequent and significantly alter peripheral resistance. These changes in vessel radius are the result of contraction and relaxation of vascular smooth muscle. As radius and resistance are inversely related a slight change in the radius of a vessel brings about a notable change in blood flow (Klabunde, 2004).

2.5 Mechanical Forces Experienced by the Endothelium

Mechanical forces are important modulators of endothelial cells. The endothelium responds rapidly and sensitively to the mechanical conditions created by blood flow. As blood flows through a vessel, it exerts a physical force on the vessel wall. The mechanical stresses acting on the vessel wall include cyclic strain and shear stress (Vane et al., 1990).

Cyclic strain is the circumferential stress, which acts along the vessel wall perimeter to cause stretching and is caused by variations in blood pressure (Lelkes, 1999). The endothelium is greatly affected by cyclic strain, as it causes changes in cell properties, function and vessel homeostasis (Chien et al., 1998). In particular, cyclic strain regulates the production of a number of factors, which directly impact on the endothelium, the vasodilator nitric oxide (NO) and nitric oxide synthase (NOS) which are central to vessel diameter (Cheng et al., 2001), vascular endothelial growth factor (VEGF), which is central to endothelial proliferation (Zheng et al., 2001) and intercellular adhesion molecule-1 (ICAM-1), which is central for barrier function (Collins et al., 2006).

Cyclic strain has also been reported to increase release of reactive oxygen species (Cheng et al., 1998) and to increase expression of adhesion molecules including ICAM, selectin and monocyte chemoattractant protein (MCP-1) (Yun et al., 1999). These observations are consistent with many reports in the literature that indicate that chronic increases in blood pressure are associated with impaired endothelial function and increased progression of atherosclerosis (Laughlin et al., 2008).

Fluid shear stress is the frictional force imposed on the vessel wall when blood flows through (Lelkes, 1999). This frictional drag is referred to as shear stress and is defined in terms of blood viscosity and velocity. Under normal shear stress endothelial cell stability and survival is promoted and the cells align in the direction of flow. This laminar unidirectional flow is known as mean positive shear stress (MPSS). This laminar shear promotes the release of factors from endothelial cells that are responsible for the inhibition of coagulation, migration of leukocytes, and smooth muscle cell proliferation (Michiels, 2003). This positive laminar shear stress promotes release of atheroprotective factors, such as NO, from endothelial cells (White and Frangos, 2007; Harrison et al., 2006; Chien, 2007). Research has also shown that increasing brachial

artery wall shear stress values is associated with increasing vasodilation in the same artery as assessed by flow-mediated vasodilation (Irace et al., 2001).

The patterns of these stresses are different between the straight part of the arterial tree versus the branch points and curvatures (Chien, 2007). In the straight part, the shear stress and stretch have well-defined directions. Flow at these sites is primarily unidirectional and mean positive shear stress is high, the occurrence of plaque formation within these regions is respectively very low (White and Frangos, 2007). Departures from unidirectional flow occur mainly around branch points. Lesions are distributed mainly along the outer walls of the branch points, where mean wall shear stress is relatively low. In these curvature or branch point regions of the vasculature, blood flow is also turbulent. This type of shear results in attenuation of NO production along with an increase in reactive oxygen species (ROS) production. ROS are very small molecules that include oxygen ions, peroxides and free radicals. During time of turbulent shear stress ROS levels can increase dramatically and can cause significant damage to cell structures (Lynch, 2003). This cumulates into a situation know as oxidative stress. Thus, locations where perturbed shear stress is predominant are implicated in initiation of endothelial dysfunctional cardiovascular disease states (Davies et al., 1995).

2.5.1 Mechanotransduction

As they are located at the interface between blood flow and the vessel wall, endothelial cells are in a position to sense shear stress and cyclic strain and convert the mechanical signal to an intracellular response. This ability of the endothelium to sense and respond to shear is mediated through mechanotransduction signalling mechanisms, which include integrins, ion channels, G-proteins and receptor tyrosine kinases (Davies, 2007).

Integrins are membrane receptors that are involved in cell-cell interactions. A study by Muller et al (1997) showed that flow-mediated vasodilation in coronary arteries, which is mediated by NO release, could be blocked with peptides that compete for integrin interactions. In addition, similar attenuation of flow-mediated vasodilation was obtained if using integrin blockers such as integrin antibodies, supporting the hypothesis that integrins are involved in the mechanotransduction of fluid shear stress (Muller et al., 1997; Bhullar et al., 1998).

Another mechanism identified as a potential shear stress receptor is mechanosensitive ion channels. Two different mechanosensitive ion channels have been identified in endothelial cells that are responsive to hemodynamic forces, these are fluid shear stress-responsive potassium channels and stretch-activated calcium channels (Davies, 1995). Studies have shown that blocking the potassium (K^+) channels also blocks the shear mediated increases in NO production, thus suggesting that ion channels are important mediators of the endothelial cell response to fluid shear stress (Berk and Traub, 1999).

The search for very rapid events that occur after exposing endothelial cells to shear stress has led to the identification of G-proteins as primary mechanosensors in endothelial cells. Research by Gudi et al. (1996) demonstrated that activation of G-proteins in endothelial cells could occur as quickly as one second after the onset of shear stress.

Receptor tyrosine kinases, a class of membrane proteins, also take part in mechanotransduction. It has been shown that they play a role in the phosphorylation of epidermal growth factor. Research by Iwasaki et al. (2000) in which protein synthesis induced in stretched vascular smooth muscle cells had been exposed to be blocked when the cells are incubated in a receptor antagonist, suggests that receptor tyrosine kinases are involved in the mechanotransduction of fluid shear stress (Iwasaki et al., 2000).

2.6   Cardiovascular Disease

Cardiovascular disease (CVD) refers to any abnormal condition characterised by the dysfunction of the heart or blood vessels (Harris et al., 2005). It includes diseases such as hypertension, atherosclerosis, coronary heart disease, and cerebrovascular disease (WHO, 2009). CVD is the primary cause of mortality and morbidity worldwide (WHO, 2009). The World Health Organisation estimates that cardiovascular disease contributed to a third of global deaths in 1999, rising to 17.5 million in 2005 and it is estimated that by 2010 CVD will be the leading cause of death in developing countries (WHO, 2009).

Presently, CVD is the number one cause of death in Ireland, it accounts for 36% of all deaths nationally. Approximately 10,000 people die from cardiovascular disease, each year (Irish Heart Foundation, 2009). While numbers remain high they are in fact

declining at a steady rate. The improving figures are due not only to advances in treatment and preventative strategies, but also to greater public awareness. It is recognised that CVD is linked to the lifestyle of the individual, a fact that is well known and heavily advertised through the media. In America, the 80's saw an increase in information on CVD provided by the media and public health organisations. Frank et al (1993) conducted research, which highlighted that from 1980 to 1990, through a greater understanding of the risk factors of CVD, those individuals whom believed that nothing could be done in relation to cardiovascular disease risk decreased from 3.6% to 0.9%. Along with this, as many as 64.1% stated that, within the last year, they had made a change(s) to improve their health, (Frank et al., 1993).

Risk factors for the disease include smoking, elevated cholesterol levels, obesity, high blood pressure, and stress (Frayn and Stanner, 2005). Numerous measures can be taken to protect against these factors, all of which correspond to a healthier lifestyle, cessation of smoking, regular exercise and a healthy diet. For this reason much research has focused on the major markers of disease risk, which includes high blood cholesterol levels and smoking. Although this approach has had some success, it has also been recognised that these markers do not account for all cardiovascular risk (Frayn and Stanner, 2005). This prompted a search for other indicators of risk of CVD. Over the past few years, a number of such risk markers have emerged. These include factors associated with inflammation and with clotting, lowered resistance to oxidative stress and impaired functioning of blood vessels (Frayn and Stanner, 2005). All of the major modifiable CVD risk factors cause endothelial activation and impaired endothelial dependent dilation. Preventing and controlling the established risk factors are associated with preserved endothelial function and reduced risk of CVD.

2.7   Atherosclerosis

Atherosclerosis constitutes the single most important contributor to this growing burden of cardiovascular disease. It is a slowly progressive disease of elastic and muscular arteries (Fuster et al., 1996). It can affect all large and medium sized arteries, including the coronary, carotid, and cerebral arteries, the aorta, its branches and major arteries of the extremities. Atherosclerosis is the principal precursor to myocardial infarction, stroke, claudication and aneurysm formation (Fuster et al., 1996). The risk factors

include dyslipidemia, diabetes, cigarette smoking, family history, sedentary lifestyle, obesity and hypertension (Fuster et al., 1996).

Atherosclerosis mainly affects the branch points or inner curvatures of the vascular tree. These areas of turbulent or oscillatory blood flow have been shown to inhibit endothelial production of NO and lead to impaired endothelial dependent dilation (Galley and Webster, 2004). Atherosclerosis involves lipid accumulation within the wall of muscular and elastic arteries, causing plaque formation [Figure 2.3 & 2.4]. These plaques consist mainly of foam cells, which are macrophages that have taken up lipid particles, necrotic cell debris, cholesterol esters, and calcium deposits (Frayn and Stanner, 2005).

Ross proposed the response to injury hypothesis of atherosclerosis suggesting atherosclerosis begins with injury to the arterial wall leading to endothelial denudation (Ross and Glomser, 1973). Atherosclerosis is no longer considered a disorder due to abnormalities in lipid metabolism; inflammation develops concurrently with accumulation of lipids in the arterial wall. Atherosclerosis is now generally considered an inflammatory disease, inflammation being the cause of both initiation and progression of the lesion (Libby, 2002).

2.7.1   Pathophysiology of Atherosclerosis

Atherosclerosis is a complex, multi-step process involving many different aspects of vascular biology. Lesion formation in humans occurs as early as childhood, symptoms develop when growth or rupture of the plaque reduces or obstructs blood flow (Becker, 1997). The two most common lesions associated with atherosclerosis are the fatty streak and the fibrous plaque or atheroma (Lilly, 1992).

The diagrams by Hansson (2005) will be used to demonstrate the pathophysiology of atherosclerosis. During the initiation of atherosclerosis lipoprotein particles that are small enough, that is, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) accumulate in the sub-endothelial matrix [Figure 2.3]. The LDL may become oxidised (oxLDL) by interaction with the products of cell processes, such as reactive oxygen species (ROS). Ox-LDL is known to increase pro-inflammatory activation, platelet aggregation and impair endothelial dependent dilation (Simon, Cunnigham and

Cohen, 1990; Tsimikas and Witzum, 2001). The LDL may also be taken up by macrophages, which ultimately become foam cells, the typical component of the fatty streak [Figure 2.3]. Uptake of the oxidised LDL renders the macrophages less mobile, thereby promoting the accumulation of these lipid-laden cells in the intima.

Figure 2.3   Infiltration of LDL and VLDL

LDL & VLDL

Adhesion Molecules
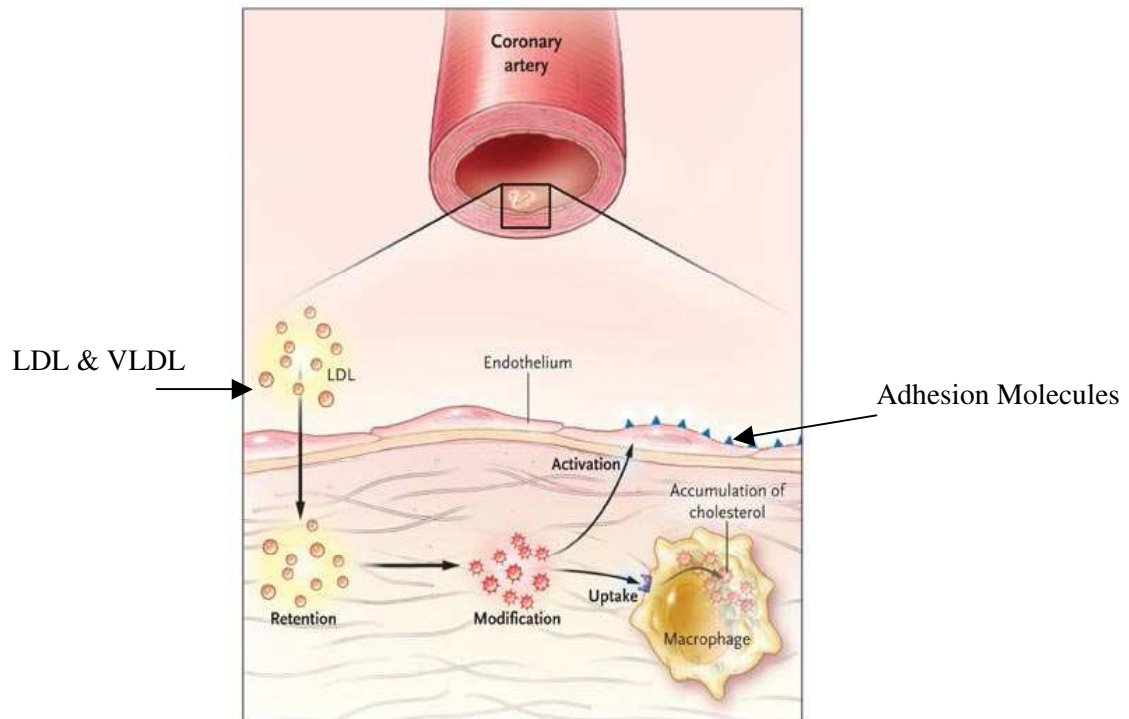
Source: Hansson, G. (2005)

LDL and VLDL pass through the endothelium and are taken up by macrophages, which evolve into foam cells.

The subsequent evolution of the fatty streak has been shown to be dependent on continuing inflammation (De Caterina and Libby, 2007). Oxidised LDL is toxic to the endothelial cells and the endothelium becomes activated by atherogenic and proinflammatory stimuli [Figure 2.4]. As a result, the expression of adhesion molecules is up regulated (De Caterina and Libby, 2007). Adhesion molecules are pro-inflammatory molecules that mediate the adhesion of leukocytes to the endothelium by binding to specific ligands on the leukocytes (Muller, 2003). These adhesion molecules include vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin and P-selectin.

Increased expression of adhesion molecules increases the recruitment of monocyte and lymphocytes (T-cells). Transmigration into the arterial wall is mediated through chemoattractants such as monocyte chemotactic protein. This leads to accumulation of inflammatory macrophages and T-cells within the arterial wall (Hansson, 2001). Once in the intima, monocyte-derived macrophages secrete pro-inflammatory chemokines, such as, chemo-attractant protein-1 (MCP-1) and express cytokines, such as tumour-necrosis factor-α (TNFα that amplify the local inflammatory response in the lesion (Libby, 2002). TNFα induces VCAM-1 expression in endothelial cells (Libby, 2002) and MCP-1 allows the recruitment of monocytes to areas of inflammation (Capoccia et al., 2008) all of which continues and exacerbates the process of atherosclerosis.

Figure 2.4   Activated Endothelium

Adhesion molecules bind with monocytes and T-cells

Monocyte-derived Macrophage

MCP-1
TNFα
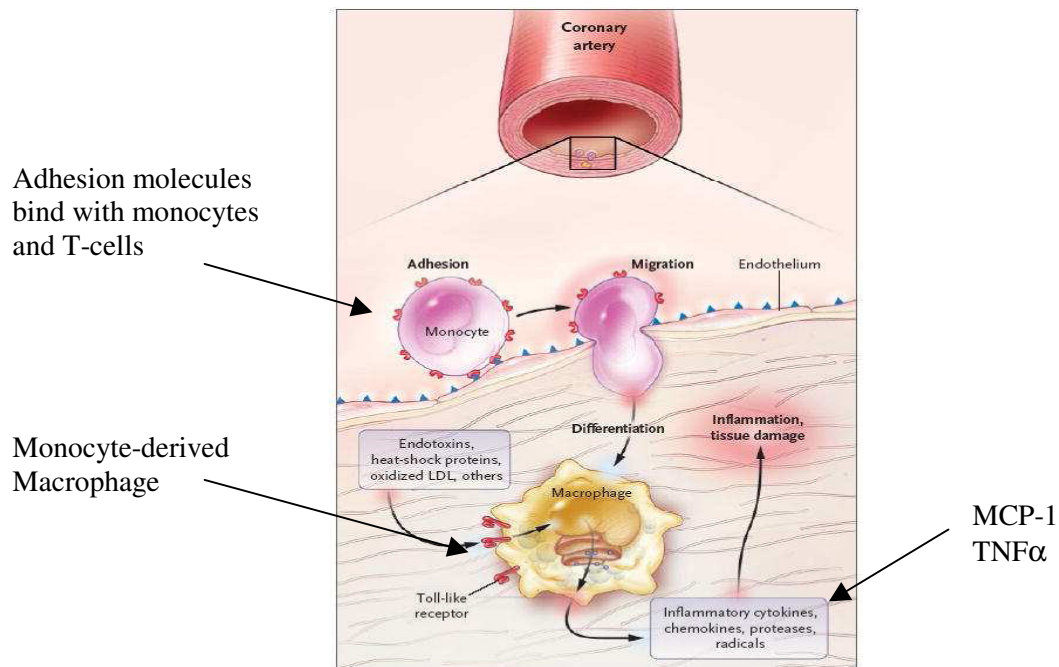
Source: Hansson, G. (2005)

LDL becomes oxidised resulting in the macrophages releasing inflammatory cytokines and an increase in the expression of adhesion molecules. Source: Hansson, G. (2005)

The early development of the fatty streak involves mainly endothelial cells, some T-cells and macrophages. Activated leukocytes release proteolytic enzymes and a variety of growth factors and cytokines that stimulate endothelial cells and macrophages [Figure 2.5]. One such cytokine, CD40 and its ligand CD40L, expressed on several inflammatory cells, including macrophages, T-cells and endothelial cells, result in the

production of pro-inflammatory cytokines and adhesion molecules (Schonbeck and Libby, 2001).

Within the atheroma, the main type of T-cell is CD4$^+$, in response to cytokines CD4$^+$ can polarise into those secreting generally pro-inflammatory cytokines, known as Th1 cells (Daugherty and Rateri, 2002) [Figure 2.5]. The cytokines and growth factors secreted by the macrophages and T-cells also stimulate smooth muscle cells proliferation and migration, which leads to thickening of the vascular wall. Accumulation of foam cells and smooth muscle cells lead to the formation of a necrotic core. Continuous smooth muscle cell proliferation and migration in conjunction with the inflammatory response leads to a complex, atherosclerotic plaque that becomes covered by a fibrous cap (De Caterina and Libby, 2007).

Figure 2.5   T-cell Activation and Development of Atherosclerotic Plaque

T-Cell (CD4$^+$) Polarised into Th1

Cytokines Stimulate Smooth muscle cells

Source: Hansson, G. (2005)
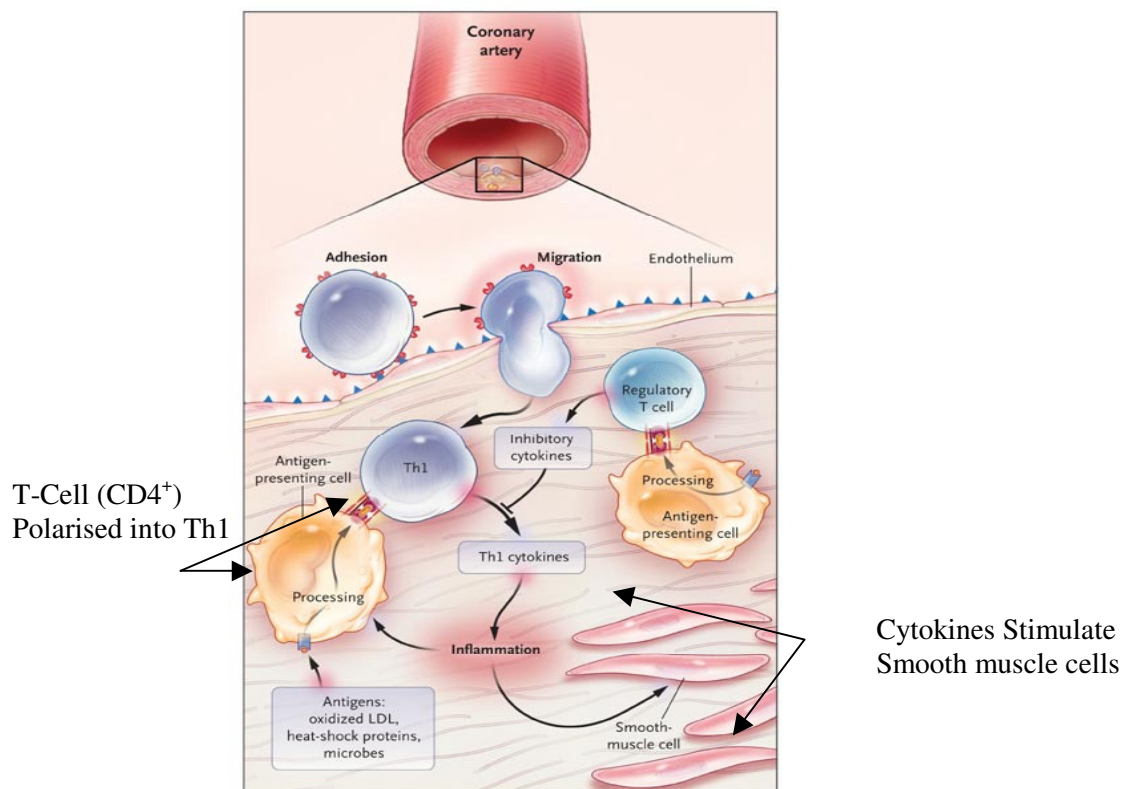
The cytokines released from both the T-cells and macrophages stimulate smooth muscle cell growth and development. The build up of the foam cells and smooth muscle cells leads to the atherosclerotic plaque.

The primary pro-inflammatory cytokines, interferon-γ interleukin-1 and TNF-α bring about the expression of other cytokines from activated leukocytes within the plaque.

One such cytokine, interleukin-6 (IL-6) is considered to be the most valuable circulating marker of endothelial dysfunction and consequently a useful marker of risk (Erzen et al., 2007). IL-6 is mainly responsible for the synthesis of acute- phase reactant proteins, namely, C-reactive protein (CRP) produced by the liver (Libby, 2002) [Figure 2.6]. Plasma CRP concentrations can increase several hundred-fold in response to inflammation (Pasceri, Willerson and Yeh, 2000).

Figure 2.6   Production of CRP
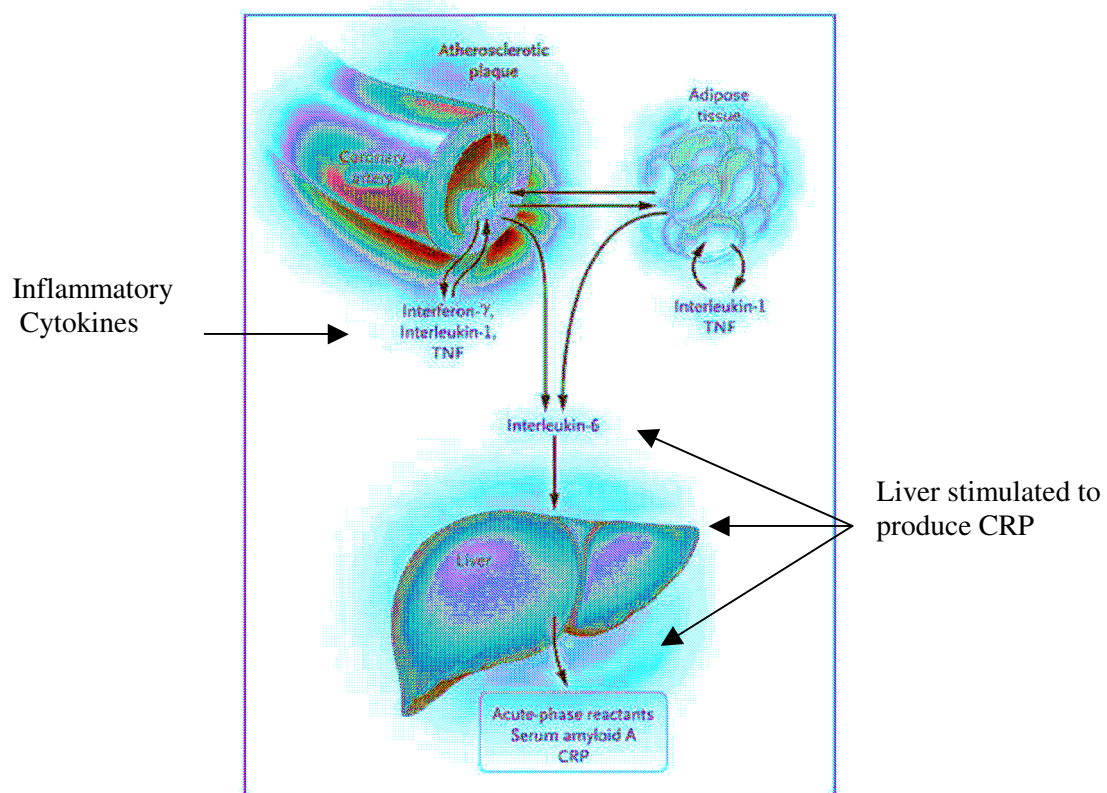
Source: Hansson, G. (2005)

The inflammatory cytokines stimulate the release of interleukin-6, which stimulates the liver to release the inflammatory marker C-reactive protein (CRP).

Beyond its role as a proven marker of inflammation, numerous adverse effects of CRP have now been described [Table 2.1]. CRP has been identified as not only a risk marker but also an actual mediator of risk (Verma and Yeh, 2003). This has led to the identification of CRP as playing a direct role in promoting the inflammatory component of atherosclerosis.

Table 2.1 CRP as a Mediator of Risk

Facilitates LDL uptake by macrophages
Increases expression of adhesion molecules (ICAM & VCAM)
Increases release of chemoattractant chemokines (MCP-1)
Recruits monocytes into the arterial wall
Decreases endothelial NO production
Stimulates smooth muscle cell migration and proliferation

(Verma and Yeh, 2003)

2.8 The Endothelium and Vascular Integrity

The endothelium is now recognised as not simply being an inert barrier that separates flowing blood from underlying tissue, but as a dynamic cellular interface (Dvorak, 2007; Mehta and Malik, 2006; Sagripanti and Carpi, 2000). The endothelium plays a key role in vascular homeostasis through the release of a variety of substances. In addition the endothelium participates in immune and inflammatory reactions, presents a non-thrombogenic surface for blood flow and contains a semi permeable barrier (Mehta and Malik, 2006; Sagripanti and Carpi, 2000)

A prominent feature of endothelial cells is the presence of many pinocytotic vesicles, which are involved in the process of transport of substances from one side of the cell to the other (Dvorak, 2007). The transport functions of the endothelium include the transport of essential circulating molecules across endothelial cells to the subendothelial space to meet the metabolic needs of the surrounding tissue cells. The efficient transfer of many water insoluble substances from the blood into the interstitial space relies on endothelial permeability, and often on specific carrier proteins (Mehta and Malik, 2006). Endothelial transport can be thought of in a general sense as occurring via paracellular and transcellular pathways (Mehta and Malik, 2006).

The maintenance by the endothelium of a semi-permeable barrier is particularly important in controlling the passage of molecules and fluid between the blood and interstitial space. This is especially important for the maintenance of regional oxygen delivery. Oxygen diffuses directly to the endothelium where it is transported to the surrounding tissue (Friedman, 2008). The endothelial barrier is described as restrictive, in that only small molecules can move through gaps formed by the opening of junctions

between individual endothelial cells, that is, via the paracellular route (Mehta and Malik, 2006). The majority of these substances are low in weight (< 3 nm) and higher in concentration in the plasma than in the interstitial space; therefore passive diffusion is the main transport mode. The vessel wall is restrictive to high-molecular weight substances such as proteins, most notably albumin; these are actively transported via the transcellular pathway (Mehta and Malik, 2006). Caveolae are vesicle carriers that mediate the transcellular transport (Fuster et al, 2004).

The most important function of the endothelial cells lining the tunica intima is modulation of vessel tone and maintenance of homeostasis (Vane, Anggard and Botting, 1990). It does so by producing vasodilators, vasoconstrictors, pro- and anti- coagulants, pro- and anti-inflammatory molecules and anti-oxidants, which is highlighted in Figure 2.7.

Figure 2.7   Endothelial Cells and Cellular functions

Vasodilators
Nitric Oxide
Prostacyclin

Anticoagulant Factors
Heparin
Thrombomodulin
Plasminogen activator

Growth Factors
Colony stimulating factor
Insulin like growth factor
Vascular endothelial growth factor

Endothelial Cell

Procoagulant Factors
Thromboxane A2
Platelet activating factor
von Willebrand factor
Plasminogen activator inhibitor

Vasoconstrictors
Thromboxane A2
Angiotensin II
Endothelin

Inflammatory Modulators
Intercellular adhesion molecule-1 (ICAM-1)
Vascular cell adhesion molecule-1 (VCAM-1)
E-Selectin
Interleukin -1, -6 & -8
Leukotrienes

As shown in Figure 2.7, endothelial cells are involved in many cellular functions throughout the body. Vascular tone is regulated by a number of vasodilator and vasoconstrictor substances produced by the endothelium, including nitric oxide (NO),

endothelin, and angiotensin (Verma and Anderson, 2002). A critical balance between endothelial-derived relaxing and contracting factors maintains vascular homeostasis. Nitric oxide (NO) is the most important substance produced by the endothelium. It is synthesised from L-arginine by the action of the endothelial isoform of nitric oxide synthase (NOS), that is, NOS3 (Ballingand and Mayer, 2000). Figure 2.8 outlines the molecular structure of NO.

Figure 2.8   Molecular Structure of Nitric Oxide
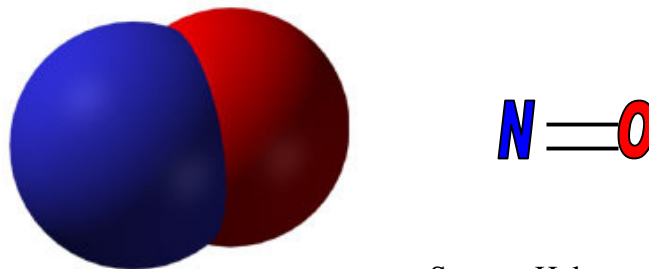
Source: Helmenstine, A. (2009).

NO has one unpaired electron, which therefore means it is a free radical species (Ignarro, 2000). NO has the ability to react with other species with unpaired electrons such as oxygen and superoxide (Ignarro, 2000). NO only acts in the area in which it is formed, once in the blood its half-life is limited as it is quickly neutralised in the circulating blood by haemoglobin (Gardner & Fox, 2000). NO plays a pivotal role in the maintenance of vascular tone and reactivity (Vane, Anggard and Botting, 1990). It activates smooth muscle cell guanylate cyclase, which relaxes smooth muscle (Ballingand and Mayer, 2000). Katzung (2007) and Jugdutt (2004) have described many more functions of NO, which are revealed in Table 2.2.

Table 2.2   Functions of Nitric Oxide

Regulates vascular tone
Maintains vascular smooth muscle relaxation
Protects against thrombosis and atherogenesis
Inhibits platelet aggregation and adhesion
Inhibits proliferation and migration of vascular smooth muscle
Inhibits leukocyte adhesion and expression of adhesion molecules
Regulates endothelial integrity and permeability
Regulates blood flow and blood pressure

(Katzung, 2007; Jugdutt, 2004)

Oxidised LDL can compromise NO production (Ignarro, 2000; Safar and Frohlich, 2000). Oxidised LDL down regulates NOS expression (Ignarro, 2000) and reduces the uptake of L-arginine into endothelial cells (Safar and Frohlich, 2000), both of which lead to decreased nitric oxide production. The bioavailability of nitric oxide can also be affected. Decreased NO bioavailability may either reflect a drop in NO due to a decreased biosynthesis, impaired availability of bioactive NO or enhanced NO inactivation (Loscalzo and Vita, 2000). Impaired bioavailability and bioactivity of NO may further be due to enhanced degradation by increased free radicals (Loscalzo and Vita, 2000). The free radical superoxide interferes with NO thus limiting its biological activity (De Caterina and Libby, 2007) and resulting in the formation of a powerful oxidant, peroxynitrite (Pacher et al., 2007). The peroxynitrite anion can lead to cellular damage and cytotoxity (Pryor and Squadrito, 1995). If NO production is impaired or its bioavailability is reduced then vasoconstriction, thrombosis and inflammation can occur (De Caterina and Libby, 2007).

Another important function of endothelial cells is host defence; this is achieved through the production of adhesion molecules, cytokines, and growth factor (Kerr, 1999). It is thought that disruptions in endothelial function may have an important role in cardiovascular disease, hypertension, hyperglycaemia, diabetes, and thrombosis (De Caterina and Libby, 2007). A disturbance in endothelial cell function may increase transendothelial leukocyte migration and impair endothelial dependent dilation (De Caterina and Libby, 2007).

2.9 Endothelial Dependent Dilation (EDD)

Endothelial dependent dilation refers to the dilation that results from the endothelium secreting vasodilators and controlling the release of vasoconstrictors. While endothelial independent dilation (EID) refers to the action of the vasodilators and vasoconstrictors on the vascular smooth muscle cell (Veves & Freeman, 2007).

In 1980, Furchgott and Zawadzki first reported that the endothelium releases a substance that relaxes blood vessels, for which they termed endothelium-derived relaxing factor (Davies, 2007). This was later understood to be NO. Ludmer and colleagues (1986) initially reported the importance of NO in regulating vasomotor tone (cited in Loscalzo and Vita, 2000). It was found that infusion of ACh caused

vasodilation in smooth-appearing coronary arteries but vasoconstriction in atherosclerotic arteries (Ignarro, 2000).

Impaired EDD is characterised by a change of the activities of the endothelium toward vasoconstriction, prothrombic properties, and a pro-inflammatory state, with the aforementioned mechanical forces playing a central role in the process. Turbulent or oscillatory flow at curvatures in the blood vessels and in arterial branches can trigger signals leading to impaired EDD (Hwang et al., 2003; McNally et al., 2003; Hsiai et al., 2003; Gambillara et al., 2006). Oscillatory flow has been shown to attenuate NO production and increase reactive oxygen species (ROS) production (Hwang et al., 2003). ROS production is generally balanced by antioxidant generation, however irregular shear stress upsets the balance leading to an excess of ROS and an oxidative stress state (Behrendt and Ganz, 2007). ROS can transform the endothelium from an anti-inflammatory surface to one that is pro-inflammatory and inhibit NO (Behrendt and Ganz, 2007) resulting in loss of its vasoprotective functions.

Although impaired EDD occurs in many different disease processes, oxidative stress is recognised as being a frequent contributor (Griendling and Fitzgerald, 2003a, 2003b). Increased oxidative stress is the most important mechanism of impaired endothelium-derived NO bioavailability, as a result of inactivation (De Caterina and Libby, 2007). Growing evidence suggest the critical involvement of increased oxidative stress in the pathogenesis of many CVDs (Zalba, Beaumont, San Jose, et al, 2000). Impaired EDD has been implicated in the pathophysiology of different forms of cardiovascular diseases, including hypertension, coronary artery disease, peripheral artery disease, diabetes and atherosclerosis. In conjunction with a cardiovascular risk factor impaired endothelial dependent dilation can be detected before there is any angiographic evidence of disease (Schachinger and Zehier, 2001).

2.10   Endothelial Dependent Dilation Assessments

Several methods have been used to assess vascular function in humans. Invasive techniques, which involve intracoronary or intrabrachial infusions of vasoactive agents, are still considered to be the gold standard for detection of EDD (Felmeden and Lip, 2005). The intra-arterial infusion of nitric oxide agonists, such as acetylcholine (ACh) demonstrates the important role of nitric oxide release.

2.10.1 Intracoronary Infusions of Vasoactive Agents

The most important invasive method is the evaluation of the coronary endothelial function. Coronary artery EDD is assessed using quantitative angiography or intravascular ultrasound in conjunction with the infusion of agonists. During coronary angiography, quantitative angiography is performed before and after the infusion of acetylcholine. Coronary arteries with normal endothelial function dilate in a dose dependent way to the infusion of ACh, on the other hand, while in the presence of impaired EDD, acetylcholine may lead to vasoconstriction (Felmeden and Lip, 2005). However, the invasive nature of this makes it unsuitable for some populations (Moyna and Thompson, 2004).

2.10.2 Intrabrachial Infusions of Vasoactive Agents

Given the systemic nature of EDD, the question arises as to whether peripheral vascular function parallels that of the coronary arteries and thus, may serve as a surrogate marker to identify individuals with impaired coronary EDD. Many studies have shown a correlation between coronary and peripheral arterial function and therefore, assessment of peripheral endothelial function can be used as a proxy for coronary artery endothelial function (Anderson et al, 1995; Takase et al, 1998). EDD may be assessed on forearm resistance arteries, the infusion of vasoactive agents can also be applied to the brachial artery, which is easily accessible and has fewer problems. The gold standard is mercury filled strain-gauge plethysmography in response to intra-arterial infusions of endothelial dependent agonists, such as ACh (Ishibashi et al., 2006). However, this method is not suitable for repeated measures (Ishibashi et al., 2006).

Although the intracoronary and intrabrachial methods have been used to investigate EDD in humans, they are not available for routine measurement or screening tests in the general population as they are invasive techniques, requiring catheter insertion into the artery and they take several hours to perform (Ishibashi et al., 2006; Felmeden and Lip, 2005). Consequently, several methods have been developed to non-invasively assess endothelial function.

2.10.3 Flow-Mediated Vasodilation

EDD is most commonly assessed in the literature by measuring changes in brachial artery conduit vessel diameter in response to arterial occlusion. Arterial occlusion triggers a vasodilatory response in the forearm resistance vessels with this increase in blood flow triggering an upstream increase in the conduit vessel diameter 5 seconds after cuff release (Anderson, 1999).

The vasodilatory response of the brachial artery to increased shear stress is called flow-mediated dilation (FMD), and reflects the ability of the vascular endothelium to produce NO (Anderson and Mark, 1989). Thus, impaired dilation reflects reduced production of nitric oxide, which can be regarded as an important marker of EDD. Anderson et al. (1995) have shown that brachial artery FMD correlates with measures of coronary EDD. When using high-resolution ultrasound the subject's arm is positioned and rested for the brachial artery to be imaged. The diameter of the brachial artery is determined at rest and after the release of the upper arm occlusion cuff (Anderson, 1999). This technique is attractive because it is non-invasive and allows repeated measurements. However, despite its widespread use, there are technical and interpretive limitations (Felmeden and Lip, 2005). The complexity of the high-resolution ultrasound procedure involves specialised expensive equipment and it requires a suitably trained operator to produce valid, reproducible data (Felmeden and Lip, 2005), it also requires a high level of patient co-operation and has relatively poor resolution relative to arterial size (Tousoulis et al., 2005).

2.10.4 Reactive Hyperaemia

Resistance vessel vascular function can be assessed by measuring increase in limb blood flow after occlusion that is reactive hyperaemia, using strain gauge plethysmography in response to arterial occlusion. This non-invasive method is considered useful for assessing resistance artery endothelial function (Higashi et al., 2001a), and the flow response results from many endothelium-derived vasoactive substances, such as NO (Tagawa et al., 1994), prostaglandin (Carlsson and Wennmalm, 1983) and adenosine (Carlsson et al., 1987), all of which are capable of modulating endothelium dependent vasodilatation.

Reactive hyperaemia is typically measured in the forearm but also in the legs and calf (Thijssen, et al., 2005). Resting forearm blood flow (FBF) is measured using mercury filled strain gauge plethysmography. As described by Sinoway et al. (1986), the strain gauge is placed on the upper forearm, at the position with the maximum diameter, approximately 10cm distal to the olecranon process. A cuff is placed on the upper arm and one on the wrist to exclude hand blood flow. The blood flow is determined at rest (FBF) and after arterial occlusion (reactive hyperaemia). As recommended by Groothuis et al. (2003) the upper arm cuff is inflated to 50 mmHg for assessment of resting blood flow. For the assessment of reactive hyperaemia the upper arm cuff is inflated to suprasystolic pressure for five minutes, to produce ischaemia. Immediately before the blood flow measurements hand circulation is occluded for one minute by inflating the wrist cuff to 240 mmHg (Kerslake, 1949).

Figure 2.9 is an example of blood flow measurements obtained in the present research, at rest and after ischaemia using strain gauge plethysmography. The graph highlights the results for each time point during reactive hyperaemia, which was measured for approximately 3 minutes. Reactive hyperaemia is the response that occurs after a period of tissue ischaemia.

As highlighted in Figure 2.9, various indices are used in relation to vascular function as assessed by strain gauge plethysmography. These indices are:
1. Peak Reactive Hyperaemia
2. Total Reactive Hyperaemia
3. Duration of Reactive Hyperaemia

Peak reactive hyperaemia is the maximum blood flow measurement observed during reactive hyperaemia. The duration of reactive hyperaemia is the time from the peak RH until blood flow returns to the resting blood flow measurement. The final index is Total RH, which is represented by the area under the blood flow vs. time curve (AUC). The area under the curve of the flow response during reactive hyperaemia represents the overall dilatory capacity of resistance arteries, and it is also considered to be an indirect index of endothelial function (Higashi, 2001b; Tousoulis et al., 2003). The extent to which these dilatory responses are indeed endothelial dependent is open to debate.

Figure 2.9   Forearm Blood Flow Assessed by Strain Gauge Plethysmography
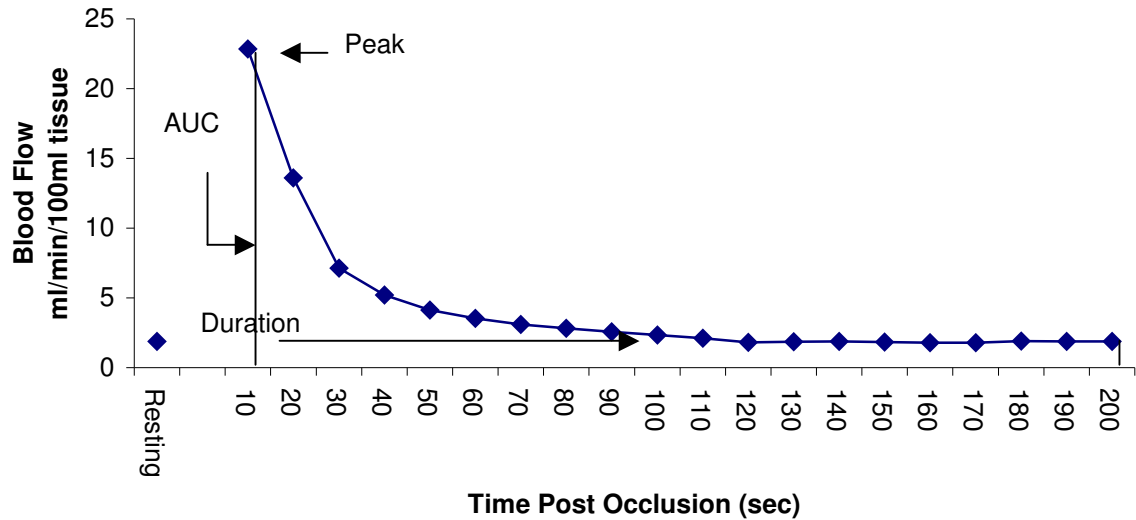
Source: Results obtained in the present research

Peak reactive hyperaemia is the maximum blood flow measurement observed during reactive hyperaemia (RH). Duration is the time from the peak RH until blood flow returns to the resting blood flow measurement. Total RH is represented by the area under the blood flow vs. time curve (AUC).

2.11   Relationship of EDD as Determined by Reactive Hyperaemia and Other Methods.

Research has shown high correlations between endothelial function as determined by reactive hyperaemia and flow-mediated dilation (Joyner et al., 2001; Tschakovsky et al., 1995). Tschakovsky and colleagues (1995) reported a high correlation ($r^2 = 0.87$-0.98) between both methods across a wide range of flows. Evidence of this correlation is important, as a number of studies have compared endothelial function as assessed by flow-mediated dilation and the gold standard, that is, strain-gauge plethysmography in response to intra-arterial infusions of particular agonists. There are varied positions in relation to whether they correlate or not. Researches such as Eskurza et al. (2001) and Lind et al. (2000) found no correlation between the two methods. Additionally, research conducted by Green et al. (2004b; 2006) found no correlation between flow-mediated dilation (FMD) as assessed by Doppler ultrasound and reactive hyperaemia (RH) as assessed by strain gauge plethysmography in response to acetylcholine (ACh) infusions. In both studies, significant improvements were found in FMD and RH in response to the exercise training intervention administered, however, there were no statistically

significant correlations between changes in conduit (FMD) and resistance vessel (FBF) endothelium-dependent function.

Irace et al. (2001) studied 6 healthy male subjects and ten male subjects with either hypertension or obesity. Again flow-mediated vasodilation was utilised as well as strain gauge plethysmography in conjunction with the infusion of acetylcholine (ACh). In this study a strong and statistically significant correlation was found in endothelial function assessed by both methods. Green et al. (2002b) used both methods in 8 young healthy male volunteers. The data collected from both these methods revealed that the differences in baseline flow measured with each technique was not significantly different and was highly correlated (294 ± 34 Vs 206 ± 15%, r = 0.83, p< 001). Green et al. (2002b) also reported on the data from both these methods after reactive hyperaemia, which were also highly correlated (r = 0.83, p< 0.01), although in absolute terms they were significantly different.

Several explanations have been put forward for the lack of association between conduit and resistance vessel assessment. One possibility is that assessments using strain-gauge plethysmography differ to those using high-resolution ultrasound (Green et al., 2006). A second possibility is that the mechanisms responsible for exercise training-induced adaptations in the vasculature may differ according to the vascular territory involved (Green et al., 2004b). Eskurza et al. (2001) suggested that differences in the release of vasodilators other than NO might relate to the lack of association between conduit and resistance vessel responses.

Very little research has directly assessed the correlation between endothelial function as determined by reactive hyperaemia assessed with mercury filled strain-gauge plethysmography and the gold standard, that is, mercury filled strain-gauge plethysmography in response to intra-arterial infusions of particular agonists. However, Higashi and colleagues (2001a) found a strong correlation in a mixed population of hypertensive and healthy adults between the peak reactive hyperaemic responses as assessed with the plethysmograph and the gold standard. Higashi et al. (2001a) identified the time course of the forearm vascular response during reactive hyperaemia and the dose-response curve for forearm blood flow to ACh to be similar in each subject assessed. In this study it was also reported that peak blood flow during reactive hyperaemia (Peak RH) was correlated with blood flow to maximal ACh dose

(30 µg/min; r = 0.91, p< 0.001) (Higashi et al., 2001a). This indicates that this non-invasive method is a useful alternative to the intra-arterial infusion of vasoactive agents for assessing resistance vessel endothelial function (Higashi et al., 2001a).

2.12　　　Importance of Endothelial Factors in Regulation of Reactive Hyperaemia

The contribution of nitric oxide (NO) and the endothelium to the post-occlusion increase in limb blood flow is open to debate. The reactive hyperaemic response has been shown to depend on the local production of non-endothelial dependent vasodilators, such as, adenosine (Loscalzo & Vita, 1994), and endothelial dependent vasodilators, such as endothelium-derived nitric oxide (Higashi et al., 2001a).

Adenosine has been a leading candidate for metabolic vasodilation for many years as it is a vasodilator, which has been shown to be produced by skeletal muscle contraction (Hester et al., 1993). Hellsten et al. (1998) reported a strong correlation (r = 0.98) between adenosine concentrations and leg blood flow during exercise. Additionally, infusion of an adenosine receptor antagonist has been shown to reduce the exercise-induced increases in blood flow (Duncker et al., 1995; Radegran & Calbet, 2001), which suggests that adenosine plays a role in causing vasodilation. Higashi et al. (2001a) reported that infusions of NG-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase blocker, decreased the forearm blood flow responses to reactive hyperaemia and ACh in all participants (Higashi et al., 2001a), suggesting that NO plays a role in the blood flow response to reactive hyperaemia.

On the other hand, in a study by Tagawa et al. (1994) intra-arterial infusion of L-NMMA reduced blood flow only during the mid-to-late phase of reactive hyperaemia and decreased the total reactive hyperaemic flow (AUC). These results suggest that NO plays a significant role in the mid-to-late phase of reactive hyperaemia and that NO plays a minimal role in the peak reactive hyperaemic response (Peak RH) in the human forearm. Contrary to this, Meredith et al. (1996) found both the peak reactive hyperaemic response (Peak RH) and total reactive hyperaemia (AUC) to be reduced in the presence of L-NMMA. These results suggest that the endothelium-derived vasodilator, nitric oxide, plays a role in both the peak reactive hyperaemia and in the maintenance of the hyperaemic response following ischaemia in the forearm.

2.13   Traditional Cardiovascular Risk Factors and Reactive Hyperaemia

Many studies have reported that cardiovascular risk factors impair reactive hyperaemia. For example, impaired reactive hyperaemia has been associated with higher blood pressure, old age, male gender, smoking, insulin resistance, obesity, and lipid abnormalities (Gokce et al., 2002). In relation to lipid abnormalities, elevated free fatty acid levels have been shown to impair endothelium-dependent vasodilation in humans (Watanabe et al., 2005). Dietary fat is broken down into lipids (cholesterol and triglycerides), which are important cellular building blocks. Low-density lipoprotein (LDL) transports cholesterol and triglycerides to the cells for utilisation, but when they are released they are vulnerable to oxidation and can build up in the arteries that feed the heart and brain, forming plaques (Marieb and Hoehn, 2007). High-density lipoprotein (HDL) carries cholesterol away from the plaque and to the liver for removal; however, high triglyceride levels can increase the atherogenicity of HDL and LDL (Marieb and Hoehn, 2007).

Total cholesterol and triglycerides are associated with a decreased peak reactive hyperaemic response (Peak RH) in relatively young and healthy individuals (Antoniades et al., 2006). After ingestion of a high fat meal, the peak reactive hyperaemia (Peak RH) and the total reactive hyperaemic response (AUC) were both negatively correlated with postprandial increases in free fatty acid concentrations, but not with insulin, glucose, total cholesterol or HDL cholesterol (Shimabukuro et al., 2007). Whereas, Raitakari et al. found the total reactive hyperaemic response (AUC) correlated with postprandial increases in insulin and triglycerides (Raitakari et al., 2000).

Higashi et al. (2001b) found the reactive hyperaemic response to be impaired in patients with essential hypertension compared with normotensive subjects. Lieberman et al. (1996) and Celermajer et al. (1992) found reactive hyperaemia assessed by FMD was significantly lower in individuals with coronary artery disease, with the latter also highlighting reactive hyperaemia to be significantly reduced in smokers and children with familial hypercholesterolaemia (Celermajer et al., 1992). In addition to both reactive hyperaemia and flow-mediated dilation being lower among those with cardiovascular risk factors, Huang et al. (2007) revealed that they are strong predictors for cardiovascular events. Lower reactive hyperaemia is associated with a greater

incidence of any event in relation to cardiovascular disease, including a higher incident of unstable angina and a significantly greater incident of death (Huang et al., 2007).

2.14   Endothelial Activation

Related to the concept of impaired EDD is the concept of endothelial activation. Although no uniform definition exists it has been explained in terms of a change in endothelial phenotype or function in response to particular stimuli, including, among others cytokines and oxidants (Zimmerman et al., 1999).

Under resting conditions, endothelial cells provide a non-adhesive surface and regulate vascular permeability and tone by release of NO and other agents to maintain smooth blood flow in the vasculature (Bombeli et al., 1997). Endothelial activation involves changes toward a procoagulant, pro-adhesive state, promoting adhesion of blood cells, coagulation and inflammation. This induces expression of adhesion molecules (Libby, 2002) and shedding of endothelial microparticles (vanWijk et al., 2003).

2.14.1   Endothelial Microparticles

Circulating endothelial microparticles (EMPs) are membranous vesicles, which are shed by endothelial cells (Jimenez et al., 2003). Microparticles can be formed during cell activation by many agonists such as tumor necrosis factor TNF-α (Jimenez et al., 2003; VanWijk et al., 2003) or from apoptosis (Jimenez et al., 2003). Apoptosis is a cell death process, which involves the acquisition of surface changes by dying cells. It was shown that cells undergoing apoptosis break up the phospholipid asymmetry of their plasma membrane and expose phospholipid-like phosphatidylserine to the outer layer of the membrane (Davis, 2002). Annexin V has proven to be a useful tool in detecting apoptotic cells since it binds to negatively charged phospholipids on the outer microparticle membrane (Davis, 2002). Therefore it is possible to distinguish cells at different stages of apoptosis.

The process of microparticle release from apoptosis appears to follow an earlier stage of membrane blebbing before pinching off as free particles [Figure 2.10]. Microparticles formed by apoptosis may differ from microparticles formed by cell activation in size, lipid and protein composition and pathological and/or physiological effects (VanWijk et

al., 2003). During cell activation by agonists or stress, including apoptosis modifications of the plasma membrane, and an increase in bleb formation take place [Figure 2.10] (VanWijk et al., 2003). All cell types subjected to activation can release the microparticles, generated from blebs.

Figure 2.10   Microparticle Release
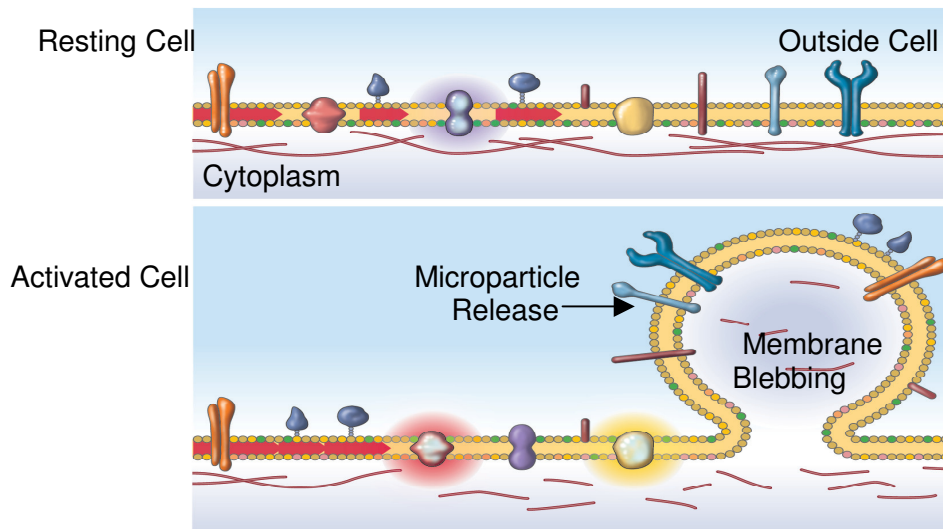
Source: Hugel, B. et al. (2004)

Endothelial cells shed Microparticles. The cell membrane blebs before pinching off as a microparticle.

Microparticle membranes consist mainly of lipids and proteins. Their composition depends on the cellular origin and the cellular processes triggering their formation (VanWijk et al., 2003). They have been shown to be elevated in a range of vascular disease states including atherosclerosis, angina, hypertension, pre-eclampsia and vasculitis (Van Wijk et al., 2003). It should be noted that a range of cells, including platelets, endothelial cells, leukocytes and erythrocytes shed microparticles. They are also elevated in non-vascular disease states including cancer (Martinez et al., 2005; Goon et al., 2006). Currently, there is considerable interest in their biological function and in their putative role as sensitive markers of disease activity (Malat et al., 2000; Bernal-Mizrachi et al., 2003).

EMPs carry with them many of the membrane antigens and phospholipids of the parent cell (Jimenez et al., 2003). These identification antigens are always present on the cell surface, and enable the determination of their cellular source (Jimenez et al., 2003). As they carry markers of the parent cell, including those induced by the activation or

apoptosis, EMPs can provide valuable information on the status of the parent cell (Malat et al., 2000; Jimenez et al., 2003; Martin et al., 2004). Microparticles of endothelial origin carry CD31 or CD146, with CD62E present on E-selectin, whereas CD4, CD3 or CD8 is present at the surface membrane of leukocyte microparticles (Malat et al., 2000; Martin et al., 2004). Furthermore, in vitro studies showed that EMPs from apoptotic endothelial cells were distinct from those from activated endothelial cells; in that activated endothelial cells release predominantly CD62E and CD54 EMPs whereas apoptotic EC are rich in CD31 EMPs (Jimenez et al., 2003).

Microparticles have been reported to be increased in patients with acute coronary syndromes (Malat et al., 2000; Bernal-Mizrachi et al., 2003), the metabolic syndrome (Arteaga et al., 2006) and obesity (Esposito et al., 2006). In addition, endothelial microparticles have been demonstrated to directly affect endothelial dependent dilation (Koga et al., 2005; Brodsky et al., 2004), and nitric oxide production (Brodsky et al., 2004).

2.14.2   Cellular Adhesion Molecules

Cellular adhesion molecules include E-selectins, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). VCAM-1 and ICAM-1 normally play an important role in homeostasis and host defence (Muro, 2007). However, under pathological conditions, dysregulation in their expression and/or function may intensify tissue damage and disease progression, as occurs in atherosclerosis (Muro and Muzykantov, 2005). E-selectin is a cell surface-bound leukocyte adhesion molecule specific to endothelial cells. It mediates the interaction between leukocytes, platelets and the endothelium (Milstone, 2007). Whereas, VCAM-1 and ICAM-1 are proteins expressed on the surface of activated endothelial cells, part of them are shed into the blood and can be detected as soluble VCAM (sVCAM) and ICAM (sICAM) (Cybulsky and Gimbrone, 1991).

Adhesion molecules are involved in the attachment and transmigration of leukocytes across the endothelial surface (De Caterina and Libby, 2007). Elevated levels of cellular adhesion molecules represent an early marker of several pathogenic processes associated with endothelial activation and the potential development of cardiovascular disease (Meigs et al., 2004). An increase in their expression on the endothelial surface

causes increased adhesion of leukocytes, particularly monocytes. It is well known that this is one of the first steps in the process leading to atherosclerosis (Libby, 2002; Hansson, 2005; De Caterina and Libby, 2007). Each adhesion molecule has its own specific role to play in the development of endothelial activation and the initiation of atherosclerosis. Their functions vary from binding monocytes and fibrinogen to allowing transendothelial migration of leukocytes.

Cellular adhesion molecules are frequently investigated to improve knowledge on endothelial function. Evidence exists that increased levels reflect an alteration of endothelial function. Gearing and Newman (1993) recognised that endothelial cells do not express E-selectin under normal physiological conditions. An increase in E-selectin on the endothelial surface is likely to be due to endothelial activation (Felmeden and Lip, 2003). DeCaterina et al. (1997a) found plasma concentrations of cellular adhesion molecules to be elevated in individuals with atherosclerosis and hypertensive individuals. Furthermore, levels of all these adhesion molecules in plasma have been shown to correlate to its membrane-bound expression (Leeuwenberg et al., 1992; Schram and Stehouwer, 2005). Therefore, they could be utilised as a surrogate marker of endothelial activation (DeCaterina et al., 1997a).

2.15 Exercise and Endothelial Function

The influence of exercise training has been investigated previously in healthy and clinical cohorts using a variety of methodologies including flow-mediated vasodilation, reactive hyperaemia, ACh mediated forearm blood flow and ACh mediated coronary blood flow. These studies are summarised in Table 2.3.

Exercise training appears to have relatively little or no impact when endothelial function is normal at baseline (Green et al., 1994; Kingwell et al., 1997; Maiorana et al., 2001) [Table 2.3; Table 2.4]. Endothelial function assessed in the forearm of healthy young men without cardiovascular risk factors did not change following 4 weeks of either daily handgrip training (Green et al., 1994) or cycling training (Kingwell et al., 1997). Similar findings were reported in healthy sedentary men and women after performing eight weeks of whole body exercise training (Maiorana et al., 2001). In contrast, Clarkson et al. (1999) conducted a 10-week combined aerobic and anaerobic training

programme with young healthy military men, which resulted in significant increases in flow-mediated dilation (Clarkson et al., 1999).

When endothelial function is impaired at baseline, exercise-training studies have demonstrated a positive effect on endothelial function [Table 2.3]. Rinder et al. (2000) found that endothelial-dependent dilation was greater in older active adults compared with age-matched healthy sedentary men. Moreover, regular aerobic exercise has also been shown to prevent the age related decline in endothelium-dependent vasodilation and restore the loss in endothelial-dependent vasodilation in middle-aged men (DeSouza, 2000). However, Goto et al. (2003) found that only moderate intensity exercise (50% $\dot{V}O_{2max}$) resulted in improvements in endothelial function.

Hambrecht et al. (2000; 2003) found that 4 weeks of aerobic exercise training improved endothelial function in patients with coronary artery disease. After 12 weeks of exercise training endothelial function significantly improved in hypertensive individuals (Higashi et al., 1999), asymptomatic men (Lavrencic et al., 2000), and post heart transplant individuals (Pierce et al., 2008). Hambrecht and colleagues also noted that coronary interventions, such as stenting, only treat a short segment of the vascular tree, whereas exercise exerts beneficial effects on endothelial function and disease progression in the entire arterial bed (Hambrecht et al., 2004).

Murray et al. (2006), Pierce et al. (2008) and O' Sullivan (2002) assessed endothelial dependent dilation by reactive hyperaemia. Differences were found in relation to resting forearm blood flow; Murray et al. (2006) demonstrated an increase in resting blood flow after two weeks of exercise training, while O'Sullivan (2003) reported no change after 5 weeks of training. There was more of a consensus in relation to peak reactive hyperaemic response. After one week of cycle training the peak conductance recorded during reactive hyperaemia increased by approximately 60%. After the second week of training the overall improvement was around 200% of that seen at pre-training (Murray et al. 2006). Peak reactive hyperaemic blood flow significantly increased from 7.8 ± 0.7 to 19.3 ± 3.3 ml/min/100ml tissue (p<0.01) in sedentary individuals after five weeks of cycling training (O'Sullivan, 2003). Similar results were found in the peak calf blood flow of post heart transplant patients (Pierce et al., 2008).

Table 2.3   Exercise and Endothelial Function Training Studies

Reference	Participants	Findings	Assessment of EDD
Higashi et al. (1999)	27 mild-mod hypertension 20-exercise, 7-controls	Increase in peak reactive hyperaemia after 5/7 X 30 min week^{-1} of brisk walking for 12 weeks with no change in controls	ACh-FBF (SGP)
Hambrecht et al. (2000)	19 elderly men with CAD	Increase in EDD of coronary artery after 6 x 10 min day^{-1} cycling at 80% MHR for 4 weeks.	ACh (CBF)
Murray et al. (2006)	30 males (21±2 y)	Increase in reactive hyperaemia by 60% after week 1 and to 200% after wk 2, with no further changes after 3 x 30 min cycling in week 1 at 60% $\dot{V}O_{2max}$ & 4 X 30 min sessions in the in following 3 wks	RH (SGP)
Maiorana et al. (2001)	16 Type II diabetics	Increase in FMD after 3 x 1hr sessions of whole body exercise for 8 week	ACh-FBF (SGP) & FMD
Maiorana et al. (2001)	17 Healthy sedentary men & 2 women; 47± 2 y	No change in FMD after 3 X 1h sessions of whole body exercise for 8 weeks	ACh-FBF (SGP) & FMD
Lavrencic et al. (2000)	30 asymptomatic men aged 40-60 y.	Increase in EDD after 3 X 50 min week^{-1}, cycling at 70% MHR for 12 weeks	FMD
Pierce et al. (2008)	20 subjects 8 weeks post heart transplant	Increase in peak reactive hyperaemia in the calf after 35-40 min of treadmill walking for 12 weeks.	RH (SGP)
Linke et al. (2001)	22 men ≤ 70 years with Chronic Heart failure	Increase in EDD after 6 x 10 min day^{-1} cycling at 70% $\dot{V}O_{2max}$ for 4 weeks.	FMD
Thijssen et al. (2005b)	10 SCI individuals 9 men and 1 woman	Increase in reactive hyperaemia in the thigh and increase in femoral artery diameter after 2/3 x 30 min whole body exercise training with 30 min FES of legs for 4 weeks	RH (SGP) & FMD

RH (SGP), Reactive Hyperaemia as assessed by strain gauge plethysmography; FMD, Flow Mediated Vasodilation; ACh (CBF) Acetylcholine mediated coronary blood flow as assessed by angiography; SCI, Spinal Cord Injuries; MHR, Maximum Heart Rate; CAD, Coronary Heart Disease; HRR, Heart Rate Reserve; FES, Functional electrical stimulation.

Table 2.3 Cont.

Reference	Participants	Findings	Assessment of EDD
Gokce et al. (2002b)	45 men and 14 women with CAD	Increase in EDD in tibial artery after 3 x 30-40 min week^{-1} cardiac rehabilitation - 45-85% HRR, for 10 weeks	FMD
DeSouza et al. (2000)	13 middle-aged and older sedentary men	Increase in forearm vascular conductance response to ACh improved after walking 40-45 min x 5-6 day at 70-75% for 3 months.	ACh-FBF (SGP)
O' Sullivan. (2003)	34 males; 18 fit and 16 sedentary.	Increase in peak reactive hyperaemia after 3 x 30 min week^{-1} cycling at 60% $\dot{V}O_2$ max for 5 weeks	RH (SGP)
Hambrecht et al. (2003)	35 males; 17 training 18 control	Increase in EDD in response to ACh after 3 x 10mins rowing and 3 x 10 min cycling daily for 4 weeks, with no change in controls	ACh (CBF)
Hambrecht et al. (1998)	Men over 70 years with Heart failure	Increase in EDD after 6 x 10 min day^{-1} cycling at 70% MHR for initial 3 weeks - 2 x 45 min session daily for 6 months	ACh-FMD
Goto et al. (2003)	26 men: 3 intensity groups	Increase in reactive hyperaemia in response to ACh after 12 wks 5-7 x 20 min Mod-intensity cycling. No change after mild- or high-intensity	ACh-FBF (SGP)
Green et al. (2004b)	15 Type 2, 8 CAD, 20 hypercholesterolemic	Increase in FMD and reactive hyperaemia in response to ACh after 3 x 45-60 min circuit training for 8 weeks in all patient types.	ACh-FBF (SGP) & FMD
Clarkson et al. (1999)	35 military males 20 matched civilian controls	Increase in FMD in brachial artery after aerobic and anaerobic exercise for 10 weeks, with no change in the controls	FMD
Kingwell et al. (1997)	13 Healthy men	No change in reactive hyperaemia after 3 x 1hour combined aerobic and resistance exercise for 4 weeks	ACh-FBF (SGP)

RH (SGP), Reactive Hyperaemia as assessed by strain gauge plethysmography; ACh-FBF (SGP), Acetylcholine mediated blood flow as assessed by strain gauge plethysmography; FMD, Flow Mediated Vasodilation; ACh (CBF) Acetylcholine mediated coronary blood flow as assessed by angiography; ACh-FMD Acetylcholine mediated blood flow as assessed by Flow Mediated Vasodilation; CAD, Coronary Heart Disease; HRR, Heart Rate Reserve; MHR, Maximum Heart Rate.

2.16   Exercise and Endothelial Activation

Few studies have examined the relationship between regular physical activity and markers of inflammation and activation. A cross-sectional study reported the highest versus the lowest level of activity associated with approximately 43% lower CRP, and 8% lower ICAM-1 (Mora et al., 2006). Moreover, the inverse relationship between physical activity and CVD risk was primarily explained by a decrease in inflammatory markers (Mora et al., 2007).

Two training studies have reported decreases in cellular adhesion molecules as a result of exercise training. Zoppini et al. (2006) reported that plasma ICAM-1 concentrations were clearly reduced; however the pro-inflammatory cytokine CRP did not change after 6 months of progressive aerobic exercise training. Adamopolous et al. (2001) reported a correlation between regular exercise and a chronic anti-inflammatory effect, with moderate reductions (20 to 30%) in CRP and soluble ICAM and VCAM (Adamopolous et al., 2001).

However, many of the studies reporting on the influence of exercise training on endothelial activation have shown no change in cellular adhesion molecules (Jilma et al., 1997; Pierce et al., 2008; Smith et al., 2000). Circulating levels of E-selectin, VCAM-1 and ICAM-1 have been shown to be unaffected by moderate exercise (Jilma et al., 1997) or resistance exercise (Smith et al., 2000) in healthy men. Moreover, Pierce et al. (2008) reported that 12 weeks of aerobic exercise training in heart transplant recipients resulted in no change in the plasma concentrations of ICAM-1 or CRP.

One study showed an increase in sICAM-1 after strenuous exercise (Akimoto et al., 2002). Research has shown that acute bouts of exercise result in a transient, mostly pro-inflammatory, increase in acute phase reactants and cytokines, proportional to the amount of exercise and muscle injury (Kasapis et al., 2005). Das (2004) reported an initial increase in pro-inflammatory cytokines such as TNF-α and IL-6 to be the stimulus for the increased generation of anti-inflammatory cytokines and reduction in cellular adhesion molecules (Das, 2004). To date, no study has examined the influence of exercise training on microparticles.

2.17   Mechanisms for Putative Exercise Effects

Prior studies have demonstrated favourable effects of physical activity on traditional cardiovascular risk factors (Mora et al., 2007). A large body of studies have shown exercise to reduce resting blood pressure, total body fat and abdominal fat, increase serum HDL cholesterol and decrease triglycerides, reduce blood platelet adhesiveness and aggregation, and reduce insulin needs and improve glucose tolerance (ACSM, 2006). It is plausible that the increase in endothelial dependent dilation may relate to a positive exercise influence on blood lipids.

Changes in endothelial function have been reported in the presence of improvements in traditional cardiovascular risk factors. An exercise induced decrease in forearm vascular resistance was accompanied with lowered serum concentrations of total cholesterol and LDL cholesterol (Higashi et al., 1999). In a cross-sectional study Kingwell et al. (1996) reported that endurance-trained athletes had greater vasodilatory responses as compared to a sedentary control group. These differences were linked to the level of serum cholesterol indicating that training-induced changes in endothelium function might be the result of reduction in total cholesterol level (Kingwell et al., 1996).

Changes in endothelial function have also been reported in the absence of improvements in traditional cardiovascular risk factors. Linke et al. (2001) reported that flow-mediated dilation increased by 50% and the vasodilatory response to acetylcholine (ACh) increased 2.5 fold after four weeks of cycle ergometry exercise in the absence of serum cholesterol, blood glucose and blood pressure changes. Others (Maiorana et al., 2001; Green et al., 2003) have also demonstrated an improvement in vascular function in the absence of changes in blood lipid concentrations. Green et al. (2003) found that exercise training improved reactive hyperaemia without improvements in lipids, blood pressure, and blood glucose or body mass index. These data suggest that the effects of exercise on cardiovascular risk factors do not solely mediate improvements in vascular function.

2.17.1   Exercise Training and Mechanical Forces

It is also possible that exercise can positively influence endothelial function by lipid-independent mechanisms. One possible mechanism by which exercise augments endothelial function is by repeated bouts of increases in vascular shear stress and cyclic

strain on the arterial endothelium resulting from the increase in cardiac output and arterial blood pressure that accompany whole-body exercise. Awolesi et al. (1995) conducted research on cultured aortic endothelial cells, which showed that cyclic strain up-regulates the expression of endothelial nitric oxide synthase (eNOS) transcripts (Awolesi et al., 1995).

Regular exposure to increased shear stress on endothelial cells that results form increases in blood flow during exercise in considered by many to be the primary signal for exercise training-induced adaptations of endothelial function and phenotype (Clarkson, et al., 1999; Harrison et al., 1996; Miller & Burnett, 1992). The increased stress of blood shearing against the vessels is a signal that causes vasodilation to reduce stress against the vascular wall. However, an intact endothelium is necessary for optimal regulation of shear stress (Niebauer, 1996). Stress of increased blood flow shearing against the endothelium layer produces vasodilatory factors, including NO, from the endothelium resulting in vasodilation of the smooth muscle cells (Joannides et al., 1995; White and Frangos, 2007; Harrison et al., 2006; Chien, 2007).

2.18   Existing Local vs. Generalised Exercise Effects in Endothelial Function

It is uncertain whether any improvement in endothelial-dependent dilation following exercise training is experienced by all arteries (generalised effect) or is confined to the arteries that serve the exercising muscle (local effect). This is an important question as vascular beds such as those in the carotid artery, which are prone to atherosclerosis (Talusan et al., 2005) may not experience increases in blood flow from exercise.

It is clear that intensive exercise training in isolated limbs results in considerable local EDD improvements, which have been highlighted through many handgrip training studies [Table 2.4]. Four weeks of handgrip training resulted in a significant improvement in the trained arm (Hornig et al., 1996; Alomari & Welsch, 2007) with a 19% increase in reactive hyperaemia been reported (Alomari et al., 2007). Similar results were reported after 8 weeks of handgrip training (Katz et al., 1997), with evidence to suggest that as little as one week of handgrip training exercise can improve reactive hyperaemia (Alomari & Welsch 2007).

Table 2.4   Handgrip Training and Endothelial Function Studies

Reference	Participants	Findings	Assessment of EDD
Green et al. (1994)	17 healthy young men	Increase in FMD of trained forearm after handgrip exercise 30 min day^{-1} for 4 weeks	FMD
Alomari & Welsch (2007)	17 males; 22.6 ± 3.5 y	Increase in reactive hyperaemia (19%) after 5 x 20 min handgrip training 1 contraction every 4 s @ 60% max, for 4 weeks	RH (SGP)
Katz et al. (1997)	10 men and 2 women	Increase in reactive hyperaemia and no change in untrained arm after 30 min day^{-1} 20 contractions min^{-1} @ 70% for 8 weeks	ACh-FBF (SGP)
Hornig et al. (1996)	12 with chronic heart failure, 7 age-matched controls 41± 8 y	Increase in FMD in trained arm - no change in untrained arm after 30 min x day^{-1} at 70% maximal workload for 4 weeks	FMD
Bank et al. (1998)	11 healthy, 7 HF patients	Increase in peak reactive hyperaemia in healthy only. But there was no change in heart failure patients after 30 min of handgrip x 4 days for 4-6 weeks.	ACh-FBF (SGP)

43

RH (SGP), Reactive Hyperaemia as assessed by strain gauge plethysmography; ACh-FBF (SGP), Acetylcholine mediated blood flow as assessed by strain gauge plethysmography; FMD, Flow Mediated Vasodilation; HF, heart failure

Table 2.5 Exercise Training Studies Indicating the Primacy of a Localised Exercise Effect

Reference	Participants	Findings	Assessment of EDD
Pierce et al. (2008)	20 patients 8 weeks post heart transplant	Increase in peak reactive hyperaemia in calf muscle after 35-40 mins of treadmill walking for 12 weeks. No change in forearm.	RH (SGP)
Motohiro et al. (2005)	44 patients with uncomplicated MI	Increase in reactive hyperaemia in the calf after 3 weeks exercise training. No change in the forearm	RH (SGP)
Kobayashi et al. (2003)	28 patients with Chronic HF	Increase in FMD in the tibial arteries after 2/3 d per week of cycle ergometry training for 3 months. No change in brachial artery.	FMD
Demopoulos et al. (1997)	16 patients with Congestive HF	Increase in peak reactive hyperaemia in the calf after cycle exercise 1 h x day, 4 times a week, for 12 weeks. No change in forearm	RH (SGP)

RH (SGP), Reactive Hyperaemia as assessed by strain gauge plethysmography; FMD, Flow Mediated Vasodilation; MI, myocardial infarction; HF, heart failure; CAD, coronary artery disease; HRR, heart rate reserve

Some researchers (Pierce et al., 2008; Motohiro et al., 2005; Demopoulos et al, 1997; Kobayashi et al., 2003) have reported the effect of exercise to be localised only and not experienced by all arteries [Table 2.5]. Improvements in endothelial function were seen in the calf but not in the forearm after 12 weeks of walking in heart transplant patients (Pierce et al., 2008) and cycling in patients with severe congestive heart failure (Demopoulos et al., 1997). Kobayashi et al. (2003) reported a significant increase in pre to post FMD in the lower limbs (3.64 ± 0.26 vs. 6.44 ± 0.56%, p<0.01) with no change in the upper limbs (4.34 ± 0.45 vs. 4.56 ± 0.43%) after three months cycle ergometry exercise training.

Research has also reported improvements in brachial artery EDD as a result of lower limb exercise training [Table 2.6] (Higashi et al., 1999; Goto et al., 2003; O'Sullivan, 2003; Maiorana et al., 2000; Murray et al., 2006). These studies were not designed to test for a generalised effect, but they may be interpreted to indicate a generalised effect of exercise training in EDD. An increase in forearm reactive hyperaemia was reported after 12 weeks of moderate intensity cycle ergometry training (Goto et al., 2003) and brisk walking (Higashi et al., 1999), with one study showing a 200% increase in forearm reactive hyperaemia after two weeks of cycle ergometry training at 60% $\dot{V}O_{2max}$ (Murray et al., 2006).

Thijssen and Hopman (2008) note that studies that showed an improvement in endothelial function in the inactive forearm after lower limb exercise mainly involved walking and running exercise, which involves upper body movement, hence, the forearm vascular bed is somewhat active (Thijssen & Hopman, 2008). These authors argue that exercise does not bring about vascular adaptations beyond the active muscles. However the results of a recent study indicate that increases in blood flow during exercise can occur in perfectly still inactive limbs. Tanaka et al. (2006) assessed the changes in femoral blood flow and brachial blood flow from an exercise test using an arm cycle ergometer and a leg cycle ergometer, respectively. Each test was adjusted to minimise any involuntary movement of the non-exercising limbs. There was a 3.5 fold increase in femoral blood flow during the arm cycle test, while during the leg cycle test there was a 4-fold increase in brachial blood flow.

From the results of the aforementioned exercise training studies, it is still not clear whether the exercise induced effects are generalised or localised. What is evident from

the above literature is that all the exercise-training studies have either reported on the effect of lower limb exercise on the upper vasculature or the localised effect of one arm handgrip training. To date, no study has compared the changes in forearm EDD that result from a combined generalized and local exercise stimulus to changes resulting from a generalized stimulus only, while eliminating all movement in the unexercised limb.

Table 2.6   Exercise Training Studies Indicating an Increase in EDD in Inactive Vascular Beds

Reference	Participants	Findings	Assessment of EDD
Clarkson et al. (1999)	35 military males 20 matched civilian men	Increase in FMD in brachial artery after 10 weeks of daily 3-mile runs	FMD
Goto et al. (2003)	26 men: 3 intensity groups	Increase brachial artery response to ACh only after 5-7 x 20 mins moderate intensity cycling for 12 weeks	ACh-FBF (SGP)
Hambrecht et al. (2003)	35 males; 17 training 18 control	Increase in EDD in response to ACh after 3 x 10mins rowing and 3 x 10 min cycling daily for 4 weeks	ACh (CBF)
Higashi et al. (1999)	27 mild-mod hypertension 17 Normotensive	Increase in peak reactive hyperaemia in the forearm after 5/7 d X 30 mins week^{-1} of brisk walking for 12 weeks	ACh (SGP)
Maiorana et al. (2001)	16 Type II diabetics	Increase in FMD in the brachial artery after 3 x 1hr sessions of exercise concentrating on the lower limbs, for 8 weeks	ACh-FBF (SGP) & FMD
DeSouza et al. (2000)	13 middle-aged and older sedentary men	Increase in forearm vascular conductance response to ACh in both middle-aged and older sedentary men after walking 40-45 mins x 5-6 day at 70-75% for 3 months.	ACh-FBF (SGP)
O' Sullivan (2003)	34 males; 18 fit controls and 16 sedentary.	Increase in peak forearm reactive hyperaemia in sedentary men after 3 x 30 minutes week^{-1} cycling at 60% $\dot{V}O_2$ max for, 5 weeks	RH (SGP)

RH (SGP), Reactive Hyperaemia as assessed by strain gauge plethysmography; ACh-FBF (SGP), Acetylcholine mediated blood flow as assessed by strain gauge plethysmography; ACh (CBF) Acetylcholine mediated coronary blood flow as assessed by angiography; FMD, Flow Mediated Vasodilation; HF, heart failure; EDD, Endothelial dependent dilation.

Table 2.6 cont.

Reference	Participants	Findings	Assessment of EDD
Lavrencic et al. (2000)	30 asymptomatic men aged 40-60 y.	Increase in forearm EDD after 3 X 50 min week^{-1}, cycling at 70% MHR for 12 weeks	FMD
Linke et al. (2001)	22 men ≤ 70 y with Chronic HF	Increase in EDD in radial artery after 6 x 10 min day^{-1} cycling at 70% $\dot{V}O_2$max for 4 weeks.	FMD
Murray et al. (2006)	30 males (21 ± 2 y)	Increase in reactive hyperaemia in the forearm to 200% after wk 2, with no further changes after 3 x 30 min cycling in week 1 at 60% $\dot{V}O_2$max & 4 X 30 min sessions in the in following 3 wks	RH (SGP)

RH (SGP), Reactive Hyperaemia as assessed by strain gauge plethysmography; ACh, Acetylcholine; FMD, Flow Mediated Vasodilation; HF, heart failure; MHR, Maximum Heart Rate; CAD, Coronary artery disease; EDD, Endothelial dependent dilation

2.19   Summary of Exercise Evidence

In summary, exercise has been shown to play an important role in relation to endothelial cell function.   Accordingly exercise training has been highlighted as improving endothelial dependent dilation, primarily when it is impaired at baseline. However, the mechanism for the exercise induced effects remains unclear. One possible method is an increase in shear stress and cyclic strain on the arterial endothelium resulting from the increase in blood flow, cardiac output and arterial blood pressure that accompany exercise. Uncertainties surround whether any improvement in endothelium-dependent dilation following exercise training is experienced by all arteries (generalised effect) or is confined to the arteries that serve the exercising muscle (local effect).   It is possible that the improvements in EDD may relate to a positive exercise influence on traditional cardiovascular risk factors, improvements have been reported with and without exercise induced improvements in risk factors. The balance of studies reporting on the influence of exercise training on endothelial activation have shown little or no change in cellular adhesion molecules, with no study having examined the influence of exercise training on microparticles.

Chapter 3

Methodology

3.0 Purpose of Study

The purpose of these studies were to:

1. Establish a protocol for the assessment of reactive hyperaemia with a strain gauge plethysmograph and to determine the reproducibility of this technique.

2. Examine the hypothesis that any exercise effect in endothelial dependent dilation is localised to vascular beds in the exercised limb.

3. Determine the effect of short-term aerobic training on the concentrations of adhesion molecules and cell-derived microparticles.

3.1 Overview of Work

The preliminary work for this study involved the development and validation of the protocol for the assessment of reactive hyperaemia. The initial experiments were in relation to the adjustment of various settings, including time intervals and occlusion pressures. A reproducibility of blood flow measurements was then undertaken. This study involved 15 volunteers that were assessed twice on the first testing day, fifteen minutes apart, for determination of short-term reproducibility and again a week later to assess medium-term reproducibility.

The primary work for this study involved a novel research design involving two weeks of two legged cycle-ergometry exercise training combined with one arm handgrip exercise. Reactive hyperaemia was assessed before and after the exercise intervention, in both the handgrip trained arm and the control arm. During all the exercise sessions the control arm was kept perfectly still. Blood samples were also taken before and after the training intervention, with the post-training samples taken on the morning after the last exercise session. These samples were used for the determination of lipid concentrations and biomarkers of endothelial activation.

3.2 Inclusion and Exclusion Criteria

Subjects were recruited to participate in this study, at Waterford Institute of Technology, as a result of posters [Appendix A], emails and word of mouth in the local community. Overweight men (BMI > 25 kg/m^2) between the ages of 30 and 45 y who

were self reported as inactive (< 20 min of moderate physical activity, 3 times per week) were recruited to participate. Smokers, and individuals with more than one cardiovascular disease risk factor, or with the known presence of cardiovascular disease, and those taking medication known to influence carbohydrate or lipid metabolism were excluded.

Young, lean, highly active individuals were excluded as endothelial function may only be improved following exercise training in individuals with impaired endothelial dependent dilation. Impaired reactive hyperaemia has been associated with higher blood pressure, old age, male gender, insulin resistance, obesity, and lipid abnormalities (Gokce et al., 2002).

3.3   Ethics, Screening and Consent

Ethical approval of all methods and procedures detailed herein was obtained from the Research Ethics Committee at Waterford Institute of Technology. Before conducting any preliminary tests, participants were screened. Along with the standard screening protocols, a health questionnaire was completed to exclude cardiorespiratory, musculoskeletal or haematological abnormalities and to ensure there were no contraindications to maximal exercise testing (Appendix B). Activity levels were assessed using a physical activity questionnaire (Appendix C). After comprehensive explanation of the proposed study, its benefits, inherent risks and expected commitments, all participants signed an informed consent (Appendix D). Each participant gave full, free and informed consent to take part in this research. Each participant was given a copy of the signed informed consent form and an information sheet containing a brief explanation of the procedures and a reminder of what testing was taken place on each day (Appendix E).

3.4   Characteristics of Subjects

Ten men participated in this study; from this nine completed the research (n=9). One participant did not complete the study due to lack of compliance.

Table 3.1   Subject Characteristics

	Pre-Training	Range
Age (y)	34 ± 2	30 - 44
Height (cm)	177.1 ± 2.2	164 - 186
Weight (kg)	94.1 ± 5.2	69 - 118
BMI (kg$\cdot$m^{-2})	29.85 ±1.4	26 - 39
Waist Circumference (cm)	99.6 ± 2.8	91 - 117
HR$_{rest}$ (bpm)	102 ± 4.9	96 - 137
$\dot{V}O_2{}_{max}$ (ml$\cdot$min$^{-1}\cdot$kg^{-1})	33.5 ± 1.6	27 - 43
SBP$_{rest}$ (mmHg)	132 ± 3	120 – 140
DBP$_{rest}$ (mmHg)	83.1 ± 1.5	78 – 90
% Body Fat	22.8 ± 1.4	17.4 – 30.8
Values are mean ± SEM; BMI, body mass index; HR$_{rest,}$ heart rate at rest; SBP$_{rest,}$ Systolic blood pressure at rest; DBP$_{rest,}$ Diastolic blood pressure at rest.		

3.5   Measurement Techniques

Waist circumference was determined by placing a measuring tape around the abdomen, above the hipbone ensuring that the tape measure was horizontal and snug but not causing compressions on the skin. In order to assess body mass index, height was recorded with a SECA Leicester Portable Height Measure (Seca Ltd. Birmingham) and weight was measured (kg) using a SECA 700 mechanical column scale (Seca Ltd. Birmingham). From these two measurements Body Mass Index (BMI) was calculated, Weight (kg) / [height (m)^2].

3.5.1   Dual Energy X-Ray Absorptiometry (DEXA)

Percentage body fat was determined for each subject using this gold standard method from a low radiation DEXA scan (Norland XR-46, Norland Medical Systems, NY, USA). DEXA technology, originally developed to measure bone mineral density, can be used for direct assessment of fat mass. The attenuation by tissues of two X-ray intensities is determined and compared to known values for fat and lean tissue.

The subject was placed lying supine on the DEXA bed, ensuring that they were within the scanning limits [Figure 3.1]. A laser diode (red 670 nm, < 0.2 mW) was used to mark a point 1 cm above the centre of the subject's head. The laser dot was then positioned at a point on the abdomen adjacent to the spine and midway between the lowest rib and the iliac crest. The position was marked in the area of maximum soft tissue and no bone. The scan was started and the participant was scanned from head to toe. The scan took between four and five minutes. Figure 3.2 illustrates a body scan obtained using the DEXA from a participant with a percentage body fat of 30%.

Figure 3.1.   Subject positioned on DEXA
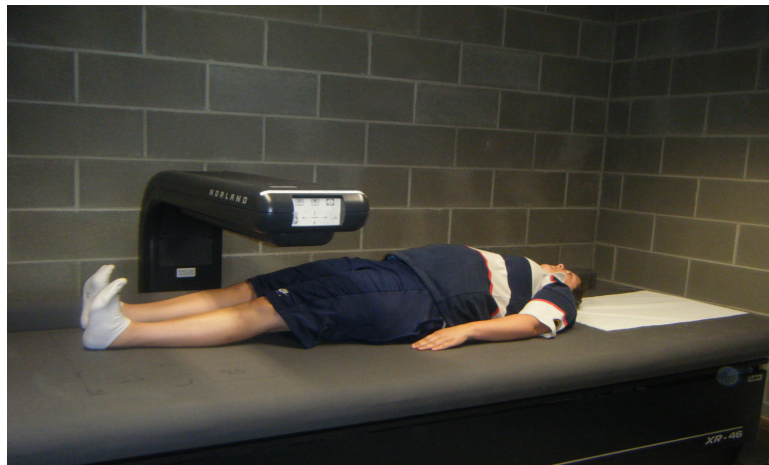
Figure 3.2   DEXA Scan
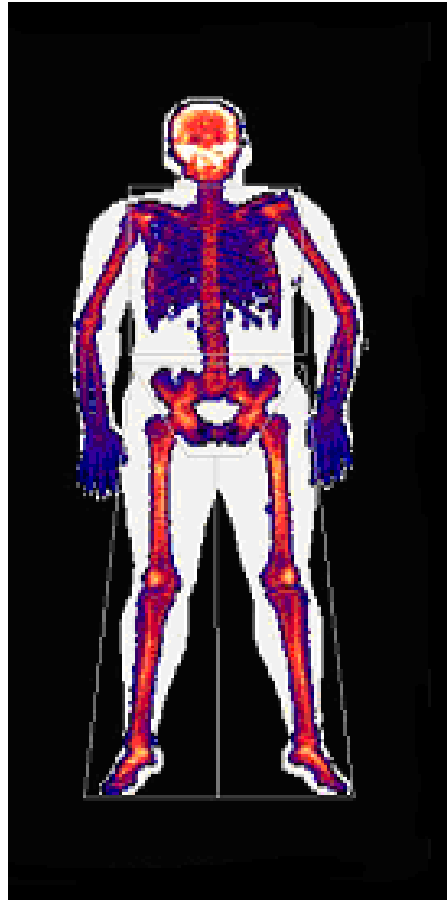
3.5.2   Blood Pressure

Blood Pressure (BP) was measured indirectly by auscultation. For this, a stethoscope and a sphygmomanometer consisting of a blood pressure cuff and a mercury column were used. The participant was seated in a quiet room for approximately five minutes. The cuff was wrapped firmly around the bare upper arm so that the lower edge of the cuff was about 2.5cm above the antecubital fossa. The head of the stethoscope was then placed over the brachial pulse and the cuff was inflated. The valve was then slightly opened and released slowly. The first clear Korotkoff sound was noted as the systolic pressure and when the sound disappeared the diastolic pressure was recorded.

3.5.3 Maximum Oxygen Uptake

Maximal oxygen uptake is the highest $\dot{V}O_2$ value recorded during maximal exercise. $\dot{V}O_{2max}$ can be reported in absolute terms (L/min) or relative to body mass (ml·min^{-1}·kg^{-1}). Participants performed a maximal incremental exercise test on a cycle ergometer before and after the exercise intervention. Before the test commenced the procedure was explained and participants were instructed to continue exercising until exhaustion. Participants were instructed not to consume caffeine or alcohol in the twenty-four hours before testing, which took place between 09.00-14.00h at an ambient temperature of 20-22°C.

Maximum oxygen uptake was determined using an incremental exercise test on a cycle ergometer (Corival, Cycle Ergometer). After adjustment of the seat height for individual requirements, the headgear and heart rate monitor were put in place and a nose clip was used to ensure all the air was being inspired and expired through the metabolic system. Figure 3.3 illustrates an individual ready to perform a maximal exercise test. Participants rested on the cycle ergometer for five minutes before the testing commenced to ascertain resting values.

Figure 3.3   Subject in Position for a Maximal Exercise Test

Expired air was collected continuously for determination of VO_2 and VCO_2 via a metabolic system, a MOXUS modular metabolic system with a mixing chamber (AEI Technologies, Naperville, IL). The participant wore headgear, which contains a Hans Rudolph non-re-breathing valve. Before each test was conducted the calibration of the oxygen and carbon dioxide analysers, using room air and a known gas concentration (16% O_2 and 4% CO_2) was performed.

The initial workload and increments were individualised so that each test was 8 - 12 min in duration. The test consisted of a sub-maximal section with 3 min stages. A Borg scale [a scale of 6-20 where 6 means "no exertion at all" and 20 means "maximal exertion" (Borg, 1998)] was used to measure perceived exhaustion at each stage. When the participant reached 15 on the Borg scale the stages were changed to 2 min increments, exercising at increasing work rates, until they reached maximal exercise capacity. The purpose of this was to calculate 70% of the $\dot{V}O_{2peak,}$ which was determined from the relationship between the workload and the oxygen uptake during the submaximal section of the exercise test. This protocol was also used for the post-intervention assessment of maximal oxygen uptake.

The most widely accepted criterion for the achievement of $\dot{V}O_{2max}$ is a plateau in $\dot{V}O_2$ as the work rate continues to increase (Maud and Foster, 2006). However, less than 50% of subjects to undergo testing demonstrate a plateau (Maud and Foster, 2006). Subjects were regarded as achieving a maximum if at test termination, the respiratory exchange ratio was greater than 1.10 and heart rate was within 10 beats of age-predicted HR max.

3.5.4 Handgrip Strength Testing

Prior to and at the end of the two-week intervention, participants underwent handgrip strength testing in the dominant and non-dominant arm. Handgrip strength was evaluated using a calibrated handgrip dynamometer (Takei, model T.K.K. 5101, Tokyo, Japan), with the participant upright, with their arms by their side. The test involved an all out gripping effort, without movement of the arm, held for 3 s. The average of three consecutive trials was used as the measure of strength.

3.6 Measurement of Forearm Blood Flow

Vascular function indices were obtained in the dominant and non-dominant forearms using mercury strain gauge plethysmography before and after the exercise intervention. Forearm blood flow is assessed with this method by placing an occlusion cuff on the upper arm, inflated to a pressure greater than venous pressure and lower than diastolic blood pressure that is 50 mmHg.

This allows blood flow into the arm but restricts outflow. An occlusion cuff is also placed on the wrist to restrict blood flow to the hand, ensuring that all blood flow is to the muscle in the forearm. Strain gauges connected to the EC6 plethysmograph (Hokanson Inc, Bellevue, WA) are placed on the widest part of the forearm to detect changes in diameter and measure percent change in volume that occur as a result of the increases in blood flow. Figure 3.4 illustrates an individual's arm prepared for the forearm blood flow measurement using strain gauge plethysmography.

Figure 3.4   Subject in Position for Measurement of Forearm Blood Flow
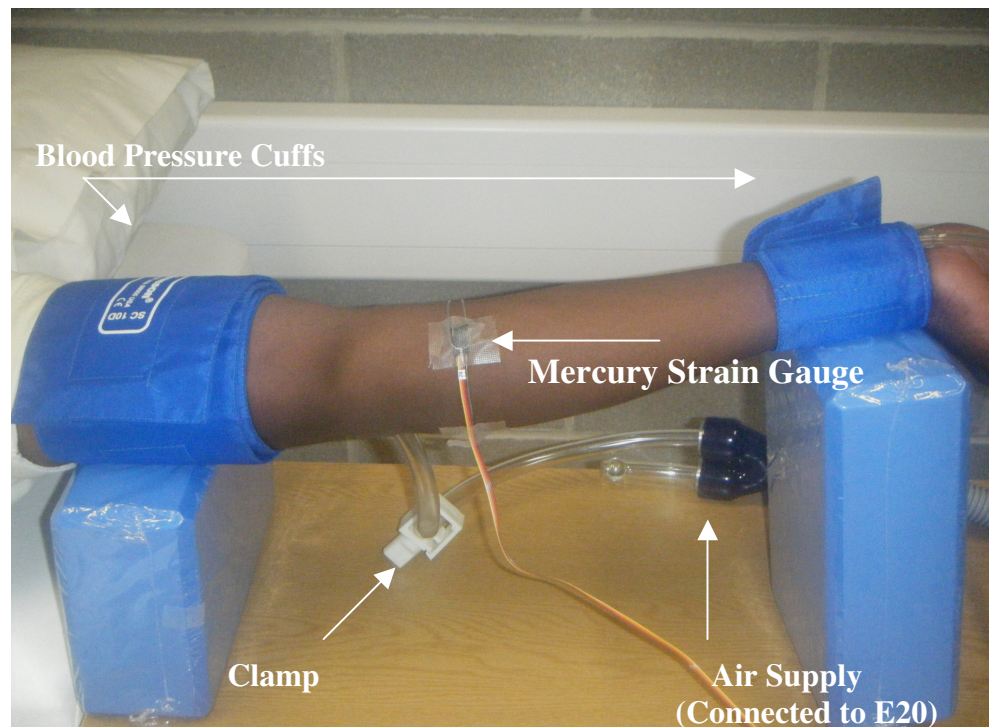
The forearm is extended and slightly supinated using Styrofoam blocks so the elbow is at heart level and the hand is at a comfortable height to allow forearm venous drainage. Blood flow is recorded every 10 s, with the cuff inflated and the percent change in volume recorded for 6 s and then deflated for 4 s.

Immediately before the resting blood flow measurements are recorded, hand circulation is occluded for one minute by inflating the wrist cuff to 240 mmHg. The upper arm cuff is then inflated to 50 mmHg and resting forearm blood flow is recorded, this procedure is repeated 10 times and the mean of the results is used.

3.6.1   Determination of Reactive Hyperaemia

Arterial occlusion results in considerable increases in forearm blood flow when cuff deflation occurs, termed reactive hyperaemia. Arterial forearm blood flow returns to resting values over a 1-3 minute period. Blood flow is measured at rest and every 10 s post occlusion for 200 s using mercury-filled strain gauge plethysmography.

Figure 3.5 is an example of blood flow measurements obtained at rest and after ischaemia using strain gauge plethysmography. The graph highlights the results for each time point during reactive hyperaemia, which was measured for 200 s. This figure also highlights the various indices, which are used in relation to vascular function as assessed by strain gauge plethysmography. These indices are:

1.   Peak Reactive Hyperaemia
2.   Total Reactive Hyperaemia
3.   Duration of Reactive Hyperaemia

Figure 3.5   Forearm Blood Flow Assessed by Strain Gauge Plethysmography (n=1)
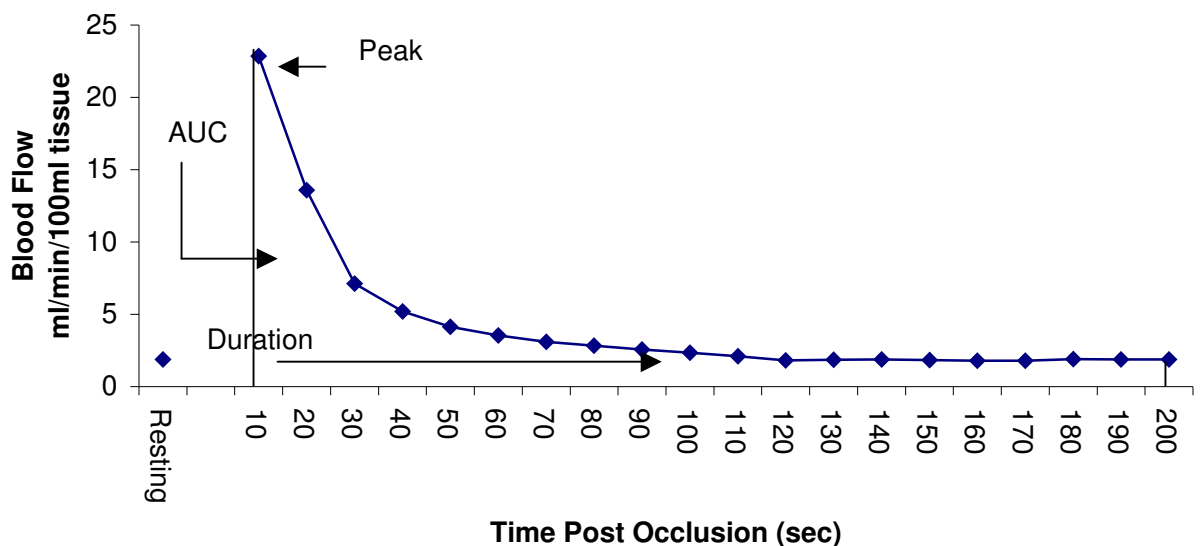
Peak reactive hyperaemia is the maximum blood flow measurement observed during reactive hyperaemia, usually the initial post-occlusion reading. The duration of reactive hyperaemia is the time from the peak reactive hyperaemic response until blood flow returns to the resting blood flow measurement. Total reactive hyperaemia represents the area under the blood flow vs. time curve (AUC) and reflects both peak reactive hyperaemia and the duration of reactive hyperaemia. Higher flow values indicate better vascular function.

3.6.2 Technique for Reactive Hyperaemia Measurements

The protocol for measuring reactive hyperaemia and resting forearm blood flow can be found in Appendix F. This method of strain gauge plethysmography is similar to that shown by Sinoway et al. (1986) to be optimal for the assessment of vascular function. Participants were reminded to refrain from activities for the days prior to the pre-training measurements. All assessments were conducted in a controlled laboratory between 08:00 and 11:00h at an ambient temperature of 20-22°C. The following steps were taken:

After resting forearm blood flow (FBF) was measured, a five-minute rest period was observed, before assessing reactive hyperaemia.

1. The upper arm cuff was then inflated to 30 mmHg above the systolic blood pressure for five minutes to induce ischaemia.

2. After the first minute the cuff was clamped [Figure 3.4] to maintain the suprasystolic pressure and the E20 rapid cuff inflator (Hokanson Inc, Bellevue, WA) was reset to 50 mmHg, for the next set of measurements.

3. Again, immediately before the blood flow measurements hand circulation was occluded for one minute by inflating the wrist cuff to 240 mmHg.

4. On the fifth minute mark the clamp was removed and flow measurements were recorded for the subsequent 200 s to assess peak and duration of reactive hyperaemic blood flow.

The two procedures were repeated after a fifteen-minute rest period was observed and the measurements were averaged. The measurements were then conducted in the participants opposite arm.

3.7   Exercise Intervention

Following the completion of the initial assessments, each participant commenced a two-week exercise intervention. The intervention involved 10 exercise sessions, 5 of which took place during week one. During the second week of the intervention subjects exercised on 5 consecutive days leading into the final post-training measurements. The exercise sessions involved two legged cycling combined with one-arm handgrip training. Thus both forearms were subjected to a generalised exercise effect, with one forearm also subjected to a localised effect. Vascular function was measured in both the handgrip-trained forearm and the control arm pre and post the intervention.

3.7.1   Exercise Session

Each exercise session took place at the exercise physiology laboratory at Waterford Institute of Technology, between 14:00 and 18:00. During the first week the participants exercised for 30 min at 70% $\dot{V}O_{2peak}$, which increased to 45 min at 70% $\dot{V}O_{2peak}$ during the second week. The 70% $\dot{V}O_{2peak}$ workload was determined from the relationship between the workload and oxygen uptake from the maximal exercise test.

The exercise session involved cycling on a cycle ergometer (SECA Cardiotest 100, Cranlea, UK) combined with simultaneous handgrip exercises using a F990 Max Calibrated Hand Grip (Country Technology Inc, Wisconsin). Figure 3.6 depicts a research participant using the handgrip during an exercise session with the untrained arm being rested and unused. Each participant performed the handgrip exercises during every exercise session for at least half of the total duration. Therefore, the handgrip was handed to the participant every 5 minutes and used for two and a half minutes. The handgrip exercises were conducted at a cadence of 1 contraction every 2 s at an intensity of 40% of maximum handgrip strength. The allocation of the dominant and non-dominant arm to the handgrip training or control condition was counterbalanced.

Figure 3.6   Subject Using the Handgrip During an Exercise Session

3.7.2   Calorie Compensation and Meal Timing

The participants completed dietary questionnaires [Appendix G] for the three days prior to the initiation of the present study. This dietary protocol was then repeated over the 3 d prior to the post testing. The final exercise session was set at a time to ensure subjects consumed one meal after the last exercise bout in advance of the post-testing.

In order to replenish the calories from the final exercise sessions, biscuits were given to each participant that corresponded to their individual calorie loss from the exercise session. Each biscuit used was 70 kcal; biscuits corresponding to half of the calories lost were consumed before the last exercise session with the other half eaten after the exercise session.

To ascertain the caloric cost of each participants final exercise session the ACSM metabolic equation for leg cycling was utilised (ACSM, 2006), which is shown as Equation 3.1.

Equation 3.1:

$\dot{V}O2$ (ml·min^{-1}·kg^{-1}) =   1.8 (work rate) / (Body Mass) + 3.5 ml·min^{-1}·kg^{-1} + 3.5 ml·min^{-1}·kg^{-1}

3.7.3 Outcome Measures

The outcome measures for the present study were:

1. Indices of vascular function (Peak reactive hyperaemia, duration of reactive hyperaemia and total reactive hyperaemia)

2. Blood lipids and glucose

3. Biomarkers of endothelial activation

3.8 Blood Sampling and Storage

Venous blood samples were taken before and after the two-week exercise intervention. Fasting blood samples were obtained from the antecubital vein between 08.00 and 09.30 h, using a 21 G needle. Blood samples were obtained with participants in a seated position for 5 min and with legs uncrossed. As suggested by Biro et al. (2004) prolonged use of a tourniquet was avoided for the later analysis of microparticles, hence each tourniquet was removed as soon as the last vacutainer was attached to the luer.

Post-training fasting blood samples were taken approximately 16 hours after the last exercise session. Thus, the post-training sample will reflect both the effect of training, albeit short-term, and the effect of the last exercise bout. Ten exercise sessions are too few in any case to reflect a "true" training effect. The vascular health of regularly active individuals likely relates to the effects of recent exercise bouts in a trained vasculature, hence the post-training assessments. The partitioning of exercise effects into acute and chronic is primarily relevant to changes in lipid profiles, which have been demonstrated to be affected acutely by exercise (Crouse et al., 1997). No evidence exists that reactive hyperaemia; endothelial microparticles or cellular adhesion molecules are subject to a last bout affect.

Serum vacutainers (Vacutainer systems, Becton Dickinson) were allowed to stand for 30 min before centrifugation. Within two hours the serum and EDTA vacutainers (Becton Dickinson) were centrifuged (Sigma 3-16PK, Germany) at 3000 rpm (1500 g) for 15 min and then stored rapidly at -80°C, for between 2 and 4 months.

Sodium citrate vacutainers (Becton Dickinson) for microparticle analysis were collected, prepared and stored according to the double centrifugation method described by Dignat-George et al. (2004). Within 2 hours, whole blood samples were centrifuged

for 15 min at 1,500 g at room temperature to prepare platelet rich plasma (PRP). This was harvested from the top leaving a 0.5 cm layer of plasma undisturbed close to cell debris. The PRP was further centrifuged for 2 min at 13000 g to obtain platelet-poor plasma (PPP). Samples were aliquoted and then immediately frozen at -80°C until used. This protocol allows delayed analysis on -80°C frozen plasma, and reduces variability of samples due to microparticle loss (Dignat-George et al., 2004).

3.9   Biochemical Analysis

Blood to be used for a complete blood count was collected into a vacutainer tube containing EDTA (Vacutainer systems, Becton Dickinson) and analysed by an automated haematology analyser (A^{c}Tdiff, Beckman Coulter, USA). The protocol for the haematology analysis can be found in Appendix H.

3.9.1   Blood Chemistries - Clinical Chemistry System

Serum triglycerides, total cholesterol, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and glucose were determined via spectrophotometric assays, performed on an automated clinical chemistry system (ACE®, Alfa Wassermann B.V., Netherlands) using appropriate reagents, calibrators and controls (Randox Laboratories, UK). The protocol for the use of calibrators and controls can be found in Appendix I.

Calibrators were used to establish the relationship between the optical density and analyte concentrations. Once all the calibrator and control results were within the acceptable ranges the analyser was then ready to be operated. The coefficient of variation for all spectrophotometric assays was less than 3%.

3.9.2   Biomarkers of Endothelial Activation - Assay

Serum concentrations of soluble VCAM-1 (sVCAM-1), sICAM-1, CRP, sE-selectin, sP-Selectin, and Thrombomodulin were determined in duplicate using multi-array immunoassay technology, Vascular Injury Panel I and II from MSD Systems (Meso Scale Discovery (MSD), Maryland, USA). This immunoassay system uses electrochemiluminescence signals to detect binding events on 96 well multi-spot plates. The coefficients of variation (CoV) for these assays are shown in Table 3.3a and 3.3b.

Table 3.2a Mean Coefficient of Variation for Duplicates - Vascular Injury Panel I

Analyte	CoV (%)
Thrombomodulin	5.4
sICAM-3	5.09
sE-Selectin	6.9
sP-Selectin	8.5

Table 3.2b Mean Coefficient of Variation for Duplicates - Vascular Injury Panel II

Analyte	CoV (%)
Serum Amyloid A	3.75
C-Reactive Protein	3.9
sVCAM-1	3.7
sICAM-1	7.1

The protocols for both plates can be found in Appendix J.

3.9.3   Microparticles - Flow Cytometry

Microparticle counts were quantified by flow cytometry (Cytomics FC500, Beckman Coulter). Individual cells/particles held in fluid are passed through laser beams that cause light to scatter and fluorescent dyes to emit light at various frequencies. The light scatter is differentiated into forward and side scatter and is used for preliminary identification of cells. Forward scatter (FS) is an indication of cell size, whereas side scatter (SS) is as a result of cell granularity. Fluorescent labelling allows investigation of microparticle membrane content. Based on the proteins it is possible to distinguish microparticles from different parent cells. In addition, the use of Annexin V, a fluorescent dye that binds to negatively charged phospholipids can determine if the cell shedding the microparticle is undergoing apoptosis.

3.9.4   Flow Cytometer Standardisation

Flow cytometry protocols vary with each flow cytometer and laboratory. Thus, in an effort to standardise the flow cytometer settings, the method proposed by Robert et al.

(2008) and currently being used by the International Society for Thrombosis and Haematosis in the attempt to standardise microparticle counts across instruments, was used in this research.

The standardisation protocol was set up as detailed by Robert et al. (2008) [Appendix K]. In brief, to measure microparticles between 0.5 and 1 μm, fluorescent Megamix beads (Biocytex, Marseille, France) of 0.5 μm and 0.9 μm diameters were used to establish a gate based on size.

Approximately two thirds of the beads in the mix are 0.5 μm in diameter [Figure 3.7]. The discriminator on the forward scatter is set to 1 to ignore small events. The voltage on the FS is then adjusted downwards moving the cluster of 0.5 μm beads into the discriminator region. Eventually the cytometer will detect a ratio of 50%: 50% between 0.5 and 0.9 μm. The histogram of the distribution of both beads was then developed and can be seen in Figure 3.8.

Figure 3.7   Selection of Megamix Beads Subsets
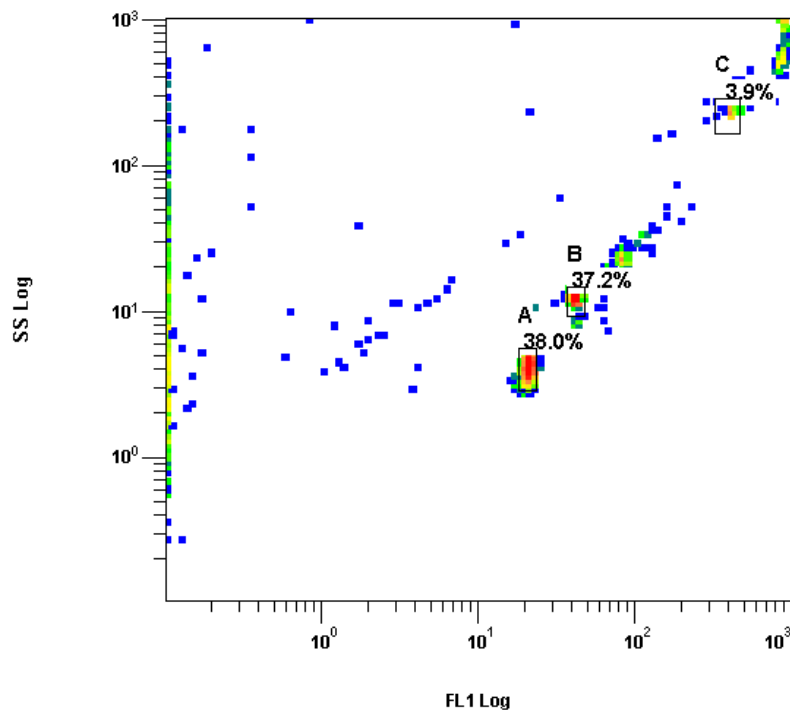
Figure 3.8   Forward Scatter Distribution of Megamix Beads
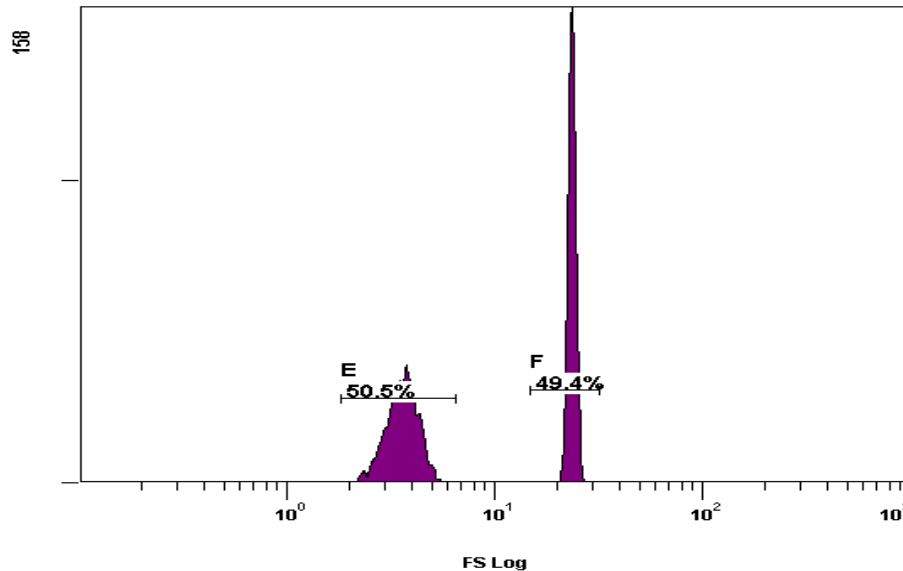
This is the optimal middle ground between the best possible microparticle detection and background excess with an acceptable range of 48-52%. As is evident form Figure 3.8 the results 50.5% 0.5 μm and 49.4% 0.9 μm are within the accepted range. This is a means of ensuring that all flow cytometers are measuring the same.

3.9.5   Microparticle Analysis

In the present research microparticles were identified in platelet poor plasma based on size and fluorescence. The method used is the 2-colour combination of phycoerythrin (PE)-labelled anti-CD62E (Becton Dickinson) with fluorescein iosthiocyanate (FITC) labelled Annexin V (Becton Dickinson) and PE - labelled anti-CD41 (Becton Dickinson).

The procedure for the platelet microparticle and endothelial microparticle analysis is similar except for the use of different antibodies, which is CD41 for platelet microparticles and CD62E for endothelial microparticles. The procedure involved adding 10 μL of the specific antibody and 20 μL of Annexin V (FITC) diluted with binding buffer, (10 μL Annexin V and 10 μL binding buffer), to 30 μL of sample. These samples were then incubated for 30 minutes in the dark before adding 500 μL of binding buffer. The samples were then added to the carousel run on low speed, which took 60 s per sample.

The flow cytometer is set to express counts per minute; therefore in order to express microparticle counts as absolute numbers per micro litre of sample, a flow count was undertaken. In order to do this 30 µL of Flow Count Beads with a known concentration of 972 beads per µL (Flow Count™ Fluorospheres, Beckman Coulter) was added to the sample. The calculated flow rate, at low flow was 10.11 µL per minute.

3.10 Statistical Analysis

Parametric tests were used for all analyses as the differences between pre-intervention and post-intervention data were normally distributed, using the Kolmogorov-Smirnov test.

The significance of summary reactive hyperaemia changes (i.e. peak reactive hyperaemia, duration of reactive hyperaemia and AUC) in the handgrip-trained arm relative to the changes in the control arm was determined via the interaction term of a fully repeated measures (arm x time) 2-way analysis of variance. Where this term was significant, the influence of the intervention was determined separately for the handgrip trained and control arms using post-hoc pairwise comparisons with Bonferroni correction for multiple comparisons.

The significance of changes in timepoint blood flow values in the handgrip-trained arm relative to the changes in the control arm was determined using a three way repeated measures analysis of variance (timepoint [timepoint post-occlusion] x trial [pre- or post-intervention] x arm [control or intervention arm]). Where the 3-way interaction term was significant, a two way repeated measures analysis (timepoint x trial) was performed separately for the control and intervention arms. Where the 2-way interaction term was significant, post-hoc pairwise comparisons were undertaken at each timepoint with Bonferroni correction for multiple comparisons.

The significance of changes in lipid and biomarker concentrations was determined by paired t-tests.

Although haemoglobin and hematocrit were not significantly different between pre- and post-training, concentrations of lipids and cellular adhesion molecules and microparticle

counts were adjusted for individual changes in plasma volume, determined by the Dill and Costill (1974) method.

Relationships between selected variables were determined using Pearson correlations.

Data are reported as mean ± SE and significance was set at $p<0.05$.

3.11   Preliminary testing – Development of method

Prior to the initiation of the present study, blood flow measurements were performed in a cross section of the population.   The main purpose was to determine the methodological and technical aspects regarding the examination of vascular function using mercury in-Silastic strain gauge plethysmography.

Experiment 1: Recording time intervals & Resting forearm blood flow measurements

This experiment was designed to examine relationships between forearm arterial and venous function indexes and the differing durations between and during measurements. After much adjustment, a reading interval of 10 s and an inflow time of 6 s were also selected. The reading interval of 10 s was a sufficient amount of time between readings for normal blood flow to resume. Six seconds of inflow was an adequate amount of time to ensure that a viable measurement could be gained from each waveform. If there was movement of the gauge or an unusual blip in the measurement 6 s allowed for a long enough waveform to be recorded which included at least four heartbeats, hence the waveform would include enough peaks (heartbeats) to gain a measurement.

It was noted in the present research that the first resting forearm blood flow reading was somewhat different from each measurement. The decision was made to remove the first initial reading for the resting forearm blood flow. As it was decided to remove the initial reading, which appeared to be the highest, the lowest result was also eliminated.

Experiment 2: Effect of varying venous and arterial occlusion pressures and time on vascular indexes.

As is evident from Table 3.3, there have been a variety of occlusion pressures used throughout the research in this area. All of which are below diastolic blood pressure for venous occlusion and above systolic blood pressure for arterial occlusion. Table 3.3 below reveals that there is somewhat of a consensus to set the venous occlusion pressure between 40 and 50 mmHg. Initially, the pressures of 50 mmHg and 7 mmHg below diastolic were used for the venous occlusion and 200 mmHg for the arterial occlusion. However, after experiment one, in the preliminary testing, it became evident that both the 7 mmHg and the 200 mmHg pressures were unsuitable.

It became clear that 7 mmHg below was too close to the diastolic pressure, so much so that it impinged on the reproducibility of the results. After much deliberation and further testing around these pressures, it was resolved to set the venous occlusion pressure at 50 mmHg.

There have also been a variety of pressures used to occlude arterial blood flow [Table 3.3]. As mentioned above an occlusion pressure of 200 mmHg was initially used, in the present experiment. This pressure proved too uncomfortable for a few individuals, so much so, that the occlusion had to be stopped before the five minutes elapsed. For those with normal blood pressure the occlusion pressure was approximately 80 mmHg above systolic pressure, whereas for others it was anywhere between 60 and 70 mmHg above systolic. It is necessary to have individuals as comfortable as possible throughout measurements to ensure they are relaxed and remain still. Especially so as both arms were measured twice pre- and post-training.

Table 3.3   Occlusion Pressures Used in Previous Research

Researcher	Venous Occlusion (mmHg)	Arterial Occlusion (mmHg)
Ridout et al., 2005	45	80-100 > Systolic
Ishibashi et al., 2006	40-50	20-30 > Systolic
Dagre et al., 2007	50	50 > Systolic

Shimabukuro et al., 2007	40	200
Addor et al., 2008	40	200
Higashi et al., 1999	40	280
Raitakari et al., 2000	45	250
Schulte et al., 1988	40	220
Wassmann et al., 2002	50	200
Thijssen et al., 2005b	50	220
Alomari et al., 2007	7 < Diastolic	240

In Table 3.3 the majority of studies used pressures of 200 mmHg and greater to occlude arterial flow. To ensure that arterial blood flow was completely occluded pressures between 30 mmHg above systolic and 200 mmHg were used. Results proved there to be no difference between the lowest and the highest occlusion pressure. Consequently, the lowest pressure, 30 mmHg above systolic, was chosen for this subject population, to ensure the least upset as possible throughout measurements. However, if the pressure was not, for any reason, occluding the arterial flow completely, the baseline waveform pointer would move and the pressure could be increased slightly, before the hose was clamped.

Experiment 3: Determination of Reproducibility of Blood Flow Measurements

After completion of the above experiments and fine-tuning technical hitches, blood flow measurements were performed on 15 healthy subjects. The purpose of this was to assess short-term and medium-term reproducibility of the baseline forearm blood flow and post occlusion reactive hyperaemia.

Previous studies [Table 3.4] have reported varied results in relation to the short-term and medium-term reproducibility of forearm blood flow (FBF) and an index of vascular function, peak reactive hyperaemia (Peak RH).

Table 3.4 CoVs for the Reproducibility Reported in Previous Studies

	CoV % for Resting FBF	CoV % for Peak RH
Roberts et al. (1986)	10.5**	9.8*
Altenkirch et al. (1989)	24.9*	10.5*
Engelke et al. (1996)	8.2*	
Petrie et al. (1998)	39-31**	
Walker et al. (2001)	16-19**	
Thijsen et al. (2005a)	16.9*	6.1*
Thijsen et al. (2005a)	21.2**	8.6**
CoV, Coefficient of Variation; *Short-term reproducibility – On one day; **Medium term reproducibility –days-weeks.		

Source: Thijsen et al., 2005a

In the present experiment, subjects were measured on the first day twice, fifteen minutes apart, and approximately seven days later. Short-term (15 minutes; Test 1 & Test 2) reproducibility was assessed by calculating the coefficient of variation (CoV) from the two measurements. Medium-term (1 week; Day 1 & Day 2) reproducibility was based on the duplicate measures made on Day 1 and Day 2 and by calculating the CoV from the two measurements. Equation 3.2 below is that used for the calculation of the CoV.

Equation 3.2:

CoV = Standard Deviation of repeated measures / mean of repeated measures * 100

Chapter 4

Presentation of Results

4.1 Reproducibility of Reactive Hyperaemia Measurements

Test 1 and Test 2 were conducted on the same day, fifteen minutes apart and Day 1 and Day 2 measurements were conducted one week apart.

Figure 4.1 Short Term Reproducibility of Resting Forearm Blood Flow (n=15)
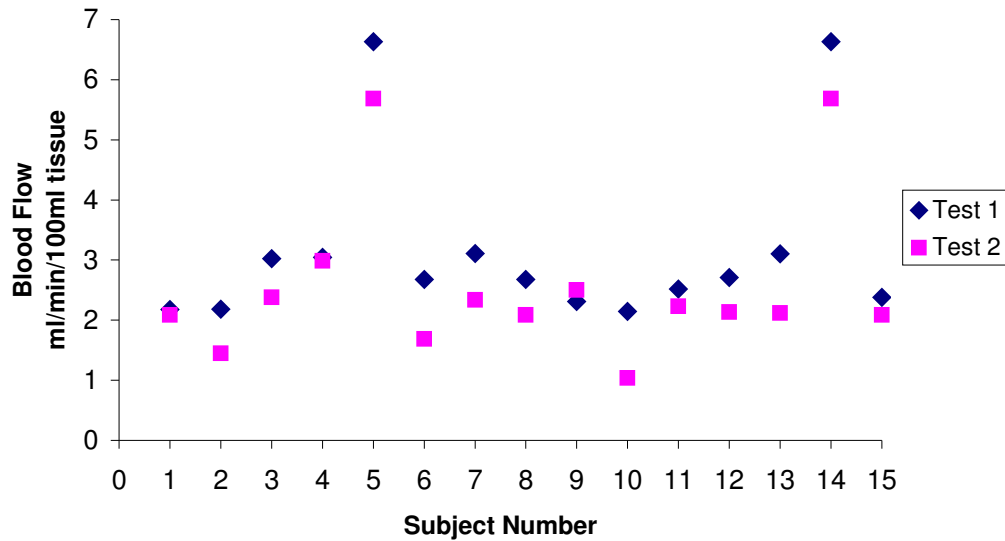
Resting forearm blood values were not significantly different between Test 1 and Test 2 (3.2 ± 0.4 vs. 2.6 ± 0.4 ml/min/100ml tissue, p = 0.67). The correlation between Test 1 and Test 2 was r = 0.96 and the CoV was 16%

Figure 4.2   Medium Term Reproducibility of Resting Forearm Blood Flow (n=15)
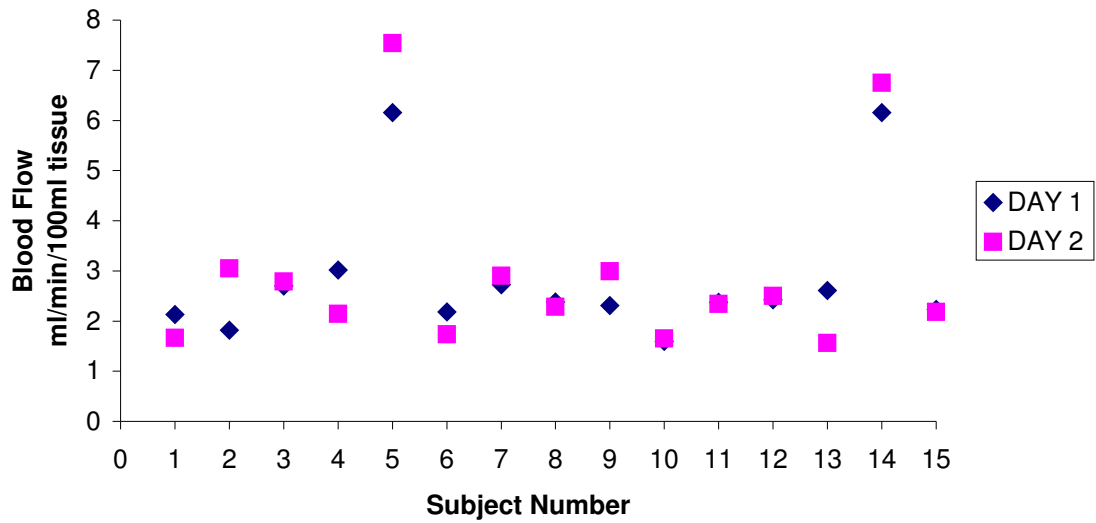
Resting forearm blood values were not significantly different between Day 1 and Day 2 (2.7 ± 0.4 vs. 2.9 ± 0.5 ml/min/100ml tissue, p = 0.63). The correlation between Day 1 and Day 2 was r = 0.94 and the CoV was 12%

Figure 4.3   Short Term Reproducibility of Total Reactive Hyperaemia (AUC) (n=15)
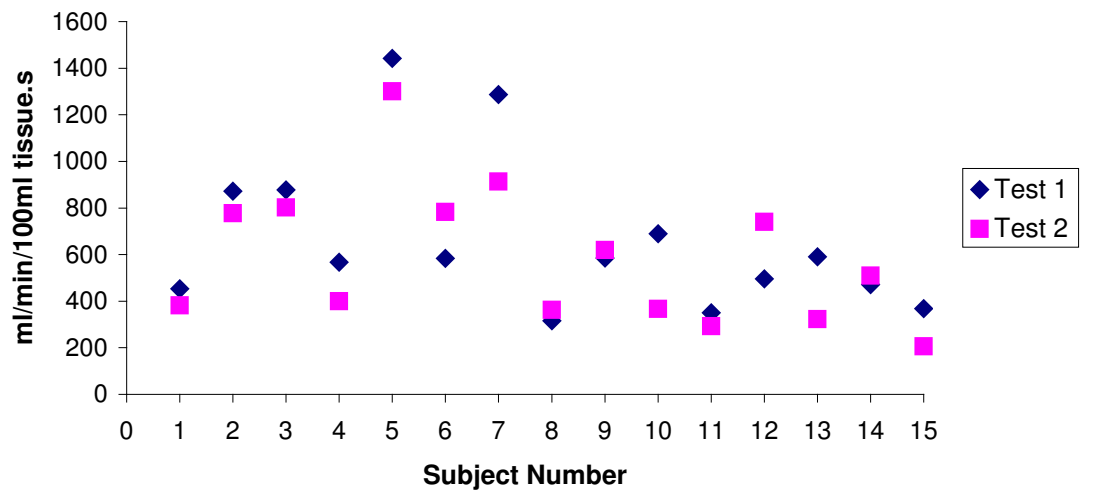
Total reactive hyperaemia [area under the blood flow vs. time curve (AUC)] was not significantly different between Test 1 and Test 2 (663 ± 85 vs. 505 ±61 ml/min/100ml tissue.s, p = 0.17). The correlation between Test 1 and Test 2 was r = 0.72 and the CoV was 20%

Figure 4.4 Medium Term Reproducibility of Total Reactive Hyperaemia (AUC) (n=15)
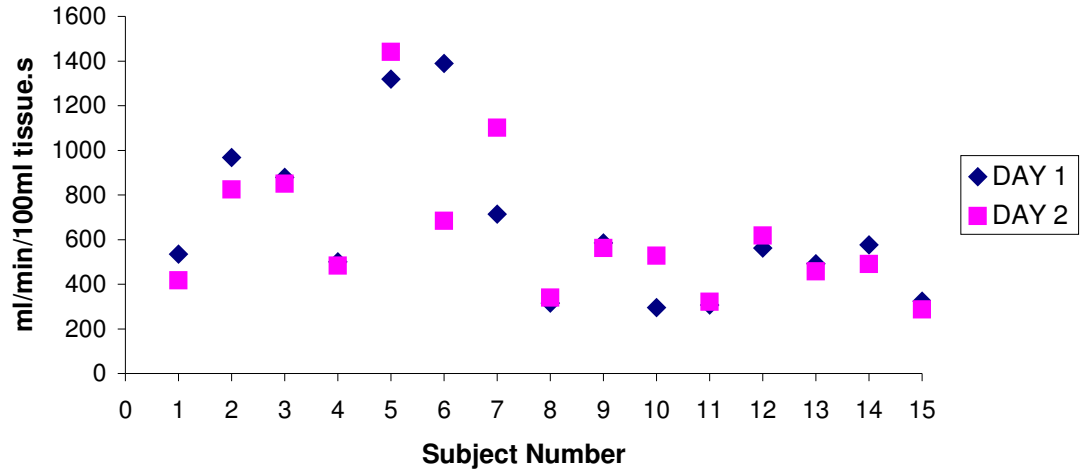
Total reactive hyperaemic [area under the blood flow vs. time curve (AUC)] was not significantly different between Day 1 and Day 2 (638 ± 95 vs. 615 ± 86 ml/min/100ml tissue.s, p = 0.74). The correlation between Day 1 and Day 2 was r = 0.75 and the CoV was 15%.

Figure 4.5 Short Term Reproducibility of Peak Reactive Hyperaemia (n=15)
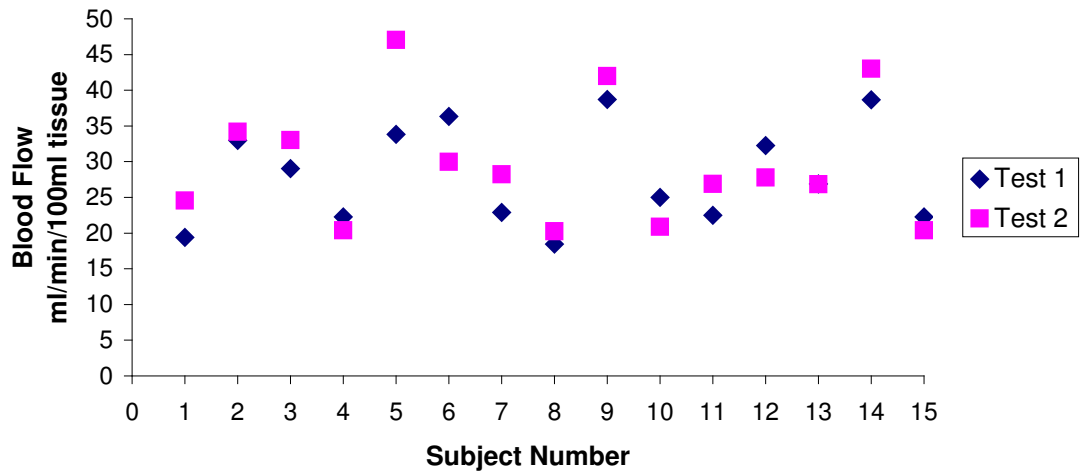
Peak reactive hyperaemia was not significantly different between Test 1 and Test 2 (28 ± 2 vs. 29 ± 2 ml/min/100ml tissue, p = 0.39). The correlation between Test 1 and Test 2 was r = 0.78 and the CoV was 10%

Figure 4.6   Medium Term Reproducibility of Peak Reactive Hyperaemia (n=15)
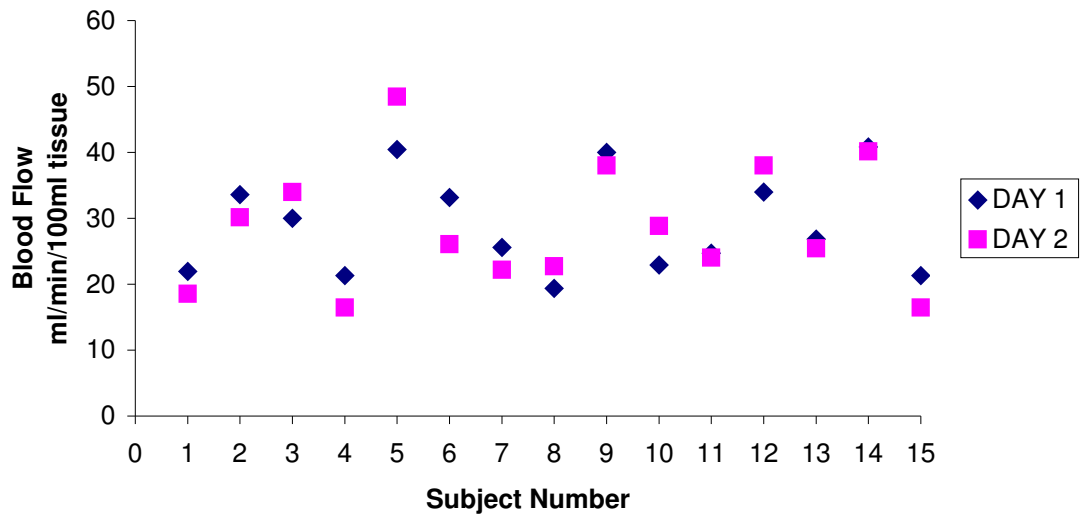
Peak reactive hyperaemia values were not significantly different between Day 1 and Day 2 (28 ± 2, 26 ± 3 ml/min/100ml tissue, p = 0.45). The correlation between Day 1 and Day 2 was r = 0.88 and the CoV was 10%

Figure 4.7   Short Term Reproducibility of Duration of Reactive Hyperaemia (n=15)
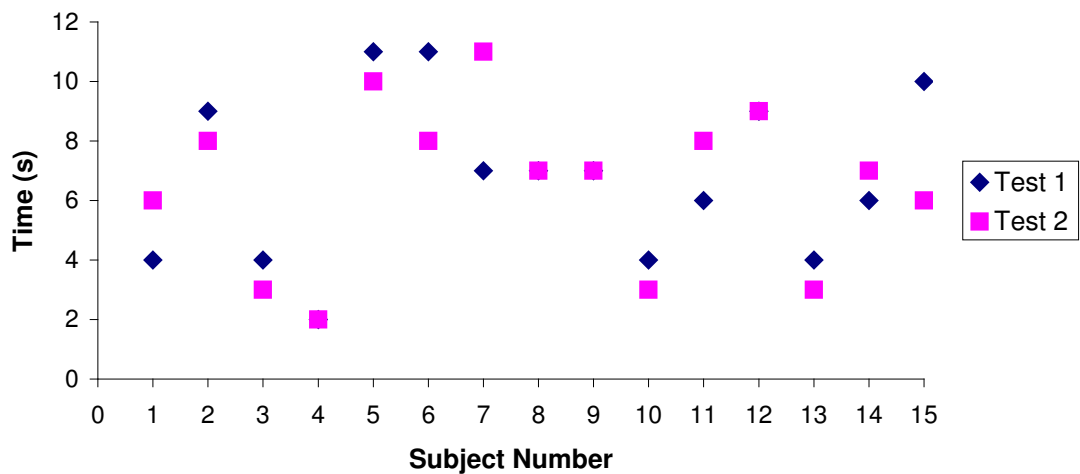
Duration of reactive hyperaemia was not significantly different between Test 1 and Test 2 (6.7 ± 0.7 vs. 6.8 ± 0.7 s, p = 0.79). The correlation between Test 1 and Test 2 was r = 0.72 and the CoV was 15%

Figure 4.8 Medium Term Reproducibility of Duration of Reactive Hyperaemia (n=15)
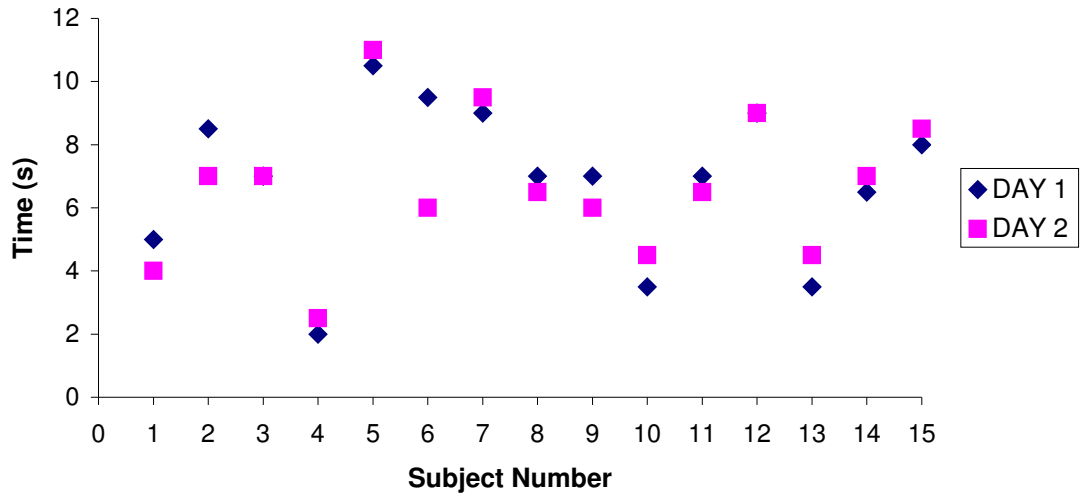
Duration of reactive hyperaemia was not significantly different between Day 1 and Day 2 (6.8 ± 0.7 vs. 6.7 ± 0.6 s, p = 0.58). The correlation between Day 1 and Day 2 was r = 0.88 and the CoV was 10%

Table 4.1 Summary of Coefficient of Variation Statistics for Various Vascular Measurements (n = 15)

Measurement	Short-Term	Medium-Term
FBF[a] ml/min/100ml tissue	16%	12%
Peak RH[b] ml/min/100ml tissue	10%	10%
Total RH (AUC[c]) ml/min/100ml tissue.s	20%	15%
Duration RH[b] Time (s)	15%	10%
[a] **FBF:** Resting forearm blood flow; [b] **RH:** Reactive hyperaemia; increase in blood flow after release of occlusion cuff; [c] **AUC:** Area under the blood flow vs. time curve		

The short-term reproducibility varied from 10-20% and the medium term reproducibility varied from 10-15%

4.2 Descriptive Analysis of Participants

Table 4.2   Influence of Short-Term Exercise Training* on Anthropometric, Cardiovascular and Exercise Measures (n = 9)

Variable	Pre-Training	Post-Training
Weight (kg)	94.1 ± 5.2	93.8 ± 5.2
BMI (kg·m^{-2})	29.9 ± 1.4	29.8 ± 1.4
Waist Circumference (cm)	99.6 ± 2.8	99.2 ± 2.8
Body Fat (%)	21.6 ± 1.6	20.7 ± 1.5
SBP$_{rest}$ (mmHg)	132 ± 3	131 ± 3
DBP$_{rest}$ (mmHg)	83.1 ± 1.5	82 ± 1.2
MAP$_{rest}$ (mmHg)	99.4 ± 1.6	98.3 ± 1.5
$\dot{V}O_{2peak}$ (ml·min^{-1}·kg^{-1})	33.5 ± 1.6	34.1 ± 1.9
Handgrip (HG) Strength HG trained arm (kg)	42.1 ± 2.1	44.6 ± 1.9
HG Strength control arm (kg)	41.9 ± 2.7	41.8 ± 2.7
Values are mean ± SEM; *Exercise Training consisted of 2 weeks of two legged cycle ergometry and one arm handgrip training; SBP$_{rest,}$ Systolic blood pressure at rest; DBP$_{rest,}$ Diastolic blood pressure at rest; MAP$_{rest,}$ Mean arterial blood pressure at rest; HG**,** Handgrip.		

There were no significant changes from pre-training to post-training measurements in any of the variables of interest.

4.3 Pre-Training Comparison of Handgrip Trained Arms and Control Arms

Table 4.3 Comparison of Pre-Training Scores on Various Blood Flow Measurements in Handgrip Trained and Control Arms (n = 9)

Measurement	HG Trained	Control	P Value
FBF[a] ml/min/100ml tissue	1.89 ± 0.2	2.1 ± 0.3	0.94
Peak RH[b] ml/min/100ml tissue	22.9 ± 0.8	23.6 ± 2.1	0.76
Duration of RH[b] time (s)	75.6 ± 11.1	93.9 ± 13.4	0.10
AUC[c] ml/min/100ml tissue.s	701 ± 41	836 ± 119	0.21
[a] **FBF:** Resting Forearm Blood Flow; [b] **RH:** Reactive Hyperaemia; increase in blood flow after release of occlusion cuff; [c] **AUC:** Area under the blood flow vs. time curve			

There was no significant difference, thus the handgrip trained and control arms were equivalent at pre-training.

4.4 Effect of Exercise Training on Vascular Function

Table 4.4 Comparison of Pre- and Post- Intervention[I] Indices of Vascular Function in Handgrip Trained and Control Arms (n = 9)

Measurement	Handgrip Trained Arms		Control Arms		
	Pre-Training	Post-Training	Pre-Training	Post-Training	P Value (Interaction[II])
FBF[a] ml/min/100ml tissue	1.9 ± 0.2	2.4 ± 0.3	2.1 ± 0.3	2.1 ± 0.2	0.43
Peak RH[b] ml/min/100ml tissue	23 ± 1	34 ± 2*	24 ± 2	27 ± 3*	0.003
Duration of RH[b] Time (s)	76 ± 11	104 ± 11*	94 ± 13	92 ± 10	0.02
Total RH (AUC[c]) ml/min/100ml tissue.s	701 ± 41	1055 ± 118*	836 ± 119	835 ± 113	0.02

[I]Intervention: consisted of 2 weeks of 10 exercise session - two legged cycle ergometry and one arm handgrip training; [II]Interaction: The significance of changes in the handgrip-trained arm relative to the changes in the control arm was determined via the interaction term of a fully repeated measures (arm x trial) 2-way analysis of variance; [a] **FBF:** Resting Forearm Blood Flow; [b] **RH:** Reactive Hyperaemia; increase in blood flow after release of occlusion cuff; [c] **AUC:** Area under the blood flow vs. time curve; . *Significant difference from pre-training measurement, p< 0.05, pair-wise post-hoc comparison following significant arm x trial interaction.

The pre to post changes in reactive hyperaemia, duration of reactive hyperaemia and total reactive hyperaemia in the handgrip-trained arm relative to the changes in the control arm differed significantly between pre- and post- intervention (significant interaction). Consequently separate comparisons of pre- and post- intervention scores are justified.

There was a 48% increase in the peak reactive hyperaemia (p = 0.008), a 46% increase in the duration of reactive hyperaemia (p = 0.004) and a 50% increase in the total reactive hyperaemia (p = 0.006) in the handgrip-trained arm. There was a 16% increase

in the peak reactive hyperaemia (p = 0.047) in the control arm. The increase in the peak reactive hyperaemia in the handgrip-trained arm was significantly greater (p < 0.05 for interaction). There was no difference in total reactive hyperaemia (AUC) (p = 0.81) or duration of reactive hyperaemia (p = 0.73) in the control arm.

Figure 4.9a   Resting and Post Occlusion Blood Flow Measurement in the Handgrip Trained Arm, Pre- and Post-Training (n = 9)
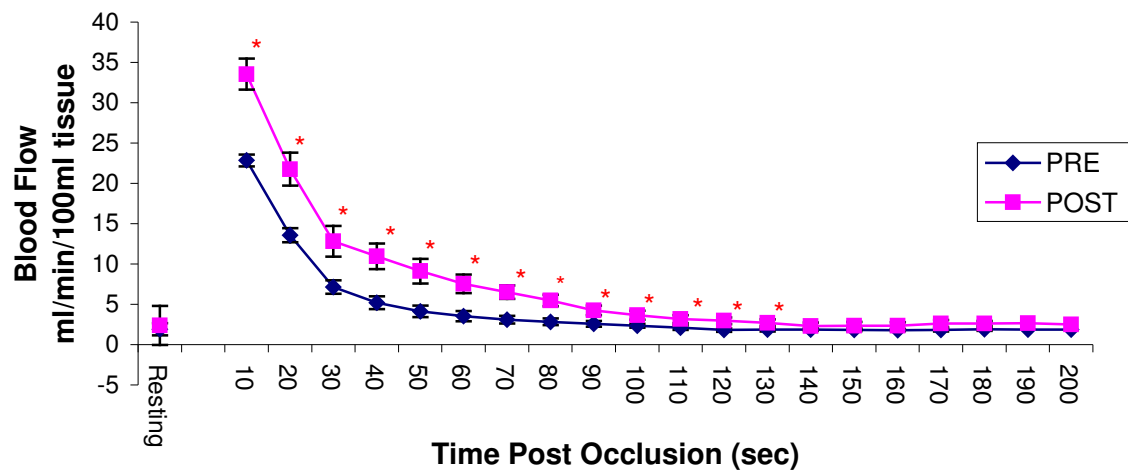
*p<0.05, Compared to pre-training, post-hoc pairwise comparisons following significant timepoint x trial interaction

Figure 4.9b   Resting and Post Occlusion Blood Flow Measurement in the Control Arms, Pre- and Post-Training (n = 9)
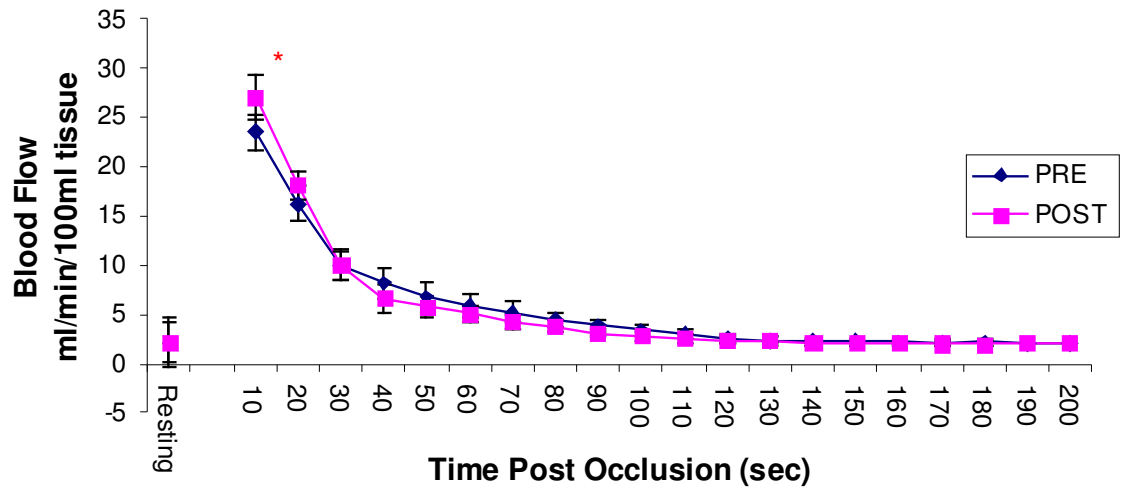
*p<0.05, Compared to pre-training, post-hoc pairwise comparisons following significant timepoint x trial interaction.

Forearm blood flow was greater post-occlusion in the handgrip-trained arm at all time-points up to 130 s post occlusion. Forearm blood flow was greater only at the first post occlusion time point in the control arm.

4.5　　　Effect of Exercise Training on Blood Chemistries

Table 4.5　　　Comparison of Pre- and Post-Training* Blood Chemistries (n = 9)

Measurement	Pre-Training	Post-Training	P Value
Glucose mmol/L	5.18 ± 0.22	5.49 ± 0.37	0.55
Cholesterol mmol/L	5.43 ± 0.34	5.09 ± 0.3	0.02
HDL-C mmol/L	1.14 ± 0.07	1.07 ± 0.07	0.08
LDL-C mmol/L	3.86 ± 0.31	3.67 ± 0.35	0.15
Triglyceride mmol/L	1.69 ± 0.35	1.48 ± 0.43	0.56
Cholesterol:HDL-C ratio	4.42 ± 0.24	4.45 ± 0.3	0.74
*Training consisted of 2 weeks of two legged cycle ergometry and one arm handgrip training			

Total cholesterol was 6% lower post-training.　Serum glucose, HDL-C, LDL-C, Triglyceride and the cholesterol:HDL-C ratio were not influenced by the exercise training.

4.6   Effect of Training on Biomarkers of Endothelial Activation

Table 4.6   Comparison of Pre- and Post-Training Values on Various Biomarkers of Endothelial Activation

Measurement	Pre-Training[I]	Post-Training[I]	P Value
sE-Selectin ng/ml	19.7 ± 0.7	20.1 ± 1.2	0.9
sICAM[a]-3 ng/ml	3.8 ± 0.3	3.7 ± 0.3	0.9
Thrombomodulin ng/ml	3.6 ± 0.3	3.8 ± 0.2	0.5
SAA[b] mg/ml	1.2 ± 0.2	1.4 ± 0.2	0.35
CRP[c] mg/ml	1.3 ± 0.3	1.8 ± 0.4	0.34
sICAM[a]-1 ng/ml	242 ± 7	253 ± 13	0.61
sVCAM[d]-1 ng/ml	517 ± 32	569 ± 47*	0.05
[I]Training consisted of 2 weeks of two legged cycle ergometry and one arm handgrip training *Significant difference from pre-training measure, $p < 0.05$, paired t-test; [a] **ICAM:** Intercellular Adhesion Molecule; [b] **SAA:** Serum Amyloid A; [c] **CRP:** C-Reactive Protein; [d] **VCAM:** Vascular Cell Adhesion Molecule			

Soluble VCAM-1 was 11% higher post-training. There was no change in any other biomarker following exercise training.

4.7 Effect of Training on Microparticles

Table 4.7 Comparison of Pre- and Post-Training* Microparticle Events

Measurement	Pre-Training	Post-training	P Value
AnnexinV$^+$ counts/μL	1491 ± 928	844 ± 220	0.47
CD41$^+$ AnnexinV$^+$ counts/μL	242 ± 52	244 ± 53	0.51
CD41$^+$ counts/μL	244 ± 53	303 ± 94	0.59
CD62E$^+$ AnnexinV$^+$ counts/μL	151 ± 82	72 ± 16	0.40
CD62E$^+$ counts/μL	529 ± 117	1306 ± 985	0.42
*Training consisted of 2 weeks of two legged cycle ergometry and one arm handgrip training; $^+$ positive;			

Exercise did not influence endothelial microparticle or platelet microparticle counts, whether defined with or without AnnexinV positivity.

4.8   Interrelationships Between Indices of Reactive Hyperaemia and Biomarkers of Endothelial Activation

Table 4.8   Interrelationships Between Directly Measured Vascular Function (AUC) and Biomarkers of Endothelial Activation

	Total RH	AnnexinV+	CD41+ AnnexinV+ events	CD41+	CD62+	CD62+ AnnexinV+	sE-Selectin	CRP	sICAM-1	sVCAM-1
Total RH	1	-.427	-.142	-.013	-.796(*)	-.796(*)	-.714(*)	.317	.198	.285
AnnexinV+ events	-.427	1	.701	.709	.670	.642	.485	.074	.736	.513
CD41+ AnnexinV+ events	-.142	.701	1	.573	.188	.171	.545	.613	.875(**)	.696
CD41+ events	-.013	.709	.573	1	.464	.469	.010	.478	.789(*)	.548
CD62+ events	-.796(*)	.670	.188	.464	1	.998(**)	.357	-.412	.235	-.030
CD62+ AnnexinV+ events	-.796(*)	.642	.171	.469	.998(**)	1	.343	-.412	.224	-.021
sE-Selectin	-.714(*)	.485	.545	.010	.357	.343	1	-.208	.031	.202
CRP	.317	.074	.613	.478	-.412	-.412	-.208	1	.607	.365
sICAM-1	.198	.736	.875(**)	.789(*)	.235	.224	.031	.607	1	.733(*)
sVCAM-1	.285	.513	.696	.548	-.030	-.021	.202	.365	.733(*)	1

** Correlation is significant at the 0.01 level (2-tailed)   * Correlation is significant at the 0.05 level (2-tailed)

Total reactive hyperaemia (AUC) was related to endothelial microparticle counts defined as $CD62E^+$ (r = -0.796, p<0.05) and $CD62E^+AnnexinV^+$ (r = -0.796, p<0.05). Total reactive hyperaemia was also related to sE-Selectin (r = -0.714, p<0.05).

Chapter 5

Discussion of Results

5.0   Summary of Findings

This is the first study to simultaneously assess the effect of lower limb exercise, in the form of two legged cycle ergometry, in combination with one-arm handgrip training, with the control arm kept perfectly still, on endothelial dependent dilation in the handgrip trained and control arms.

The principal finding of the present study is that short-term exercise training resulted in considerable increases in all indices of reactive hyperaemia in the handgrip-trained arm. Apart from a small increase in peak reactive hyperaemia (+ 16%), short-term exercise training had no effect in the control arm. This increase was significantly less than the increase in the handgrip-trained arm (p = 0.003 for interaction). These results point towards the primacy of a localised effect of exercise training. The measurements of forearm blood flow as assessed by strain gauge plethysmography were reproducible with CoV ranging from 10% to 20% for the various indices of reactive hyperaemia. Exercise training resulted in a decrease in total cholesterol but no change in any other blood lipid. Exercise training resulted in a small but significant increase in sVCAM-1 but did not influence any other biomarker of activation or inflammation, that is, sICAM-1, sICAM-3, sE-selectin, thrombomodulin, SAA, CRP. Exercise training did not influence endothelial or platelet microparticle counts.

5.1   Reproducibility Study

Previous reproducibility studies have reported a wide range of results, which vary from a good short-term reproducibility of peak reactive hyperaemia with a coefficient of variation (CoV) of 6.1% (Roberts et al., 1986) to poor medium-term reproducibility, with a CoV of 39% (Walker et al., 2001). The reliability measures used in the present study confirm adequate consistency between tests (short-term) and days (medium-term) for blood flow measures. The absolute values for the indices of reactive hyperaemia, that is, peak reactive hyperaemia, duration of reactive hyperaemia and total reactive hyperaemia observed in the present study are consistent with those reported in the literature using similar measuring techniques (Thijssen et al., 2005; Murray et al., 2006; O'Sullivan, 2003). Reproducibility in this study was better than that observed by others (Thijssen et al., 2005; Roberts et al., 1986; Walker et al., 2001). The reproducibility results, although acceptable and not at the top end of the reproducibility result scale, does show room for improvement. Given that the primary objective of this study was to

identify changes from exercise training in the order of 10%, the decision was made to perform all the reactive hyperaemia measurement in duplicate. Additional work is needed to identify the factors that influence the reproducibility of this technique. However, the reproducibility for this technique and the results of the present work is still better than that observed with FMD, which has CoVs as high as 29% and 50% (West et al., 2004; deRoos et al., 2003). These results suggest that reactive hyperaemia is less variable thus making it easier to detect small changes in endothelial function.

5.2 Reactive Hyperaemia and EDD

In the present study, reactive hyperaemia, as assessed by strain gauge plethysmography, was employed to examine changes in EDD. There is some controversy over its use (Lind et al., 2000; Green et al., 2006), however, we feel there is sufficient evidence from previous studies that indices of reactive hyperaemia are at least in part endothelial dependent (Meredith et al., 1996; Higashi et al., 2001a; Kingwell et al., 1997). Although it was not measured in the present study, endothelium-derived nitric oxide has been shown to contribute to reactive hyperaemia, as shown by reductions in both the peak and total reactive hyperaemic response from the infusion of L-NMMA, an inhibitor of nitric oxide synthase (Meredith et al., 1996). On the other hand, Tagawa et al. (1994) reported reductions in only the mid-to-late phase of reactive hyperaemia to the infusion of L-NMMA. This suggests that NO plays a minimal role in peak reactive hyperaemia in the human forearm. Muscle hypoxia during arterial occlusion stimulates blood flow and causes vasodilation. This initial surge in flow, that is peak reactive hyperaemia, triggers NO release from endothelial cells, therefore the late phase reactive hyperaemia maybe more reflective of endothelial function. Exercise studies that used the infusion of L-NMMA showed a significant decrease in the exercise-induced blood flow (Higashi et al., 2001a; Kingwell et al., 1997), with L-NMMA causing a greater vasoconstriction after training (Kingwell et al., 1997). These results suggest that production of nitric oxide occurs as a result of exercise, suggesting that exercise induced increases in reactive hyperaemia are in part endothelial dependent. However, it must be noted that this increase in NO can inadvertently lead to an increase in peroxynitrite, a powerful oxidant, which can lead to cellular damage (Pryor and Squadrito, 1995).

Some studies have suggested that reactive hyperaemia maybe in part non-endothelial dependent. Reactive hyperaemia has been described as a complex response that reflects

dilation of micro vessels by non-endothelium dependent vasodilators generated during local ischemia, including adenosine (Loscalzo & Vita, 1994). A strong correlation was reported between adenosine concentrations and blood flow (Hellsten et al., 1998), with infusions of adenosine receptor antagonists been shown to reduce the exercise-induced increases in blood flow (Duncker et al., 1995; Radegran & Calbet, 2001), suggesting that indices of reactive hyperaemia are in part endothelial independent. Thus, exercise-induced changes in reactive hyperaemia may be both endothelial dependent and endothelial independent.

5.3   Exercise and Endothelial Function

The results of this study lend weight to the primacy of a local exercise effect. The research design involved two legged cycle-ergometry exercise training combined with one arm handgrip exercise. It has been shown that the small muscle mass used with handgrip exercise creates lower heart rates and aerobic metabolic demands than exercises involving larger muscles (McArdle, Katch and Katch, 2007) and overall produces less cardiovascular demands (Dutton, 2008). Hence there was a need for two-legged cycle ergometry exercise to provoke substantial hemodynamic increases throughout the vasculature. The exercise training protocol in overweight, sedentary men, resulted in considerable improvements in all the indices of reactive hyperaemia, but in the handgrip trained arm only. The improvements observed in the present study are in line with other handgrip training studies. Studies of young healthy men (Green et al., 1994; Alomari & Welsch, 2007; Katz et al., 1997; Bank et al., 1998) have all reported that intensive exercise training in isolated limbs results in considerable local EDD improvements. All of these studies reported no change in reactive hyperaemia in the control arms.

The results of the present study support other studies that argue for the primacy of local exercise effect (Pierce et al., 2008; Motohiro et al., 2005; Demopoulos et al, 1997). All of these studies were carried out with diseased individuals from patients post heart transplant (Pierce et al., 2008) to patients with heart failure (Demopoulos et al, 1997; Kobayashi et al., 2003). Each study reported EDD improvements in the vasculature of the active limb only. To date, no study has shown a localised effect of exercise training in healthy individuals or in sedentary men.

The results of the present study do not support the argument that short-term exercise training results in increases in EDD in inactive vascular beds. The present study found little or no improvements in EDD in the control arm as assessed by reactive hyperaemia. Four possible explanations can be forwarded to account for the findings of generalised exercise effects in some studies but not in the present study. Firstly a number of previous studies have used more clinical populations including hypertensive (Higashi et al., 1999) and patients with chronic heart failure (Hambrecht et al., 1998; Maiorana et al., 2000). Secondly a number of studies reporting effects in upper limb endothelial function from lower limb exercise have utilised different techniques to assess FMD, including FMD and ACh mediated blood flow (Higashi et al., 1999; Goto et al., 2003; DeSouza et al., 2000; Maiorana et al., 2000; 2001; Clarkson et al., 1999; Hambrecht et al., 1998). Thirdly, interventions have been considerably longer in some studies. These studies involved exercise interventions of durations varying from 8 weeks (Maiorana et al., 2000) to 6 months (Hambrecht et al., 1998), much longer than that used in the present study. Finally, it should be noted that the results of the present study indicating the primacy of a localised exercise effect contradict two other studies (O'Sullivan, 2003; Murray et al., 2006) that used the same assessment technique (RH (SGP)), a comparable cohort and a similar duration of the exercise intervention (O'Sullivan, 2003; Murray et al., 2006). Both Murray et al. (2006) and O' Sullivan (2003) reported considerable improvements in reactive hyperaemia in the forearm vasculature after cycling training. However, both studies failed to specify the level of involvement of the upper limbs. It is possible that the arms in these studies were subject to a localised exercise effect as gripping of the handlebars will stimulate the forearm. It is also worth noting that researchers (Maiorana et al., 2000) incorporated whole body exercise training interventions while others (Higashi et al., 1999) used walking as the choice of activity. Both types of activity involve some use of the arms and thus improvements would be expected in the forearms. It would not be possible to distinguish between a localised and a generalised exercise effect in these situations. All of the studies reporting improvements in inactive vascular beds either stated that the exercise training was predominantly in the lower limbs or failed to specify the exact involvement of the upper limbs. In the present study the control arm was kept perfectly still. This level of experimental control was not reported in other studies.

The length of exercise intervention that is necessary for putative exercise effects to be evident is open to debate. Previous research has shown positive exercise effects after 4

weeks to 6 months of exercise training. The majority of previous studies have reported improvements after 4 (Hambrecht et al, 200; 2003; Linke, et al., 2001; Thijssen et al, 2005b; Kingwell et al., 1997) and 12 weeks (Higashi et al., 1999; Lavrencic et al., 2000; Pierce et al., 2008; DeSouza et al., 2000; Goto et al., 2003) exercise training. However, research has shown improvements in EDD after as little as one week of exercise training (Murray et al., 2006).

The type of cohort necessary for putative exercise effects to be evident is also open to debate. Generalised exercise effects have been highlighted as occurring primarily when EDD is impaired at baseline. The studies supporting the argument that short-term exercise training results in increases in EDD in inactive vascular beds have primarily being carried out with diseased individuals from hypertensive (Higashi et al., 1999) to individuals with chronic heart failure (Hambrecht et al., 1998; Maiorana et al., 2000). The cohort used in the present study were overweight sedentary men, who had no clinical signs of overt atherosclerosis and no more than one cardiovascular risk factor. It is possible that exercise interventions in more diseased populations in whom the atherosclerotic process is at a later stage would have generated different results. However, exercise induced improvements have been reported in hypertensive individuals (Higashi et al., 1999), asymptomatic men (Lavrencic et al., 2000), post heart transplant individuals (Pierce et al., 2008) and in young healthy men (Clarkson et al., 1999).

We cannot be certain that exercise does not result in a generalised increase in EDD. It is possible that exercise interventions of longer durations and involving a more diseased population in whom the atherosclerotic process is at a later stage would have generated different results. Rather the results point to a local exercise effect that occurs with only two weeks of training and is apparent in less clinical populations. Mean pre- to post-intervention differences in total reactive hyperaemia and the duration of reactive hyperaemia were only 3.1% and 2.3% respectively in the non-handgrip trained arm. With the pre- to post-intervention variation in these parameters of considerably greater magnitude (medium term co-efficient of variations were 15% and 10% for total reactive hyperaemia and the duration of reactive hyperaemia), statistical significance for these small mean changes would not have been reached, even with a considerably larger sample size.

Increased shear stress on endothelial cells may be the primary signal for exercise training-induced adaptations of endothelial function. Cyclic strain acts along the vessel wall perimeter to cause stretching and is caused by variations in blood pressure with fluid shear stress being the frictional force imposed on the vessel wall when blood flows through. Cyclic strain has been reported to up-regulate the expression of eNOS transcripts (Awolesi et al., 1995) with shear stress increasing the production of vasodilatory factors from the endothelium (Joannides et al. 1995; White and Frangos, 2007; Harrison et al. 2006; Chien, 2007). However, increases in cyclic strain secondary to an increase in cardiac output and blood pressure would have been experienced in both arms. The local increase in shear stress in the handgrip-trained arm may be a more plausible explanation for our results. It should be noted that NO can be converted to the powerful oxidant peroxynitrite in the presence of the free radical superoxide, potentially inhibiting vasodilation. Further investigations are needed to clarify the mechanisms by exercise-induced increases in shear stress and cyclic strain influence NO metabolism and vessel tone.

The factors responsible for the small increase in peak reactive hyperaemia in the control arm are open to debate. Forearm blood flow was higher post-training at the first time point post occlusion but at no other time point. It is possible that small increases in blood flow occurred in the control arm during exercise despite it being kept perfectly still. Tanaka et al. (2006) reported a 4-fold increase in forearm blood flow during leg exercises and a 3.5-fold increase in femoral blood flow during arm cycling (Tanaka et al., 2006). Alternatively, the increase in the peak reactive hyperaemia of the control arm may have been endothelial independent. Tagawa et al. (1994) reported reductions in only the mid-to-late phase of reactive hyperaemia to the infusion of L-NMMA. This suggests that NO plays a minimal role in peak reactive hyperaemia in the human forearm. Therefore, it appears that the increase in peak reactive hyperaemia found in the control arm may possibly have involved other endothelial or endothelial independent factors, as total reactive hyperaemia and duration of reactive hyperaemia did not change.

Improvements in EDD reported in the present study coincided with a decrease in total cholesterol, similar to what has been reported by others (Kingwell et al., 1996; Higashi et al., 1999). However, improvements in endothelial function have been found in the absence of any improvement in traditional cardiovascular risk factors, which suggests

that the exercise induced improvements in cardiovascular risk factors do not solely mediate improvements in vascular function. As in the present study, if the decrease in cholesterol mediated the increase in EDD, then the control arm would have benefited also.

The lack of a non-exercise control group may be seen as a limitation within the present study. As the study design involved two-legged cycling and one-arm handgrip work (non-handgrip arm perfectly still), the non-handgrip trained arm served as a control with respect to the hypothesis that the effects of exercise are primarily local in nature. Had significant vascular changes been found in the non-handgrip trained arm following the two-week intervention, the absence of a non-exercise control group may have threatened the validity of such a finding. It should be noted that total reactive hyperaemia, peak reactive hyperaemia and the duration of reactive hyperaemia did not change over the course of the reproducibility study, confirming the stability of these parameters in a similar cohort over a similar time frame.

5.4   Exercise and Biomarkers of Endothelial Activation and Inflammation

Exercise training did not result in a reduction in any biomarker of endothelial activation or inflammation. These results are largely in line with the few previous studies that have examined the influence of exercise training on endothelial activation (Jilma et al., 1997; Pierce et al., 2008; Smith et al., 2000). Of the two training studies that have reported reductions in cellular adhesion molecules (Zoppinin et al., 2006; Adamopolous et al., 2001) the training interventions were of longer durations from 3 to 6 months with diseased individuals (type II diabetics and patients with chronic heart failure). It may be a possibility that the intervention in the present study was not of a long enough duration to incur any improvements in endothelial activation. However, John et al (2000) found that cellular adhesion molecules were not elevated in hypercholesterolaemic patients with significantly impaired endothelium-dependent vasodilation. The results of that study indicate that impaired EDD may not be clinically useful to indicate endothelial activation.

In the present study an increase in the concentrations of sVCAM-1 was reported. This is not the only study to show changes in markers of endothelial activation acutely after exercise. Research has shown that acute bouts of exercise result in a brief change to a

pro-inflammatory phenotype proportional to the amount of exercise and muscle soreness (Kasapis et al., 2005). Das (2004) suggests that an initial increase in a pro-inflammatory phenotype may be necessary during the early stages of training before a decrease in cellular adhesion molecules is observed. One previous study (Akimoto et al., 2002) reported an increase in sICAM-1 after level and downhill running but not after prolonged cycling. The authors suggest that the running sessions perhaps caused too much muscle injury and/or inflammation (Akimoto et al., 2002).

Currently microparticles are being investigated for their role as sensitive markers of disease activity. They have been found to be elevated in many disease states, including obesity (Esposito et al., 2003) and coronary syndromes (Bernal-Mizrachi et al., 2003). This is the first study to report on the effect of an exercise training intervention on microparticle counts. The present study found no effect of exercise training on endothelial or platelet derived microparticles. It is also possible that the negative effects of acute or short-term exercise may balance the positive effects; shear stress has been reported to increase the release of microparticles (Miyazaki et al. 1996). However, the present study focused on a patient population early in the process of developing atherosclerosis. These individuals were overweight with high LDL cholesterol and total cholesterol to HDL ratio of 4.4. These individuals had no clinical signs of overt atherosclerosis and no more than one cardiovascular risk factor. An area of future research could possibly look at the exercise interventions in more diseased populations in whom the atherosclerotic process is at a later stage. The identification of sensitive and specific biomarkers will allow for a more effective monitoring of endothelial cell status in vivo. It may be interesting to measure markers of endothelial activation at different time points after the last training session in conjunction with markers of oxidative stress and muscle damage to identify possible associations

It is also possible that biomarkers in general will never be a sensitive indicator of exercise training adaptations. The beneficial effects of exercise may be confined to the active arteries only, whereas the whole vasculature including the venous system and the capillaries release microparticles and adhesion molecules. Any reduction in the release of these biomarkers from the arterial endothelium may be lost in the noise associated with their release from other vascular beds.

Interesting inverse correlations were observed between sE-selectin, the microparticles bearing E-selectin (CD62E) and total reactive hyperaemia. It has been shown that activated endothelial cells predominantly release CD62E endothelial microparticles (Jimenez et al., 2003). The results of the present study suggest that the endothelial microparticle CD62E and its soluble E-selectin are lower in individuals with higher reactive hyperaemia. Thus E-selectin may be the most promising biomarker linking impaired EDD to endothelial activation.

5.5   Conclusion

The results of this study point to the primacy of localised vascular changes in exercised limbs. This localised response illustrates the importance of whole-body exercise training programs to ensure all vascular beds, in particular those, which are prone to atherosclerosis; experience increases in shear stress from the increases in blood flow from exercise.

Reactive hyperaemia can be used to measure EDD with adequate reproducibility between tests and days for blood flow measures. Even though reproducibility appears better for reactive hyperaemia than FMD, future work should address the factors that influence the reproducibility of this technique to ensure it is less variable thus making it easier to detect small changes in endothelial function.

Despite the considerable research interest in biomarkers, this study does not support their use as sensitive biomarkers of exercise induced changes in vascular health status. Future work should address the effect of a longer duration exercise intervention with a more diseased population.

Bibliography

ACSM. (2006). *Guidelines for exercise testing and prescription (7th Ed).* Philadelphia. Lippincott Williams & Wilkins.

ADAMOPOLOUS, S., PARISSIS, J., KROUPIS, C. GEORGIADIS, M., KARATZAS, D., KARAVOLIAS, G., KONIAVITOU, K., COATS, A.J. & KREMASTINOS, D.T. (2001). Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. *Eur Heart J* 22: 791-797.

ADDOR, G., DELACHAUX, A., DISCHL, B., HAYOZ, D., LIAUDET, L., WAEBER, B. & FEIHL, F. (2008). A Comparative Study of Reactive Hyperemia in Human Forearm Skin and Muscle. *Physiol Res* 57: 685-692.

AKIMOTO, T., FURUDATE, M., SAITOH, M., et al. (2002). Increased plasma concentrations of intercellular adhesion molecules-1 after strenuous exercise associated with muscle damage. *Eur J Appl Physiol* 86: 185-190.

ALOMARI, M., SOLOMITO, A., REYES, R., KHALIL, S.M., WOOD, R., & WELSCH, M. (2004). Measurements of vascular function using strain-gauge plethysmography: technical considerations, standardization, and physiological findings. *Am J Physiol Heart Circ Physiol* 286: 99-107.

ALOMARI, M., & WELSCH, M. (2007). Regional changes in reactive hyperemic blood flow during exercise training: time-course adaptations. *Dynamic Medicine* 6: 1-6.

ALTENKIRCH, H.U., FRANSSON, L. & KOCH, G. (1989). Assessment of arterial and venous circulation in upper and lower extremities by venous occlusion strain gauge plethysmography. Normal values and reproducibility. *Vasa* 18: 140-145.

ANDERSON, E.A., & MARK, A.L. (1989). Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation* 79: 93-100.

ANDERSON, T.J. (1999). Assessment of Endothelial Function. *JACC* 34: 631-638.

ANDERSON, T.J., UEHATA, A. & GARHARD, M.D. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *JACC* 26(5): 1235-1241.

ANTONIADES, C., TOUSOULIS, D., MARINOU, K., STEFANADI, E., NTARLADIMAS, I., LATSIOS, G., KONNIARI, K., PAPAGEORGIOU, N., SIASOS, G. & STEFANADIS, C. (2006). Effects of lipid profile on forearm hyperemic response in young subjects. *Hellenic J Cardiol* 47: 152-157.

ARTEAGA, R. B., CHIRINOS, J.A., SORIANO, A.O., JY, W., HORSTMAN, L., JIMENEZ, J.J., et al. (2006). Endothelial microparticles and platelet and leukocyte activation in patients with the metabolic syndrome. *Am J Cardiol* 98: 70-74.

AWOLESI, M.A., SESSA, W.C. & SUMPIO, B.E. (1995). Cyclic strain upregulates nitric oxide synthase in cultured bovine aortic endothelial cells. J Clin Invest 96(3): 1449–1454.

BALLINGAND, J., & MAYER, B. (2000). *Nitric Oxide.* United States, Springer.

BANK, A., ROGER, M.D., SHAMMAS, A., MULLEN, K., PETER, R.N. & CHUANG, P. (1998). Effects of short-term forearm exercise training on resistance vessel endothelial function in normal subjects and patients with heart failure. *J Card Fail* 4(3): 193-201.

BECK, N. (2008). *Diagnostic Haematology.* United States, Springer.

BECKER, R.C. (1997). *Textbook of coronary thrombosis and thrombolysis.* United States, Springer.

BEHRENDT, D., & GANZ, P. (2007). Oxidative stress and vascular disease. In De Caterina, R., and Libby, P. (2007). *Endothelial Dysfunctions and Vascular Disease.* UK, Blackwell Publishing.

BERK, B. & TRAUB, O. (1999). Shear stress-mediated regulation of ERK1/2 in endothelial cells: signal transduction pathways. In Catravas, J., Callow, A. & Ryan, U. (1999). *Vascular endothelium: Mechanisms of cell signalling.* Netherlands, IOS Press.

BERNAL-MIZRACHI, L., JY, W., JIMENEZ, J.J., PASTOR, J., MAURO, L.M., HORSTMAN, L.L., DEMARCHENA, E. & AHN, Y.S. (2003). High levels of circulating endothelial microparticles in patients with acute coronary syndromes. *Am Heart J* 145: 962-970.

BHULLAR, I.S., LI, Y.S., MIAO, H., ZANDI, E., KIM, M., SHYY, J.Y. & CHIEN, S. (1998). Fluid shear stress activation of IkB Kinase is integrin-dependent. *J Biol Chem* 273(46): 30544-30549.

BIRO, E., NIEUWLAND, R. & STURK, A. (2004). Measuring circulating cell-derived microparticles. *J Thromb Haemost* 2: 1843-1844.

BOMBELI, T., MUELLER, M. & HAEBERLI, A. (1997). Anticoagulant properties of the vascular endothelium. *Thromb Haemost* 77: 408-423.

BORG, G. (1998). *Borg's perceived exertion and pain scales.* US, Human Kinetics.

BRAITH, R.W., SCHOFIELD, R.S., HILL, J.A., CASEY, D.P. & PIERCE, G.L. (2008). Exercise training attenuates progressive decline in brachial artery reactivity in heart transplant recipients. *J Heart Lung Transplant* 27(1): 52-59.

BRODSKY, S., ZHANG, F., NASJLETTI, A. & GOLIGORSKY, M. (2004). Endothelium-derived microparticles impair endothelial function in vitro. *Am J Physiol Heart Circ Physiol* 286: H1910-1915.

CAPOCCIA, B.J., GREGORY, A.D. & LINK, D.C. (2008). Recruitment of the inflammatory subset of monocytes to sites of ischemia induces angiogenesis in

a monocyte chemoattractant protein-1-dependent fashion. *J Leukocyte Biol* 84: 760-768.

CARLSSON, I., SOLLEVI, A. & WENNMALM A. (1987). The role of myogenic relaxation, adenosine and prostaglandins in human forearm reactive hyperaemia. *J Physiol* 389: 147– 161.

CARLSSON, I. & WENNMALM, A. (1983). Effect of different prostaglandin synthesis inhibitors on post-occlusive blood flow in human forearm. *Prostaglandins* 26**:** 241 – 252.

CELERMAJER, D.S., SORENSEN, K.E., GOOCH, V.M., SPIEGELHALTER, D.J., MILLER, O.I., SULLIVAN, I.D., LLOYD, J.K. & DEANFIELD, J.E. (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340: 1111-1115.

CHENG, T. H., SHIH, N. L., CHEN, S. Y., LOH, S. H., CHENG, P. Y., TSAI, C. S., LIU, S. H., WANG, D. L. & CHEN, J. J. (2001). Reactive oxygen species mediate cyclic strain-induced endothelin-1 gene expression via Ras/Raf/extracellular signal regulated kinase pathway in endothelial cells. *J Mol Cell Cardiol* 33**:** 1805-1814.

CHENG, J.J., WUNG, B.S., CHAO, Y.J. & WANG, D.L. (1998). Cyclic strain-induced reactive oxygen species involved in ICAM-1 gene induction in endothelial cells. *Hypertension* 31: 125-130.

CHIEN, S. (2007). Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. *Am J Physiol Heart Circ Physiol* 292: H1209-1224.

CHIEN, S., LI, S. & SHYY, Y. J. (1998). Effects of mechanical forces on signal transduction and gene expression in endothelial cells. *Hypertension* 31**:** 162-169.

CLARKSON, P., MONTGOMERY, H.E., MULLEN, M.J., et al. (1999). Exercise training enhances endothelial function in young men. *JACC* 33(5): 1379-1385.

COLLINS, N. T., CUMMINS, P. M., COLGAN, O. C., FERGUSON, G., BIRNEY, Y. A., MURPHY, R. P., MEADE, G. & CAHILL, P. A. (2006). Cyclic strain mediated regulation of vascular endothelial occludin and ZO-1: influence on intercellular tight junction assembly and function. *Arterioscler Thromb Vasc Biol* 26**:** 62-68.

CROUSE, S.F., O'BRIEN, B.C., GRANDJEAN, P.W., LOWE, R.C., ROHACK, J.J. & GREEN, J.S. (1997). Effects of training and a single session of exercise on lipids and apolipoproteins in hypercholesterolemic men. *J Appl Phsiol* 83: 2019-2028.

CURTIS, A.M., ZHANG, L., MEDENILLA, E., GUI, M., WILKINSON, P., HU, E., GIRI, J., DORASIWAMY, V., GUNDA, S., BURGERT, M., EDELBERG, J. & MOHLER, E III. (2008). Ratio of microparticles to endothelial progenitor cells is increased in a diabetic population. *Circ* 118(18): S_ 1113.

CYBULSKY, M.I. AND GIMBRONE, M.A. (1991). Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* 251: 788-791.

DAGRE, A. G., LEKAKIS, J.P., PROTOGEROU, A.D., COURIDAS, G.N., PAPAIOANNOU, T.G., TRYFONOPOULOS, D.J., PAPMICHAEL, C. M. & ALEVIZAKI, M. (2007). Abnormal endothelial function in female patients with hypothyroidism and borderline thyroid function. *I J Cardiol* 114(3): 332-338.

DAROVIC, G. (2002). *Hemodynamic Monitoring Invasive and Non-invasive Clinical Application*. United States, Saunders.

DAUGHERTY, A., & RATERI, D. (2002). T Lymphocytes in Atherosclerosis The Yin-Yang of Th1 and Th2 Influence on Lesion Formation. *Circ Res*. 90:1039-1040.

DAS, U. N. (2004). Anti-inflammatory nature of exercise. *Nutrition* 20: 323-326.

DAVIDOVITS, P. (2007). *Physics in Biology and Medicine*. United States. Academic Press.

DAVIES, P. (1995). Flow-mediated endothelial mechanotransduction. *Physiol Rev* 75: 519-560.

DAVIES, P. (2007). Hemodynamics in the determination of endothelial phenotype and flow mechanotransduction. In Aird, W. *Endothelial Biomedicine*. United Kingdom, Cambridge University Press.

DAVIES, M.J., GORDON, J.L., GEARING, A.J.H et al (1993). The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol* 171: 223-229.

DAVIES, P. F., ROBOTEWSKYJ, A. & GRIEM, M. L. (1994). Quantitative studies of endothelial cell adhesion. Directional remodeling of focal adhesion sites in response to flow forces. *J Clin Invest* 93**:** 2031-8.

DAVIS, M.A. (2002). *Apoptosis methods in pharmacology and toxicology: approaches to measurement and quantification*. US. Humana Press.

DE CATERINA, R. & BASTA, G. (1997a). Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. *Arterioscle Thromb Vasc Biol* 17: 2646-2654.

DE CATERINA, R., BASTA, G., LAZZERINI, G. et al. (1997b). Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. *Arterioscler Thromb Vasc Biol* 17: 2646-2654.

DE CATERINA, R. & LIBBY, P. (2007). *Endothelial Dysfunctions and Vascular Disease*. UK, Blackwell Publishing.

DEMOPOULOS, L., BIJOU, R., FERGUS, I., JONES, M., STROM, J. & LEJEMTEL, T.H. (1997). Exercise training in patients with severe congestive heart failure:

enhancing peak aerobic capacity while minimizing the increase in ventricular wall stress. *J Am Coll Cardiol* 29: 597-603.

DE ROOS, N.M., BOT, M., SCHOUTEN, E.G. & KATAN, M.B. (2003). Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. *Ultrasound Med Biol* 29(3): 401-406.

DE SOUZA, C. A., SHAPIRO, L. F., CLEVENGER, C. M., DINENNO, F. A., MONAHAN, K. D., TANAKA, H. & SEALS, D. R. (2000). Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* 102: 1351-1357.

DIGNAT-GEORGE, F., SABATIER, F., CAMOIN-JAU, L., SAMPOL, J. (2004). Measuring circulating cell-derived microparticles. *J Thromb Haemost* 2: 1844-1845.

DILL, D.B. & COSTILL, D.L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247-248.

DINENNO, F.A., TANAKA, H., MONAHAN, K.D., CLEVENGER, C.M., ESKURZA, I., DESOUZA, C.A. & SEALS, D.R. (2001). Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men. *J Physiol* 534: 287–295.

DUNCKER, D.J., VANZON, N.S., PAVEK, T.J., HERRLINGER, S.K. & BACHE, R.J. (1995). Endogenous adenosine mediates coronary vasodilation in response to exercise after K1ATP channel blockade. *J Clin Invest* 95: 285–295.

DUTTON, M. (2008). Orthopaedic examination, evaluation and intervention. United States, McGraw Hill Professional.

DVORAK, A (2007). Electron Microscopic – Facilitated Understanding of Endothelial Cell Biology. In Aird, W. *Endothelial Biomedicine*, United Kingdom, Cambridge University Press.

ENGELKE, K.A., HALLIWILL, J.R., PROCTOR, D.N., DIETZ, N.M. & JOYNER, M.J. (1996). Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm. *J Appl Physiol* 81: 1807-1814.

ERZEN, B., SABOVIC, M., SEBESTJEN, M., KEBER, I. & POREDOS, P. (2007). Interleukin-6 correlates with endothelial dysfunction in young post-myocardial infarction patients. *Cardiology* 107: 111-116.

ESKURZA, I., SEALD, D.R., DESOUZA, C.A. & TANAKA, H. (2001). Pharmacologic versus flow-mediated assessments of peripheral vascular endothelial vasodilatory function in humans. *Am J Cardiol* 88: 1067-1069.

ESPOSITO, K., CIOTOLA, M., SCHISANO, B., GUALDIERO, R., SARDELLU, L., MISSO, L., GIANNETTI, G. & GIUGLIANO, D. (2006). Endothelial

microparticles correlate with endothelial dysfunction in obese women. *JCEM* 91(9): 3676-3679.

FELMEDEN, D. & LIP, G. (2005). Endothelial function and its assessment. *Expert Opin. Investig. Drugs.* 14(11): 1319-1336.

FOX, S. (1993). *Human Physiology (4ᵗʰ Ed.).* United States, Brown Publishers.

FRANK, E. et al (1993). Cardiovascular Disease Risk Factors: Improvements in Knowledge and Behaviour in the 1980s. *Am J Public Health.* 83(4): 590-593.

FRAYN, K. & STANNER, S. (2005). *Cardiovascular Disease: Diet, Nutrition and Emerging Risk Factors.* England, Blackwell Publishing.

FRIEDMAN, M. (2008). *Principles and models of biological transport.* United States, Springer.

FUSTER, V., et al (2004). *Hurst's The Heart (11ᵗʰ Ed.)* United States. McGraw-Hill Professional.

FUSTER, V., ROSS, R., & TOPOL, E.J. (1996). *Atherosclerosis and Coronary Artery Disease.* Philadelphia: Lippincott Raven.

GALLEY, H.F. & WEBSTER, N.R. (2004). Physiology of the Endothelium. *British Journal of Anaesthesia.* 93(1): 105-113.

GAMBILLARA, V., CHAMBAZ, C., MONTORZI, G., ROY, S., STERGIOPULOS, N. & SILACCI, P. (2006). Plaque-prone hemodynamics impair endothelial function in pig carotid arteries. *Am J Physiol Heart Circ Physiol* 290:H2320-2328.

GARDNER, A.M.N. & FOX, R.H. (2000). *The venous system in health and disease.* Netherlands, IOS Press.

GEARING, A.J. & NEWMAN, W. (1993). Circulating adhesion molecules in disease. *Immunol Today* 14: 506–512.

GOKCE, N. & VITA, J.A. (2002a). Clinical manifestations of endothelial dysfunction. In Loscalzo, J. & Schafer, A.I. (Eds) *Thrombosis and Hemorrhage.* Philadelphia, Lippincott, Williams and Wilkins.

GOKCE, N., VITA, J.A., et al. (2002b). Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *Am J Cardiol* 90: 124-127.

GOON, P.K.Y., LIP, G.Y.H., BOOS, C.J., STONELAKE, P.S. & BLANN, A.D. (2006). Circulating endothelial cells, endothelial progenitor cells, and endothelial microparticles in cancer. *Neoplasia* 8(2): 79-88.

GOTO, C., HIGASHI, Y., KIMURA, M., NOMA, K., HARA, K., NAKAGAWA, K., KAWAMURA, M., CHAYAMA, K., YOSHIZUMI, M. & NARA, I. (2003). Effect of different intensities of exercise on endothelium-dependent

vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 108: 530-535.

GRAVES, J. et al. (2006). Anthropometry and Body Composition Measurement. In Maud, P., and Foster, C. (2006). *Physiological Assessment of Human Fitness*. US, Human Kinetics.

GREEN, D.J., BILSBOROUGH, W., NAYLOR, L., REED, C., WRIGHT, J., O'DRISCOLL, G. & WALSH, J. (2005). Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *J Physiol* 562: 617-628.

GREEN, D.J., CABLE, N.T., FOX, C., RANKIN, J.M. & TAYLOR, R.R. (1994). Modification of forearm resistance vessels by exercise training in young men. *J Appl Physiol* 77:1829-1833.

GREEN, D.J., CHEETHAM, C., MAVADDAT, L., WATTS, K., BEST, M., Taylor, R.R. & O'DRISCOLL, G. (2002a). Effect of lower limb blood flow on forearm vascular function: Contribution of nitric oxide. *Am J Physiol Heart Circ Physiol* 283: H899-907.

GREEN, D.J., CHEETHAM, C., REED, C., DEMBO, L. & O'DRISCOLL, G. (2002b). Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *J Appl Physiol* 93: 361-368.

GREEN, D. J., MAIORANA, A. J. & CABLE, N. T. (2008). Point: Exercise training does induce vascular adaptations beyond the active muscle beds. *J Appl Physiol* 105(3): 1002-1004.

GREEN, D.J., MAIORANA, A. J, O'DRISCOLL, G. & TAYLOR, R. (2004a). Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol* 15: 1-25.

GREEN, D. J., MAIORANA, A. J., TSCHAKOVSKY, M., PYKE, K., WEISBROD, C. & O'DRISCOLL, J.G. (2006). Relationship between changes in brachial artery flow-mediated dilation and basal release of nitric oxide in subjects with Type 2 diabetes. *Am J Physiol Heart Circl Physiol* 291: H1193-1199.

GREEN, D. J., WALSH, J. H., MAIORANA, A., BEST, M. J., TAYLOR, R. R. & O'DRISCOLL, J. G. (2003). Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *Am J Physiol Heart Circ Physiol* 285: H2679-2687.

GREEN, D. J., WALSH, J. H., MAIORANA, A., BURKE, V., TAYLOR, R. R. & O'DRISCOLL, J. G. (2004b). Comparison of resistance and conduit vessel nitric oxide-mediated vascular function in vivo: effects of exercise training. *Am J Physiol* 97: 749-755.

GRIENDLING, K.K. & FITZGERALD, G.A. (2003a). Oxidative stress and cardiovascular injury. Part I: Basic mechanisms and in vivo monitoring of ROS. *Circulation* 108: 1912-1916.

GRIENDLING, K.K. & FITZGERALD, G.A. (2003b). Oxidative stress and cardiovascular injury. Part II: Animal and human studies. *Circulation* 108: 2034-2040.

GROOTHUIS, J., VAN VLIET, L., KOOIJMAN, M., & HOPMAN, M. (2003). Venous cuff pressures from 30mmHg to diastolic pressure are recommended to measure arterial inflow by plethysmography. *J Appl Physiol* 95: 342-347.

GUDI, S.R.P., CLARK, C.B.& FRANGOS, J.A. (1996). Fluid Flow Rapidly Activates G Proteins in Human Endothelial Cells Involvement of G Proteins in Mechanochemical Signal Transduction. *Circ* 79: 834-839.

GUYTON, A.C. (1991). *Text Book of Medical Physiology. (8ᵗʰ Ed.).* United States, W. B. Saunders.

HANSSON, G. (2001). Immune Mechanisms in Atherosclerosis. *Arterioscler Thromb Vasc Biol* 21:1876-1890.

HANSSON, G. (2005). Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med.* 352: 1685-1695.

HAMBRECHT, R., ADAMS, V., ERBS, S., LINKE, A., KRANKEL, N., SHU, Y., BAITHER, Y., GIELEN, S., THIELE, H., GUMMERT, J.F., MOHR, F.W. & SCHULER, G. (2003). Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 107(25): 3152-3158.

HAMBRECHT. R., FIEHN, E., WEIGL, C., GIELEN, S., HAMANN, C., KAISER, R., YU, J., ADAMS, V., NIEBAUER, J. & SCHULER, G. (1998). Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* 98: 2709-2715.

HAMBRECHT, R., WALTHER, C., MOBIUS-WINKLER, S., GEILEN, S., LINKE, A., CONRADI, K., ERBS, S., KLUGE, R., KENDZIORRA, K., SABRI, O., SICK, P. & SCHULER, G. (2004). Percutaneous coronary angioplasty compared with exercise training in patients with stable coronary artery disease: A randomized trial. *Circulation* 109: 1371-1378.

HAMBRECHT, R., HILBRICH, L., ERBS, S., et al. (2000). Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med* 342: 454-460.

HAMBURG, N., MCMACKIN, C., HUANG, A., SHENOUDA, S., WIDLANSKY, M., SCHULZ, E., GOKCE, N., RUDERMAN, N., KEANEY, J.F. JR. & VITA, A. (2007). Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. *Arterioscler Thromb Vasc Biol* 27: 2650-2656.

HARRIS, P., NAGY, S. & VARDAXIS, N. (2005). *Mosby's dictionary of medicine, nursing and health professions.* United States, Elsevier-Mosby.

HARRISON, D.G., SAYEGH, H., OHARA, Y., INOUE, N. & VENEMA, R.C. (1996). Regulation of expression of the endothelial cell nitric oxide synthase. *Clin Exp Pharmacol Physiol* 23: 251-255.

HARRISON, D.G., WIDDER, J., GRUMBACH, I., CHEN, W., WEBER, M., & SEARLES, C. (2006). Endothelial mechanotransduction, nitric oxide and vascular inflammation. *Journal of Internal Medicine* 259: 351-363.

HELLSTEN, Y., MACLEAN, D., RADEGRAN, G., SALTIN, B. & BANGSBO J. (1998). Adenosine concentration in the interstitium of resting and contracting human skeletal muscle. *Circulation* 98: 6–8.

HELMENSTEIN, A. (2009). Nitric Oxide. Available at: *http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---N/Nitric -Oxide.htm* Accessed on 24th August 2009.

HESTER, R.L., ERASLAN, A. & SAITO, Y. (1993). Differences in EDNO contribution to arteriolar diameters at rest and during functional dilation in striated muscle. *Am J Physiol Heart Circ Physiol* 265: H146–151.

HIGASHI, Y., SASAKI, S., KIEGO, N., et al. (2001b). Effect of angiotensin-converting enzyme inhibitor imidapril on reactive hyperemia in patients with essential hypertension: relationship between treatment periods and resistance artery endothelial function. *J Am Coll Cardiology* 37: 863-870.

HIGASHI, Y., SASAKI, S., NAKAGAWA, K., MATSUURA, H., KAJIYAMA, G., & OSHIMA, T. (2001a). A non-invasive measurement of reactive hyperemia that can be used to assess resistance artery endothelial function in humans. *Am J of Cardiology* 87: 121-125.

HIGASHI, Y., SASAKI, S., SASAKI, N., NAKAGAWA, K., UEDA, T., YOSHIMIZU, A., KURISU, S., MATSUURA, H., KAJIYAMA, G. & OSHIMA, T. (1999). Daily aerobic exercise improves reactive hyperemia in patients with essential hypertension. *Hypertension* 33: 591-597.

HORNIG, B., MAIER, V. & DREXLER, H. (1996). Physical training improves endothelial function in patients with chronic heart failure. *Circulation* 93: 210-214.

HIMBURG, HA., DOWD, SE., & FRIEDMAN, MH. (2007). The frequency dependent response of the vascular endothelium to pulsatile shear stress. *Am J Physiol Heart Circ Physiol* 293: H645-H653.

HSIAI, TK., CHO SK., WONG, PK., ING, M., SALZAR, A., SEVANIAN, A., NAVAB, M., DEMER, LL., & HO, C-M. (2003). Monocyte recruitment to endothelial cells in response to oscillatory shear stress. *FASEB* J 17:1648-1657.

HUANG, A.L., SILVER, A.E., SHVENKE, E., SCHOPFER, D.W. et al. (2007). Predictive value of reactive hyperemia for cardiovascular events in patients with peripheral arterial disease undergoing vascular surgery. *Arterioscler Thromb Vasc Biol* 27: 2113-2119.

HUGEL, B., MARTINEZ, M. C., KUNZELMANN, C. & FREYSINET, J-M. (2004). Membrane Microparticles: Two Sides of the Coin. *Physiology* 20: 22-27.

HWANG, J., ING, MH., SALAZAR, S., LASSEGUE, B., GRIENDLING, K., NAVAB, M., SEVANIAN, A., & HSIAI, TK. (2003). Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: Implication for native LDL oxidation. *Circulation Res* 93: 1225-1232.

IGNARRO, L.J. (2000). *Nitric oxide: biology and pathophysiology.* Missouri, Academic Press.

IRACE, C., CERAVOLO, R., NOTARANGELO, L., CRESCENZO, A., VENTURA, G., TAMBURRINI, O., PERTICONE, F. & GNASSO, A. (2001). Comparison of endothelial function evaluated by strain gauge plethysmography and brachial artery ultrasound. *Atherosclerosis* 158: 53-59.

IRISH HEART FOUNDATION (2009). Facts on Heart disease and stroke. Available at: *http://www.irishheart.ie/iopen24/facts-heart-disease-stroke-t-7_18.html.* Accessed on 9th March 2009.

ISHIBASHI, Y. et al. (2006). Short duration of reactive hyperaemia in the forearm of subjects with multiple cardiovascular risk factors. *Circulation* 70: 115-123.

IWASAKI, H., EGUCHI, S., UENO, H., Marumo, F. & Hirata, Y. (2000). Mechanical stretch stimulates growth of vascular smooth muscle cells via epidermal growth factor receptor. *Am J Physiol Heart Circ Phsiol* 278: H521-529.

JILMA, B., EICHLER, HG, STOHLAWETZ, P., et al. (1997). Effects of exercise on circulating vascular adhesion molecules in healthy men. *Immunobiology* 197: 505-512.

JIMENEZ, J., JY, W., MAURO, L. et al (2003). Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. *Thromb Res* 109: 175-180.

JIMENEZ, J., JY, W., MAURO, L., HORTSMAN, L.L, BIDOT, C.J. & AHN, Y.S. (2005). Endothelial microparticle (EMP) as vascular disease markers. *Adv Clin Chem* 39: 131-157.

JOANNIDES, R., HAEFELI, W.E., LINDER, L., et al. (1995). Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 91: 1314-1319.

JOHN, S., JOHANNES, J., DELLES, C., SCHLAICH, M.P., ALTER, O. & SCHMIEDER, R.E.. (2000). Plasma soluble adhesion molecules and endothelium-dependent vasodilation in early human atherosclerosis. *Clinical Science* 98: 521-529.

JOYNER, M.J., DIETZ, N.M. & SHEPHERD, J.T. (2001). From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *J Appl Physiol.* 91:2431–2441.

JUGDUTT, B. (2004). *The Role of Nitric Oxide in Heart Failure*. United States. Springer.

KASAPIS, C. & THOMPSON, P.D. (2005). The effects of physical activity on serum C-reactive protein and inflammatory markers: a systemic review. *J Am Coll Cardiol* 45: 1563-1569.

KATZ, S. D., YUEN, J., BIJOU, R. & LEJEMTEL, T. H. (1997). Training improves endothelium-dependent vasodilation in resistance vessels of patients with heart failure. *J Appl Physiol*, 82, 1488-1492.

KATZUNG, B. (2007). *Basic and Clinical Pharmacology*. United States. McGraw-Hill Professional.

KERR, J. (1999). Cell adhesion molecules in the pathogenesis of and host defence against microbial infection. *J Clin Pathol: Mol Pathol*. 52: 220-230.

KERSLAKE, D.M. (1949). The effect of the application of an arterial occlusion cuff to the wrist on the blood flow in the human forearm. *J. Physiol* 108: 451-461.

KINGWELL, B.A., TRAN, B., CAMERON, J.D., et al. (1996). Enhanced vasodilation to acetylcholine in athletes is associated with lower plasma cholesterol. *Am J Physiol.* 270: H2008-2013.

KINGWELL, B.A., SHERRARD, B., JENNINGS, G.L. & DART, A.M. (1997). Four weeks of cycle training increases basal production of nitric oxide from the forearm. *Am J Physiol* 272: H1070–H1077.

KLABUNDE, R. (2004). *Cardiovascular Physiology Concepts*. United States. Lippincott Williams and Wilkins.

KOBAYASHI, N., TSRUYA, Y., IWASAWA, T., IKEDA, N., HASHIMOTO, S., YASU, T., UEBA, H., KUBO, N., FUJII, M., KAWAKAMI, M. & SAITO, M. (2003). Exercise training in patients with chronic heart failure improves endothelial function predominantly in the trained extremities. *Circ J* 67: 505-510.

KOGA, H., SUGIYAMA, S., KUGIYAMA, K., WATANABE, K., FUKUSHIMA, H., TANAKA, T., SAKAMOTO, T., YOSHIMURA, M., JINNOUCHI, H. & OGAWA, H. (2005). Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol* 45:1622–1630.

LAUGHLIN, M.H., NEWCOMER, S.C. & BENDER, S.B. (2008). Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol* 104: 588-600.

LAVRENCIC, A., SALOBIR, B. & KEBER, I. (2000). Physical training improves flow-mediated dilation in patients with the polymetabolic syndrome. *Arterioscler Thromb Vasc Biol* 20: 551-555.

LEEUWENBERG, J.F., SMEETS, E.F., NEEFJES, J.J. et al. (1992). E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. *Immunology*. 77: 543-549.

LELKES, P. (1999). *Mechanical forces and the endothelium.* United States, CRC Press.

LI, H., CYBULSKY, MA. & LIBBY, P. (1993). An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arterioscler Thromb*.13(2): 197-204.

LIBBY, P. (2002). Inflammation in atherosclerosis. *Nature* 420: 868-874.

LIEBERMAN, E.H., GERHARD, M.D., UEHATA, A., SELWYN, A.P., GANZ, P., YEUNG, A.C. & CREAGER, M.A. (1996). Flow induced vasodilation of the human brachial artery is impaired in patients < 40 years of age with coronary artery disease. *Am J Cardiol*. 78(11): 1210-1214.

LILLY, L. (1992). *Pathophysiology of Heart Disease*. Philadelphia, Lea & Febiger.

LIND, L., HALL, J., LARSON, A., ANNUK, M., FELLSTROM, B. & LITHELL, H. (2000). Evaluation of endothelium-dependent vasodilation in the human forearm peripheral circulation. *Clin Physiol* 20: 440-448.

LINKE, A., SCHOENE, N., GIELEN, S., et al. (2001). Endothelial dysfunction in patients with chronic heart failure: systemic effects of lower-limb exercise training. *J Am Coll Cardiol* 37: 392-397.

LOSCALZO, J. & VITA, J.A. (1994). Ischemia, hyperemia, exercise and nitric oxide. Complex physiology and complex molecular adaptations. *Circulation* 90: 2556-2559.

LOSCALZO, J. & VITA, J.A. (2000). *Nitric Oxide and the Cardiovascular System*. Totawa, NJ, Humana Press.

LYNCH, J. P. III (2003). *Idiopathic pulmonary fibrosis.* London. Informa Health Care.

MARIEB, E. & HOEHN, K. (2007). *Human Anatomy & Physiology*. United States, Pearson-Benjamin Cummings.

MAIORANA, A., O'DRISCOLL, G., CHEETHAM, C., DEMBO, L., STANTON, K., GOODMAN, C., TAYLOR, R. & GREEN, D. (2001). The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J Am Coll Cardiol* 38: 860-866.

MAIORANA, A., O'DRISCOLL, G., DEMBO, L., CHEETHAM, C., GOODMAN, C., TAYLOR, R. & GREEN, D. (2000). Effect of aerobic and resistance exercise training on vascular function in heart failure. *Am J Physiol Heart Circ Physiol* 279: H1999-2005.

MALAT, Z., BENAMER, H., HUGEL, B., BENESSIANO, J., STEG, P.G., FREYSSINET, J.M. & TEDGUI, A. (2000). Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral

circulating blood of patients with acute coronary syndromes. *Circulation* 101: 841-843.

MANO T, MASUYAMA T, YAMAMOTO K, et al. (1996). Endothelial dysfunction in the early stage of atherosclerosis precedes appearance of intimal lesions assessable with intravascular ultrasound. *Am Heart J*. 131:231–238.

MARTIN, S., et al (2004). Shed membrane particle from T lymphocytes with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation* 109: 1653-1659.

MARTINEZ, MC., et al (2005). Shed membranes microparticles from circulating and vascular cells in regulating vascular function. *AJP-Heart* 288: 1004-1009.

MAUD, P., and FOSTER, C. (2006). *Physiological Assessment of Human Fitness*. US, Human Kinetics.

MCARDLE, W.D., KATCH, F.I. & KATCH, V.L. (2007). *Exercise physiology: energy, nutrition and human performance.* United States, Lippincott Williams & Wilkins.

MCNALLY, JS., DAVIS, ME., GIDDENS, DP., SAHA, A., HWANG, J., DIKALOV, S., JO, H, & HARRISON DG. (2003). Role of xanthine oxiodoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol Heart Circ Physiol* 285: H2290-2297.

MEHTA, D., & MALIK, A. (2006). Signaling Mechanisms Regulating Endothelial Permeability. *Physiol Rev* 86:279-367.

MEIGS, J.B., HU, F.B., RIFAI, N. & MANSON, J.E. (2004). Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *JAMA* 291(16): 1978-1986.

MEREDITH, I.T., CURRIE, K.E., ANDERSON, T.J., RODDY, M.A., GANZ, P. & CREAGER, M.A. (1996). Postischemic vasodilation in human forearm is dependent on endothelium-derived nitric oxide. *Am J Physiol Heart Circ Physiol* 270: H1435-1440.

MICHIELS, C. (2003). Endothelial cell functions. *J Cellular Physiol* 196: 430-443.

MILLER, V.M. & BURNETT, J.C., Jr. (1992). Modulation of NO and endothelin by chronic increases in blood flow in canine femoral arteries. *Am J Physiol*. 263: H103-108.

MILSTONE, D. (2007). E-Selectin. In Aird, W. *Endothelial Biomedicine*, United Kingdom, Cambridge University Press.

MOORE, K., AGUR, A., & DALLEY, A. (2006). *Essential Clinical Anatomy*. United States. Lippincott Williams & Wilkins.

MORA, S., COOK, N., BURING, J., RIDKER, P. & LEE, I. (2007). Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation* 116: 2110-2118.

MORA, S., LEE, I.M., BURING, J.E. & RIDKER, P.M. (2006). Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. *JAMA* 295: 1412-1419.

MOTOHIRO, M., YUASA, F., HATTORI, T., SUMIMOTO, T., TAKEUCHI, M., KAIDA, M., JIKUHARA, T., HIKOSAKA, M., SUGUIRA, T. & IWASAKA, T. (2005). Cardiovascular adaptations to exercise training after uncomplicated acute myocardial infarction. *Am J Phys Med Rehab* 84(9): 684-691.

MOYNA, N. & THOMPSON, P. (2004). The effect if physical activity on endothelial function in man. *Acta Physiol Scand.* 180: 113-123.

MULLER, J.M., CHILIAN, W.M. & DAVIES M.J. (1997). Integrin signaling transduces shear stress-dependent vasodilation of coronary arterioles. *Circulation Res*.80:320–326.

MULLER, W.A. (2003). Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. *Trends Immunol* 24: 327-334.

MURO, S. (2007). Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. In Aird, W. *Endothelial Biomedicine*. United Kingdom, Cambridge University Press.

MURO, S., & MUZYKANTOV, V.R. (2005). Targeting of antioxidant and antithrombotic drugs to endothelial cell adhesion molecules. *Curr Pharm Des*. 11(18): 2383-2401.

MURRAY, A., DELANEY, T., & BELL, C. (2006). Rapid onset and offset of circulatory adaptations to exercise training in men. *Journal of Human Hypertension*. 20:193-200.

NIEBAUER, J. (1996). Cardiovascular effects of exercise: role of endothelial shear stress. *J Am Coll Cardiology*. 28(7): 1652-1660.

NOBLE, A., JOHNSON, R., & THOMAS, A. (2005). *The Cardiovascular System*. United States Elsevier Health Sciences.

O'LEARY, D. S. (1991). Regional vascular resistance vs. conductance: which index for baroreflex responses? *Am J Physiol Heart Circ Physiol* 260: H632-637.

O'SULLIVAN, S.E. (2003). The effects of exercise training on markers of endothelial function in young healthy men. *Int J Sports Med*. 24:404-409.

PACHER, P., BECKMAN, J.S. & LIAUDET,L. (2007). Nitric oxide and peroxynitire: in health and disease. Physiological Reviews 87(1): 315-424.

PASCERI, V., WILLERSON, T., & YEH, E. (2000). Direct proinflammatory Effect of C-Reactive Protein on Human Endothelial Cells. *Circulation* 102: 2165-2168.

PETRIE, J.R., UEDA, S., MORRIS, A.D., MURRAY, L.S., ELLIOTT, H.L. & CONNELL, J.M. (1998). How reproducible is bilateral forearm plethysmograpohy? *Br J Clin Pharmacol*. 45: 131-139.

PIERCE, G. L., SCHOFIELD, R. S., CASEY, D. P., HAMLIN, S. A., HILL, J. A. & BRAITH, R. W. (2008). Effects of exercise training on forearm and calf vasodilation and proinflammatory markers in recent heart transplant recipients: a pilot study. *Eur J Cardiovasc Prev Rehabil*. 15: 10-18.

PLOWMAN, S. & SMITH, D. (2007). *Exercise physiology for health, fitness and performance*. United States, Lippincott Williams and Wilkins.

PRYOR, W.A. & SQUADRITO, G.L. (1995). The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol Lung Cell Mol Physiol* 268: 699-722.

RADEGRAN, G. & CALBET, J.L.A. (2001). Role of adenosine in exercise-induced human skeletal muscle vasodilation. *Acta Physiol Scand* 171: 177–185.

RAITAKARI, O.T., LAI, N., GRIFFITHS, K., MCCREDIE, R., SULLIVAN, D. & CELERMAJER, D.S. (2000). Enhanced peripheral vasodilation in humans after a fatty meal. *J Am Coll Cardiol* 36: 417-422.

RIDKER, P.M., HENNEKENS, C.H., ROITMAN-JOHNSON, B., STAMPFER, M.J. & ALLEN, J. (1998). Plasma concentrations of soluble intercellular adhesion molecule-1 and risks of future myocardial infarction in apparently healthy men. *Lancet* 351: 88-92.

RIDOUT, S.J., PARKER, B.A. & PROCTOR D.N. (2005). Age and regional specificity of peak limb vascular conductance in women. *J Appl Physiol* 99: 2067–2074.

RINDER, M.R., SPINA, R.J. & EHSANI, A.A. (2000). enhanced endothelium-dependent vasodilation in older endurance-trained men. *J Appl Physiol* 88: 761-766.

ROBERT, S., PONCELET, P., LACROIX, R., ARNAUD, L., GIRAUDO, L., HAUCHARD, A., SAMPOL, J. & DIGANT-GEORGE, F. (2008). Standardization of platelet-derived microparticle counting using calibrated beads and a Cytomics FC500 routine flow cytometer: a first step towards multicenter studies? *J Thromb Haem*, 7: 190–197.

ROBERTS, D.H., TSAO, Y. & BRECKENRIDGE, A.M. (1986). The reproducibility of limb blood flow measurements in human volunteers at rest and after exercise by using mercury-in-silastic strain gauge plethysmography under standardised conditions. *Clin Sci* 70: 635-638.

ROSS, R., & GLOMSER, J.A. (1973). Atherosclerosis and the arterial smooth muscle cell: proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. *Science* 180:1332-1339,

ROWELL, L.B. (1986). *Human Circulation: Regulation During Physical Stress*. New York, NY: Oxford University Press.

SAFAR, M. & FROHLICH, E.D. (2000). Atherosclerosis, large arteries and cardiovascular risk. Switzerland, Karger.

SAGRIPANTI, A. & CARPI, A. (2000). Antithrombotic and prothrombotic activities of the vascular endothelium. *Biomed Pharmacother*. 54**:** 107-111.

SCHACHINGER, V. & ZEHIER, A.M. (2001). Prognostic implication of endothelial dysfunction: does it mean anything? *Coronary Artery Disease*. 12: 435-443.

SCHONBECK, U., & LIBBY, P (2001). CD40 Signalling and Plaque Instability. *Circ. Res.* 89:1092-1103.

SCHRAM, M.T. & STEHOUWER, C.D.A. (2005). Endothelial dysfunction, cellular adhesion molecules and the metabolic syndrome. *Hormone and Metabolic Research. 37 Supplement*. 1:49-55.

SCHULTE, K.L., BRAUN, J., MEYER-SABELLEK, W., WEGSCHEIDER, K., GOTZEN, R. & DISTLER, A. (1988). Functional versus structural changes of forearm vascular resistance in hypertension. *Hypertension* 11: 320-325.

SHERWOOD, L. (2005). *Fundamentals of Physiology: a human perspective*. United States. Cengage Learning.

SHIMABUKURO, M., CHINEN, I., HIGA, N., TAKASU, N., YAMAKAWA, K. & UEDA, S. (2007). Effects of dietary composition on postprandial endothelial function and adiponectin concentrations in healthy humans: a crossover controlled study. *Am J Clin Nutr* 86: 923-928.

SHIMABUKURO, M., HIGA, N., TOMOHIRO, A., OSHIRO, Y., TAKASU, N., TAGAWA, T., UEDA, S., SHIMOMURA, I., FUNAHASHI, T. & MATSUZAWA, Y. (2003). Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *JCEM* 88(7): 3236-3240.

SIMON, BC., CUNNINGHAM, LD., & COHEN, RA. (1990). Oxidised low density lipoproteins cause contraction and inhibit endothelium-dependent relaxation in the pig coronary artery. *J Clin Invest* 86(1): 75-79.

SINOWAY, L.I. et al (1986). Enhanced maximal metabolic vasodilation in the dominant forearms of tennis players. *Journal of Applied Physiology* 61: 673-678.

SMITH, J., & KAMPINE, J. (1990). *Circulatory Physiology*. United States, Williams & Wilkins.

SMITH, L.L., ANWAR, A., FRAGEN, M., RANANTO, C., JOHNSON, R. & HOLBERT, D. (2000). Cytokines and cell adhesion molecules associated with high intensity eccentric exercise. *Eur J Appl Physiol* 82: 61-67.

TAGAWA, T., IMAIZUMI, T., ENDO, T., SHIRAMOTO, M., HARASAWA, Y. & TAKESHITA, A. (1994). Role of Nitric Oxide in reactive hyperemia in human forearm vessels. *Circulation* 90: 2285-2290.

TAKASE, B., UEHATA, A., AKIMA, T., et al. (1998). Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. *American Journal of Cardiology.* 82(12): 1535-1539.

TALUSAN, P., BEDRI, S., YANG, S., KATTAPURAN, T., SILVA, N., ROUGHLEY, P.J. & STONE, J.R. (2005). Analysis of intimal proteoglycans in atherosclerosis-prone and atherosclerosis-resistant human arteries by mass spectrometry. *Moll Cell Proteomics* 4(9): 1350-1357.

TANAKA, H., SHIMIZU, F., OHMORI, Y., MURAOKA, M., KUMAGAI, M., YOSHIZAWA, M. & KAGAYA, A. (2006). *Med. Sci. Sports Exerc.,* 38(1): 81-85.

THIJSSEN, D., BLEEKER, M., SMITS, P. & HOPMAN, M. (2005a). Reproducibility of blood flow and post-occlusive reactive hyperemia as measured by venous occlusion plethysmography. *Clinical Science* 108: 151-157.

THIJSSEN, D. H., HEESTERBEEK, P., VAN KUPPEVELT, D. J., DUYSENS, J. & HOPMAN, M. T. (2005b). Local vascular adaptations after hybrid training in spinal cord-injured subjects. *Med Sci Sports Exerc* 37: 1112-1118.

THIJSSEN, D. H. & HOPMAN, M. T. (2008). Counterpoint: Exercise training does not induce vascular adaptations beyond the active muscle beds. *J Appl Physiol* 105(3): 1004-1006.

THOMPSON, P.D., CROUSE, S.F., GOODPASTER, B., KELLEY, D., MOYNA, M. & PESCATELLO, L. (2001). The acute versus the chronic response to exercise. *Med Sci Sports Exerc.* 33: S438-445.

TOUSOULIS, D., ANTONIADES, C. et al. (2003). Antioxidant vitamins C and E administration in smokers: effects on endothelial function and adhesion molecules. *Atherosclerosis* 170: 263-269.

TOUSOULIS, D., ANTONIADES, C. & STEFANADIS, C. (2005). Evaluating endothelial function in humans: A guide to invasive and non-invasive techniques. *Heart* 91: 553-558.

TOUSOULIS, D., CHARAKIDA, M. & STEFANADIS, C. (2008). Endothelial function and inflammation in coronary artery disease. *Postgrad Med J* 84:368-371.

TSCHAKOVSKY, M.E., SHOEMAKER, J.K. & HUGHSON, R.L. (1995). Beat-by-beat forearm blood flow with Doppler ultrasound and strain gauge plethysmography. *J Appl Physiol* 79: 713–719.

TSIMIKAS, S. & WITZTUM, J.L. (2001). Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. *Circulation* 103:1930-1932.

VANE, J.R., ANGGARD, E.E. & BOTTING, R.M. (1990). Regulatory functions of the vascular endothelium. *New England Journal of Medicine.* 323(1): 27-36.

VANWIJK, M.J., VANBAVEL, E., STURK, A. & NIEUWLAND, R. (2003). Microparticles in cardiovascular diseases. *Cardiovasc Res.* 59:277-287.

VERMA, S. & ANDERSON, T.J. (2002). Fundamentals of endothelial function for the clinical cardiologist. *Circulation.* 105: 546-549.

VERMA, S., & YEH, E. (2003). C-reactive protein atherothrombosis – Beyond a biomarker: an actual partaker of lesion formation. *Am J Physiol Regul Integr Comp Physiol.* 285: 1253-1256.

VEVES, A. & FREEMAN, R. (2007). Diagnosing endothelial cell dysfunction. In Aird, W. *Endothelial Biomedicine*, United Kingdom, Cambridge University Press.

WALKER, H.A.J.G., RITTER, J.M. & CHOWIENCZYK, P.J. (2001). Assessment of forearm vasodilator responses to acetylcholine and albutrol by strain gauge plethysmography: reproducibility and influence of strain gauge placement. *Br J Clin Pharmacol* 51: 225-229.

WANNAMETHEE, S.G., LOWE, G.D., WHINCUP, P.H., RUMLEY, A., WALKER, M. & LENNON, L. (2002). Physical activity and hemostatic and inflammatory variables in elderly men. *Circulation* 105: 1785-1790.

WASSMANN, S., HILGERS, S., LAUFS, U., BÖHM, M. & NICKENIG, G. (2002). Angiotensin II Type 1 Receptor Antagonism Improves Hypercholesterolemia-Associated Endothelial Dysfunction. *Arterio Thromb Vas Biol* 22:1208-1212.

WATANABE, S., TAGAWA, T., YAMAKAWA, K., SHIMABUKURO, M. & UEDA, S. (2005). Inhibition of the renin-angiotensin system prevents free fatty acid-induced acute endothelial dysfunction in humans. *Arterioscler Thromb Vasc Biol* 25: 2376-2380.

WAUGH, A., & GRANT, A. (2006). *Ross and Wilson anatomy and Physiology: in health and illness*. Edinburgh. Churchill Livingston.

WEST, S.G., WAGNER, P., SCHOEMER, S.L., HECKER, K.D., HURSTON, K.L., LIKOS KRICK, A., BOSESKA, L., ULBRECHT, J. & HINDERLITER, A.L. (2004). Biological correlates of day-to-day variation in flow-mediated dilation in individuals with Type 2 diabetes: a study of test-retest reliability. *Diabetologia* 47(9): 1625-1631.

WHITE, C.R. & FRANGOS, J.A. (2007). The shear stress of it all: the cell membrane and mechanochemical transduction. *Phil Trans R Soc B* 362: 1459-1467.

WHO (2009). Cardiovascular diseases. Available at: *http://www.who.int/topics/ cardiovascular_diseases/en/*. Accessed on 12th March 2009.

YUN, .J.K, ANDERSON, J.M. & ZIATS, N.P. (1999). Cyclic-strain-induced endothelial cell expression of adhesion molecules and their roles in monocyte-endothelial interaction. *J Biomed Materials Res* 44: 87-97.

ZALBA, G., BEAUMONT, J., SAN JOSE, G et al (2000). Vascular oxidant stress: molecular mechanisms and pathophysiological implications. *J Physiol Biochem* 56(1): 57-64.

ZHENG, W., SEFTOR, E. A., MEININGER, C. J., HENDRIX, M. J. & TOMANEK, R. J. (2001). Mechanisms of coronary angiogenesis in response to stretch: role of VEGF and TGF-beta. *Am J Physiol Heart Circ Physiol*. 280**:** H909-917

ZIMMERMAN, G.A., ALBERTINE, K.H., CARVETH, H.J., GILL, E.A.,et al. (1999). Endothelial Activation in ARDS. *Chest* 116: 185-245.

ZOPPINI, G., TARGHER, G., ZAMBONI, C., VENTURI, C., CACCIATORI, V., MOGHETTI, P. & MUGGEO, M. (2006). Effects of moderate-intensity exercise training on plasma biomarkers of inflammation and endothelial dysfunction in older patients with type 2 diabetes. *Nutr Metab Cardiovasc Dis*. 16: 543-549.

Appendices

Appendix A

Recruitment Poster
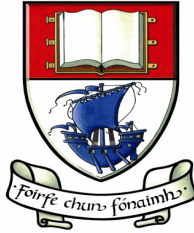
RESEARCH VOLUNTEERS REQUIRED
To determine the influence of exercise training on vascular health.

How can you help?

• Each volunteer will be asked to attend the Exercise and Health Department at Waterford Institute of Technology. During this visit you will be asked to complete consent forms and health questionnaires and blood samples and blood flow measurements will be taken.

• As a volunteer you will be asked to give your full commitment for the duration of the research.

• You will be asked to abstain from exercise for seven days prior to the pre-training measurements.

• You will be asked to participate in 10 supervised exercise sessions (30-60min) over a two-week period - this will take place in WIT.

What you will receive:

As a volunteer of this research you will receive a printed report detailing your results in relation to your:

- Total Cholesterol   - HDL Cholesterol   - LDL Cholesterol

- % Body Fat   - Triglycerides   - Glucose

- Hand Grip strength   - Body Mass Index   - Blood Pressure

- Aerobic Fitness

You will also receive a breakdown of the norm values for each of the above.

How can you get involved?

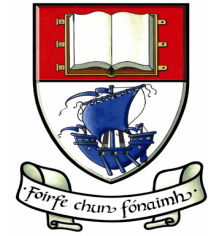If you are male, a non-smoker and aged 30-45 years, please contact:

Ms Deirdre Upton 087-9388434
Email: dupton@wit.ie

All information will be kept strictly confidential.

Appendix B

Health History Questionnaire

Health History Questionnaire

Please read the following carefully and answer all truthfully. Information will be treated in strictest confidence.

Name: _____   Gender: _____

 Date of birth: _____   Current Age: _____

Have you ever had a heart problem such as a heart attack, hypertrophic cardiomyopathy, congenital abnormality, heart valve defect, heart failure or heart rhythm disturbance?	Yes / No
Have you ever received treatment for a heart problem such as heart surgery, the fitting of a pacemaker / defibrillator, coronary angioplasty or heart transplantation?	Yes / No
Are you currently taking medications for your heart?	Yes / No

Do you currently or have you ever suffered from any of the following?

Arthritis, osteoporosis or any other bone or joint problem	Yes / No
Asthma, bronchitis or any other lung problem	Yes / No
Blood problems including coagulation disorders	Yes / No
Diabetes (type I or type II)	Yes / No
Epilepsy	Yes / No
High Blood pressure	Yes / No
Liver or gastrointestinal problems	Yes / No
Kidney problems	Yes / No
Infectious disease such as HIV, hepatitis or glandular fever?	Yes / No

Do you experience any of the following?

Chest discomfort with exertion	Yes / No
Unreasonable breathlessness	Yes / No
Dizziness, fainting, blackouts	Yes / No

Palpitations or skipped heart beats   Yes / No

Unusual levels of fatigue   Yes / No

Please indicate if any of the following are true:
You have a close blood male relative (father or brother) who has had a heart
attack before age 55 or a close blood female relative (mother or sister)   Yes / No
who has had a heart attack before age 65?

You have elevated levels of cholesterol or are on lipid lowering
medication?   Yes / No

You are a cigarette smoker or have quit within the last six months?   Yes / No

You have elevated levels of blood glucose?   Yes / No

You are completely inactive (do not take 20 minutes of moderate physical
activity such as walking, three times per week).   Yes / No

Are you currently taking any medications?   Yes / No
If Yes please give details:

**Have you any other condition that may be relevant to an individual
undertaking strenuous exercise?**   Yes / No
If Yes please give details:

Physical Measurements
Blood Pressure: _____mmHg

BMI: _____Kg/m^2

Waist Circumference: _____cm

Declaration:
I have understood all of the questions put to me and that my replies are correct to the
best of my knowledge. I understand that this information will be treated with the
strictest confidence.

Signed: _____   Date: _____

Appendix C

Physical Activity Questionnaire

General Practice Physical Activity Questionnaire

Date _____   Name _____

Question 1
Please tell us the type and amount of physical activity involved in your work. Please tick one box that best corresponds with your present work from the following possibilities:

 Please tick one box only

a.   I am not in employment (e.g. retired, retired for health reasons, unemployment, full-time carer, etc).   ☐

b.   I spend most of my time at work sitting (such as in an office).   ☐

c.   I spend most of my time at work standing or walking. However, my work does not require much intense physical effort, (e.g. shop assistant, hairdresser, security guard, childminder, etc).   ☐

d.   My work involves definite physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter, cleaner, hospital nurse, gardener, postal delivery worker, etc).   ☐

e.   My work involves vigorous physical activity including handling of very heavy objects (e.g. scaffolder, construction worker, refuse collector, etc).   ☐

Question 2
How would you describe your normal walking pace?   Please tick one box only

a.   Slow pace (i.e. less than 3mph)   ☐

b.   Steady average pace   ☐

c.   Brisk pace   ☐

d.   Fast pace (i.e. over 4 mph)   ☐

Question 3
During the <u>last week</u>, how many hours did you spend on each of the following
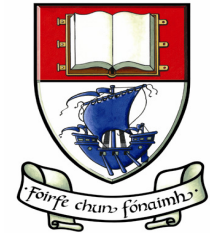activities? Please tick one box only on each row.

	None	Some but less than 1 hour	1 hour but less than 3 hours	3 hours or more
a. Physical exercise such as swimming, jogging, aerobics, football, tennis, gym workout, etc.	☐	☐	☐	☐
b. Cycling, including cycling to work and during leisure time.	☐	☐	☐	☐
c. Walking, including walking to work, shopping, for pleasure, etc.	☐	☐	☐	☐
d. Housework/Childcare	☐	☐	☐	☐
e. Gardening/DIY	☐	☐	☐	☐

Key

Inactive
Sedentary job and no recreational physical activity

Moderately inactive
Sedentary job and some but less than 1 hour recreational physical
activity per week OR
Standing job and no recreational physical activity

Moderately active
Sedentary job and 1-2.9 hours recreational physical activity per week
OR
Standing job and some but less than 1 hour recreational physical
activity per week OR
Physical job and no recreational physical activity

Active
Sedentary job and 3 hours or more recreational physical activity per
week OR
Standing job and 1-2.9 hours recreational physical activity per week
OR
Physical job and some but less than 1 hour recreational physical
activity per week OR
Heavy manual job

Ref: NICE guideline – PH12 Physical activity: Implementation advice 2006
Comprehensive patient resources are available at www.patient.co.uk

Appendix D

Informed Consent

**WATERFORD INSTITUTE OF TECHNOLOGY
RESEARCH - INFORMED CONSENT FORM**

I. Project Title:

Effect of exercise training on vascular function in active and inactive vascular beds.

II. Introduction to this study:

Exercise is known to have a positive influence on the health of the major arteries. However, we do not fully understand why this is so. It is unclear if the beneficial effects of exercise are evident in all arteries or only in the arteries that flow through exercised limbs. The physical force of blood flowing across the cells that line the artery wall may have a positive influence on cardiovascular health. Blood flow increases considerably during exercise. However, exercise reduces the level of fat in the bloodstream and increases the level of "good cholesterol". These lipid changes may be beneficial to all artery beds.

III. I am being asked to participate in this research study. The study has the following purposes:

To determine the influence of two weeks of exercise training on indicators of cardiovascular health.

To compare the effects of exercise training on indicators of vascular reactivity in exercised and non-exercised limbs.

IV. This research study will take place at the Human Performance Laboratories, Waterford Institute of Technology

V. This is what will happen during the research study:

1. During a preliminary visit, you will have your aerobic fitness determined and body fat assessed. This will involve exercising at increasing work rates on a cycle ergometer, until you reach maximal exercise capacity. For this, and all subsequent exercise tests, you will be fitted with a mouthpiece connected to a machine to measure the composition of gases in your breath. Your handgrip strength will be measured using a

Hand Dynamometer. Your body fat will be measured using an X-ray scanner (DXA). The radiation exposure during a DXA scan is trivial (< 0.3 micro sieverts) and is considered to be of minimum risk.

2. After this visit you will undertake two weeks of supervised aerobic training on the cycle ergometer (30-60 minutes of "moderate intensity" exercise on five days of each week). During the training sessions, one arm must remain perfectly still, and you will be asked to perform handgrip exercises intermittently with the other arm. This must take place in WIT.

3. Before and after the two week training intervention, measurements will be taken that will indicate if any changes in cardiovascular health have taken place. Blood flow will be measured in the forearm at rest and then after blood supply to the lower arm has been cut off for 5 minutes by inflating a blood pressure cuff. The increase in forearm blood flow, that occurs when the cuff is deflated, is an indicator of cardiovascular health.

   A blood sample will also be taken before and after the first and second week training intervention, to determine changes in blood lipids and other indicators of cardiovascular health.

4. Volunteers in will be asked to abstain from exercise for seven days prior to the pre- training measurements. They will also be asked to abstain from alcohol and follow a set diet for two days before the pre-training and post-training measurements.   These restrictions are necessary because diet and alcohol can also influence test results.

VI.Sometimes there are side effects from studies of this nature. 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 abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Because you will be asked to give a maximum effort, you may experience some muscle soreness in your arms and legs following each maximal exercise test.

2. Discomfort may be experienced during the cardiovascular health test, during the 5 minutes of forearm blood flow restriction. In addition, pins and needles may be experienced after the blood pressure cuff is deflated and normal blood flow resumes. However, there is no lasting damage associated with this procedure.

3. You may feel slight pain when the blood-drawing needle is inserted and you may develop a bruise where the blood sample was obtained. The pain and bruising is usually mild and a person trained in blood drawing will obtain your blood.

VII. <u>There may be benefits from my participation in this study. These are:</u>

1. You will receive a printed report detailing your aerobic fitness, percentage body fat, and blood concentrations of cholesterol, HDL cholesterol (good cholesterol), triglycerides and glucose.

VIII. <u>My confidentiality will be guarded:</u>

Waterford Institute of Technology will protect all the information about you

and your part in this study. Your identity or personal information will not be

revealed, published or used in future studies. The study findings will form

the basis for preparation of a postgraduate thesis, academic publications,

conference papers and other scientific publications.

IX. <u>If I have questions about the research project, I am free to call Dr. Michael Harrison at 051-302161:</u>

X. <u>Taking part in this study is my decision</u>.

If you do agree to take part in the study, you may withdraw at any point. There will be no penalty if you withdraw before you have completed all stages of the study. However, once you have completed the study you will not be allowed to have your personal information and results removed from the database.

XI.   <u>Signature:</u>

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 consent to take part in this research project entitled:

"Effect of exercise training on vascular function in active and inactive vascular beds."

Signed:

_____

Date:

_____

Witness:

_____

Appendix E

Information Sheet

INFORMATION SHEET

BACKGROUND
Vascular health refers to the well being of the heart and the blood vessels, also called the cardiovascular system. As part of this system, the heart pumps blood through the arteries to all parts of the body. Vascular health can be affected when there is a reduced arterial blood supply to a part of the body, such as the legs or brain. Arteries can become diseased through a process called atherosclerosis.

PURPOSE OF THE STUDY
The purpose of the study is to determine the influence of exercise training on indicators of cardiovascular health and to compare the effects of exercise training on indicators of vascular reactivity in exercised and non-exercised limbs.

PROCEDURES
Before attending the college for testing you are asked to refrain from exercising for seven days prior to the pre-training measurements (DAY 1). You are also asked to abstain from alcohol and follow a set diet for three days before the pre-training and post-training measurements (DAY 1 & 15). These restrictions are necessary because diet and alcohol can also influence test results.

DAY 1 & 15 → 2.5 Hours
Before and after the two-week training intervention blood flow will be measured in the forearm at rest and then after blood supply to the lower arm has been cut off for 5 minutes by inflating a blood pressure cuff. A blood sample will also be taken before and after the first and second week training intervention. You are required to attend both sessions in a **fasted state**.

DAY 2 & 16 → 1 Hour
On your second and final visit you will have your aerobic fitness determined and body fat assessed. This will involve exercising at increasing work rates on a cycle ergometer, until you reach maximal exercise capacity. To obtain a true result it is necessary that you continue cycling until complete exhaustion. Your body fat will be measured using an X-ray scanner (DXA) on DAY 2 and Bioelectrical Impedance Analysis (BIA) on DAY 2 and 16.

DAY 3–14 → 10 sessions - 30-60 minutes
After your second visit you will undertake two weeks of supervised aerobic training on the cycle ergometer (30-60 minutes of "moderate intensity" exercise on five days of each week). During the training sessions, one arm must remain perfectly still, and you will be asked to perform handgrip exercises intermittently with the other arm.

If you have any questions or concerns about the research, please feel free to contact

Deirdre Upton 087-9388434, Email: dupton@wit.ie

Appendix F

Protocol for Blood Flow Measurements

Protocol for Measuring Forearm Blood Flow with an EC6 Plethysmograph and Hokanson NIVP3 Software

Setup:

- Start the NIVP3 program and enter the subject's information and select Inflow.

- Position the subject on the table in a supine position, (or seated) with the forearm extended and slightly supinated.

- Attach the SC10D cuff just above the subjects elbow, and connect it to the Y-connector of the E20.

- Attach a cuff, which can be inflated with a sphygmomanometer, to the wrist to exclude hand flow during measurements.

- Measure the circumference at the widest point of the forearm (~10cm distal to the olecranon process) and select a strain gauge that is 2 to 3cm smaller.

- Place the strain gauge around the arm by holding the head of the gauge in place and bringing the loop under the arm and bringing it back to attach it to the head, ensuring the two strands are parallel as they go around the arm.

- Plug the strain gauge into the EC6.

- The gauge should be fixed to the skin by placing a piece of tape over the head of the gauge and another over the cable a couple of inches away. This is to prevent the gauge from moving if the cable is disturbed.

- The elbow and wrist should be supported with Styrofoam blocks so that the strain gauge does not touch anything other than the arm, and also to elevate the forearm above the heart.

Measuring Forearm Blood Flow at Rest:

- Ensure the Balance is not set to Auto Balance, if it is the plethysmograph will reset the baseline whenever the signal goes off scale, which could upset the readings.

- Set the Range of the EC6 to 0.5%/cm and verify the Range on the PC.

- Set the Reading Interval to 10sec and the Inflow Time to 6sec on the PC.

- Test that everything is working correctly by selecting Instrument Check on the PC. Balance the plethysmograph and verify that the trace is moving across the screen near the bottom and that the settings agree with the plethysmograph.

- Turn the rapid inflator (E20) and the Air Source (AG101) on. Relieve the pressure in the AG101 – presetting the pressure on the E20 to anything above zero and then pressing the Mode switch on the E20 to Cuff Mode can accomplish this.

- With the E20 in Preset Mode, preset the pressure to 50mmHg.

- Inflate the wrist cuff to 240mmHg for one minute. Ensuring the pressure remains up.

- Select Start Readings on the PC.

- Turn the EC6 on; select the 1% switch and press the Balance switch downward to adjust the baseline.

- At the one-minute mark select OK on the PC, to initiate measurements.

- If the waveform does not completely return to baseline, press the Balance switch prior to the next measurement.

- Select Done with Readings on the PC, when finished.

- Deflate the wrist cuff and turn the EC6 off.

- Allow the subject to rest for 5 minutes before starting the next measurements.

Measuring Forearm Blood Flow after Hyperaemia:

- Select Create Next Visit on the PC.

- Ensure the Balance is not set to Auto Balance.

- Set the Range on the EC6 to 0.5%/cm and verify the Range on the PC.

- Turn the E20 and the AG101 on and relieve the pressure in the AG101.

- Press the Mode button of the E20 to Cuff Mode and increase the pressure to 240mmHg, for five minutes.

- Turn the EC6 on; select the 1% switch and press the Balance switch downward to adjust the baseline.

- If the baseline is steady after the first minute, the hose may then be clamped. (If the baseline is not steady then the pressure will need to be increased, before it may be clamped.)

- Once the hose is clamped press the Mode button of the E20 to Preset and set the pressure to 50mmHg. Turn the EC6 off.

- At the fourth minute mark, inflate the wrist cuff to 240mmHg.

- Select Start Readings on the PC.

- Turn the EC6 on; select the 1% switch and press the Balance switch downward to adjust the baseline.

- At the fifth minute mark remove the clamp and select OK on the PC, to initiate the measurements.

- If the waveform does not completely return to baseline, press the Balance switch prior to the next measurement.

- Select Done with Readings on the PC, when finished

- Deflate the pressure in the wrist cuff and turn the EC6 off.

Appendix G

Dietary Questionnaire
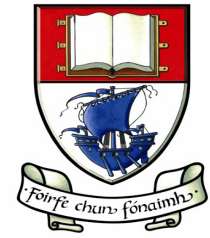
3 Day Dietary Intake Recall Form

Instructions for recording your intake:

♦ Please record everything, even bites, sips, etc.
♦ Include WATER.
♦ Be specific regarding serving sizes and portions. Example: If you eat a large bowl of cereal with milk, please record as: 3 cups of Cornflakes with 1 cup of 2% milk.
♦ Include the brand name of the food, if pre-prepared (i.e. Frozen dinner).
♦ Don't forget foods such as condiments.
♦ Take this form with you throughout the day to help you give the most accurate recall.
♦ **PLEASE TURN IN THIS COMPLETED FORM ON THE DAY OF YOUR APPOINTMENT.**

Name: _____

Day: _____

Breakfast   _____

_____

_____

Morning Snack   _____

_____

_____

Lunch   _____

_____

_____

Afternoon Snack _____

_____

_____

Dinner _____

_____

_____

Evening Snack _____

_____

_____

Day: _____

Breakfast _____

_____

_____

Morning Snack _____

_____

_____

Lunch _____

_____

_____

Afternoon Snack _____

_____

_____

Dinner _____

_____

_____

Evening Snack _____

_____

_____

Day: _____

Breakfast _____

_____

_____

Morning Snack _____

_____

_____

Lunch _____

_____

_____

Afternoon Snack _____

_____

_____

Dinner _____

_____

_____

Evening Snack _____

_____

_____

Appendix H

Protocol for Haematology Analysis

Haematology Analysis

Before any samples or controls were run the values from the table of expected results in the assay sheet (supplied with controls) were entered. The corresponding lot number, expiration date and values for each lot of controls, low (L), medium (N) and high (H) were entered and saved into the instrument.

1. Before running each sample, the controls were used. Each control was warmed at room temperature and inverted gently before use.

2. The correct control level (L, N, H) was then selected on the screen and the control bottle was presented to the probe, ensuring the tip of the probe was well into the bottle, and the aspirate switch was pressed.

3. This process was repeated for each level of control and if the results were in the expected range they were then saved.

The hematology analyser was then ready to examine a whole blood sample.

4. To run a whole blood sample the whole blood mode and patient range (patient range 1) were selected.

5. The system used was auto-sequenced; therefore the sample ID was recorded.

6. As with the controls, the sample was brought to room temperature and inverted approximately 8 times.

7. The cap was then removed from the vacutainer and presented to the probe before the aspirate switch was pressed.

8. The sample results were automatically saved and a print out was filed with each subjects records.

Appendix I
Protocol for Lipids & Glucose

Lipids & Glucose

Protocol for the use of calibrators and controls with an automated clinical chemistry system (ACE®, Alfa Wassermann B.V., Netherlands).

Before analysing samples, calibrators and controls were used.

1. Cal Serum level-3 (2351, Randox Laboratories) was used for the calibration of triglycerides and glucose. Direct HDL/LDL cholesterol calibrator calibrated HDL and LDL cholesterol.

2. The calibrator values listed on the information sheets provided were then inputted into the analyser.

3. The calibrators were mixed with 1 ml of distilled water and allowed to stand for 30 minutes.

4. Calibrators were then added to the cups and positioned into the segments into the machine.

5. After selecting all the tests that were to be completed, in the panel section, the calibration was initiated.

Once all calibration reports were passed, verifying the accuracy of results, the quality control tests were started.

6. Randox Lipid Control Level 2 was used for the testing of total cholesterol, HDL and LDL cholesterol and triglycerides

7. Randox Multi Sera Level 2 was used for the testing of glucose.

8. Once again the tests were selected in the panels section and names were assigned to each test.

9. Once the segment number was inputted, the cups containing the control samples were placed into the segment and the testing was initiated.

10. The results (Table A3) were compared with the acceptable ranges that were provided with the controls, via information sheets and compact discs.

Table A3 Controls Requisition Report

Test	Result (mmol/L)	Acceptable Range	Control
Glucose	6.1	6.03 - 7.37	Randox Multi-Sera Level 2
Cholesterol	5.92	5.18 - 6.72	Randox Lipid Control Level 2
HDL-C	1.08	1.00 - 1.35	Randox Lipid Control Level 2
LDL-C	3.62	2.99 - 4.05	Randox Lipid Control Level 2
Triglycerides	1.89	1.67 – 2.31	Randox Lipid Control Level 2

Appendix J

Protocol for Immunoassays

Protocol for Immunoassays

1. A 5% blocking solution was added to both plates and incubated overnight at 4°C.

2. Serum samples were removed from the freezer and allowed to defrost.

3. A wash solution of phosphate buffered saline with 0.05% Tween-20 (PBS-T) was then prepared.

4. The calibrator dilution series was prepared using Human Vascular Injury I Calibrator Blend and Assay Diluent GF2 for the Vascular Injury Panel I and using Human Vascular Injury II and DiluentV for the Vascular Injury Panel II.

5. The preparation of the calibrator was identical; A seven point calibration curve was used - firstly the calibrator blend was used as the top of the curve and then 10 μL of the solution was added to 60 μL of the particular diluent and this was repeated five more times to make calibrator solutions as those shown in Table A1.

Table A1   Sample plate layout with Calibrator Solutions

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000											
B	143											
C	20											
D	2.9											
E	0.42											
F	0.06											
G	0.008											
H	0											
Blended Calibrators			Samples									

6. The 96-well plates were then washed three times with 200 μL per well of PBS-T.

7. Following these washes kit I and II had 10 μL of calibrator, prepared with Diluent GF2 and Diluent V respectively, added to wells A1-A2 through to H1-H2, as shown in Table A1. The remaining wells had 10 μL of sample added.

8. The samples were diluted with 1% Blocker A Solution (5% Blocker A solution diluted to 1% with PBS) for the Vascular Injury Panel II Assay. The plates were incubated at room temperature with shaking for 2 hours.

9. The detection antibodies were then prepared - for Panel I Anti-Human Vascular Injury I Detection Antibody Blend was combined with Assay Diluent GF2, while Anti-Human Vascular Injury II Detection Antibody Blend and Diluent V was used for Panel II.

10. After the wash procedure, 25 μL of the SULFO-TAG detection antibody solution was added to each well and again the plate was incubated at room temperature with shaking for one hour.

11. The Sector Imager was then prepared; firstly the plate layouts were edited. The four analytes on each plate were selected from the lists on the Sector Imager and assigned the exact position on the assay section of the layout, as they are found on the plates, as shown in Figure A1.

Figure A1   Analyte locations on Vascular Injury Panel I and Panel II
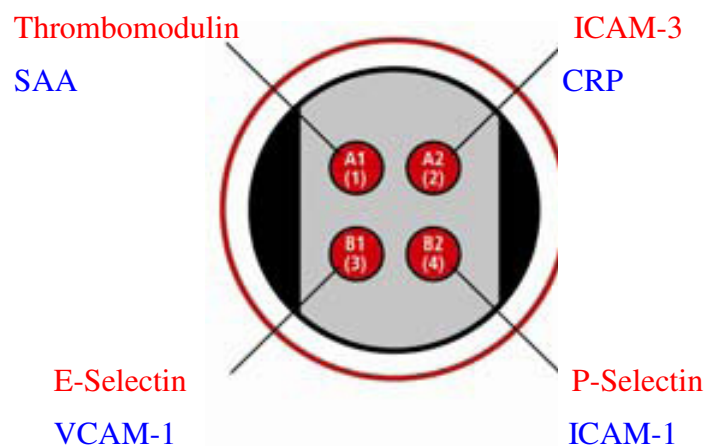
The analytes above in red are those on the Vascular Injury Panel I Assay, those in blue are found on the Vascular Injury Panel II Assay.

12. In the sample section of the plate the standards were assigned to the wells where the blended calibrators had been added, as shown in Table A1. Each other well were assigned as an unknown. The plate layouts were then saved as Vascular I and Vascular II for the analysis of the corresponding plates.

13. The plates were then washed as above to remove any unbound antibody enzyme reagent. Read buffer, diluted with deionised water, was added and the plate was analysed on the MSD reader.

Appendix K

Protocol for Standardisation of Flow Cytometry

Flow Cytometry Standardisation Protocol

As described by Robert et al. (2008, pp192).

First Step

1. Run Megamix beads with discriminator on FL1
2. Gate each individual bead subset on SS x FL1 cytogram (Figure 3.7)
3. Build FS histogram for 0.5 and 0.9 µm beads (Figure 3.8)

Second Step

4. Run megamix beads with discriminator on FS
5. Monitor percentage of 0.5 µm beads (left peak) and optimise FS settings to approach 50% as in Figure 3.10
6. Visualise SS X FS cytogram. Create/actualise an elliptical autogate on 0.9 µm beads. Set up the MP gate to tangent the autogate.

Final Step

7. Run plasma sample stained with AnnexinV - FITC/CD41-PE
8. Condition FL1 x FL2 cytogram on the MP gate and measure microparticle counts.