Clinical Investigation of Subclinical Vascular Disease in Psychosocial Stress and Dyslipidaemia

by

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DECLARATION

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Abstract

Cardiovascular disease is a major cause of death and ill health across the world. Psychosocial factors are increasingly being recognised as potential cardiovascular risk factors, contributing to the development and progression of atherosclerotic disease. However, the pathways between psychosocial factors and cardiovascular disease are not yet fully understood.

The aims of this thesis were to explore the associations between psychosocial factors (both chronic and acute stress) and measures of subclinical vascular disease, and to further develop and validate, a new method of assessing vasomotor function.

Women with both depression and anxiety were found to have increased carotid intima-media thickness and this relationship was found to be influenced by the presence of dyslipidaemia. When looking at the inflammatory responses to an acute mental stress challenge it was shown that those participants who had an elevated fibrinogen response 45 minutes after the stress challenge had poorer endothelial function 3 years later, as assessed by flow-mediated dilatation. These findings suggest that both chronic and acute stress may play a role in the development of cardiovascular disease.

Good reproducibility was demonstrated with the new method for assessing vasomotor function following further development of the protocol for the method. The technique was able to detect differences in vasomotor function between a group of patients with Familial Hypercholesterolaemia compared with age and gender matched controls. In addition it was also able to detect improvement in vasomotor function following a single lipoprotein apheresis treatment in patients with Familial Hypercholesterolaemia undergoing long-term treatment. These studies demonstrated the potential of this method for use in non-specialist vascular research laboratories and out in the field for the assessment of vasomotor function.

In memory of Ann Donald

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Definitions and abbreviations

5-HT	5-hydroxytryptamine serotonin
ACE	Angiotensin-converting enzyme
ACH	Acetylcholine
AIx	Augmentation index
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
Anx	Anxiety
ApoA	Apolipoprotein A
ApoB	Apolipoprotein B
ARH	LDL receptor adaptor protein 1 gene
AT I	Angiotensin I
AT II	Angiotensin II
AUC	Area under curve
BK	Bradykinin
BL dia	Baseline diameter
BLVTI	Baseline velocity time integral
BMI	Body mass index
BP	Blood pressure
CES-D	Centre for epidemiological studies depression scale
CRH	Corticotrophin releasing hormone
CRP	C-reactive protein
CV%	Coefficient of variation
CVD	Cardiovascular disease
DALI	Direct adsorption of lipoprotein
DBP	Diastolic blood pressure
DC	Distensibility coefficient
Dep	Depression
DSA	Dextran sulphate adsorption
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
EDHF	Endothelium-derived hyperpolarizing factor
EDTA	Ethylenediaminetetraacetic acid
eNOS	Endothelial nitric oxide synthase

ET-1	Endothelin-1
FBF	Forearm blood flow
Fbg	Fibrinogen
FH	Familial Hypercholesterolaemia
FMD	Flow-mediated dilatation
FMD_{ABS}	Absolute flow-mediated dilatation
$FMD_{\%}$	Percentage change flow-mediated dilatation
FMS	Flow-mediated slowing
FMS_{ABS}	Absolute flow-mediated slowing
$FMS_{\%}$	Percentage change flow-mediated slowing
GHQ	General Health Questionnaire
GMP	Guanosine monophosphate
GTN	Glyceryl trinitrate
HDL	High density lipoprotein
HELP	Heparin extracorporeal LDL precipitation
HPA	Hypothalamic-pituitary-adrenal axis
HR	Heart rate
IDL	Intermediate density lipoprotein
IL-6	Interleukin 6
IMT	Intima-media thickness
LA	Lipoprotein apheresis
LDL	Low density lipoprotein
L-NMMA	L-N^G-monomethyl Arginine citrate
LP	Lipoprotein
MRI	Magnetic resonance imaging
NHS	National Health Service
NO	Nitric oxide
nonHDL	non high density lipoprotein
NOS	Nitric oxide synthase
O2-	Superoxide ion
PAT	Pulse amplitude tonometry
PCA	Pulse contour analysis
PCSK9	Proprotein convertase subtilisin/kexin type 9
PGF-2α	Prostaglandin F-2α
PGI2	Prostacyclin
PFBA	Polyacrylate full blood adsorption

PS	Post stress
PWA	Pulse wave analysis
PWV	Pulse wave velocity
R	Receptor
RH%	Reactive hyperaemia expressed as a percentage
SBP	Systolic blood pressure
SES	Socio-economic status
SMC	Smooth muscle cell
SNS	Sympathetic nervous system
SP	Substance P
TC	Total cholesterol
TC/HDL	Total cholesterol to high density lipoprotein ratio
Trigs	Triglycerides
Trigs/HDL	Triglycerides to high density lipoprotein ratio
TNFα	Tumour necrosis factor
TxA2	Thromboxane A2,
UCL	University College London
UK	United Kingdom
VLDL	Very low density lipoprotein
VTI	Velocity time integral
WHII	Whitehall II study

Chapter 1. General Introduction

1.1 Biology of atherosclerosis and cardiovascular risk

1.1.1 Epidemiology

Cardiovascular disease (CVD), which encompasses coronary heart disease, heart failure and stroke amongst others, is the leading cause of death by a non-communicable disease worldwide. In 2012, it was responsible for 17.5 million deaths, with an estimated 7.4 million of these deaths due to ischaemic heart disease, and 6.7 million due to stroke[1]. It has been projected that by 2030, over 23 million people will die annually from CVD[2].

There is still a substantial morbidity and mortality burden due to CVD within the United Kingdom (UK), but some good progress is being made in the prevention of CVD. In 2012-2013 CVD was overtaken by cancer as the leading cause of death within the UK. Cancer was responsible for 29% of all deaths, ahead of CVD with 28%. However, in women, CVD remains the major cause of death ahead of cancer (28% vs 27%), whilst in men CVD has fallen behind cancer (CVD 29% cancer 32%)[3]. It is not only in the UK where a decline in mortality from CVD has been seen; between 1998 and 2008 the annual rate of deaths due to CVD declined by 31% in the United States[4].

The reduction in mortality is in part explained by improved treatment of acute and chronic cardiovascular diseases such as myocardial infarction and chronic heart failure. Additionally, changes in risk factors such as smoking habits and treatments for high cholesterol and hypertension have also helped reduce cardiovascular mortality[5]. However, many people are also living with CVD. Within the UK 2.3 million people are estimated to be living with coronary heart disease. The prevalence of cardiovascular conditions increases with age and is greater in men than women, with men 3 times more likely to have had a myocardial infarction than women. CVD was also the third highest main diagnosis in men (at 10%) on discharge from NHS hospitals in 2012/2013, behind cancer and digestive system diseases[3]. The economic impact of these high levels of CVD is great. In 2012-2013, £6.8 billion was spent within the NHS in England on treating CVD, whilst in Wales the cost was £442.3 million[3].

Alongside the differences in mortality and prevalence of CVD between the genders, there is also a socio-economic gradient within the UK. Scotland has an age-standardised total CVD mortality rate of 347per 100,000 population whereas in South West England it is lower at 269/100,000. Within England there is a further North/South divide with those in the North having higher mortality rates[3]. Prevalence of CVD also varies within the UK. Data from the Quality and Outcomes Framework has shown that England has the lowest prevalence for all cardiovascular conditions except hypertension. Scotland has the highest prevalence of CHD, stroke and peripheral arterial disease whilst hypertension, heart failure and atrial fibrillation are highest in Wales. As with mortality there is also a North/South divide within England, with the prevalence of CVD being greater in the less advantaged North[3].

It is not only where someone lives that increases the likelihood of whether they die or suffer from CVD. Social position, as determined by educational level, occupation, income, ethnicity and race and gender, also influences an individual's risk of CVD; with manual workers at greater risk than those in professional/managerial occupations[6]. These socio-economic factors are linked to many other psychosocial factors such as access to health care, dietary quality, stress and other lifestyle behaviours (smoking, alcohol) that contribute to the increased risk of CVD. It is therefore important to try and gain a deeper understanding of how psychosocial and biological factors mediate CVD risk in order to develop new approaches to CVD prevention.

So although there is evidence that good progress has been made in the overall reduction of the impact of CVD, there is still a need for further investigation into the inequalities in mortality and morbidity within the psychosocial environment and how they contribute to CVD.

1.1.2 Initiation and development of atherosclerosis

A healthy artery wall is made up of three layers; the intima, media and adventitia (figure 1.1A). The intima is the innermost layer which consists of the endothelial cells which lie on a basement membrane containing type IV collagen and a few smooth muscle cells[7]. It is separated from the media by an internal elastic lamina. Within the media are elastin, collagen, glycoproteins and smooth muscle cells. In elastic arteries such as the aorta there are several layers of elastic lamina between which are the smooth muscle cells and other

structural components, whilst in more muscular arteries smooth muscle cells are arranged in spiralling layers[7, 8]. The adventitia is the outermost layer, and consists mainly of connective tissue, fibroblasts, smooth muscle cells, microvessels and nerve endings.

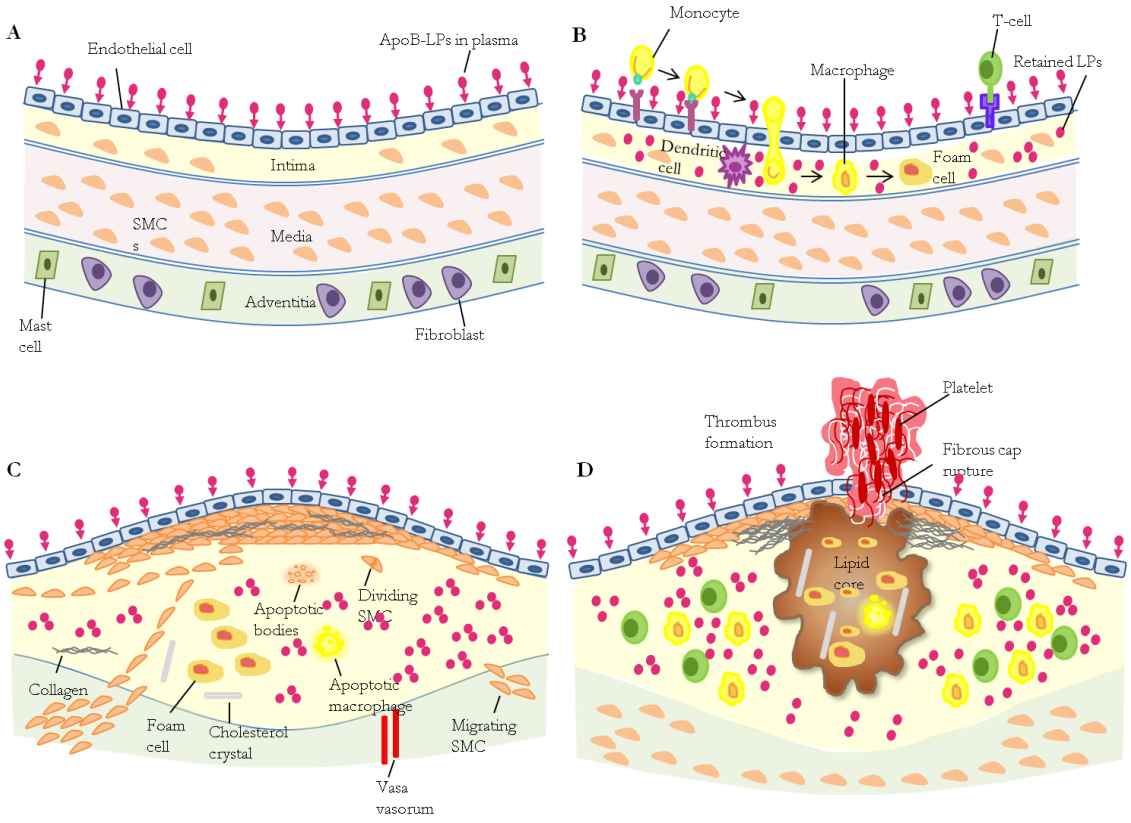

Figure 1.1: Development of the atherosclerotic plaque. **A** normal artery; **B** the initial steps of atherosclerosis; **C** progression of the atherosclerotic lesion; **D** atherosclerotic plaque with thrombosis. Further explanation is within the text of section 1.1.2. LP = lipoprotein; SMC = smooth muscle cell. Adapted from Libby et al[9]

Exposure of the artery wall to cardiovascular risk factors such as hypertension, hypercholesterolaemia, hyperglycaemia and smoking, and their associated physiological and biochemical stressors activates the endothelium. This results in a cascade of inflammatory processes including innate and cellular immune processes. These in turn encourage greater transit and retention of LDL into the intima which, along with mononuclear cells, which take up these lipoproteins, form foam cells. This in turn triggers further inflammatory processes, initiating changes within the extracellular matrix and inducing migration of vascular smooth muscle cells from the media to the intima and ultimately resulting in the development of a plaque. The plaque progresses over time and may eventually reach such a size that it occludes the lumen or may become unstable and ulcerate/rupture, which can cause clinical events such as angina, myocardial infarction or stroke (figure 1.1). Although

the clinical consequences of atherosclerosis usually occur later in life, the disease process has often been silently progressing from much earlier in life and in extreme cases even before birth[10, 11].

1.1.2.1 The healthy endothelium

A thin single celled monolayer, the endothelium plays a key role in vascular homeostasis. It is involved in numerous processes such as thrombus prevention, cellular adhesion, regulation of the transport of macromolecules, inflammation, smooth muscle cell proliferation and importantly, vascular tone.

Regulation of vascular tone plays an important role in the balance of tissue oxygen supply and metabolic demand. The tone and variation in diameter of the vessel is controlled through the synthesis and release of vasoconstrictors and vasodilators from the endothelium and by its response to, and modification of, circulating vasoactive mediators.

Endothelial cells were first demonstrated to be essential for vascular smooth muscle relaxation by Furchgott & Zawadzki by the administration of acetylcholine to rabbit aortas with and without intact endothelium [12]. Initially known as endothelium-derived relaxing factor, the substance responsible for the relaxation was later identified as Nitric Oxide (NO)[13]. NO is synthesised from the conversion of L-Arginine to L-Citrulline by endothelial Nitric Oxide Synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin (BH4). It then diffuses through to the vascular smooth muscle cells where it activates guanylate cyclase leading to relaxation of the smooth muscle through cyclic guanosine monophosphate production[14]. Production of NO is regulated through a number of pathways such as increases in shear stress which enhances eNOS expression[15]. Other signalling molecules which influence NO production are bradykinin, adenosine and vascular endothelial growth factor[16]. The endothelium also produces other vasodilators which are NO independent, for example endothelium derived hyperpolarizing factors (EDHF) and prostacyclin[17, 18]. Vascular tone is also modulated by the endothelium through vasoconstrictors such as angiotensin II and endothelin-1(figure 1.2)[19, 20].

Finally, NO is also involved in the inhibition of inflammation, cell proliferation and thrombosis; all actions which help to maintain a healthy endothelium[21].

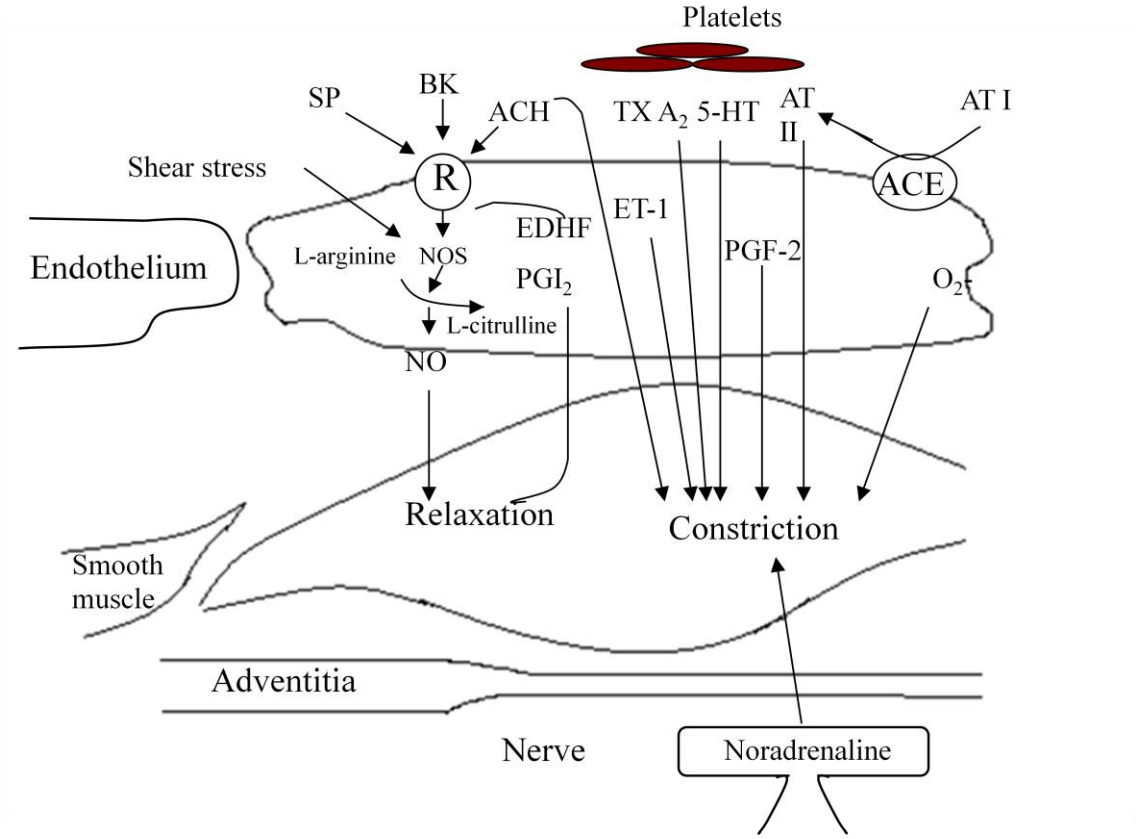

Figure 1.2: Endothelium-derived vasodilators and endogenous vasoconstrictors. ACH, Acetylcholine; BK, bradykinin; SP, substance P; NOS, nitric oxide synthase;PGI2, prostacyclin; NO, nitric oxide; ACE, angiotensin-converting enzyme; TxA_2,thromboxane A_2; 5-HT, 5-hydroxytryptamine serotonin; AT I, angiotensin I; AT II,angiotensin II; EDHF, endothelium-derived hyperpolarizing factor; ET-1, endothelin-1;O2-, superoxide ion; PGF-2α, prostaglandin F-2α; R, receptor. Adapted from Ellins & Halcox[22]

1.1.2.2 Endothelial activation and atherogenesis

Atherogenesis is thought to be triggered by the interaction between the activated endothelium and subendothelial lipoprotein transit, retention and cellular uptake[23]. Activation of the endothelium can occur in response to the effects of cardiovascular risk factors, such as hypercholesterolaemia and hypertension. Furthermore, atherogenesis typically develops most favourably within the intima, in regions of disturbed blood flow[23]. Changes in shear stress along the vessel wall can trigger chemical responses driving the endothelium into a proatherogenic dysfunctional state in vulnerable sections of the arterial tree.

Activation of the endothelium increases its permeability which, alongside changes within the subendothelial extracelluar matrix, facilitates ApoB lipoproteins to enter into and be retained in the subendothelial space where they can be further modified by oxidation[24]. The activated endothelium may be further activated by the retention and oxidation of lipoproteins. This may also initiate proinflammatory responses, characterizing the production of both chemokines and inflammatory cytokines which further stimulate the endothelium to produce selectins and adhesion molecules which attract and interact with mononuclear cells. These molecules facilitate the attraction and adhesion of mononuclear cells, particularly monocytes and T lymphocytes, to the endothelium which migrate into the sub-intimal space. Once within the vessel wall, monocytes mature into macrophages which ingest both native and modified lipoproteins through a combination of phagocytosis, scavenger receptor-mediated uptake and fluid phase pinocytosis to become cholesterol laden foam cells[24]. In addition, monocytes and T lymphocytes also secrete inflammatory cytokines encouraging further endothelial activation and monocyte attraction, adhesion and infiltration[9].

Smooth muscle cell migration is also an important component of atheroma formation. These cells are already scantily present in the intima, but during atherogenesis, more smooth muscle cells migrate in from the media and proliferate in response to mediators. They also produce extracellular matrix molecules such as collagen and elastin, and form a fibrous cap over the atheroma. This cap often covers foam cells, which, upon dying, free lipids which accumulate extracellularly in the subintima. This can contribute to the formation of the plaque's necrotic core, which is a lipid-rich pool formed from the build up of debris from cells and the extracellular lipids[9].

With this continuous cycle of inflammatory processes, lipid accumulation, foam cell formation and changes within the extracellular matrix, the plaque continues to develop increasing in size and complexity. This causes remodelling of the artery. Initially the plaque causes expansion of the artery wall whilst the lumen remains clear with no intrusion. Eventually the plaque may begin to intrude into the lumen of the artery where it can become a blood flow-limiting stenosis leading to ischaemia of the surrounding tissue. Alternatively, the plaque can be breached, either through rupture or erosion of the endothelium. Plaques that are prone to rupture (vulnerable plaques) have a weakened fibrous cap typically due to the actions of inflammatory cells which produce cytokines and metalloproteinase that break down collagen decreasing the tensile strength of the cap. Inflammatory cytokines also enhance cell death and prothrombotic activity within the

plaque. The cap eventually is unable to withstand the mechanical forces of blood pressure and fissures appear within the cap exposing the inner core of the plaque[25]. The mechanisms of endothelial erosion are not fully understood but recent work has suggested that innate immunity may play a role in this process[25]. Either way, both rupture or erosion results in exposure of thrombogenic material within the plaque to platelets and coagulation factors in the lumen which activates thrombus formation at the site of the breach. This may lead to obstruction of local blood flow, or (mirco) embolism which can lodge in distal branch arteries both of which processes can result in ischaemia, stroke or myocardial infarction.

1.2 Cardiovascular risk factors and vascular function

Epidemiological studies have shown that alongside age and gender, there are a number of factors that increase a person's risk of suffering from CVD or mortality from a cardiovascular event. The INTERHEART study, a large study of 12,461 cases and 14,637 controls from 52 countries demonstrated that 90.4% of the population attributable myocardial infarction (MI) risk was accounted for by 9 modifiable risk factors. In fact just five of these risk factors abnormal lipids, smoking, hypertension, diabetes and obesity, were responsible for 80.2% of the estimated population attributable MI risk[26].

1.2.1 Smoking

Both smoking and passive smoking have been shown to increase the risk of having a cardiac event in men and women[27, 28]. The exact causative pathways determining the relationship between smoking and CVD are not fully understood. However, cigarette smoke contains numerous constituents including nicotine, carbon monoxide and free radicals and it is thought that these and other toxic components within the smoke promote vascular dysfunction, atherogenesis and thrombosis in multiple vascular beds [29].

Smoking has a number of effects on the cardiovascular system including increasing blood pressure, heart rate and cardiac output[30]. In addition it creates a favourable environment for the development and progression of atherosclerosis through increased numbers of free radicals and oxidative stress, inflammatory cytokines and oxidation of LDL[29]. These can all contribute to the endothelial dysfunction seen in response to both active smoking and environmental exposure to smoke (passive smoking)[31, 32]. Smoking is also associated with

the development of subclinical arterial disease, for example increased arterial stiffness and carotid artery intima-media thickness (IMT)[33, 34].

Smoking cessation can reduce the relative risk of mortality in those with coronary heart disease by 36%[35]. In the UK, there has been a concerted effort by Public Health bodies to reduce cigarette consumption within the population and the number of smokers in England has declined from 26% of adults aged 16 and over in 2003, to 19% in 2013[36]. Legislation banning smoking in the work place and indoor public spaces has also made an impact on the risk of cardiac events. A meta-analysis of 31 studies looking at the impact of smoking bans on cardiovascular events showed an overall 12% reduction in hospitalizations from acute coronary events[37].

1.2.2 Hypertension

High blood pressure (currently defined as ≥140/90 mmHg) is a chronic multifactorial systemic disorder[38]. Blood pressure increases with age and environmental, pathological and genetic factors all play an important role in both the control of and the development of increased blood pressure; the relative influence of which can vary between individuals and populations[39]. High blood pressure is considered a major independent risk factor for CVD but is also commonly seen with obesity, insulin resistance and over activity of the sympathetic nervous system[40-44]. Hypertension is very common, with current estimates putting its prevalence at 26.4% of the adult global population, which is projected to increase to 29.2% by 2025, and makes it the leading risk factor for mortality[45, 46]. In addition to their greater risk of CVD, people with hypertension are also at increased risk for kidney disease and stroke.

Hypertension has also been shown to be related to adverse subclinical markers of vascular structure and function. One such marker is endothelial function, which has been shown to be diminished in patients with hypertension[47, 48]. The presence of endothelial dysfunction as assessed by flow-mediated dilatation may identify those patients with hypertension who were at greatest risk of non fatal and fatal CVD events[49]. Diminished endothelium-dependent dilatation may be due to a range of factors notably the reduced bioavailability of NO in response to increased oxidative stress[50].

Hypertension may also result in the remodelling of both large and small arteries. Alterations in the extracellular matrix and to collagen and elastin fibres within the artery wall can lead to changes to the lumen/wall ratio and lumen diameter[51]. These changes in the structure of the artery walls can result in increased intima-media thickness and arterial stiffness; which can further increase blood pressure, creating a vicious cycle[52]. Hypertension-induced changes to the arteries also impact on the heart, for example, increased arterial stiffness is associated with left ventricular hypertrophy, a known risk factor for cardiovascular events[53, 54].

Reduction of blood pressure through the use of antihypertensive medication such as ACE inhibitors, calcium antagonists, β-blockers, angiotensin receptor antagonists and thiazide diuretics, reduces cardiovascular morbidity and mortality with a 30-40% reduction in the risk of fatal and non-fatal stroke and 20% for coronary events[38]. A meta-analysis looking at the use of blood pressure lowering drugs and CVD prevention showed that a significant reduction in risk could be achieved with medication in those with and without CVD regardless of the pre treatment blood pressure, (down to a systolic of 110 mmHg and diastolic blood pressure of 70 mmHg)[55]. In addition, endothelial function and pulse wave velocity (PWV) have both been shown to improve in response to antihypertensive therapy, whilst there is also evidence of both IMT regression and the slowing of its progression in patients on blood pressure lowering medications[56-63]. However, there is still room for improvement as many patients under medical care still have uncontrolled hypertension and their risk of cardiovascular events could be lowered further with better control of their blood pressure[64].

1.2.3 Lipids

Elevated levels of lipoproteins are a major risk factor for CVD and as discussed previously play a key role in the development of atherosclerosis. Lipoproteins are globular, micelle like particles containing a hydrophobic core of triglycerides and cholesterol esters surrounded by an amphipathic coat of protein, phospholipid and cholesterol. The apolipoproteins on the surface help solubilise the lipids and act as cellular targeting signals. The main function of lipoproteins is to transport triglycerides, cholesterol and phospholipids around the body but also have a number of other biological properties. They are commonly classified into 5 types of lipoprotein based on their physical and functional properties; chylomicrons, very

low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

Chylomicrons

These triglyceride rich particles are synthesised in the large intestine in response to dietary fat intake and are the largest and least dense (0.95g/ml) lipoprotein. They transport dietary triglycerides from the intestines to other tissues, predominantly skeletal muscle and adipose tissue, and also move triglycerides and cholesterol to the liver. Chylomicrons contain a number of apolipoproteins which are crucial to lipoprotein metabolism. Apolipoproteins have a number of functions including a structural role, acting as ligands for lipoprotein receptors, guiding the formation of lipoproteins and activating and inhibiting enzymes involved in lipoprotein metabolism. ApoB-48, a core structural protein is one of the key apolipoproteins in chylomicrons. Triglycerides from the chylomicrons are hydrolysed by lipoprotein lipase, freeing fatty acids and becoming smaller particles called chylomicron remnants. These are richer in cholesterol and are potentially pro-atherogenic[65].

VLDL, IDL and LDL

Triglyceride rich VLDLs are synthesised in the liver and transport triglycerides and cholesterol from the liver to other tissues, mainly skeletal muscle and adipose tissue. They also contain the proatherogenic apolipoprotein ApoB-100 which is a structural protein and acts as a ligand for the LDL receptor. As with chylomicrons, lipoprotein lipase acts on triglycerides releasing fatty acids which are taken up for storage. In parallel, exchange of triglycerides for cholesterol esters between these ApoB containing lipoproteins and HDL further enriches their cholesterol content. As a consequence, the size of these lipoproteins decreases and they become VLDL remnants and eventually IDL particles, some of which will be taken up by the liver. The remaining IDL are converted to LDL which are cholesterol rich, contain ApoB-100 and are the main source of cholesterol to the tissues[65]. LDL receptors play a key role in the delivery of cholesterol to tissues and consequently plasma levels. Genetic defects which result in reduced numbers or loss of function of the receptors commonly result in patients with these conditions having very high levels of LDL[66]. Other factors also contribute to levels of LDL such as dietary habits and in women postmenopausal status[67]. LDL particles vary in size due to differences in the amounts of cholesterol esters and triglycerides contained within them. Those with the lowest number of cholesterol esters and greater triglyceride content are small dense LDL. These are the

most proatherogenic as they have less affinity for the LDL receptor on the liver which prolongs their time in the circulation. They also enter the arterial wall relatively easily and are retained within the intima and are more susceptible to oxidation, all key features of the atherogenic process. Higher levels of small dense LDL are commonly seen in association with high triglycerides, low HDL levels, obesity, type 2 diabetes and in inflammatory states [65].

HDL

HDL is a complex lipoprotein with a density of 1.06-1.21 g/ml. It is formed from the apolipoprotein ApoA1, which is synthesised in the liver and intestine. The lipid poor ApoA1 protein initially acquires lipids through interaction with the cholesterol-phospholipid transporter ATP Binding Cassette A1 forming a nascent HDL particle. Further lipids and additional apolipoproteins are acquired from the degradation of other triglyceride rich lipoproteins. Cholesterol within HDL is converted into cholesterol esters forming the core of the mature HDL particle. These cholesterol esters are then either taken up directly by the liver or transferred, often in exchange for triglycerides, to ApoB containing lipoproteins[68]. Reverse cholesterol transport from peripheral tissues to the liver is the main function of HDL, but it also has other important roles, which can be viewed as atheroprotective. These include inhibition of cytokine-induced expression of adhesion molecules, reduction of the adhesion of monocytes to the endothelium and inhibition of oxidation of LDL and its transit into the intima[69-72]. HDL levels are affected by both clinical and environmental factors including obesity, type 2 diabetes, inflammation, smoking, alcohol, exercise and thyroid hormone[68].

1.2.3.1 Dyslipidaemia

Changes in lipid metabolism can lead to elevated levels of lipoproteins and alterations in their function. These lipid abnormalities may be a result of other conditions, lifestyle and environmental factors or genetic disposition and both individually or in combination with other risk factors contribute to the development of atherosclerosis.

Obesity related dyslipidaemia

A large proportion of people who are obese have dyslipidaemia. This is mainly due to a poor diet combined with a sedentary lifestyle in those with a poor genetic and environmental disposition. Obesity related dyslipidaemia manifests itself as an atherogenic lipid triad of high levels of triglyceride rich, ApoB containing lipoproteins such as VLDL and small dense LDL, plus low levels of HDL[73, 74]. Additionally postprandial triglycerides are often raised in those with obesity, which typically become proatherogenic chylomicron remnants.

Familial hypercholesterolaemia

Familial hypercholesterolaemia (FH) is a genetic disorder characterised by elevated levels of LDL resulting in premature coronary heart disease. Defects in the hepatic uptake and the degradation of LDL lead to poor clearance of LDL and its increased levels in the plasma. These defects are due to genetic mutations mainly in the LDL receptor gene but also in the gene coding ApoB, the gene that encodes proprotein convertase subtilisin/kexin type 9 (PCSK9) and the LDL receptor adaptor protein 1 gene (ARH)[75]. FH can either be heterozygous with patients often having one of the first three listed gene mutations or homozygous due to homozygous or compound heterozygous mutations in the LDL receptor or ARH genes[76].

The prevalence of heterozygous FH has been estimated to be 1 in 500 in white Europeans, however, a recent analysis of the US based National Health and Nutrition Examination Survey has put that figure at 1 in 250[77]. Homozygous FH is considerably rarer with a prevalence of 1/1 000 000[76]. Patients with untreated heterozygous FH commonly have total cholesterol levels of 8-15 mmol/L and, if untreated, typically develop coronary heart disease before the age of 55 in men and 60 in women. In homozygous FH, total cholesterol levels are as high as 12-30 mmol/L. Patients typically develop coronary heart disease very early in life and if untreated commonly die before they are 20[76].

In the UK, FH is often diagnosed using the Simon Broome criteria. Patients have to meet a number of different criteria (as described in table 1.1), and can be given a definite diagnosis or a probable diagnosis depending on which criteria they meet[78]. Two other groups have also developed diagnostic tools; these are the Dutch Lipid Clinic Network criteria and US MedPed program criteria[79, 80]. However, not all individuals diagnosed as

having definite or probable FH using these criteria are found to have an identifiable gene mutation [81, 82]. Modifications of these criteria are being tested to improve identification of hypercholesterolaemia patients for genetic testing[83].

	Criteria
Definite Diagnosis	Adult: total cholesterol >7.5 mmol/l or LDL cholesterol above 4.9 mmol/l Child <16 years: total cholesterol > 6.7 mmol/l or LDL cholesterol above 4.0 mmol/l (Levels either pre-treatment or highest on treatment) **Plus** Tendon xanthomas in patient, or in 1st degree relative (parent, sibling, child), or in 2nd degree relative (grandparent, uncle, aunt) **Or** DNA-based evidence of an LDL receptor mutation, familial defective ApoB-100, or a PCSK9 mutation.
Probable Diagnosis	Adult: total cholesterol >7.5 mmol/l or LDL cholesterol above 4.9 mmol/l Child <16 years: total cholesterol >6.7 mmol/l or LDL cholesterol above 4.0 mmol/l (Levels either pre-treatment or highest on treatment) **Plus at least one of the following** A family history of myocardial infarction: at <50 years in a 2nd degree relative or <60 years in a 1st degree relative **Or** A family history of raised total cholesterol: >7.5 mmol/l in a adult 1st or 2nd degree relative or >6.7 mmol/l in a child or sibling aged younger <16 years.

Table 1.1 : Diagnostic criteria for Familial Hypercholesterolaemia as defined by the Simon Broome Criteria[78]

1.2.3.2 Lipids and cardiovascular risk

Low levels of HDL have been associated with increased risk of CVD[84]. However, this finding has not followed through to clinical trials of drugs to raise HDL levels. Three trials, ILLUMINATE, AIM-HIGH and dal-outcomes, have all successfully raised HDL with pharmacological agents but did not see a reduction in cardiovascular events[85-87]. It has therefore been suggested that it is not HDL "cholesterol" per se that is protective but the function of the HDL particles. Thus future pharmacological agents may need to address HDL functions rather than HDL cholesterol concentration[68].

LDL is associated with increased cardiovascular risk both individually and in combination with other lipid parameters such as low HDL and high triglycerides[67]. Where lipid levels are

raised by genetic determinants such as FH the risk of cardiovascular disease is even greater. In individuals with treated FH the odds ratio for coronary artery disease was 10.3 (7.8-13.8) in comparison to non-FH participants, the risk was even in greater in those with FH but not on lipid lowering therapy (13.2 [10.0-17.4])[88].

Hypercholesterolaemia and subclinical vascular disease

Prior to the development of premature CVD, patients with hypercholesterolaemia are more likely to demonstrate significant subclinical vascular disease. Individuals with hypercholesterolaemia have been shown to have poorer endothelial function than those with normal cholesterol levels in a number of studies and clearly demonstrated in a meta-analysis in FH patients[89-92] . A meta-analysis by Masoura and colleagues also demonstrated that structural atherosclerotic disease as defined by carotid IMT was increased in patients with FH[92]. Increased arterial stiffness has also been associated with hypercholesterolaemia[93, 94]. These findings may reflect the impact of the high levels of circulating lipoproteins and their uptake and retention into the arterial wall promoting inflammation and oxidative stress with consequential impact on the endothelial availability of NO.

1.2.3.3 Treatment of dyslipidaemia

Pharmacological agents

The reduction of lipid levels with pharmacological agents has played a major role in lowering the cardiovascular risk of patients with hypercholesterolaemia. Statins are one of the most commonly used lipid lowering drugs. They decrease LDL cholesterol and other ApoB rich lipoproteins through their actions on the hydroxymethylglutaryl coenzyme A pathway, decreasing synthesis of LDL but also increasing LDL receptor expression, further enhancing the lipid lowering effect. A meta-analysis of 27 randomized statin trials demonstrated a reduction in the risk of major vascular events of 21% per mmol/L decrease in LDL[95]. A reduction in risk of coronary heart disease has been shown in FH patients by Versmissen et al, who found an overall risk reduction of 76% in FH patients on statin therapy. In addition their risk of myocardial infarction was found to be not dissimilar to that of an age matched group from the general population[96]. Statin therapy has also been shown to improve endothelial function and reduce IMT[92, 97, 98].

Other pharmacological agents which are effective at lowering LDL are resins and cholesterol adsorption inhibitors which act through different pathways to statins but may also contribute to the reduction in cardiovascular risk[99]. There are other drugs such as fibrates and nicotinic acid, which lower triglycerides and can increase HDL. Fibrates have been shown to reduce major CVD events particularly in those with increased triglycerides and low HDL[100]. As with statins, these drugs have also been shown to improve endothelial function, slow progression and promote regression of IMT[101-103].

Lipoprotein apheresis

Lipoprotein apheresis (LA) is often used in combination with lipid lowering agents for reducing LDL levels. In the UK, it is normally used for patients with homozygous or heterozygous FH, who have evidence of progressive and symptomatic coronary heart disease, despite maximal drug therapy, or are intolerant to drug therapy[104]. LA is an extracorporeal treatment that lowers LDL and other ApoB containing particles and is similar in concept to renal dialysis. Observational studies in homozygous FH patients have shown a reduction in LDL of up to 72% with LA treatment[105]. There are five techniques which are used clinically for LA:

Dextran sulphate adsorption (DSA). Dextran sulphate covalently bound to cellulose beads selectively binds VLDL and LDL. Originally this method separated out the plasma to remove the lipoproteins but this can now also be achieved using whole blood[106].

Heparin extracorporeal LDL precipitation (HELP) system. This method adds heparin to isolated plasma which then causes precipitation of the LDL particles which are removed by filtration. The filtered blood is then run through an adsorber to remove any excess heparin before being ultradialyzed to restore physiological pH and remove excess fluid[107].

Polyacrylate full blood adsorption (PFBA) or direct adsorption of lipoprotein (DALI). This technique uses a non-haemolytic adsorber. This was the first method which was able to adsorb LDL directly from the patients whole blood without the need to isolate the plasma[108].

Immunoadsorption. In this method plasma is pumped through two columns containing polyclonal sheep antibodies to human ApoB-100[109].

Filtration plasmapheresis. This method involves two filters. Plasma is separated from blood cells by the first filter and the second selectively removes molecules with larger molecular weights such as LDL whilst retaining HDL and other useful components[110].

LA is a challenging treatment for patients with some suffering nausea, vomiting, abdominal pain and headaches. Each session takes a few hours and patients have to regularly attend fortnightly. It is also an expensive treatment with an estimate of£39,000 per patient per year and is therefore limited to those patients most in need[105].

In addition to its LDL lowering effects, decreases in major adverse cardiovascular events and the need for myocardial revascularisation have also been attributed to this treatment, although definitive large scale randomized control trials have not been performed [111, 112]. In addition a number of studies have demonstrated improved endothelial and vasomotor function in both the micro and macro coronary vasculature following apheresis treatment[113-115]. Improvement in endothelial function has also been shown in the peripheral microvasculature immediately after apheresis treatment[116]. The one study looking at endothelial function within a conduit artery assessed by flow-mediated dilatation (FMD) in the brachial artery in patients with FH, found that patients undergoing long term apheresis treatment had similar FMD to controls but did not see an additional improvement in FMD immediately following apheresis treatment[117]. An improvement in FMD was seen by Morimoto and colleagues, 4 weeks after the tenth and final apheresis treatment in a group of patients with peripheral arterial occlusive disease[118]. Improvements have also been shown in other subclinical vascular measures. In a group of patients undergoing lipid apheresis treatment IMT progression was slowed in those with homozygous FH whilst a reduction in IMT was seen in those patients with heterozygous FH[119]. However, there are currently no studies that have seen an improvement in arterial stiffness either immediately after a session of apheresis or after a year of regular treatment[120, 121].

1.2.4 Obesity

Obesity, defined as a BMI greater than 30 kg/m^2 or a waist circumference of more than 102 cm for males and 88 cm females, is an increasing problem. Between 1993 and 2012 the proportion of men in England with a BMI ≥ 30 kg/m^2 rose from 13.2% to 24.4%, whilst in women it increased from 16.4% to 25.1%. The proportion of those with a raised waist circumference has also risen from 23% to 39%[122]. Being overweight and obesity have both been associated with increased risk of CVD[123-125]. Where the fat is stored (either subcutaneously or intra-abdominally) is also important, as visceral fat has been shown to be associated with metabolic abnormities such as insulin resistance and dyslipidaemia which are related to increased cardiovascular risk[126]. Hypertension is also commonly associated with obesity and the clustering of this risk factor plus insulin resistance and dyslipidaemia, with obesity as determined by waist circumference (an indicator of abdominal obesity), have been termed the metabolic syndrome and is also associated with increased cardiovascular risk[127].

Endothelial dysfunction has been associated with obesity and metabolic syndrome[128-131]. This is likely due to the impact of increased free fatty acids, oxidative stress, inflammatory cytokines on the availability of NO and the increased presence of vasoconstrictors[132]. Arterial stiffness and IMT have also been shown to be increased in those with obesity or metabolic syndrome[133-135].

Weight loss and lifestyle interventions reduce blood pressure and prevent or delay the onset of type 2 diabetes lowering the risk of cardiovascular disease[136, 137]. A dietary and exercise intervention in obese children demonstrated an improvement in FMD after 6 weeks and a regression in carotid IMT after 1 year in those who continued with the exercise program[138].

1.3 Non-invasive assessment of subclinical vascular function and disease

With the greater understanding of the processes responsible for atherogenesis, such as the role of the endothelium, and the changes that occur to the structure and function of the arteries throughout the development of atherosclerosis, a variety of methods have been developed for assessing endothelial function, carotid intima-media thickness and arterial stiffness. Assessing subclinical vascular disease has provided insights into the

pathophysiology of atherosclerotic disease at an early stage, enabled the study of the impact of interventions and helped identify individuals who are potentially at greater risk of future cardiovascular events.

1.3.1 Endothelial function testing

Based on the findings of Furchgott and Zawadzki who demonstrated the importance of the endothelium for vascular smooth muscle relaxation, a number of methods have been developed that assess endothelial function[12]. These encompass a range of both invasive and non-invasive techniques, which use changes in blood flow or the administration of pharmacological agents to assess endothelial function in the coronary circulation, resistance vessels or conduit arteries. These methods have been shown to be related to cardiovascular risk factors such as smoking, hypertension, diabetes and dyslipidaemia and to be predictive of cardiovascular events[31, 47, 91, 139-144].

1.3.1.1 Invasive methods

Endothelial vasomotor function was first clinically assessed in the coronary circulation. Using quantitative coronary angiography and Doppler flow-wire techniques changes in the epicardial and microvascular responses to endothelium-dependent pharmacological agents are measured during cardiac catheterization. Vasodilatation in response to acetylcholine indicates preserved epicardial coronary endothelial function. Whereas, constriction of the vessel is suggestive of the smooth muscle response to direct muscarinic receptor stimulation overwhelming the absent or depressed dilation that follows from reduced bioavailability of endothelial NO[145]. Due to the invasive nature of this technique, its use is restricted to individuals in the more advanced stages of arterial disease who have clinical indications for cardiac catheterization. Despite this limitation, coronary vascular function testing has provided important insights into the effects of atherosclerosis and its risk factors on coronary regulatory physiology and risk stratification as well as demonstrating the potential reversibility of endothelial dysfunction in response to treatments such as statins and ACE-inhibitors[142, 146, 147].

A second invasive method, which is commonly used to assess vasomotor function of the resistance vessels in the forearm, is venous occlusion plethysmography. This technique assesses changes in forearm blood flow (FBF) in response to pharmacological agents. It provides its own control by using the contralateral arm, permitting adjustments to be made

for systemic influences that affect basal flow and blood pressure in the non-infused arm. Most studies measure percentage differences in FBF and vascular resistance between the experimental and control arms following administration of endothelium-dependent and -independent agonists. Evaluation of the contribution of NO to vasomotor regulation can be made using eNOS antagonists such as L-N^G-monomethyl Arginine citrate (L-NMMA). One advantage of this technique over assessment of changes in the coronary circulation is that it can be used in healthy controls as well as patients, thus enabling study of the endothelium from early in the disease process. Additionally, vasomotor pathways other than NO can also be evaluated. However, it is still an invasive technique which limits its repeatability and also restricts its use to small studies. The clinical relevance to atherosclerosis has also been questioned, as microvascular pathophysiology may not necessarily reflect changes in the conduit arteries in which atherosclerosis develops.

1.3.1.2 Non-invasive methods

Flow-mediated dilatation

The ultrasound based technique FMD, is the current gold standard technique for non-invasive assessment of the endothelium. This method, first developed by Celemajer et al, uses a period of forearm ischemia (induced by a cuff around the forearm inflated to suprasystolic pressure) followed by reactive hyperaemia to increase brachial arterial blood flow and, consequently, local shear stress[90]. This stimulates the endothelium to generate and release NO, activating guanylyl cyclase to produce cyclic GMP in vascular smooth muscle which causes relaxation and dilatation of the artery[148, 149]. The changes in blood flow and vessel diameter can be assessed by imaging the brachial artery and measuring blood flow with high resolution 2D ultrasound and Doppler

FMD is normally used in the research laboratory as it is a technically demanding technique and is initially expensive to set up, but has been shown to have good reproducibility in this setting[150]. It can also be used with care in large epidemiological studies and is increasingly being used in clinical trials[151, 152].

Differences do exist in the methodology for carrying out assessment of FMD. The reactive hyperaemic stimulus can be affected by both cuff position and duration of the occlusion period. When the occlusion cuff is positioned above the ultrasound probe, the whole arm is made ischemic including the arterial segment being measured and a larger reactive

hyperaemic response and vasodilatation of the vessel is seen than when the cuff is placed on the forearm distal to the study segment[149, 153]. A cuff occlusion of 15 minutes also causes a larger hyperaemic response than a 5 minute period[89]. Therefore, both proximal cuff positioning and longer occlusion periods may not specifically represent NO-mediated endothelium-dependent function [149, 154]. In contrast, more distal positioning of the cuff and using a 5 minute occlusion period has been demonstrated to induce NO-mediated vasodilatation[149, 155]. There has also been much debate on whether the FMD response (normally expressed as either a percentage or an absolute change) should be normalized for the reactive hyperaemic stimulus. However, a consensus has not yet been reached regarding whether or not this should be done nor the best method for doing this.

Pulse wave analysis (PWA) and pulse contour analysis (PCA)

Endothelial function can be assessed by the administration of agents such as the β2 adrenergic receptor agonist salbutamol. These can be administered via an inhaler or IV infusion and at standard clinical doses, do not affect blood pressure[156-158]. Salbutamol causes the release of NO from the endothelium via vascular endothelial β2 receptor activation. This leads to a reduction in arterial tone and stiffness, and can be measured in the peripheral waveform with either PWA by applanation tonometry at the radial artery or PCA with digital photoplethysmography. Although the relative simplicity of this technique compared with other methods and a relatively low cost appear advantageous, there are some practical concerns regarding its reproducibility compared with FMD[150, 159]. Also, little correlation has been observed between FMD and results with these techniques, which could implicate distinct pathophysiological influences at different levels of the vasculature requiring further evaluation[150].

Endo-PAT

Another method which is technically straight forward and uses reactive hyperaemia as the stimulus to activate the endothelium is pulse amplitude tonometry (PAT). The Endo-PAT system uses a fingertip probe to measures changes in arterial pulsatile volume. It provides its own internal control as recordings are made simultaneously in the right and left index fingers both prior to and following a 5 minute period of forearm ischemia. The reactive hyperaemic PAT index is calculated as the ratio of the average amplitude of the PAT signal over a 1min time interval starting 60 seconds after cuff deflation, divided by the average

amplitude of the PAT baseline. Reactive hyperaemic-PAT index values from the study arm are normalized to the control arm.

Endo-PAT has been shown to have similar reproducibility to that of FMD and that mechanistically the vasodilatation is mediated at least in part by NO[160, 161]. However, it is not entirely NO-dependent, and there is likely to be an important interaction with the autonomic nervous system that may confound interpretation of the results from a specifically endothelial perspective. This may account for heterogeneity between the results in some studies which have included both Endo-PAT and FMD[162, 163]. A second limitation of the technique is the inability to take into account the impact of autonomic influences on endothelium-independent response to systemic glyceryl trinitrate (GTN) due to lack of a simultaneous unexposed control arm. Finally, the fingertip probes used for measuring changes in arterial pulsatile volume can only be used once which increases the cost of using this system.

Pulse wave velocity

Endothelial function can also be assessed by measurement of PWV. PWV is the time taken for a pulse waveform to travel along the artery wall; it is calculated as distance over time and provides a measurement of arterial stiffness. In the brachial artery, it reflects both arterial wall composition and smooth muscle tone. In response to a reactive hyperaemic stimulus, which increases shear stress and stimulates endothelial NO release, PWV slows due to the resultant drop in smooth muscle tone[164]. This simple technique was first developed by Naka et al and uses two cuffs, one placed at the wrist and one on the upper arm, to assess PWV over the brachio-radial tract; the reactive hyperaemic stimulus is induced by wrist cuff occlusion. In their study, Naka et al demonstrated that PWV reduced by 14.2% in the upper limb of healthy volunteers following reactive hyperaemia[164]. When they looked at the response in a group of patients with chronic heart failure the reduction in PWV was lower at 8%. However, in both groups PWV decreased similarly in response to administration of GTN indicating that the differences between the healthy and disease groups were due to endothelial dysfunction. Additionally Naka et al looked at the effects of stimulating and inhibiting NO production on PWV by the administration of acetylcholine and the nitric oxide synthase inhibitor L-NMMA. Stimulation of NO caused a decrease in PWV whilst inhibition increased PWV indicating a role of NO in mediating changes in PWV. In unpublished observations the group had also noted that L-NMMA had been shown to inhibit hyperaemic changes in PWV[164].

Other groups have since carried out further studies using this technique but have used the carotid to radial pathway, and different methods of assessing PWV. Reproducibility of the method in the carotid to radial tract has been demonstrated by two groups, one, using the Sphygmocor for measuring PWV had a coefficient of variation (CV%) of 12% whilst the second group, Graf et al, who assessed PWV with mechanotransducers, had a CV% of 9.7%[165, 166]. A further methodological discrepancy between these studies and the technique as originally published by Naka et al is with the positioning of the occlusion cuff. Naka et al placed the occlusion cuff at the wrist, Kamran et al, positioned it around the upper arm, whilst Graf et al occluded the forearm[164-166]. As discussed in the section regarding FMD the positioning of the occlusion cuff may have implications on how much the slowing in PWV following reactive hyperaemia is mediated by NO. It is also worth noting that the use of the carotid to radial arterial tract involves the more elastic carotid artery. Muscle tone has less influence on the stiffness of the carotid artery than the brachial and radial arteries which may influence the changes in pulse wave velocity following hyperaemia.

Additional studies have been carried out in groups with risk factors where the decrease in PWV following the reactive hyperaemic stimulus has been shown to be diminished in those with increasing Framingham risk scores, congestive heart failure and in pregnant and non-pregnant hypertensives[165, 167-171].

1.3.1.3 Utility of endothelial function testing

Endothelial function testing has been used in a wide range of studies from small cross sectional studies to large cohort studies. This has enabled demonstration of its role in the causal pathway of atherosclerosis by showing endothelial dysfunction in children with FH[139]. In addition, the relationships between endothelial function and cardiovascular risk factors have been seen in smaller studies and confirmed in the larger cohort studies[172, 173]. Furthermore, prospective studies have enabled the demonstration of the linkage between endothelial function and arterial disease with an inverse association between FMD and carotid IMT in the Young Finns study, whilst a second study has shown that FMD is predictive of IMT progression[174, 175]. Prospective studies have also shown the association between endothelial function and prediction of cardiovascular events[142, 176].

Endothelial function testing is also increasingly used in intervention studies as it is possible to detect changes in endothelial function relatively quickly in comparison to other longer term time points such as changes in the carotid and coronary wall or the occurrence of clinical cardiovascular events. It also enables the study of additional beneficial drugs outside of their primary aim. For example statins have been demonstrated to have a beneficial effect on endothelial function in addition to their lipid lowering properties[92].

Despite the increasing usage of endothelial function testing in a wide range of trials, there are still limitations with the current techniques in terms of equipment and expertise required to carry out these methods. There is still room for alternative methods that are cheaper, simpler to use and more easily scalable to further progress the use of endothelial function testing.

1.3.2 Measurement of arterial stiffness

Arteries stiffen with age due to changes in their shape and the composition of their major structural proteins collagen and elastin[8]. This increased stiffening may contribute to the development of cardiovascular disease by raising systolic blood pressure, increasing cardiac after load and reducing cardiac perfusion[177].

There are a number of different indices and methods for measuring arterial stiffness. Two commonly used indices are PWV (the speed at which a pulse wave travels) and distensibility (the relative diameter change for a pressure increment).

Carotid to femoral PWV is currently viewed as the "gold standard" method for assessing regional arterial stiffness. Other arterial tracts can be assessed such as the brachial to femoral arteries and the carotid to radial arteries. Common techniques for assessing PWV are applanation tonometry (Sphygmocor), mechanotransducers (Complior) and oscillometry (Vicorder). Magnetic resonance imaging (MRI) is also being increasingly used for assessing PWV and although not such an accessible or easy to use method, it has the advantage of being able to provide accurate path length measurements, this being a much debated methodological area[177].

Distensibility is a measure of local stiffness and is often measured in the carotid artery due to its accessibility. It is commonly assessed by ultrasound often with echotracking software

to assess the change in diameter over the cardiac cycle. Ideally the blood pressure local to the site being measured should be used, but brachial blood pressure is commonly used for calculating carotid artery distensibility. MRI can also be used for assessment of distensibility particularly of the aorta[177, 178].

Both increased PWV and decreased carotid distensibility have been shown to be associated with cardiovascular risk factors and predictive of cardiovascular events and stroke[93, 178-182].

1.3.3 Measurement of atherosclerosis

One of the simplest measures for assessing atherosclerosis is IMT. This is the distance between the intimal and medial layers of the artery wall. Atherosclerosis mainly occurs in the intimal layer of the vessel wall[183]. However, it is not possible to determine with ultrasound in which layer the thickening occurs, therefore some thickening of the wall may be of non-atherogenic origin[184]. Numerous studies have shown an association between IMT and its progression with cardiovascular risk factors, it is also a predictor of cardiovascular events such as stroke and myocardial infarction[52, 92, 133, 185, 186]. A thickened IMT of ≥0.9mm is considered to be a surrogate marker of generalized atherosclerosis[38].

IMT is normally assessed using B-mode high resolution ultrasound often in the common carotid artery but also in the internal carotid artery and carotid bulb. Originally the distance between the intima and media was measured from end-diastolic images using ultrasonic callipers. However, this method has now been replaced with the development of echotracking and edge detection software which allow for a more detailed and accurate measurement. Due to its relative ease of use, reliability and applicability, assessment of IMT has been used in a wide range studies including epidemiological studies and clinical trials[187, 188].

In addition to assessing IMT using ultrasound, it is also possible to identify plaques within the artery. These are defined as areas of the artery wall where the IMT is considerably greater than adjacent sites or where encroachment on the lumen can be identified[189]. The presence of plaque is related to increased risk of cardiovascular events[190]. Although ultrasound is very useful for identifying plaques and can detect some features of the plaque such as whether it is calcified or lipid rich, it does have its limitations in its ability to distinguish the constituents and morphology of plaque . Other methods have been

developed such as magnetic resonance imaging, computerised tomography and positron emission tomography that can provide more detailed information on the features and components of plaques in the carotid and coronary arteries. These methods have been reviewed by Joshi et al[191].

1.4 Mental stress and cardiovascular disease

"Stress is a state of threatened homeostasis provoked by a psychological, environmental, or physical stressor" [192].

An increasing body of work has demonstrated the effects of stress on the cardiovascular system. Stress can generally be divided into two categories, psychosocial/chronic stressors, which can lead to accelerated atherosclerosis and higher rates of coronary heart disease incidence, and acute stressors which can trigger acute cardiac events such as myocardial infarction or angina.

1.4.1 Pyschosocial/ chronic stress

There are a wide range of psychosocial factors that can impact on the development and risk of cardiovascular disease. These broadly consist of groupings such as socio-economic status and social class, occupation related stress, such as job strain or job control, social factors, such as low social support or isolation, and negative emotional states, such as depression, anxiety or hostility.

1.4.1.1 Depression

Major depression can be characterised by the presence of a depressed mood and a lack of interest in all activities which lasts for at least two weeks and is accompanied by changes in a number of areas such as appetite, sleep disturbance, feelings of guilt or worthlessness, problems concentrating and suicidal thoughts[193]. It is often diagnosed through the use of structured questionnaires administered by trained clinicians based on the description of psychiatric disorders in the Diagnostic and Statistical Manual of Mental Disorders (DSM). In a European study looking at the prevalence of common mental disorders in general practice attendees 13.9 % women and 8.5% men were identified as having a major

depressive disorder[194]. The greater prevalence of depression in women has also been seen in a study based purely on UK general practice data[195]. Additionally, in patients with known heart disease the prevalence of depression in cross-sectional studies is about 20%[196].

1.4.1.2 Anxiety

Anxiety is characterized by transient fear, uncertainty, and apprehension about the future, but individuals vary on the frequency and intensity with which they experience anxiety[197]. Anxiety disorders can include generalised anxiety disorder, panic disorder and phobias. The prevalence of anxiety disorders has been shown to be 10% in women and 5% in men within Europe[194]. In the United States, the lifetime prevalence of any anxiety disorder has been shown to be over 28%[198]. Additionally, there is a high level of co morbidity between anxiety and depression[197].

1.4.2 Acute mental stress

Acute mental stressors are stimuli or activities that can cause negative emotional states. These can include earthquakes, war, terrorist attacks, sporting events, anger and acute work stresses such as high pressure deadlines. Negative emotional states can lead to acute physiological or pathophysiological changes that in this context can trigger a cardiovascular event[199].

1.4.3 Associations with cardiovascular disease

A large number of studies have demonstrated an association between psychosocial /chronic stress and CVD. One of the largest studies to have looked at the influence of psychosocial factors on cardiovascular events is the INTERHEART study[200]. It included 11,119 patients with a first myocardial infarction and 13, 468 controls and asked four simple questions about stress at work and at home, financial stress and major life events in the past year. There was a greater prevalence of all four stress factors in those who presented with a myocardial infarction compared to controls, and the presence of psychosocial stressors increased the risk of acute myocardial infarction[200]. Increased risk of CVD has been shown to be associated with numerous individual chronic stressors including for example social isolation, care of a sick partner and job strain[201-203].

The relationship between depression and CVD is a much studied area in part due to the consistent association between the two. Patients with CVD and depressive symptoms are at greater risk of future cardiovascular events and mortality[204-206]. Whilst those who have had major depressive disorders are also at increased risk of cardiovascular disease[207]. Population based studies in participants free from CVD have shown that depression predicts subsequent CVD and cardiovascular events in both men and women[208, 209]. A meta-analysis of 11 prospective studies found that the relative risk of depressed patients developing coronary heart disease was 1.6 with the risk being greater in those with clinical depression than those with depressive mood[210].

Anxiety has also been shown to be a predictor of CVD and death. A meta-analysis of 20 studies reporting on incident of coronary heart disease in initially healthy participants, showed hazard ratios of 1.26 for risk of coronary heart disease and 1.48 for cardiac death[211]. However, in patients who already have cardiovascular disease the findings have been mixed. Some studies have demonstrated an association between higher levels of anxiety and poorer prognosis and increased recurrence of cardiovascular events, whereas other studies have suggested that anxiety may be protective in coronary patients[212-215].

Acute stressors are more difficult to assess than chronic stressors. However, through the use of retrospective data, associations between acute mental stressors and cardiac events have been seen. Increases in sudden cardiac death and acute myocardial infarction (MI) have been seen following earthquakes in Northridge, Los Angeles and Hanshin- Awaji, Japan[216, 217]. However, there was no difference in the numbers admissions for acute MI on the day of an earthquake in the San Francisco Bay area compared with the days before or after the earthquake[218]. The discrepancy in these findings has been suggested to be due to the time of day of the earthquake, with both the Northridge and Hanshin-Awaji occurring in the early morning, thereby adding a second stressor to the stress of waking, whereas the San Francisco earthquake took place in the afternoon[219].

Other events such as national side football matches in international tournaments have also been associated with an increase with cardiac events. On the day of a European Championship football match between France and Holland there was a relative risk of death from acute MI or stroke of 1.51 for Dutch men in comparison to the 5 days either side of the match. There was no change in women[220]. Other studies have also seen increases in events with other crucial matches, although, some studies have not demonstrated an effect[221-223]. Finally, individual emotional triggers such as anger and acute

stressors such as high pressure deadlines have been associated with increased risk of cardiovascular events[224, 225].

1.4.4 Mechanisms of mental stress

As discussed above, both chronic and acute emotional stressors are associated with increased risk of cardiovascular disease and events. However chronic stressors seem to increase cardiovascular risk largely through acceleration of the atherosclerotic process, whereas acute emotional stressors appear to trigger acute arrhythmic, thrombotic or mechanical events[226].

The responses to acute mental stressors are largely mediated through activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). Stimulation of the hypothalamus causes the release of corticotrophin releasing factor which acts on the anterior pituitary causing the production of adrenocorticotrophic hormone which leads to the release of cortisol from the adrenal cortex. Cortisol has a number of effects which include, increasing blood pressure, insulin resistance, anti-inflammatory effects, central obesity and increased coagulation, many of which are considered to be cardiovascular risk factors[227-230]. Activation of the SNS leads to the release of catecholamines, mainly adrenaline. The effects of these include increases in heart rate, decreased heart rate variability, the release of inflammatory cytokines such as IL-6, increases in coagulation factors and raised blood pressure[44, 231-234]. Figure 1.3 gives a summary of this process and some of the changes brought about by activation of the HPA and SNS.

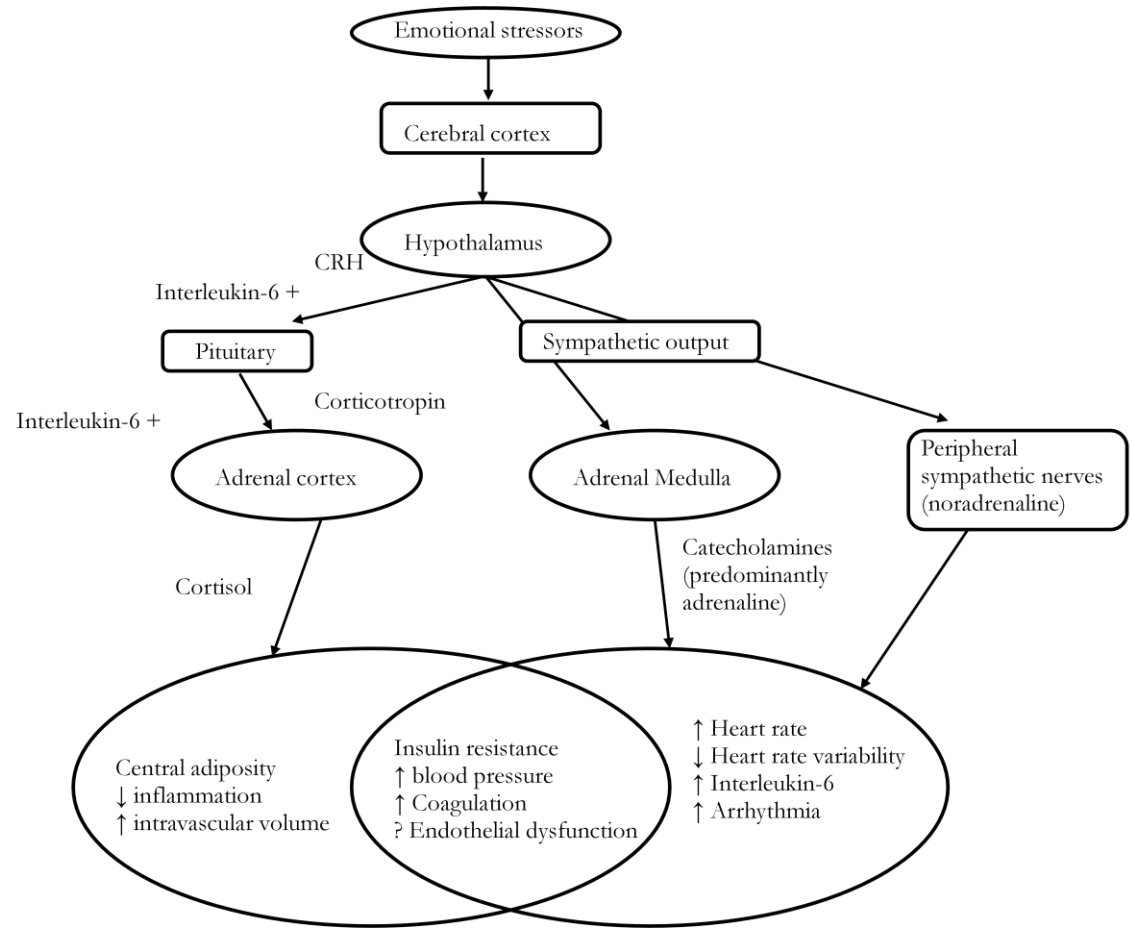

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Figure 1.3: Pathways of the stress response featuring the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. CRH = corticotrophin releasing hormone. The cardiovascular responses are at the bottom of the figure, with those responses controlled by both axes in the centre. Adapted from Brotman et al[226].

A key part of the response to acute stress which may play a role in the development of atherosclerosis is the inflammatory response. Acute stressors trigger the inflammatory acute phase response which involves the generation of the pro-inflammatory cytokine IL-6. One of the numerous roles of this cytokine is the inducement of the acute phase hepatic response which results in the production of acute phase proteins such as CRP and fibrinogen. Both of these are present in patients with coronary disease and are predictors of future coronary risk in healthy subjects[235, 236]. Fibrinogen causes endothelial and smooth muscle cell activation, increases blood viscosity, platelet activation and aggregation and immune cell recruitment which are all key in the development of atherosclerosis[237-240]. In addition, it plays a key role in the coagulation cascade where it is broken down into fibrin monomers which form a clot; fibrin is also found in atherosclerotic plaques[240, 241].

Many of the responses referred to above, such as HPA dysregulation, also relate to those found in chronic stress. Cortisol levels in both urine and plasma have been found to be increased in patients with major depression[242, 243]. In addition patients with depression have been shown to have adrenal hypertrophy, impaired suppression of cortisol after a dexamethasone challenge and blunted cortisol reactivity and recovery to psychological stress challenges[244-246]. Elevated levels of cortisol are also seen in patients with anxiety disorders[247]. The dysregulation of the HPA axis seen in psychological disorders does not only result in its hyperactivity with elevated cortisol levels, sufferers of post-traumatic stress disorder have been shown to have low levels of cortisol and diminished cortisol responses to stressors[248]. Sympathetic hyperactivity has also been recorded in those with depressive disorders evidenced by increased levels of catecholamines and decreased heart rate variability[249, 250]. In addition subjects with a high Beck depression score have shown delayed adrenaline recovery in response to a stressful task[251]. Depression and anxiety have also been associated with hypertension, dyslipidaemia, obesity and weight gain, metabolic syndrome, diabetes, increased CRP and other cytokines[252-259].

In addition to these physiological consequences of psychosocial factors such as depression and anxiety and their relationship with cardiovascular disease, behavioural changes should also be taken into account. Those with depression and anxiety are more likely to smoke, be non-compliant with medication and lead sedentary lifestyles than those without these psychosocial factors[260, 261].

1.4.5 Depression, anxiety and IMT and arterial stiffness

A number of studies have investigated the relationship between psychosocial factors and subclinical measures of atherosclerosis such as IMT and arterial stiffness to try and provide an early insight into the development of arteriosclerotic and atherosclerotic disease. However, there has been notable variation within the results.

Depression and symptoms of depression have been associated with increased IMT in a number of studies. In a study of 1505 Asian Indians, Poongothai et al found that high levels of depressive symptoms were associated with both increased IMT and augmentation index (AIx), although the relationship with AIx was lost after adjustment for cardiovascular risk factors (age, gender, BMI, glucose, cholesterol and hypertension)[262]. Similarly, Faramawi et al showed that those with depressive symptoms had increased IMT in an

elderly population, as did two other studies looking at late onset depression[263-265]. However, Kabir et al only saw an association between depression score and IMT in the carotid bulb and not in the internal or common carotid arteries in a population of young adults[266]. Further analysis also found that there was a significant negative effect of the interaction between depression and total cholesterol / HDL ratio on IMT. Sub analyses showed that those in the highest quartile of TC/HDL had a negative association between IMT and depression score, whereas in the lowest quartile it was a positive relationship. It is worth noting that only approximately 5% of the total population (n=996) were in the highest depression category which may have an impact the applicability of the findings [266]. In an analysis by Hamer et al, it was found that increased IMT was positively associated with depression score and that those with depression were more likely to have metabolic syndrome. The presence of metabolic syndrome accounted for 21% of the association between depressive symptoms and IMT[267]. However, in a study of police officers, Violanti et al initially found no relationship between IMT and depression symptoms but on stratification of the data by hypertension status showed that a higher CES-D score was associated with greater IMT but only in officers without hypertension[268].

Differences in the relationship between depressive symptoms and IMT have been seen according to gender. In the Young Finns study, depressive symptoms were associated with increased common carotid IMT in males but not females[269]. Chirinos et al did not find an association between IMT and depressive symptoms in a multivariate analysis, but found a moderating effect of gender on the relationship, with a significant association between depressive symptoms and increased IMT only in males[270]. It was suggested that the lack of association between IMT and depression in the middle-aged women may partly be due to the protective effects of oestrogen. Lee et al showed IMT was greater in Korean women with depressive symptoms; a relationship which remained after adjustment for cardiovascular risk factors, menopause and postmenopausal hormonal state. However, in this study they did not find a relationship between IMT and depression in men[271]. In another study of 336 healthy middle-aged women, those women with recurrent major depression had a 2 fold greater risk of carotid plaque in comparison to those without a lifetime history of depression. However, IMT was not increased in those with recurrent or isolated major depressive episodes[272]. A second study in a large community population also found that history of depression was related to presence of plaque but not IMT[273]. A lack of association between IMT and symptoms of depression has also been seen in other studies with both genders [274-277]. It is worth noting that in these studies a variety of questionnaires such as the CES-D scale, Beck Depression Inventory or Patient Health

Questionnaire were used to identify symptoms of depression which may partly contribute to the differences in findings.

Relationships have also been seen between symptoms of depression and the progression of IMT[278, 279]. Stewart et al found increased IMT progression with greater depressive symptoms. They also found that somatic symptoms of depression were associated with IMT progression but there was no relationship with cognitive symptoms. Additionally, they looked at the relationship with symptoms of anxiety, but saw no association with IMT progression[279]. This was in contrast to Paterniti et al who found that men with sustained anxiety (high anxiety scores at both baseline and 2 year follow up) had the greatest progression in IMT and a high risk of plaque occurrence 4 years after baseline assessment[280]. There was a similar trend in IMT progression for women but this did not reach significance ($p=0.07$)[280]. However, Paterniti et al looked at sustained anxiety, whilst Stewart et al only measured anxiety at baseline, which may account for the differences in findings[279, 280]. The majority of studies have not found an association between future or concurrent IMT and anxiety symptoms[270, 272, 275, 281-284]. One recent study in South Asians living in America, has shown that men have increased IMT with higher levels of anxiety symptoms and a second Brazilian study also found a positive association having included adjustment for gender, but in general there is less evidence of a relationship between IMT and anxiety than with symptoms of depression[277, 285].

Fewer studies have looked at the influence of depression and anxiety on arterial stiffness as a measure of subclinical atherosclerosis. Tiemeier et al found in an elderly population that those participants with greater arterial stiffness, as measured by central PWV and carotid distensibility coefficient, were more likely to have a depressive disorder[286]. Whereas, Seldenrijk et al did not find a relationship between carotid distensibility coefficient with either depression or anxiety disorders, but did show that AIx was increased in both disorders in comparison to controls[287]. Anxiety has also been found to be predictive of increased central PWV in Korean Americans independent of other confounding risk factors[288]. Other studies have also shown increased PWV in those with anxiety disorders when compared with control participants but again conflicting results have also been seen[289-292].

1.4.6 Depression, anxiety and endothelial function

The first study to show an association between depression and FMD was by Rajagopalan and colleagues in 2001[293]. FMD was reduced in a group of young depressed patients without cardiovascular risk factors when compared with matched controls. This relationship remained after multivariate analysis including adjustment for cardiovascular risk factors. This finding was closely followed by Broadley et al, who also demonstrated diminished FMD in patients with treated depression but free from CVD risk factors in comparison with healthy controls[294]. However, Taylor et al, found no difference in FMD between depressed and matched non-depressed subjects, who were all at high risk for coronary artery disease due to either elevated blood pressure or lipid levels[295]. Another study, looking at postmenopausal women who had a life time history of major depressive disorder but were free of cardiovascular disease had significantly lower FMD than never depressed controls[296]. The number of depressive episodes was also related to poorer endothelial function. The same group also found that recurrent depressive disorder was associated with lower FMD in women with type 2 diabetes[297].

Further work has looked at the relationships between symptoms of depression and anxiety as assessed by scales such as the CES-D or Beck Anxiety Inventory, and endothelial function. Hemingway et al, in data from the Whitehall II study, showed that decreased FMD was associated with mild depression but not with other psychosocial factors such as anxiety, in a healthy non-smoking middle-aged population[284]. However, lower FMD was associated with measures of both anxiety and depression symptoms in postmenopausal women and healthy adults [298, 299]. In a group of patients free of coronary heart disease but with at least two risk factors for it, those with depressive symptoms had lower FMD and increased total cholesterol and inflammatory markers compared to those without symptoms[253]. Patients with coronary heart disease or stable angina and high scores of depressive symptoms have both been shown to have impaired FMD compared with patients with these conditions but low depressive symptoms[300, 301].

Not all studies have found an association between psychosocial traits and endothelial function. In a longitudinal study of care givers who were assessed annually for 3 years, there was no relationship between depressive symptoms and FMD overtime[302]. Furthermore, in a study of 332 older adults, despite associations between suppressed anger and FMD for females and hostility and FMD for males, there was no association with symptoms of either depression or anxiety for either gender. However, the scores for both

depression and anxiety were skewed towards the lower end of the scale which may account for the lack of association in this study[303]. Another study which did not see an association between FMD and anxiety in patients with clinically diagnosed atherosclerotic vascular disease did find that both endothelium dependent and independent resistance vessel function was reduced which may suggest a different mechanism for anxiety[304].

Overall the body of evidence suggests that FMD is impaired in the presence of high depressive scores or major depressive disorders. This is further substantiated by a systematic review by Cooper et al who concluded that there was an inverse correlation between FMD and depressed mood[305]. There is less evidence for a relationship between FMD and anxiety and this remains an area for further exploration.

1.4.6.1 Acute mental stress and vascular structure and function

The assessment of cardiovascular responses to acute laboratory mental stressors allows the investigation into the potential mechanisms which underlie the development of CVD and can trigger cardiac events. These tests can include either a single stress challenge or a combination of tests such as an arithmetic test, mirror tracing task, colour word association (stroop) or a public speaking task. A number of studies have looked at how these tests or physiological responses to the challenges relate with measures of vascular structure and function which are early makers of cardiovascular disease.

As a measure of vascular structure, studies looking at the relationships between IMT and acute stress have focused on whether differences in the physiological responses to acute stressors are associated with increased IMT. Studies in adults have shown that changes in systolic, diastolic and pulse pressure have all been associated with both contemporaneously assessed and future IMT[306-309]. A recent study has also found that carotid systolic blood pressure reactivity (stress response minus baseline) to stress is positively associated with IMT in healthy adults[310]. It is not only the response to stress that has been shown to be important, but also the recovery from the stress response. A study by Steptoe et al found that delayed systolic blood pressure recovery was associated with increased IMT in those with lower socio-economic status[311]. In children, systolic blood pressure reactivity to stress has been shown to be predictive of IMT[312-314]. Another study in adolescents, where responses to mental stress challenges were assessed at two times points, demonstrated a positive association between increased diastolic blood pressure reactivity and IMT[315]. In

addition to blood pressure changes, alterations in heart rate in response to acute mental stress challenges have also been associated with IMT. Both greater heart rate reactivity and better recovery have been associated with lower IMT[316, 317]. It has also been suggested that the association between heart rate reactivity to stress and IMT is mediated through the association of heart rate reactivity with obesity and smoking[318].

A few studies have looked at the impact of acute mental stress challenges on measures of arterial stiffness. Vlachopoulos et al demonstrated that both aortic PWV and AIx (as a measure of wave reflection) were increased following a mental arithmetic test[319]. Other studies have also seen increases in these parameters and a decrease in arterial compliance either during or following mental stressors[320-322]. Other studies have looked at the association of physiological responses to stress with carotid stiffness. Lipman et al, found that greater arterial pressure responses to stress were associated with increased carotid stiffness[323]. Another study has shown that participants with the greatest inflammatory responses to acute stressors had increased carotid distensibility at a subsequent assessment 3 years later[324]. However, there is limited work in this area in both the immediate responses to acute stress and future relationships.

Studies looking at the relationship between endothelial function and acute mental stress have investigated this in two ways, by either assessing endothelial function during the stress test or at time points following the stress tasks. The first study to show a relationship between acute mental stress and endothelial function in healthy adults was by Ghiadoni et al[325]. FMD was assessed before a stress test, which involved preparing a speech to defend themselves against a false allegation of shoplifting, and at 30, 90 and 240 minutes after the stress test. It was found that FMD in healthy subjects was significantly lower at 30 and 90 minutes post stress. Importantly endothelium-independent vasodilatation as assessed by GTN was not affected. In this study they also looked at the effect of mental stress on FMD in non-insulin-dependent diabetes patients but did not see any significant changes[325]. Other studies have also seen diminished FMD post acute mental stress challenges in healthy subjects[326-331].

When endothelial function has been assessed during the acute mental stress tests the results have been more varied. Two studies saw no change in FMD; however when Lind et al normalized their data for blood flow, a significant decrease in FMD during mental stress became apparent[332, 333] . A study by Gottdiener et al also saw a reduction in brachial artery vasodilatation when expressed as the time-diameter integral (area under the curve -AUC)

when mental stress was administrated during cuff occlusion and compared with cuff occlusion alone[334]. In this study Gottdiener et al also compared the responses in subjects with and without elevated cholesterol. They found that those with elevated cholesterol had less dilatation of the brachial artery (as expressed by AUC) during a mental stress challenge alone, but there was no difference between the two groups for dilatation induced by cuff occlusion when assessed alone and concomitantly with mental stress[334]. One study has shown an improvement in FMD during a standard mental arithmetic challenge. However it is worth noting that the baseline brachial artery diameter recorded during the stress test was lower than for the pre-test FMD and may partly account for this finding. Vasoconstriction of the brachial artery during stress testing has also been shown by Ghiadoni et al[325]. A meta-analysis of the studies included here concluded that FMD did not change significantly when assessed during the mental stressor but there was a significant reduction in FMD post stress challenge[335].

There is only one study which has looked at physiological responses to acute mental stress and FMD. In the Young Finns study, participants who had better respiratory sinus arrhythmia recovery in response to a stress challenge and above median FMD assessed 2 years later, had lower IMT. Whilst those participants with low FMD and a slower pre-ejection period recovery and was related to increased IMT[336]. Therefore, there is a gap in the knowledge about whether physiological responses to acute stressors and the future development of cardiovascular disease are mediated by changes in endothelial function. Also there is limited information about whether there are differences in the responses to mental stress and how these relate to measures of subclinical vascular disease in those who already have cardiovascular risk factors.

1.5 Aims of thesis

Aim 1: To investigate the associations between stress, both chronic and acute, and measures of subclinical vascular structure and function in the Whitehall II cohort.

The evidence for an association between measures of subclinical vascular disease such as IMT and arterial stiffness and depression and anxiety is mixed. Although there is stronger evidence for an association with FMD and depression there is a paucity of evidence in those with anxiety in all measures. Furthermore it is unclear whether the presence of other cardiovascular risk factors which are commonly present in those with depression and

anxiety further exacerbates any associations between subclinical vascular disease and these chronic stressors. For acute stress, there is limited evidence on the impact of an individual's responses to acute stressors on future subclinical vascular disease and the influence of the presence of other cardiovascular risk factors. The Whitehall II cohort is a well characterised, large epidemiological study which includes measures of depression and anxiety symptoms and measures of artery structure and function. In addition, it also includes a substudy of participants who have undergone an acute mental stress challenge assessment. Thus, it is an appropriate cohort to permit exploration of the associations between chronic and mental stressors and cardiovascular disease.

Aim 2: To demonstrate the reproducibility of, and further validate a new technique for the assessment of vasomotor function.

There are a number of methods currently available for assessing endothelial function using invasive and non-invasive occlusion such as forearm plethysmography and flow-mediated dilatation respectively. However, these methods have their limitations, including the risk of invasive methods and the cost and complexity for non-invasive methods. Therefore, there is a need for a new simple and relatively inexpensive method with the potential to be used effectively by a wider range of researchers both in the research laboratory and out in the field. However, any new method will need to have been shown to be reproducible, repeatable and sensitive enough to detect differences in vasomotor function before it can be put to wider use.

Dyslipidaemia is a well known cardiovascular risk factor and is associated with increased IMT and arterial stiffness and poor endothelial function which have all been demonstrated to improve with treatment. This makes it a useful model for further investigating its impact on relationships between both chronic and acute stressors and subclinical vascular dysfunction and disease. Furthermore, it could be considered a useful paradigm for validation of a new model of endothelial function assessment. For example, by testing whether the new method is sensitive enough to detect differences in those with and without dyslipidaemia and also the impact of treatment known to improve other validated markers of subclinical disease.

Chapter 2. Whitehall II methods

2.1 Whitehall II population

The Whitehall II study consisted of 10,308 (3,413 women) nonindustrial civil servants, aged 35 to 55, who were recruited between 1985 and 1988, to investigate social and occupational influences on health and disease[337]. The study entailed repeated follow-ups every 2 to 5 years including clinic visits and self-administered questionnaires. Phase 7 (2003-2004)of the study involved a clinic visit and participants were also invited to attend the Vascular Physiology Unit at the Institute of Child Health, UCL, for an additional assessment of their carotid artery intima-media thickness. In addition, a subgroup of participants was invited to also undergo endothelial function assessment. This subgroup consisted of three groups of participants. During the previous clinical assessment at phase 5 (1997 to 1998), a random sample of non-smokers had been invited to take part in a vascular function substudy of whom 140 participants took part. A further 143 of the Whitehall II participants were identified based on glucose tolerance tests as having metabolic syndrome, glucose intolerance or glucose tolerance and also underwent vascular studies at phase 5. Both of these groups of participants were invited for a repeat endothelial function assessment at phase 7. Finally, participants from a psychobiology study were also included in the subgroup for endothelial function evaluation. Figure 2.1 gives more detail of the Whitehall II population and study phases.

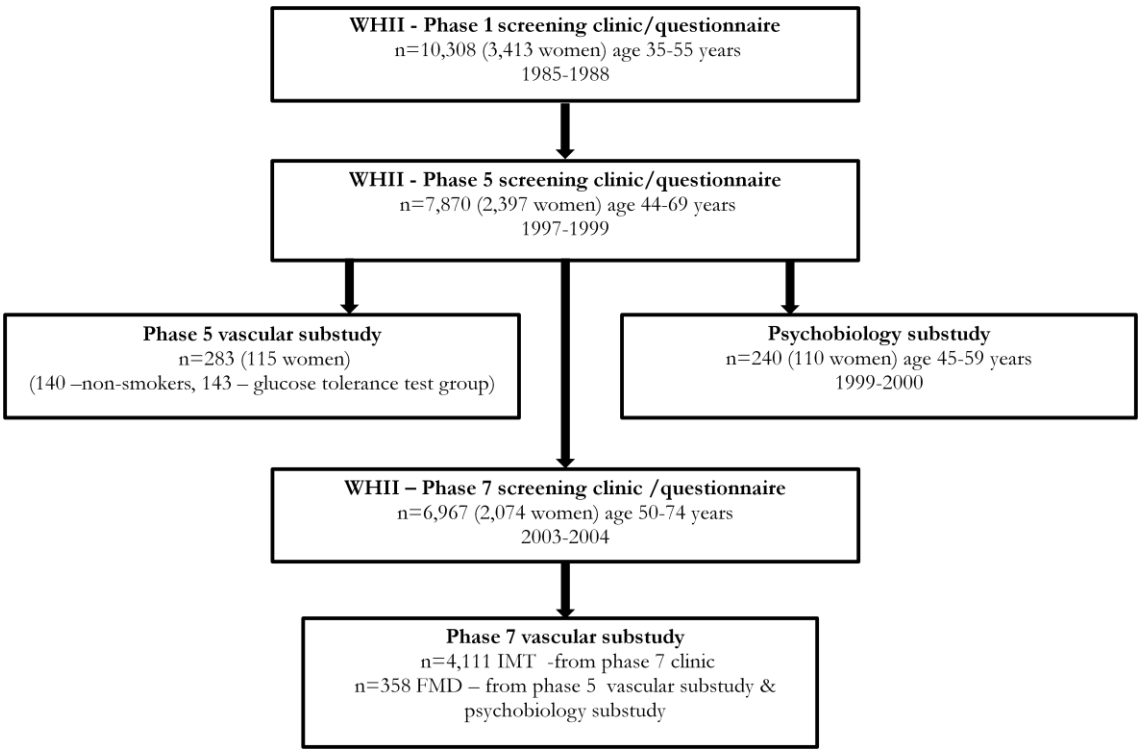

Figure 2.1: Summary of the Whitehall II study from phase 1, including the substudies that formed the population who underwent flow-mediated dilatation (FMD) assessment at phase 7. IMT = intima-media thickness.

2.2 Whitehall II phase 7 assessment

Participants attended a clinic where they underwent a wide range of clinical assessments and completed an extensive questionnaire, the link for which is included in appendix 1.

2.2.1 Identification of symptoms of depression and anxiety

Participants completed two self-administered questionnaires to identify symptoms of depression and anxiety. The first questionnaire was the 30-item General Health Questionnaire (GHQ). It is a screening instrument designed for population-based surveys and trials and is widely used in such studies[338]. Each questionnaire item enquires about a specific symptom. From the questionnaire a depression symptom score was calculated from the responses to the following four items, 'been thinking of yourself as a worthless person', 'felt that life is entirely hopeless', 'felt that life isn't worth living', and 'found at times you couldn't do anything because your nerves were too bad'. These items are a

subset of the seven items in the depression subscale of the GHQ-28[338]. They were assessed using a four point Likert scale (range 0-3). The scores for each item were added together and those with a total score of 4 or more were identified has having the presence of depressive symptoms as has been used in other studies of this cohort[339-341].

In addition, the GHQ was used to identify those with anxiety symptoms using a five-item anxiety symptom score ('lost much sleep over worry', 'felt constantly under strain', 'been getting scared or panicky for no good reason', 'found everything getting on top of you', 'been feeling nervous and strung up all the time'). These are a subset of the seven items of the GHQ-28 anxiety scale and were assessed using a Likert scale (range 0-3). As in a previous study those whose score was in the top quartile were classified as having anxiety[284].

The second questionnaire used for assessing symptoms of depression was the Centre for Epidemiologic Studies Depression Scale (CES-D)[342]. The 20 items of the CES-D measures symptoms associated with depression. Participants were asked to score the frequency of occurrence of specific symptoms during the previous week on a four point scale (0=less than one day, 1= 1-2 days, 2= 3-4 days & 3= 5-7 days). The scores for each item were summed to yield a total score between 0 & 60. Those participants scoring ≥16 were defined as having CES-D depressive symptoms[343]. A validation study of 274 Whitehall II participants demonstrated a sensitivity of 89% and specificity of 86% for CES-D symptoms when compared with a structured psychiatric interview[344].

Participants were also asked whether they had taken any medication in the last 14 days and to provide the name of the medication if they had done so. Antidepressants and anxiolytics were identified using British National Formulary codes. Finally, participants had been asked on a previous questionnaire (phase 4), whether they had ever been told that they had depression.

2.2.2 Blood pressure

Seated systolic and diastolic blood pressure was measured twice after a 5 minute rest with an automated Omron 907 device. A mean of the two readings was taken.

2.2.3 Anthropometric measures

Weight was measured using a bio impedance scale (Tanita) and was read to the nearest 0.1 kg. Height was measured to the nearest mm using a stadiometer with the participant standing straight with the head in the Frankfort plane. BMI was calculated as weight in kilograms divided by height in metres squared.

Waist and hip circumference were measured with the participants standing and unclothed, using a fibreglass tape measure at 600g tension. Waist circumference was taken as the smallest circumference at or below the costal margin and hip circumference was measured at the level of the greater trochanter. The waist to hip ratio was then calculated.

2.2.4 Blood tests

Venous blood was taken either in the fasting state or at least 5 hours after a light, fat-free breakfast. Samples were collected into plain and fluoride Sarstedt monovettes. Serum for lipid analyses was stored at -4°C and assayed within 72 hours. Cholesterol and triglycerides were measured using enzymic colorimetric methods. High-density lipoprotein cholesterol was assessed by precipitating non-HDL cholesterol with dextran sulfate-magnesium chloride using a centrifuge and measuring cholesterol in the supernatant. LDL cholesterol was calculated using the Friedewald formula[345].

Glucose was measured in fluoride plasma by an electro-chemical glucose oxidase method on a YSI model 2300 Stat Plus Analyzer (YSI Corporation, Yellow Springs, OH).

IL-6 and CRP were measured in serum that had been stored at -70^{O}C until analysis. CRP was assessed using a high sensitivity immunonephelometric assay in a BN ProSpec nephelometer (Dade Behring, Milton Keynes, UK). Values lower than the detection limit (0.154 mg/l) were assigned a value equal to half the detection limit. For assessment of short term biological variation and laboratory error a repeated sample was taken from 533 participants. Intra- and inter-assay coefficients of variation were 4.7% and 8.3%. Reliability between samples was assessed with Pearson's r correlation coefficients: r = 0.72.

Il-6 was assessed using a high - sensitivity enzyme-linked immunosorbent assay (ELISA) (R&D systems, Oxford, UK). Values lower than the detection limit (0.08pg/ml) were assigned a value equal to half the detection limit. For assessment of biological variation

and laboratory error, a repeated sample was taken from 329 participants at phase 7. Intra and inter-assay coefficients of variation were 7.5% and 8.9%. Reliability between samples was assessed with Pearson's r correlation coefficients: r = 0.63.

2.2.5 Other measures

Questions on current smoking status, socio-economic status and medications were included in the questionnaire completed by the participants at phase 7.

Socio-economic status was defined by current or last known grade of employment. The civil service consists of 12 employment grades and these were reduced to 3 categories. Group 1 consisted of the senior administrator grades, group 2 was the middle-ranking executives and group 3 was the clerical and office support grades.

The information provided by the participants on medications was coded using the British National Formulary.

2.2.6 Vascular methods

2.2.6.1 Carotid artery intima-media thickness and distensibility coefficient

Participants lay supine in a temperature-controlled vascular laboratory (22–26^{O}C). A 3-lead electrocardiogram (ECG) was attached to the participants chest and a blood pressure cuff positioned initially on the right arm (OmronM5-I sphygmomanometer). After a 10 minute period of acclimatization, the left and right carotid arteries were imaged using a Prosound 5500 ALOKA (Keymed, Southend-on-Sea, UK) with a high resolution (7.5MHz) linear array probe. A transverse image of the carotid arteries was captured to define the carotid bifurcation. The common carotid artery was then imaged longitudinally approximately 1cm proximal to the carotid bifurcation, the optimal image was then zoomed and triggered to the R-wave of the ECG and a 10 second recording made to the hard drive of the ultrasound machine and on Super VHS videotape for assessment of intima-media thickness (IMT). To measure distension the echo-tracking subsystem integral to the ultrasound machine was used[346]. Care was taken to ensure that the cursors for the echo tracking were accurately positioned and a recording made to the hard drive of the ultrasound machine. Ipsilateral blood pressure was taken immediately after each carotid measurement.

Using the images stored on the ultrasound machine, electronic callipers were used to measure the distance between the lumen-intima interface and the media-adventitia interface. Measurements were taken from three separate frames for each carotid artery and the results for the two arteries were then averaged.

Using the integral software within the ultrasound machine, distension was calculated from a minimum of 3 waveforms. The distension data and blood pressure values were then used to calculate the distensibility coefficient separately for the right and left carotid arteries using the formula (2x distension / diastolic diameter) / pulse pressure[347]. The results for the two arteries were then averaged.

Three observers were responsible for the image collection and analysis. The coefficient of variation for repeated measures of IMT was <5%.

2.2.6.2 Flow-mediated dilatation

Participants were supine and rested for 5 minutes in a temperature controlled laboratory. Blood pressure measurements were taken from the left arm both prior to and immediately following the study. A 3-lead ECG was attached to the participant's chest.

The right arm was positioned at 80^{O} to the body resting on foam supports to keep the forearm off the study table and for participant comfort. A long bladdered narrow cuff attached to a rapid cuff inflator, was positioned securely around the forearm just below the medial epicondyle. Using high resolution B-mode ultrasound (Prosound 5500 ALOKA with a 7.5MHz linear probe), the right brachial artery was imaged longitudinally. Once a straight and clear section of artery had been identified the image was magnified using the zoom function set at 2 by 2 cm. The single focus point of the ultrasound was positioned to the centre of the artery or just below the posterior wall and the B-mode gain optimised to give clear lumen/wall definition. Once a good quality and stable image was achieved the sterotactic clamp holding the ultrasound probe was tightened to fix the probe in position. Fine tuning of the image could then be made using the micrometer screw. The B-mode image was then triggered to the R-wave of the ECG so that measurements were only made on end-diastolic images. To assess changes in blood flow during the study the Doppler cursor was positioned in the centre of the artery.

Once the image was set, baseline recordings of brachial diameter and blood flow were made for 1 minute. The forearm cuff was then inflated to 300mmHg for 5 minutes, upon deflation of the cuff the recording of both the artery and blood flow continued for a further 5 minutes. Images were captured to both Super VHS videotape and via a frame grabber set to capture an image every 3 seconds, to a computer.

Brachial artery diameter was measured using the edge detection software Brachial Tools Analyzer (MIA). The recordings of the study were played through and a section of artery was selected that remained stable throughout the study. A region of interest box was positioned over the selected section ensuring that the artery walls were within the boundaries of the box. The placing of the edge detection line was then checked and adjusted if necessary to ensure that it was correctly positioned. The analysis was then run and each frame checked to make sure the edge detection line was accurately positioned at the edge of the artery wall. Baseline diameter was then calculated from the average of the first 20 frames which equated to the first minute of recording. Peak diameter was taken as the maximum diameter measurement post cuff release. Absolute change was calculated as Peak diameter – baseline diameter. Percentage change was calculated as (absolute change/baseline diameter)x100.

Doppler waveforms were traced using integral software on the ultrasound machine. Velocity time integral was used and the average of 3 waveforms from the baseline minute were taken to give baseline VTI. The largest waveform from waveforms assessed at 5, 10 and 15 seconds post cuff release was taken as peak VTI. Reactive hyperaemia was calculated as (peak VTI/baseline VTI) x 100.

2.3 Whitehall II psychobiology population

Participants were drawn from the Whitehall II epidemiological cohort for a psychobiology testing substudy led by Prof Andrew Steptoe, in which the laboratory stress testing was performed in 1999 to 2000 at UCL. Criteria for entry to the study included no history or objective signs of coronary heart disease and no previous diagnosis or treatment for hypertension, inflammatory diseases, or allergies. Selection was stratified by grade of employment to include those of higher, intermediate and lower status; all participants were

in full time employment and lived in the London area. As part of phase 7 of the wider Whitehall II study participants from the substudy were invited to undergo endothelial function assessment at the Vascular Physiology, Institute of Child Health UCL (local principle investigator, Prof John Deanfield). This took place approximately 3 years later than the stress testing.

2.3.1 Protocol

Participants were prohibited from using any antihistamine or anti-inflammatory medication 24 hours prior to testing and were rescheduled if they reported colds or other infections on the day of testing. Participants were tested individually in a light and temperature controlled laboratory. Measures of height and weight were taken using standardised techniques. A 21-gauge venous cannula was inserted. Blood pressure and heart rate were recorded using a Portapres-2. After a 30 minute rest period mental stress was induced by two 5-min tasks, administered in random order under time pressure. Blood samples were taken at the end of the 30 minute baseline, immediately after the tasks and at 45 minutes post stress.

2.3.2 Blood pressure and heart rate

Blood pressure and heart rate were continuously recorded from the fingers of the non-dominant hand using a Portapres-2. Values of SBP, DBP and heart rate were averaged across the final 5 minutes of the 30 minute rest period. Readings taken over the course of each of the two stress tasks and during the recovery period at 15-20 and 40-45 minutes post stress tasks were averaged. The results for the two stress tasks were then combined.

2.3.3 Mental stress tasks

Two tasks were used for the mental stress challenge, stroop and mirror tracing. The two tasks were administered in a random order with each task lasting for 5 minutes with a 5 minute inter-task interval. Both tests were introduced with a short and standardized explanation which was designed to also induce feelings of time pressure and of competition.

2.3.3.1 Stroop colour-word interference task

The test was a computerized version of the Stroop colour-word interference task and involved the successive presentation of target colour words (e.g., green, yellow) printed in another colour (e.g., blue, red)[348]. The names of four colours were printed in incongruous colours at the bottom of the computer screen and were selected by identified computer keys. Participants were asked to press the computer key to select the name of the colour that matched the name of the colour in which the target word was printed. The rate of presentation of stimuli was adjusted to the performance of the participant, to ensure sustained demand.

2.3.3.2 Mirror tracing

The task, which was designed to challenge and frustrate participants, involved the tracing of a star with a metal stylus, which could only be seen in a mirror image (Lafayette Instruments, Lafayette, IN, USA). A loud beep was emitted by the apparatus each time the stylus came off the star to signal a mistake and the number of mistakes was recorded in front of the participants.

2.3.4 Blood tests

A 21-gauge venous cannula was inserted into the non-dominant arm for the collection of repeated samples.

2.3.4.1 Lipids

Non fasted blood was collected in serum gel tubes and centrifuged immediately at 2,500 rpm for 10 min at room temperature. The serum was removed and snap frozen at -70 °C within 1 hour until analysis. Total cholesterol and triglycerides were measured in a centrifugal analyzer by enzymatic colorimetric methods, and HDL cholesterol was determined after dextran sulfate-magnesium chloride precipitation of non-HDL cholesterol. LDL cholesterol was computed using the Friedewald formula

2.3.4.2 Inflammatory markers

Blood was collected in tubes containing EDTA and was immediately centrifuged at room temperature at 2500 rpm for 10 minutes. The plasma was removed and frozen at -80 ^{O}C until analysis. It has previously been shown that fibrinogen responds immediately to a mental stress task whereas there is a delay in the cytokine responses[349, 350]. Therefore fibrinogen was analysed in all blood samples, whilst IL-6 and TNFα were analysed at baseline and 45 minutes post stress challenge. CRP was measured at baseline only.

IL-6 and TNFα were measured using high-sensitivity two-site ELISA assay (R&D systems, Oxford, UK). The limit of detection for IL-6 was 0.09pg/ml, and the intra- and inter-assay variability was 5.3 and 9.2%, respectively. For the TNFα assay, the limit of detection was 0.10pg/ml with intra- and inter-assay coefficients of variation of 6.9% and 8.4%. Fibrinogen was measured from plasma by an automated Clauss assay in an MDA-180 coagulometer (Oragon Teknika, Cambridge, UK). The coefficient of variation was <8%. CRP was measured using a sensitive, two-site ELISA with antibodies from Dako diagnostics (Ely, Cambs, UK). Coefficients of variation for intra and inter- assay were <10%.

2.3.5 Anthropometric measurements

Height was measured with the head in the Frankfort plane to standardize the measurement. Body weight was measured to the nearest 0.1kg. Body Mass Index (BMI) was calculated as previously described. The waist circumference was measured midway between the lowest rib and iliac crest, and the hip circumference was measured at the level of the great trochanters.

2.4 Summary

Access to the unique WHII data provides a very well characterised population with a wealth of well validated measures. This rich dataset allows an unprecedented opportunity to explore thoroughly the principal questions of this thesis, whilst adequately accounting for potentially important confounding variables.

Chapter 3. Chronic stress, lipids and subclinical vascular disease in an epidemiological cohort (Whitehall II data)

3.1 Introduction

Psychosocial factors have been associated with increased risk of cardiovascular disease (CVD) in addition to the traditional risk factors[200]. Depression in particular, has been related to increased risk of CVD within the general population and recurrent events in patients with established coronary heart disease[204, 351]. However, it is not fully understood whether these chronic stressors trigger cardiovascular events in those with underlying disease or are involved earlier in the development of atherosclerotic disease. Although a less studied area, anxiety has also been shown to be associated with increased cardiovascular risk[211]. There is high co-morbidity between anxiety and depression which has not always been taken into account in studies looking at the relationship between anxiety and CVD and may influence the findings[197].

Abnormal measures of vascular structure and function such as intima-media thickness (IMT), arterial stiffness and endothelial function, which are early indicators and predictors of cardiovascular disease and events, have been associated with the chronic stressors, depression and anxiety [263, 279, 284, 287, 293, 294]. However, there are conflicting results, for example with IMT, significant associations with depression have been found in some studies but others have found no association or a relationship has only been seen in males and not females and vice versa[263, 269-271, 274, 276, 279]. Due to the heterogeneity of these results it is important to explore further the relationships between depression, anxiety and subclinical measures of vascular structure and function.

Dyslipidaemia plays an important role in the pathogenesis of atherosclerosis. Arterial stiffness, increased IMT and endothelial dysfunction are all associated with dyslipidaemia[89, 90, 352]. Raised levels of total cholesterol (TC), low density lipoprotein (LDL) and triglycerides alongside low high density lipoprotein (HDL) have been found in those with

depression and anxiety disorders[252, 253, 353]. Previous studies looking at associations between depression and anxiety with subclinical vascular measures often adjust for the effects of lipids as a cardiovascular risk factor within their analyses but have not explored the specific impact of dyslipidaemia on these associations[263, 275]. It is therefore important to investigate the relationships between depression and anxiety and dyslipidaemia with vascular structure and function.

The Whitehall II study consists of a well characterised cohort, established to explore relationships between socio-economic status, stress and CVD. Previous findings from this study have demonstrated relationships between psychosocial factors (such as depression and social position) and cardiovascular disease[354-358] . Analyses have also shown that impaired endothelial function (low flow-mediated dilatation [FMD] of the brachial artery) is predictive of progression of atherosclerosis (change in carotid IMT between phase 5 and 7)[174]. At phase 7 of this study, a large number of participants underwent vascular assessment and completed questionnaires assessing symptoms of anxiety and depression alongside other clinical measures. This ideally places this ageing cohort for a cross-sectional analysis of the relationships between depression, anxiety, dyslipidaemia and subclinical vascular disease.

Using an array of methods for assessing sub clinical vascular disease allows a thorough investigation of the psychophysiological effects of chronic stress on different aspects of arterial pathophysiology. FMD provides insights on the potential effects on endothelial function. Assessment of IMT gives an indication of changes to the structure of the artery wall and the early development of atherosclerotic changes within the wall, whilst the distensibility coefficient provides information on local arterial stiffness and arteriosclerosis.

3.2 Aim and hypotheses

Aim: to investigate the influence of chronic stress, with and without dyslipidaemia, on endothelial function, carotid artery wall thickness and stiffness.

Hypotheses: 1. Chronic stress (depression/anxiety) is associated with greater IMT and lower distensibility coefficient (DC)

2. Chronic stress (depression or anxiety) is associated with endothelial dysfunction

3. The presence of dyslipidaemia influences the relationship between chronic stress and subclinical vascular dysfunction/disease.

3.3 Methods

3.3.1 Whitehall II cohort

Participants were from the Whitehall II cohort which originally recruited 10,308 civil servants between 1985 and 1988 (see Chapter 2). As part of the phase 7 (2003 to 2004) reassessment, participants were invited for an additional examination of their carotid arteries to assess intima-media thickness and carotid artery stiffness.

3.3.1.1 Endothelial function substudy

Participants from the phase 5 vascular substudy and the psychobiology substudy were invited to undergo endothelial function assessment at phase 7 in addition to the carotid artery assessment as detailed in Chapter 2.

3.3.2 Phase 7 assessment

Anthropometric measures such as height, weight, hip and waist measurements were taken as described in Chapter 2. In addition, a fasting blood sample was taken and TC, HDL, Trigs, LDL, glucose, CRP and IL-6 were measured and nonHDL was calculated from TC and HDL. Samples were assessed as described in Chapter 2. Seated brachial blood pressure was assessed using an automated Omron 907 device.

3.3.2.1 Measures of Chronic stress

As part of the phase 7 assessment, participants completed the 30-item General Health Questionnaire (GHQ) and the Centre for Epidemiologic Studies Depression Scale (CES-D). From the GHQ subscales were identified for depression and anxiety using factor analysis. Depression was defined as having a score of 4 or more on the subscale and anxiety was identified as having a score in the top quartile. Using the CES-D scale depression was defined as having a score of 16 or more. Participants had also been questioned on previous diagnoses of depression and medications.

3.3.3 Vascular measures

3.3.3.1 Intima-media thickness and distensibility coefficient

Participants rested in a supine position and then their right and left common carotid arteries were imaged using an ALOKA Prosound 5500 ultrasound machine. Transverse and longitudinal sections of the carotid arteries were imaged to identify the bifurcation. Longitudinal images of the common carotid artery approximately 1 cm proximal to the bifurcation, with a clearly defined far wall intima-media complex, were zoomed and triggered to the R-wave of the ECG and recorded for later analysis. A separate untriggered recording was made using an echo-tracking subsystem integral to the ultrasound machine for measurement of arterial distension[346]. Ipsilateral blood pressure measurements were taken immediately after each carotid measurement using an Omron M5-I sphygmomanometer.

IMT was measured as the distance between the leading edge of the intima and the media-adventitia border using callipers integral to the ultrasound machine. Three measurements, each from a separate frame, were taken from the left and right arteries; mean IMT was calculated from the combined results. The distensibility coefficient was calculated from arterial distension and blood pressure data using the equation (2x distension / diastolic diameter) / pulse pressure[347].

3.3.3.2 Flow-mediated dilatation

The right brachial artery was imaged longitudinally using ultrasound. Once a good quality image with clear walls was found the image was zoomed and triggered to the R-wave of the ECG. A Doppler cursor was positioned in the centre of the artery to assess blood flow. Baseline diameter and blood flow was recorded for 1 minute, after which a cuff positioned around the forearm just below the medial epicondyle was inflated to 300mmHg for 5 minutes. On release of the occlusion cuff, diameter and blood flow were recorded for a further 5 minutes. Images were acquired direct to a computer for later off-line analysis. FMD was calculated as the difference between baseline and peak diameter post cuff release as an absolute and percentage change.

3.3.4 Statistical analysis

3.3.4.1 IMT and distensibility coefficient analysis.

Participants were categorised as having depression if they met one of the following categories: had a score of 4 or more on the GHQ subscale; a score of 16 or more on the CESD scale; previously been informed they were depressed; on antidepressants. Anxiety was categorised by participants meeting one of the following criteria: in the top quartile of the GHQ questionnaire for anxiety (mean score 6.62±2.04 range 5-15) and or on anxiolytic medication.

Variables that were positively skewed were transformed using natural log transformation (Fasting glucose, Trigs, HDL, CRP & IL-6). All analyses were carried out separately for each gender. Chronic stress variables, depression and anxiety, were looked at individually and in combination. For the combination analyses participants were defined as having depression and anxiety, just depression, just anxiety and neither.

Cardiovascular risk factors were compared between the groups using independent t-tests or Chi square where appropriate. Associations between IMT or DC and cardiovascular risk factors were evaluated with Pearson correlation. The relationships between IMT or DC with chronic stress measures were investigated using analysis of covariance (ANCOVA) with IMT /DC as the dependent variable, chronic stress as the independent variable and adjustment for age included in all models. Each risk factor was studied individually within this model. Larger models were then compiled based on the Framingham risk factors (age, systolic blood pressure (SBP), TC, HDL and Glucose). Metabolic factors were then added to the model (waist, BMI & Trigs), then inflammatory factors (CRP, IL-6), social factors smoking and SES, finally heart rate (HR) was also added to the model. Relationships were looked at separately for gender and separately and combined for depression and anxiety.

This is a relatively healthy population with a low prevalence of major lipid disorders. Therefore, a pooled classification was utilised whereby those with one or more of the following were considered to have dyslipidaemia, TC >6, Trigs>1.7, HDL<1 and LDL>4 (mmol/L). Those with and without dyslipidaemia were then further divided by whether participants had depression or anxiety (chronic stress) to give a total of four groups within each gender: dyslipidaemia and chronic stress; dyslipidaemia without chronic stress; normal cholesterol and chronic stress and normal cholesterol and no chronic stress. Analyses to

investigate the influence of dyslipidaemia on relationships between measures of chronic stress and subclinical vascular disease were carried out as described above.

3.3.4.2 Endothelial function

Depression and anxiety were categorised as described above (anxiety top quartile mean score 6.81±2.07 range 5-15). Variables were checked for normality, those variables which were positively skewed were transformed using natural log transformation (fasting glucose, Trigs, HDL, CRP and IL-6). Male and female participants were not analysed separately due to the size of the cohort. Differences in risk factors between the groups and associations with FMD were carried out in the same way as in the IMT and DC analysis. For the initial ANCOVA models with adjustment for individual risk factors, all analyses were adjusted for age, gender, baseline brachial artery diameter and reactive hyperaemia. Larger models with adjustments for Framingham risk factors, metabolic, inflammatory and social factors were then run including age, gender, baseline brachial artery diameter and reactive hyperaemia. Due to the size of the cohort the larger ANCOVA models with multiple modifiable covariates were only used when investigating anxiety and depression separately.

3.4 Results

3.4.1 Chronic stress and carotid artery structure and function

4111 participants (61±6 years) attended for the vascular studies. Participants were excluded from the analysis if they were on antihypertensive (944) and/or lipid lowering therapy (451). In total 2991 participants were included in the analysis. Of this population 6.4% were smokers and 3.7% had diabetes.

3.4.1.1 Depression score

As hypothesised, depression was associated with greater IMT. However, this was relationship was only apparent in women. DC was found to be greater in women with depression but there was no difference between the groups in men.

757 (25%) of the cohort were categorised as having depression. Table 3.1 shows conventional cardiovascular risk factors in those with and without depression. Those with depression were significantly younger with lower SBP. The proportion of women in the depression group was higher than in the non-depression group (38 % vs 24 %).

There was no difference in IMT between the two groups, but DC was significantly greater in the depression group.

	Depression (757)	No depression (2234)	p
Age (yrs)	60 ± 6	61 ± 6	***0.002***
Women (%)	289 (38)	544 (24)	***<0.001***
Waist (cm)			
Men	94.56 ± 10.54	94.18 ± 10.02	*0.47*
Women	85.02 ± 13.10	85.28 ± 13.45	*0.79*
Waist/hip ratio			
Men	0.95 ± 0.06	0.95 ± 0.06	*0.05*
Women	0.85 ± 0.08	0.85 ± 0.08	*0.46*
BMI (kg/m^2)	26.00 ± 4.30	25.99 ± 3.91	*0.95*
SBP (mmHg)	124 ± 16	126 ± 16	***0.003***
DBP (mmHg)	72 ± 10	73 ± 10	*0.07*
HR (bpm)	68 ± 11	68 ± 11	*0.92*
Glucose (mmol/L)	5.28 ± 0.88	5.34 ± 0.92	*0.06*
TC (mmol/L)	5.80 ± 0.96	5.83 ± 0.97	*0.50*
HDL (mmol/L)	1.61 ± 0.45	1.61 ± 0.46	*0.90*
Trigs (mmol/L)	1.29 ± 0.79	1.27 ± 0.78	*0.56*
LDL (mmol/L)	3.60 ± 0.88	3.64 ± 0.89	*0.29*
NonHDL (mmol/L)	4.19 ± 1.01	4.22 ± 1.01	*0.47*
CRP (mg/L)	2.30 ± 4.46	2.15 ± 3.85	*0.32*
IL-6 (pg/ml)	2.13 ± 1.96	2.11 ± 1.81	*0.99*
IMT (mm)	0.78 ± 0.14	0.78 ± 0.15	*0.50*
DC (10^{-3} kPa^{-1})	15.87 ± 5.14	15.17 ± 4.75	***0.001***

Table 3.1: Participant characteristics for those with and without depression.

Depression by gender

To look at the relationships between those with and without depression and subclinical vascular measures separately in men and women, the cohort was divided by gender. Males with depression were significantly younger with lower TC, HDL and LDL than non-depression males. There was no difference in IMT or DC between the depression and non-depression males (table 3.2).

Females with depression had significantly lower heart rates and higher Trigs than those without depression, HDL was also lower in women with depression symptoms but this was at borderline significance. IMT and DC were both greater in women with depression than those in the non- depression group (table 3.2).

	Males			Females		
	Depression n= 468	No depression n=1690	p	Depression n=289	No depression n=544	p
Age (yrs)	60 ± 6	61 ± 6	***<0.001***	60 ± 6	60 ± 6	*0.91*
Waist (cm)	94.56 ± 10.54	94.18 ± 10.02	*0.47*	85.02 ± 13.1	85.28 ± 13.45	*0.79*
Waist/hip ratio	0.95 ± 0.06	0.95 ± 0.06	*0.05*	0.85 ± 0.08	0.85 ± 0.08	*0.46*
BMI (kg/m²)	26.08 ± 3.79	25.99 ± 3.58	*0.61*	25.87 ± 5.02	26.00 ± 4.79	*0.71*
SBP (mmHg)	125 ± 15	127 ± 15	***0.013***	123 ± 17	124 ± 18	*0.43*
DBP (mmHg)	73 ± 10	74 ± 10	*0.77*	71 ± 10	72 ± 11	*0.10*
HR (bpm)	67 ± 11	67 ± 11	*0.51*	68 ± 10	70 ± 10	***0.041***
Glucose (mmol/L)	5.37 ± 0.83	5.39 ± 0.92	*0.60*	5.14 ± 0.92	5.16 ± 0.91	*0.50*
TC (mmol/L)	5.67 ± 0.94	5.79 ± 0.95	***0.016***	6.01 ± 0.96	5.95 ± 1.02	*0.42*
HDL (mmol/L)	1.47 ± 0.36	1.51 ± 0.39	***0.034***	1.84 ± 0.48	1.91 ± 0.50	*0.06*
Trigs (mmol/L)	1.36 ± 0.87	1.33 ± 0.81	*0.71*	1.18 ± 0.61	1.08 ± 0.65	***0.010***
LDL (mmol/L)	3.58 ± 0.83	3.67 ± 0.88	***0.042***	3.63 ± 0.95	3.54 ± 0.94	*0.18*
NonHDL (mmol/L)	4.20 ± 0.98	4.28 ± 0.99	*0.14*	4.17 ± 1.06	4.04 ± 1.05	*0.10*
CRP (mg/L)	2.26 ± 4.86	2.01 ± 3.55	*0.39*	2.36 ± 3.74	2.58 ± 4.62	*0.80*
IL-6 (pg/ml)	2.27 ± 2.22	2.13 ± 1.84	*0.37*	1.93 ± 1.44	2.02 ± 1.72	*0.63*
IMT (mm)	0.77 ± 0.15	0.79 ± 0.16	*0.09*	0.78 ± 0.14	0.75 ± 0.13	***0.015***
DC (10^{-3} kPa^{-1})	14.94 ± 4.47	14.82 ± 4.47	*0.59*	17.36 ± 5.77	16.28 ± 5.40	***0.008***

Table 3.2: Risk factors for participants with and without depression by gender.

Males	IMT				DC			
	Depression		No depression		Depression		No depression	
	r	p	r	p	r	p	r	p
Age (yrs)	0.33	***<0.001***	0.31	***<0.001***	-0.27	***<0.001***	-0.27	***<0.001***
Waist (cm)	0.10	***0.033***	0.15	***<0.001***	-0.24	***<0.001***	-0.22	***<0.001***
Waist/hip ratio	0.11	***0.017***	0.15	***<0.001***	-0.22	***<0.001***	-0.22	***<0.001***
BMI (kg/m²)	0.09	***0.045***	0.12	***<0.001***	-0.27	***<0.001***	-0.21	***<0.001***
SBP (mmHg)	0.23	***<0.001***	0.22	***<0.001***	-0.32	***<0.001***	-0.33	***<0.001***
DBP (mmHg)	0.13	***0.004***	0.08	***0.001***	-0.35	***<0.001***	-0.35	***<0.001***
HR (bpm)	-0.04	*0.41*	-0.06	***0.010***	-0.35	***<0.001***	-0.32	***<0.001***
Glucose (mmol/L)	0.07	*0.14*	0.13	***<0.001***	-0.13	***0.007***	-0.09	***<0.001***
TC (mmol/L)	0.11	***0.022***	0.13	***<0.001***	-0.04	*0.39*	-0.06	***0.014***
HDL (mmol/L)	-0.12	***0.012***	-0.03	**0.20**	0.18	***<0.001***	0.06	***0.011***
Trigs (mmol/L)	0.08	*0.09*	0.06	***0.009***	-0.15	***0.001***	-0.10	***<0.001***
LDL (mmol/L)	0.16	***0.001***	0.13	***<0.001***	-0.08	*0.09*	-0.07	***0.007***
NonHDL (mmol/L)	0.15	***0.002***	0.14	***<0.001***	-0.11	***0.022***	-0.08	***0.001***
CRP (mg/L)	0.11	***0.016***	0.07	***0.004***	-0.18	***<0.001***	-0.10	***<0.001***
IL-6 (pg/ml)	0.12	***0.011***	0.09	***<0.001***	-0.14	***0.002***	-0.13	***<0.001***
IMT (mm)					-0.16	***0.001***	-0.08	***0.001***
DC (10^{-3} kPa^{-1})	-0.16	***0.001***	-0.08	***0.001***				

Table 3.3: Correlations between IMT and cardiovascular risk factors and DC and cardiovascular risk factors for males with and without depression.

In males IMT was correlated with all cardiovascular risk factors except for heart rate, glucose and triglycerides in the depression group and HDL in the non-depression group (table 3.3). Only TC and LDL were not correlated with DC in males in the depressed group.

In females IMT was positively correlated with age, waist, waist-hip, BMI, SBP, LDL, nonHDL and negatively correlated with DC in the depression group. In the non-depression group IMT was only correlated with age, SBP and DC. Correlations with DC were more consistent between the two groups (table 3.4).

Females	**IMT**				**DC**			
	Depression		**No depression**		**Depression**		**No depression**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Age (yrs)	0.25	***<0.001***	0.29	***<0.001***	-0.37	***<0.001***	-0.35	***<0.001***
Waist (cm)	0.12	***0.041***	0.07	*0.09*	-0.16	*0.007*	-0.09	***0.044***
Waist/hip ratio	0.13	***0.025***	0.07	*0.10*	-0.19	***0.001***	-0.06	*0.14*
BMI (kg/m²)	0.13	***0.030***	0.06	*0.17*	-0.13	***0.025***	-0.12	***0.008***
SBP (mmHg)	0.23	***<0.001***	0.28	***<0.001***	-0.44	***<0.001***	-0.39	***<0.001***
DBP (mmHg)	0.11	*0.05*	0.08	*0.06*	-0.33	***<0.001***	-0.31	***<0.001***
HR (bpm)	-0.08	*0.17*	-0.06	*0.15*	-0.23	***<0.001***	-0.25	***<0.001***
Glucose (mmol/L)	-0.06	*0.34*	0.02	*0.71*	-0.15	***0.009***	-0.02	*0.57*
TC (mmol/L)	0.15	***0.013***	0.04	*0.36*	0.00	*0.93*	-0.02	*0.64*
HDL (mmol/L)	-0.08	*0.16*	-0.05	*0.25*	0.12	***0.035***	0.05	*0.24*
Trigs (mmol/L)	0.07	*0.26*	0.03	*0.48*	-0.11	*0.06*	-0.16	***<0.001***
LDL (mmol/L)	0.17	***0.004***	0.06	*0.14*	-0.04	*0.51*	-0.01	*0.91*
NonHDL (mmol/L)	0.17	***0.004***	0.06	*0.15*	-0.06	*0.31*	-0.04	*0.31*
CRP (mg/L)	0.11	*0.07*	0.08	*0.06*	-0.04	*0.51*	-0.06	*0.14*
IL-6 (pg/ml)	0.07	*0.27*	0.08	*0.07*	-0.11	*0.08*	-0.16	***<0.001***
IMT (mm)					-0.20	***0.001***	-0.17	***<0.001***
DC (10^{-3} kPa^{-1})	-0.20	***0.001***	-0.17	***<0.001***				

Table 3.4: Correlations between IMT and cardiovascular risk factors and DC and cardiovascular risk factors for females with and without depression.

To take into account the effect of individual cardiovascular risk factors on relationships between depression and both IMT and DC, ANCOVA models were run with adjustments for risk factors. In males, there was no relationship between depression and IMT or DC. However, the significantly increased IMT and greater DC in the depression women remained after adjustment for age and individual cardiovascular risk factors (table 3.5). These relationships persisted following additional adjustment within a larger model based on Framingham risk factors (age, gender, SBP, TC, HDL & Glucose). The addition of further risk factors such as measures of obesity, inflammatory markers, smoking or SES did not alter these relationships (table 3.6 & figure 3.1).

Adjustment variable	Males				Females			
	IMT		DC		IMT		DC	
	F	p	F	p	F	p	F	p
Age (yrs)$	0.38	*0.54*	0.19	*0.67*	6.62	***0.010***	8.01	***0.005***
Waist (cm)	0.51	*0.47*	0.09	*0.77*	6.76	***0.009***	7.96	***0.005***
Waist/hip ratio	0.77	*0.38*	0.00	*0.99*	6.56	***0.011***	8.44	***0.004***
BMI	0.42	*0.52*	0.18	*0.67*	6.81	***0.009***	7.91	***0.005***
SBP (mmHg)	0.05	*0.82*	1.12	*0.29*	7.94	***0.005***	7.49	***0.006***
DBP (mmHg)	0.29	*0.59*	0.39	*0.53*	7.58	***0.006***	5.93	***0.015***
HR (bpm)	0.27	*0.60*	0.07	*0.79*	5.80	***0.016***	6.02	***0.014***
Glucose (mmol/L)	0.34	*0.56*	0.25	*0.62*	6.62	***0.010***	7.93	***0.005***
TC (mmol/L)	0.12	*0.73*	0.31	*0.58*	6.50	***0.011***	7.95	***0.005***
HDL (mmol/L)	0.61	*0.43*	0.08	*0.78*	6.12	***0.014***	8.91	***0.003***
Trigs (mmol/L)	0.40	*0.53*	0.23	*0.63*	6.63	***0.010***	9.45	***0.002***
LDL (mmol/L)	0.15	*0.70*	0.40	*0.53*	6.00	***0.015***	8.20	***0.004***
NonHDL (mmol/L)	0.15	*0.70*	0.40	*0.53*	6.09	***0.014***	8.15	***0.004***
CRP (mg/L)	0.28	*0.60*	0.05	*0.83*	7.12	***0.008***	8.41	***0.004***
IL-6 (pg/ml)	0.21	*0.65*	0.05	*0.82*	6.97	***0.008***	7.91	***0.005***

Table 3.5: Associations between depression and IMT and depression and DC in males and females adjusted for age and each individual risk factor. $ = non-adjusted.

	Males				Females			
	IMT		DC		IMT		DC	
	F	p	F	p	F	p	F	p
Model 1	0.04	*0.83*	0.81	*0.37*	6.94	***0.009***	7.74	***0.006***
Model 2	0.05	*0.83*	0.80	*0.37*	7.98	***0.005***	8.14	***0.004***
Model 3	0.02	*0.90*	0.38	*0.54*	8.64	***0.003***	8.10	***0.005***
Model 4	0.12	*0.73*	0.33	*0.57*	8.10	***0.005***	6.79	***0.009***
Model 5	0.05	*0.83*	0.15	*0.70*	7.25	***0.007***	5.91	***0.015***

Table 3.6: Relationship between depression and IMT and depression and DC with adjustments for cardiovascular risk factors for males and females.

Model 1 = Age, SBP, TC, HDL & Glucose;

Model 2 =Age, SBP, TC, HDL, Trigs, Glucose, BMI & waist;

Model 3 = Age, SBP, TC, HDL, Trigs, Glucose, BMI, waist, CRP & IL-6;

Model 4 = Age, SBP, TC, HDL, Glucose, BMI, waist, CRP, IL-6, Smoking & SES;

Model 5 = Age, SBP, TC, HDL, Glucose, BMI, waist, CRP, IL-6, HR, Smoking & SES.

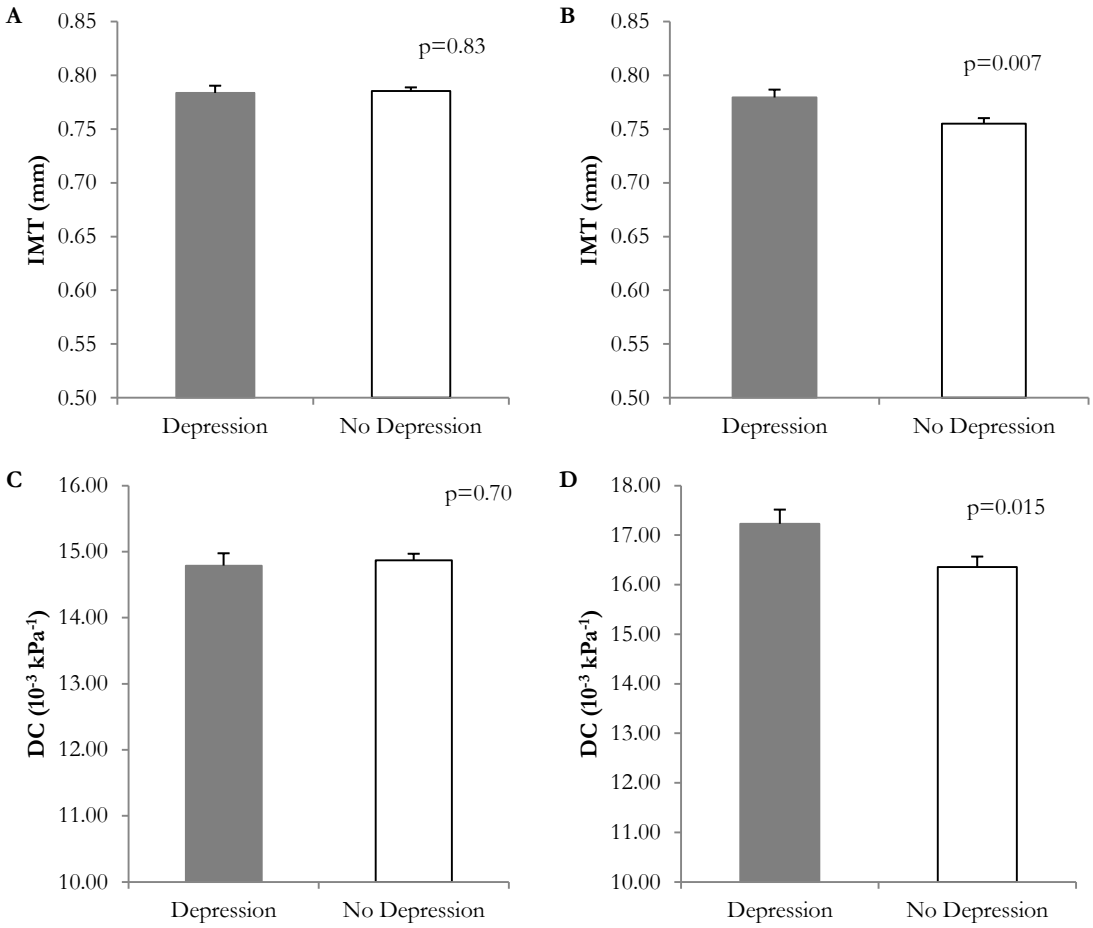

Figure 3.1: IMT in males (A) and females (B) DC in males (C) and females (D) in those with and without depression. Both IMT and DC are adjusted for age, SBP, TC, HDL, Trigs, glucose, BMI, waist, CRP IL-6, HR, smoking and SES. Data presented as mean±SEM.

3.4.1.2 Anxiety

In this section it was found that women with anxiety had greater DC, whereas DC was similar in men with and without anxiety. There was also no difference in IMT between the groups for both men and women.

827 (28%) participants met the definition for anxiety. Table 3.7 shows the participant characteristics for those with and without anxiety. Those participants with anxiety were younger, with a higher proportion of women than men in the group in comparison to those without anxiety and had lower SBP. Although anxious participants had lower IMT and greater DC, further analyses were undertaken to adjust for potential influences of age, gender, SBP and other possible determinants on the vascular outcome measures.

	Anxiety (827)	No Anxiety(2164)	p
Age (yrs)	59 ± 6	61 ± 6	***<0.001***
Women (%)	293 (35)	540 (25)	***<0.001***
Waist (cm)			
Men	94.50 ± 10.34	94.18 ± 10.07	*0.53*
Women	85.84 ± 13.73	84.83 ± 13.1	*0.30*
Waist/hip ratio			
Men	0.95 ± 0.06	0.95 ± 0.06	*0.37*
Women	0.85 ± 0.08	0.84 ± 0.08	*0.11*
BMI (kg/m^2)	26.06 ± 4.3	25.96 ± 3.9	*0.56*
SBP (mmHg)	124 ± 16	126 ± 16	***0.002***
DBP (mmHg)	73 ± 11	73 ± 10	*0.15*
HR (bpm)	68 ± 11	67 ± 11	*0.05*
Glucose (mmol/L)	5.31 ± 0.96	5.33 ± 0.89	*0.32*
TC (mmol/L)	5.79 ± 0.95	5.83 ± 0.98	*0.32*
HDL (mmol/L)	1.62 ± 0.46	1.60 ± 0.45	*0.34*
Trigs (mmol/L)	1.27 ± 0.82	1.28 ± 0.77	*0.63*
LDL (mmol/L)	3.60 ± 0.87	3.64 ± 0.90	*0.26*
NonHDL (mmol/L)	4.17 ± 0.99	4.23 ± 1.02	*0.17*
CRP (mg/L)	2.14 ± 4.11	2.21 ± 3.97	*0.52*
IL-6 (pg/ml)	2.05 ± 1.79	2.14 ± 1.87	*0.18*
IMT (mm)	0.77 ± 0.15	0.78 ± 0.15	***0.005***
DC (10^{-3} kPa^{-1})	15.84 ± 4.88	15.16 ± 4.84	***0.001***

Table 3.7: Participant characteristics for those with and without anxiety.

Anxiety by gender

To investigate differences in the relationships between those with and without anxiety and subclinical vascular measures by gender, the cohort was divided into males and females with and without anxiety. Both men and women with anxiety were significantly younger than those without anxiety. Anxious males had greater heart rates and lower systolic blood pressure as well as decreased IMT. Females with anxiety had higher DC than non-anxious women (table 3.8).

	Males			Females		
	Anxiety n= 534	**No anxiety n=1624**	**p**	**Anxiety n=293**	**No anxiety n=540**	**p**
Age (yrs)	59 ± 5	61 ± 6	***<0.001***	59 ± 6	61 ± 6	***0.012***
Waist (cm)	94.5 ± 10.34	94.18 ± 10.07	*0.53*	85.84 ± 13.73	84.83 ± 13.10	*0.30*
Waist/hip ratio	0.95 ± 0.06	0.95 ± 0.06	*0.37*	0.85 ± 0.08	0.84 ± 0.08	*0.11*
BMI (kg/m²)	26.14 ± 3.78	25.96 ± 3.58	*0.32*	25.92 ± 5.11	25.97 ± 4.73	*0.89*
SBP (mmHg)	124 ± 15	127 ± 15	***0.001***	123 ± 18	123 ± 17	*0.81*
DBP (mmHg)	73 ± 11	74 ± 10	*0.55*	71 ± 11	72 ± 10	*0.42*
HR (bpm)	68 ± 11	67 ± 11	***0.012***	69 ± 10	70 ± 10	*0.19*
Glucose (mmol/L)	5.40 ± 1.01	5.38 ± 0.86	*0.83*	5.14 ± 0.84	5.17 ± 0.96	*0.57*
TC (mmol/L)	5.70 ± 0.92	5.78 ± 0.96	*0.07*	5.96 ± 0.99	5.98 ± 1.01	*0.85*
HDL (mmol/L)	1.48 ± 0.36	1.51 ± 0.4	*0.11*	1.88 ± 0.49	1.89 ± 0.5	*0.83*
Trigs (mmol/L)	1.35 ± 0.9	1.33 ± 0.79	*0.87*	1.12 ± 0.64	1.11 ± 0.64	*0.70*
LDL (mmol/L)	3.61 ± 0.83	3.67 ± 0.88	*0.21*	3.58 ± 0.94	3.57 ± 0.95	*0.89*
NonHDL (mmol/L)	4.22 ± 0.97	4.27 ± 1.00	*0.26*	4.08 ± 1.04	4.09 ± 1.07	*0.94*
CRP (mg/L)	2.12 ± 4.60	2.05 ± 3.59	*0.76*	2.17 ± 3.04	2.68 ± 4.89	*0.21*
IL-6 (pg/ml)	2.15 ± 1.97	2.17 ± 1.91	*0.68*	1.88 ± 1.42	2.05 ± 1.73	*0.23*
IMT (mm)	0.76 ± 0.15	0.79 ± 0.16	***0.001***	0.77 ± 0.14	0.76 ± 0.13	*0.31*
DC (10^{-3} kPa^{-1})	14.94 ± 4.18	14.81 ± 4.56	*0.56*	17.47 ± 5.6	16.21 ± 5.49	***0.002***

Table 3.8: Risk factors for those participants with and without anxiety by gender.

In males all risk factors were correlated with IMT except for heart rate and Trigs in those with anxiety and HDL in those without anxiety. Only TC was not correlated with DC in the male anxious group, there were no non-significant correlations in the non-anxious groups (table 3.9). For females, IMT was positively correlated with age, SBP, LDL and nonHDL and negatively correlated with DC in both groups. In addition, IMT in the anxious group was also negatively correlated with heart rate and HDL, whilst in the non-anxious group there were additional correlations with waist, waist hip, BMI, DBP, TC, CRP and IL-6. DC was associated with all risk factors except glucose, TC, LDL, nonHDL and CRP in both groups (table 3.10)

	IMT				DC			
	Anxiety		No anxiety		Anxiety		No anxiety	
	r	p	r	p	r	p	r	p
Age (yrs)	0.30	***<0.001***	0.31	***<0.001***	-0.25	***<0.001***	-0.27	***<0.001***
Waist (cm)	0.12	***0.005***	0.14	***<0.001***	-0.22	***<0.001***	-0.23	***<0.001***
Waist/hip ratio	0.13	***0.002***	0.14	***<0.001***	-0.23	***<0.001***	-0.21	***<0.001***
BMI (kg/m^2)	0.10	***0.018***	0.12	***<0.001***	-0.23	***<0.001***	-0.22	***<0.001***
SBP (mmHg)	0.21	***<0.001***	0.23	***<0.001***	-0.31	***<0.001***	-0.33	***<0.001***
DBP (mmHg)	0.11	***0.012***	0.08	***0.001***	-0.38	***<0.001***	-0.35	***<0.001***
HR (bpm)	-0.04	*0.41*	-0.06	***0.016***	-0.37	***<0.001***	-0.32	***<0.001***
Glucose (mmol/L)	0.14	***0.001***	0.11	***<0.001***	-0.09	***0.031***	-0.10	***<0.001***
TC (mmol/L)	0.13	***0.003***	0.12	***<0.001***	-0.07	*0.11*	-0.05	***0.038***
HDL (mmol/L)	-0.09	***0.043***	-0.04	*0.13*	0.15	***0.001***	0.07	***0.005***
Trigs (mmol/L)	0.08	*0.07*	0.06	***0.011***	-0.12	***0.004***	-0.10	***<0.001***
LDL (mmol/L)	0.15	***<0.001***	0.13	***<0.001***	-0.10	***0.024***	-0.06	***0.017***
NonHDL (mmol/L)	0.16	***<0.001***	0.13	***<0.001***	-0.12	***0.005***	-0.08	***0.002***
CRP (mg/L)	0.12	***0.008***	0.07	***0.008***	-0.14	***0.002***	-0.11	***<0.001***
IL-6 (pg/ml)	0.10	***0.019***	0.09	***<0.001***	-0.14	***0.001***	-0.13	***<0.001***
IMT (mm)					-0.10	***0.020***	-0.10	***<0.001***
DC (10^{-3} kPa^{-1})	-0.10	***0.020***	-0.10	***<0.001***				

Table 3.9: Correlations between IMT and cardiovascular risk factors and DC and cardiovascular risk factors for males with and without anxiety.

	IMT				DC			
	Anxiety		No anxiety		Anxiety		No anxiety	
	r	p	r	p	r	p	r	p
Age (yrs)	0.28	***<0.001***	0.29	***<0.001***	-0.39	***<0.001***	-0.33	***<0.001***
Waist (cm)	0.01	*0.85*	0.14	***0.002***	-0.12	***0.044***	-0.12	***0.007***
Waist/hip ratio	0.01	*0.89*	0.14	***0.001***	-0.13	***0.026***	-0.10	***0.019***
BMI (kg/m^2)	0.05	*0.36*	0.10	***0.019***	-0.14	***0.016***	-0.11	***0.011***
SBP (mmHg)	0.26	***<0.001***	0.26	***<0.001***	-0.48	***<0.001***	-0.36	***<0.001***
DBP (mmHg)	0.08	*0.19*	0.09	***0.028***	-0.36	***<0.001***	-0.29	***<0.001***
HR (bpm)	-0.14	*0.018*	-0.03	*0.46*	-0.22	***<0.001***	-0.26	***<0.001***
Glucose (mmol/L)	-0.10	*0.08*	0.04	*0.37*	-0.11	*0.06*	-0.05	*0.22*
TC (mmol/L)	0.07	*0.26*	0.09	***0.047***	0.00	*0.96*	-0.02	*0.73*
HDL (mmol/L)	-0.13	***0.023***	-0.03	*0.53*	0.14	***0.019***	0.03	*0.43*
Trigs (mmol/L)	0.08	*0.17*	0.03	*0.44*	-0.19	***0.001***	-0.10	***0.026***
LDL (mmol/L)	0.12	***0.041***	0.10	***0.023***	-0.02	*0.78*	-0.01	*0.81*
NonHDL (mmol/L)	0.13	***0.031***	0.09	***0.030***	-0.07	*0.24*	-0.03	*0.48*
CRP (mg/L)	0.01	*0.83*	0.13	***0.002***	-0.07	*0.21*	-0.04	*0.36*
IL-6 (pg/ml)	0.02	*0.76*	0.10	***0.016***	-0.16	***0.006***	-0.13	***0.003***
IMT (mm)					-0.21	***<0.001***	-0.15	***<0.001***
DC (10^{-3} kPa^{-1})	-0.21	***<0.001***	-0.15	***<0.001***				

Table 3.10: Correlations between IMT and cardiovascular risk factors and DC and cardiovascular risk factors for females with and without anxiety.

ANCOVA models were run with adjustment for cardiovascular risk factors to take into account the effect these factors may have on relationships between anxiety and IMT or DC. In males there was a significant association between DC and anxiety having adjusted for age and SBP and age and DBP. The previously significant finding of increased IMT in those males without anxiety was lost with adjustment for age (table 3.11). Women with anxiety had greater DC than those women without anxiety following adjustment for age and individual cardiovascular risk factors. There was a borderline association between presence or absence of anxiety and IMT (anxious women had greater IMT) notably after adjusting for age and CRP (p=0.050) (table 3.11).

	Males				Females			
	IMT		DC		IMT		DC	
	F	p	F	p	F	p	F	p
Age (yrs)$	1.19	*0.27*	2.40	*0.12*	3.19	*0.07*	5.56	***0.019***
Waist (cm)	1.43	*0.23*	2.19	*0.14*	2.87	*0.09*	6.20	***0.013***
Waist Hip	1.64	*0.20*	1.67	*0.20*	2.58	*0.11*	6.14	***0.013***
BMI	1.36	*0.24*	2.37	*0.12*	3.17	*0.08*	5.64	***0.018***
SBP (mmHg)	0.48	*0.49*	5.19	***0.023***	3.14	*0.08*	7.01	***0.008***
DBP (mmHg)	0.97	*0.33*	4.06	***0.044***	3.54	*0.06*	4.89	***0.027***
HR (bpm)	0.75	*0.39*	0.60	*0.44*	2.87	*0.09*	4.57	***0.033***
Glucose (mmol/L)	1.42	*0.23*	2.50	*0.11*	3.21	*0.07*	5.58	***0.018***
TC (mmol/L)	0.88	*0.35*	2.78	*0.10*	3.23	*0.07*	5.64	***0.018***
HDL (mmol/L)	1.35	*0.25*	2.53	*0.11*	3.19	*0.07*	5.71	***0.017***
Trigs (mmol/L)	1.17	*0.28*	2.97	*0.08*	3.22	*0.07*	5.98	***0.015***
LDL (mmol/L)	0.91	*0.34*	3.04	*0.08*	3.26	*0.07*	5.33	***0.021***
NonHDL (mmol/L)	0.88	*0.35*	3.10	*0.08*	3.20	*0.07*	5.63	***0.018***
CRP (mg/L)	1.26	*0.26*	2.22	*0.14*	3.84	*0.05*	5.64	***0.018***
IL-6 (pg/ml)	1.15	*0.28*	2.25	*0.13*	3.43	*0.06*	5.41	***0.020***

Table 3.11: Association between anxiety and IMT and also anxiety and DC adjusted for age and each individual risk factor in males and females. $ = non-adjusted.

In males, the relationship between DC and anxiety became significant following adjustments in models 1-4; however, when HR was added to the model the relationship was lost.

The association between anxiety and increased DC in women remained after adjustment using larger models based initially on Framingham risk score variables and with subsequent addition of measures of obesity, inflammation and SES (table 3.12 & figure 3.2D). The relationship between IMT and anxiety in women remained at borderline significance in all models.

	Males				Females			
	IMT		DC		IMT		DC	
	F	p	F	p	F	p	F	p
Model 1	0.50	*0.48*	5.51	***0.019***	3.03	*0.08*	6.97	***0.008***
Model 2	0.58	*0.45*	5.05	***0.025***	3.09	*0.08*	6.85	***0.009***
Model 3	0.55	*0.46*	4.53	***0.033***	3.65	*0.06*	6.81	***0.009***
Model 4	0.67	*0.41*	4.55	***0.033***	3.33	*0.07*	6.29	***0.012***
Model 5	0.13	*0.72*	1.91	*0.17*	2.98	*0.08*	5.68	***0.017***

Table 3.12: Relationships between anxiety and IMT and also anxiety and DC with adjustments for cardiovascular risk factors in males and females.

Model 1 = Age, SBP, TC, HDL & Glucose;

Model 2 =Age, SBP, TC, HDL, Trigs, Glucose, BMI & waist;

Model 3 = Age, SBP, TC, HDL, Trigs, Glucose, BMI, waist, CRP & IL-6;

Model 4 = Age, SBP, TC, HDL, Trigs, Glucose, BMI, waist, CRP, IL-6, Smoking & SES;

Model 5 = Age, SBP, TC, HDL, Trigs, Glucose, BMI, waist, CRP, IL-6, HR, Smoking & SES.

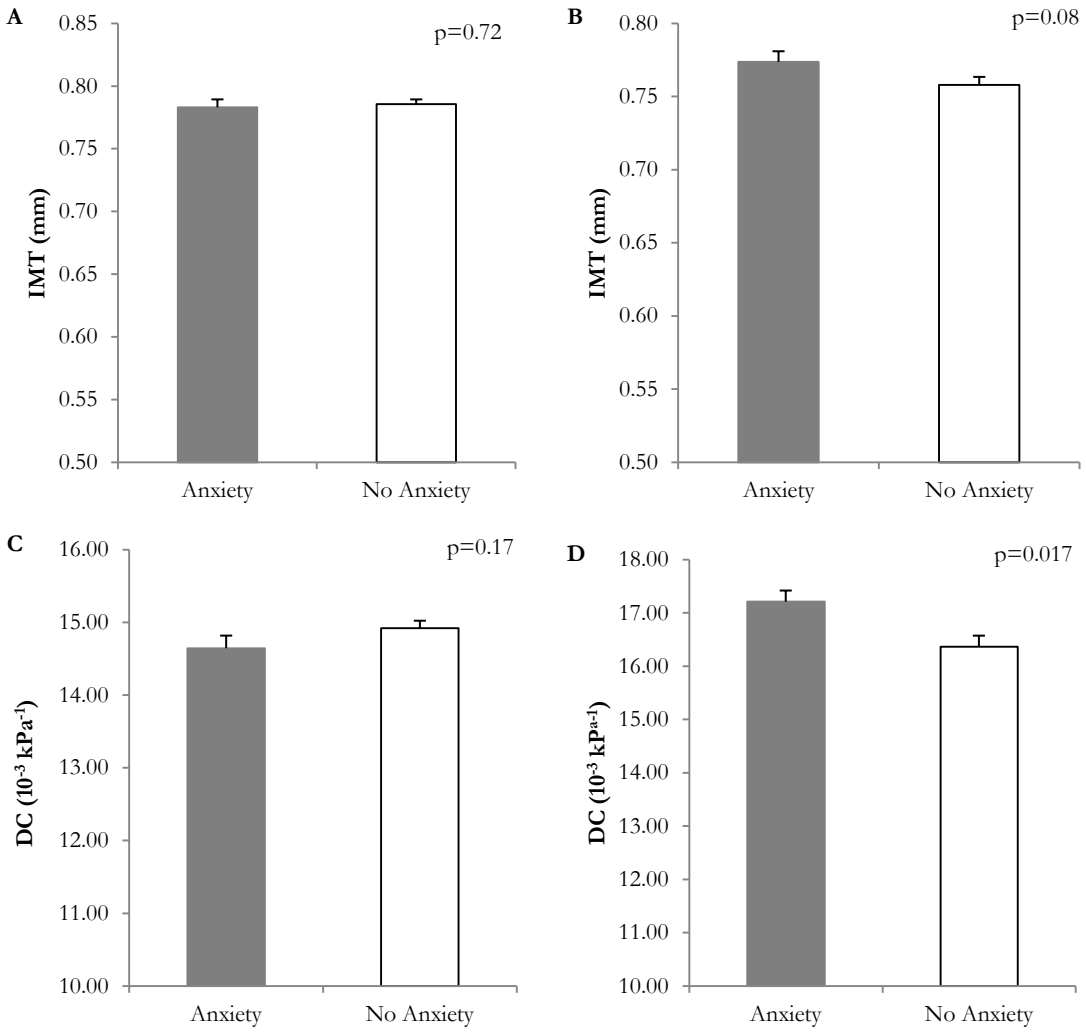

Figure 3.2: IMT for males (A) and females (B) DC males (C) and females (D) in those with and without anxiety. IMT and DC are adjusted for age, SBP, TC, HDL, Trigs, glucose, BMI, waist, CRP, IL-6, HR, smoking and SES. Data presented as mean±SEM.

3.4.1.3 Depression and anxiety

The major finding in this section was that those women with both depression and anxiety had the greatest IMT. There were no relationships between depression +/- anxiety and IMT or DC in men.

To investigate whether there were differences in vascular structure and function due to the different measures of chronic stress participants were divided into four groups. Those with depression and anxiety symptoms (D+A+), those with depression and without anxiety symptoms (D+A-), no depression but anxiety symptoms (D-A+) and neither depression nor anxiety symptoms (D-A-). Table 3.13 shows the numbers and mean IMT and DC for each group by gender. There were significant differences in IMT for males, with post hoc testing indicating this to be between men with anxiety having lower IMT than those without either depression or anxiety (p=0.024). Whilst women with either just depression or just anxiety had greater DC than those with neither (p=0.033 & p=0.045 respectively).

	Males			Females		
	n	**IMT (mm)**	**DC (10^{-3} kPa^{-1})**	**n**	**IMT (mm)**	**DC (10^{-3} kPa^{-1})**
D+A+	297	0.77 ± 0.15	14.84 ± 4.28	180	0.78 ± 0.14	17.33 ± 5.56
D+A-	171	0.78 ± 0.15	15.13 ± 4.78	109	0.77 ± 0.13	17.40 ± 6.13
D-A+	237	0.76 ± 0.15	15.07 ± 4.06	113	0.75 ± 0.14	17.69 ± 5.69
D-A-	1453	0.79 ± 0.16	14.78 ± 4.53	431	0.76 ± 0.13	15.91 ± 5.27
p		***0.006***	*0.65*		*0.08*	***0.001***

Table 3.13: Combined depression and analysis categorisation with mean ± SD for IMT and DC by gender. D+A+ = depression and anxiety, D+A- = depression only; D-A+ = anxiety only; D-A- neither depression nor anxiety. P= testing for differences between the chronic stress groups.

The relationships between measures of chronic stress and subclinical vascular measures were further tested with adjustment for cardiovascular risk factors. Following adjustment for age, the relationship between IMT and chronic stress in males did not remain (table 3.14). However, the relationship between DC and chronic stress in females remained following adjustment for age and individual cardiovascular risk factors. Additionally, the relationship between IMT and chronic stress became significant in women after adjustment for age. This association only lost significance following individual adjustments for heart rate, HDL, LDL and nonHDL. Further adjustments in models based on the Framingham risk factors, obesity, inflammatory markers and heart rate, and SES measures maintained the relationships seen between chronic stress and IMT and DC in females (table 3.15 and figure 3.3).

	Males				Females			
	IMT		DC		IMT		DC	
	F	p	F	p	F	p	F	p
Age (yrs)$	0.45	*0.71*	1.00	*0.39*	2.66	***0.047***	4.65	***0.003***
Waist (cm)	0.55	*0.65*	1.01	*0.39*	2.70	***0.045***	4.91	***0.002***
Waist Hip	0.62	*0.60*	0.87	*0.46*	2.61	***0.050***	4.92	***0.002***
BMI	0.50	*0.68*	0.97	*0.41*	2.73	***0.043***	4.72	***0.003***
SBP (mmHg)	0.22	*0.88*	1.91	*0.13*	3.09	***0.027***	5.24	***0.001***
DBP (mmHg)	0.38	*0.76*	1.67	*0.17*	2.97	***0.031***	3.91	***0.009***
HR (bpm)	0.31	*0.82*	0.34	*0.80*	2.41	*0.07*	3.71	***0.011***
Glucose (mmol/L)	0.49	*0.69*	0.94	*0.42*	2.65	***0.048***	4.74	***0.003***
TC (mmol/L)	0.33	*0.80*	1.08	*0.36*	2.62	***0.050***	4.67	***0.003***
HDL (mmol/L)	0.53	*0.66*	1.10	*0.35*	2.52	*0.06*	5.00	***0.002***
Trigs (mmol/L)	0.44	*0.73*	1.17	*0.32*	2.66	***0.047***	5.28	***0.001***
LDL (mmol/L)	0.35	*0.79*	1.22	*0.30*	2.48	*0.06*	4.54	***0.004***
NonHDL (mmol/L)	0.33	*0.80*	1.17	*0.32*	2.51	*0.06*	4.72	***0.003***
CRP (mg/L)	0.43	*0.73*	0.93	*0.42*	2.92	***0.033***	4.72	***0.003***
IL-6 (pg/ml)	0.39	*0.76*	0.92	*0.43*	2.81	***0.039***	4.53	***0.004***

Table 3.14: Association by gender between IMT and chronic stress and DC and chronic stress adjusted for age, and each individual risk factor. $ = non-adjusted.

	Males				Females			
	IMT		DC		IMT		DC	
	F	p	F	p	F	p	F	p
Model 1	0.20	*0.89*	2.03	*0.11*	2.76	***0.041***	5.35	***0.001***
Model 2	0.23	*0.88*	1.85	*0.14*	3.05	***0.028***	5.45	***0.001***
Model 3	0.21	*0.89*	1.72	*0.16*	3.41	***0.017***	5.38	***0.001***
Model 4	0.24	*0.87*	1.76	*0.15*	3.20	***0.023***	4.81	***0.003***
Model 5	0.06	*0.98*	0.88	*0.45*	3.00	***0.030***	4.21	***0.006***

Table 3.15: Relationship between chronic stress and IMT and chronic stress and DC with adjustments for cardiovascular risk factors.

Model 1 = Age, SBP, TC, HDL & Glucose;

Model 2 =Age, SBP, TC, HDL, Trigs, Glucose, BMI & waist;

Model 3 = Age, SBP, TC, HDL, Trigs, Glucose, BMI, waist, CRP & IL-6;

Model 4 = Age, SBP, TC, HDL, Trigs, Glucose, BMI, waist, CRP, IL-6, Smoking & SES;

Model 5 = Age, SBP, TC, HDL, Trigs, Glucose, BMI, waist, CRP, IL-6, HR, Smoking & SES.

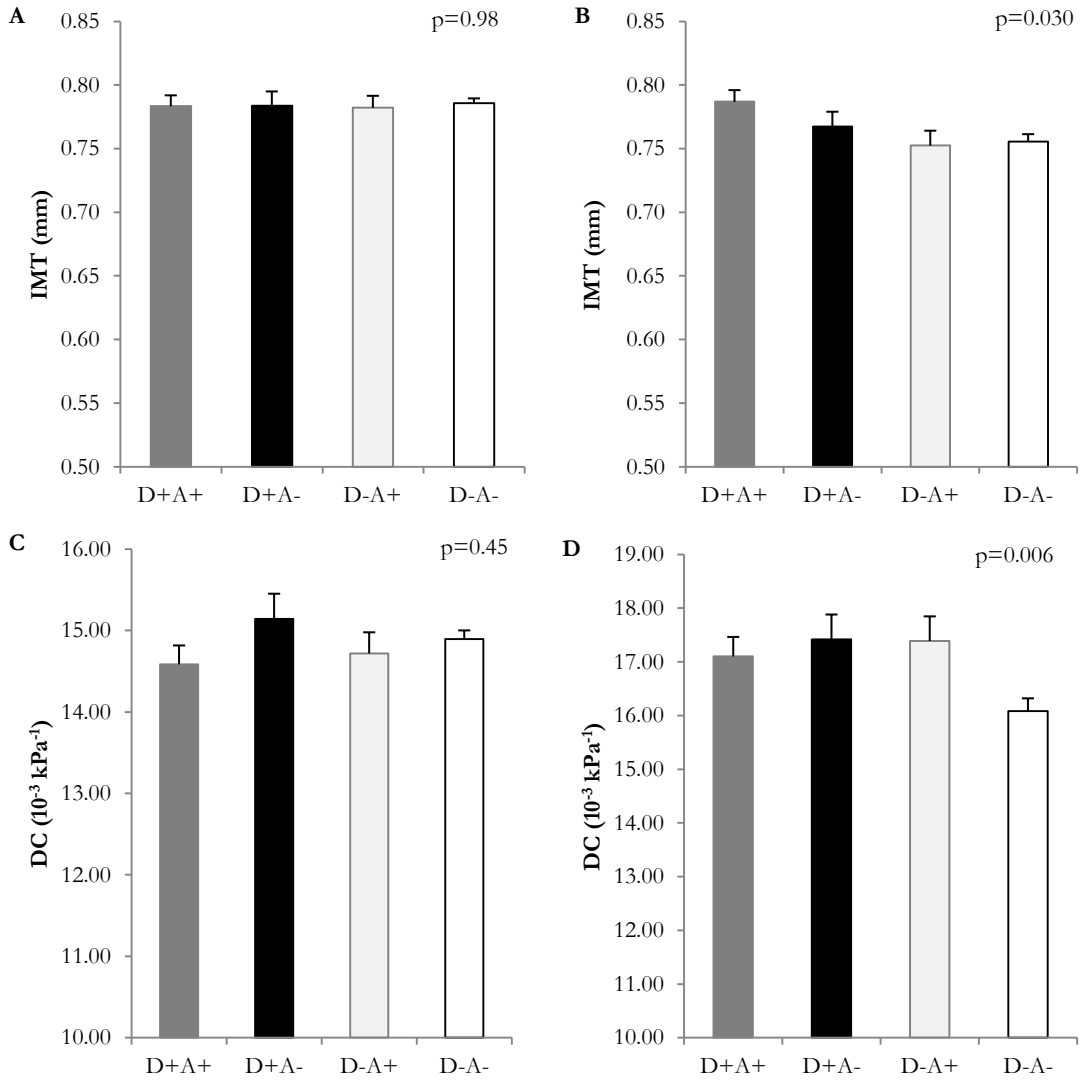

Figure 3.3: IMT in males (A) and females (B) DC males (C) and females (D) in those with and without chronic stress. Both IMT and DC are adjusted for age, SBP, TC, HDL, Trigs, glucose, BMI, waist, CRP, IL-6, HR, smoking and SES. Post hoc Bonferroni analysis showed that the difference in IMT in females was between those with both depression & anxiety and neither (p=0.029). For DC, Bonferroni post hoc tests did not reach significance. D+A+ = depression and anxiety; D+A-= depression only; D-A+ anxiety only D-A- no depression or anxiety; F = female; M= male. Data presented as mean±SEM.

3.4.1.4 Dyslipidaemia

The novel finding in this section was that dyslipidaemia adversely influenced the relationship between chronic stress and IMT, but only in women. Differences observed in IMT in men were due to dyslipidaemia and not chronic stress. Dyslipidaemia did not appear to influence the relationship between chronic stress and DC for either gender.

To investigate whether dyslipidaemia influences the relationship between chronic stress and IMT and DC, participants were categorised by presence or absence of dyslipidaemia by

meeting one or more of the criteria as set out in table 3.16. Fifty three percent of men and women were classified as having dyslipidaemia. High total cholesterol was the most commonly met criteria for both genders.

		Dyslipidaemia categories				Nº of categories met				
		TC ≥6	Trigs ≥1.7	HDL <1	LDL ≥4	1	2	3	4	Dyslipidaemia
Yes	**M**	863	497	83	749	353	544	229	16	1142
	F	401	118	5	272	148	238	56	1	443
No	**M**	1281	1646	2060	1377					
	F	430	713	826	554					

Table 3.16: Number of males and females who met each lipid category and the number of criteria for dyslipidaemia that they met (units=mmol/L).

Similar numbers of participants were classified as having depression and/or anxiety in both those with or without dyslipidaemia. Tables 3.17 and 3.18 show the cardiovascular risk factor profiles for all four groups for males and females. For males with dyslipidaemia, there were significant differences between those with and without chronic stress for age, waist/hip ratio, heart rate, HDL & triglycerides. Males without dyslipidaemia but with chronic stress were significantly younger, with lower SBP and triglycerides than those without chronic stress. In both males with and without dyslipidaemia, those who suffered with chronic stress had significantly lower IMT but there was no difference in DC.

	Dyslipidaemia			Normal		
	Dep/Anx n=356	No dep/anx n=786	p	Dep/Anx n=345	No dep/anx n=656	p
Age (yrs)	59 ± 6	61 ± 6	***<0.001***	59 ± 5	61 ± 6	***<0.001***
Waist (cm)	96.86 ± 9.8	95.65 ± 9.97	*0.06*	92.13 ± 10.53	92.38 ± 9.68	*0.70*
Waist/hip ratio	0.97 ± 0.06	0.96 ± 0.06	***0.007***	0.94 ± 0.06	0.94 ± 0.06	*0.82*
BMI (kg/m²)	26.82 ± 3.57	26.5 ± 3.6	*0.17*	25.35 ± 3.91	25.34 ± 3.36	*0.96*
SBP (mmHg)	127 ± 15	128 ± 15	*0.09*	123 ± 14	125 ± 15	***0.013***
DBP (mmHg)	75 ± 10	75 ± 10	*0.69*	72 ± 10	72 ± 10	*0.76*
HR (bpm)	70 ± 12	67 ± 11	***0.002***	66 ± 10	66 ± 11	*0.64*
Glucose (mmol/L)	5.43 ± 0.96	5.40 ± 0.78	*0.87*	5.33 ± 0.98	5.38 ± 0.96	*0.31*
TC (mmol/L)	6.29 ± 0.8	6.38 ± 0.82	*0.07*	5.08 ± 0.57	5.10 ± 0.57	*0.65*
HDL (mmol/L)	1.39 ± 0.36	1.46 ± 0.42	***0.003***	1.56 ± 0.34	1.58 ± 0.37	*0.57*
Trigs (mmol/L)	1.79 ± 1.13	1.63 ± 0.88	***0.009***	0.92 ± 0.30	0.96 ± 0.31	***0.026***
LDL (mmol/L)	4.12 ± 0.74	4.18 ± 0.79	*0.20*	3.09 ± 0.54	3.07 ± 0.55	*0.67*
NonHDL (mmol/L)	4.9 ± 0.75	4.92 ± 0.81	*0.66*	3.51 ± 0.59	3.52 ± 0.61	*0.93*
CRP (mg/L)	2.4 ± 5.05	1.92 ± 2.56	*0.12*	1.79 ± 3.25	2.21 ± 4.64	*0.10*
IL-6 (pg/ml)	2.18 ± 1.74	2.17 ± 1.87	*0.76*	2.11 ± 2.14	2.17 ± 1.97	*0.26*
IMT (mm)	0.78 ± 0.16	0.81 ± 0.17	***0.024***	0.75 ± 0.14	0.77 ± 0.15	***0.042***
DC (10^{-3} kPa^{-1})	14.58 ± 3.98	14.50 ± 4.44	*0.78*	15.39 ± 4.64	15.13 ± 4.63	*0.39*

Table 3.17: Risk factors for male participants by depression /anxiety category, and by dyslipidaemia category.

For females with dyslipidaemia, those with depression and /or anxiety had significantly lower fasting glucose than those without chronic stress. Non-dyslipidaemia females with depression and or anxiety had lower heart rate but increased triglycerides. Both IMT and DC were greater in females with dyslipidaemia and chronic stress, whilst in those without dyslipidaemia only DC was significantly greater in those with chronic stress (table 3.18).

	Dyslipidaemia			Normal		
	Dep/Anx n=209	No dep/anx n=234	p	Dep/Anx n=193	No dep/anx n=195	p
Age (yrs)	61 ± 6	61 ± 6	*0.41*	59 ± 6	60 ± 6	*0.17*
Waist (cm)	88.14 ± 12.82	86.52 ± 12.83	*0.18*	82.8 ± 14.06	82.92 ± 12.93	*0.93*
Waist/hip ratio	0.87 ± 0.08	0.85 ± 0.08	*0.06*	0.83 ± 0.08	0.83 ± 0.08	*0.75*
BMI (kg/m²)	26.58 ± 4.74	26.33 ± 4.29	*0.56*	25.25 ± 5.32	25.57 ± 5.08	*0.54*
SBP (mmHg)	124 ± 17	124 ± 17	*0.59*	122 ± 18	123 ± 18	*0.96*
DBP (mmHg)	71 ± 10	73 ± 11	*0.20*	71 ± 11	72 ± 11	*0.43*
HR (bpm)	69 ± 10	69 ± 10	*0.97*	68 ± 10	71 ± 10	***0.002***
Glucose (mmol/L)	5.10 ± 0.59	5.21 ± 0.52	***0.024***	5.20 ± 1.04	5.12 ± 1.35	*0.19*
TC (mmol/L)	6.68 ± 0.78	6.64 ± 0.81	*0.66*	5.23 ± 0.48	5.15 ± 0.55	*0.10*
HDL (mmol/L)	1.84 ± 0.51	1.92 ± 0.56	*0.13*	1.89 ± 0.43	1.9 ± 0.44	*0.87*
Trigs (mmol/L)	1.38 ± 0.73	1.30 ± 0.80	*0.12*	0.89 ± 0.29	0.82 ± 0.29	***0.016***
LDL (mmol/L)	4.23 ± 0.8	4.12 ± 0.83	*0.15*	2.93 ± 0.52	2.87 ± 0.56	*0.29*
NonHDL (mmol/L)	4.84 ± 0.86	4.73 ± 0.9	*0.18*	3.35 ± 0.54	3.25 ± 0.6	*0.11*
CRP (mg/L)	2.42 ± 3.69	2.55 ± 3.75	*0.62*	2.18 ± 3.39	2.85 ± 6.1	*0.44*
IL-6 (pg/ml)	1.9 ± 1.28	2.05 ± 1.72	*0.63*	1.90 ± 1.70	2.11 ± 1.78	*0.12*
IMT (mm)	0.79 ± 0.14	0.76 ± 0.13	***0.036***	0.75 ± 0.13	0.75 ± 0.12	*0.96*
DC (10^{-3} kPa^{-1})	17.13 ± 5.32	15.77 ± 5.09	***0.007***	17.80 ± 6.16	16.02 ± 5.5	***0.003***

Table 3.18: Risk factors for female participants by depression/anxiety category, and by dyslipidaemia category.

	Males				Females			
	IMT (mm)		DC (10^{-3} kPa^{-1})		IMT (mm)		DC (10^{-3} kPa^{-1})	
	F	p	F	p	F	p	F	p
Age (yrs)$^{\$}$	8.85	***<0.001***	4.52	***0.004***	3.71	***0.011***	4.63	***0.003***
Waist (cm)	5.89	***0.001***	1.20	*0.31*	3.20	***0.023***	5.00	***0.002***
Waist/hip ratio	5.88	***0.001***	0.95	*0.42*	3.03	***0.029***	5.01	***0.002***
BMI (kg/m²)	5.86	***0.001***	1.19	*0.31*	3.41	***0.017***	4.74	***0.003***
SBP (mmHg)	5.79	***0.001***	2.27	*0.08*	3.89	***0.009***	5.19	***0.001***
DBP (mmHg)	6.84	***<0.001***	0.80	*0.49*	3.86	***0.009***	3.92	***0.009***
HR (bpm)	9.78	***<0.001***	1.76	*0.15*	3.72	***0.011***	3.61	***0.013***
Glucose (mmol/L)	8.37	***<0.001***	4.22	***0.006***	3.68	***0.012***	4.72	***0.003***
CRP (mg/L)	8.63	***<0.001***	4.83	***0.002***	3.71	***0.011***	4.66	***0.003***
IL-6 (pg/ml)	8.80	***<0.001***	5.01	***0.002***	3.70	***0.012***	4.45	***0.004***

Table 3.19: Association between chronic stress and IMT and chronic stress and DC and the presence of dyslipidaemia adjusted for age, and each individual risk factor. $^{\$}$ = non-adjusted.

The relationships with IMT and DC were further investigated by adjusting for age and cardiovascular risk factors individually. For males, it can be seen from table 3.19 significant differences remained between the groups for IMT; whereas for DC the relationships only remained with adjustment for age, glucose and CRP individually. For females the relationships between the groups remained with both IMT and DC following adjustment.

Following adjustment for the larger risk factor models the relationships between chronic stress and dyslipidaemia with IMT remained in both males and females and with DC in females only (table 3.20).

	Males				**Females**			
	IMT (mm)		**DC (10^{-3} kPa^{-1})**		**IMT (mm)**		**DC (10^{-3} kPa^{-1})**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Model 1	5.71	***0.001***	2.23	*0.08*	3.81	***0.010***	5.23	***0.001***
Model 2	4.41	***0.004***	0.98	*0.40*	3.56	***0.014***	5.15	***0.002***
Model 3	4.76	***0.003***	1.12	*0.34*	3.59	***0.014***	4.95	***0.002***
Model 4	4.73	***0.003***	1.16	*0.33*	3.37	***0.018***	4.41	***0.004***
Model 5	5.73	***0.001***	0.36	*0.78*	3.54	***0.014***	3.82	***0.010***

Table 3.20: Relationship between chronic stress, presence of dyslipidaemia and IMT and DC by gender with adjustments for cardiovascular risk factors.

Model 1 = Age, SBP & Glucose;

Model 2 =Age, SBP, Glucose, BMI & waist;

Model 3 = Age, SBP, Glucose, BMI, waist, CRP & IL-6;

Model 4 = Age, SBP, Glucose, BMI, waist, CRP, IL-6, Smoking & SES

Model 5 = Age, SBP, Glucose, BMI, waist, CRP, IL-6, HR, Smoking & SES.

In figure 3.4A it can be seen that males with dyslipidaemia had greater IMT than those men without, irrespective of chronic stress status. Whereas in females, it was those with both dyslipidaemia and chronic stress who had the greatest IMT (figure 3.4B). Presence of depression and or anxiety appeared to have more influence on DC than dyslipidaemia in women, as DC was greatest in both of the chronic stress groups irrespective of lipid status (figure 3.4D). However, post hoc testing did not reach significance.

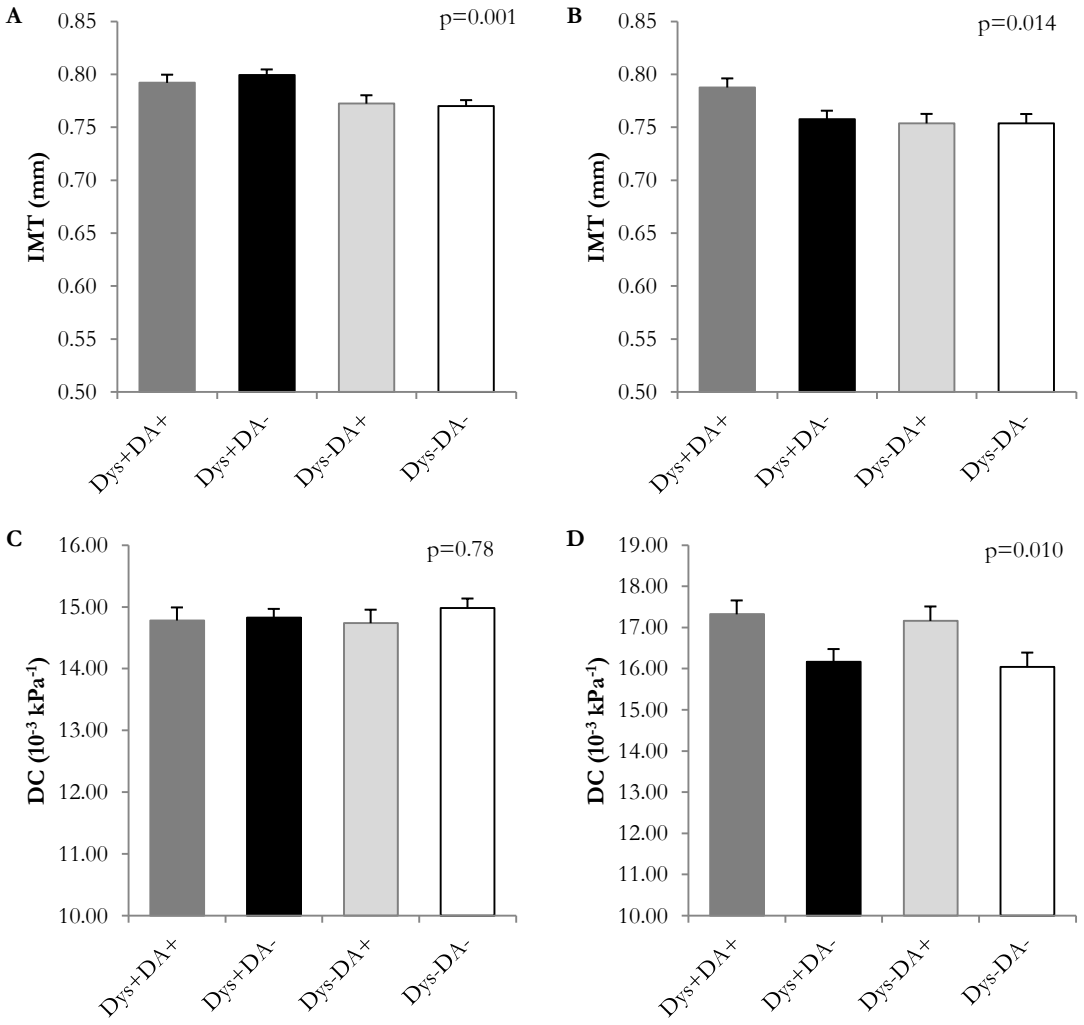

Figure 3.4: IMT for males (A) and females (B) DC males (C) and females (D) in those with and without dyslipidaemia and chronic stress. Both IMT and DC are adjusted for age, SBP, TC, HDL, Trigs, glucose, BMI, waist, CRP IL-6, HR, smoking and SES. Post hoc Bonferroni analyses showed that the differences in IMT in males were between those with dyslipidaemia without chronic stress vs Dys+ DA+ p=1.00; Dys-DA+ p=0.030; Dys-DA-p=0.001 females were between those with dyslipidaemia and chronic stress vs Dys+ DA- p=0.067; Dys-DA+ p=0.042; Dys-DA-p=0.040. For DC, Bonferroni post hoc tests did not reach significance. Dys+ = dyslipidaemia; Dys- = not dyslipidaemic; D+A+ = depression and anxiety; D+A-= depression only; D-A+ anxiety only D-A- no depression or anxiety. Data presented as mean±SEM.

3.4.2 Chronic stress and endothelial function

358 participants underwent endothelial function testing at phase 7 of the Whitehall II study. Following exclusion of those participants who were diabetic, smokers or on antihypertensive, lipid lowering, or diabetes medication, 288 participants remained in the study. Table 3.21 shows the characteristics of these participants compared with the population in the carotid artery structure and function measures analysis. The FMD cohort

was significantly younger than the IMT cohort with lower blood pressure and triglycerides and inflammatory markers. There was also a significantly greater proportion of women in the FMD cohort in comparison to the IMT group.

	FMD cohort (n=288)	IMT cohort (n=2991)	p
Age (yrs)	58.32 ± 4.94	60 ± 6	***<0.001***
Women (%)	120 (42)	833 (28)	***<0.001***
Waist (cm)			
Men	92.85 ± 10.95	94.26 ± 10.14	*0.09*
Women	84.82 ± 13.07	85.19 ± 13.33	*0.78*
Waist/hip ratio			
Men	0.94 ± 0.06	0.95 ± 0.06	***0.026***
Women	0.85 ± 0.08	0.85 ± 0.08	*0.81*
BMI (kg/m²)	25.72 ± 4.17	25.99 ± 4.01	*0.28*
SBP (mmHg)	121 ± 15	125 ± 16	***<0.001***
DBP (mmHg)	71 ± 10	73 ± 10	***<0.001***
HR (bpm)	66 ± 9	68 ± 11	***0.005***
Glucose (mmol/L)	5.2 ± 0.46	5.32 ± 0.91	***0.001***
TC (mmol/L)	5.78 ± 1.00	5.82 ± 0.97	*0.45*
HDL (mmol/L)	1.69 ± 0.47	1.61 ± 0.45	***0.004***
Trigs (mmol/L)	1.13 ± 0.80	1.27 ± 0.78	***<0.001***
LDL (mmol/L)	3.56 ± 0.91	3.63 ± 0.89	*0.22*
NonHDL (mmol/L)	4.09 ± 1.06	4.21 ± 1.01	***0.046***
CRP (mg/L)	1.66 ± 2.32	2.19 ± 4.01	***0.003***
IL-6 (pg/ml)	1.82 ± 1.55	2.11 ± 1.85	***<0.001***

Table 3.21: Comparison of characteristics of participants in the FMD and IMT cohorts.

3.4.2.1 Depression and endothelial function

In this section it was found that there was no association between depression and endothelial function.

66 participants within this cohort were classified has having depression. As can be seen from table 3.22 the only difference between the two groups was in IL-6 which was significantly higher in those with depression. There was no difference in FMD between the two groups.

	Depression (66)	No depression (222)	p
Age (yrs)	59± 5	58 ± 5	*0.17*
Women (%)	34 (52)	86 (39)	*0.06*
Waist (cm)			
Men	94.92 ± 13.24	92.37 ± 10.33	*0.24*
Women	84.23 ± 12.89	85.05 ± 13.21	*0.76*
Waist/hip ratio			
Men	0.96 ± 0.07	0.94 ± 0.06	*0.09*
Women	0.84 ± 0.07	0.85 ± 0.08	*0.55*
BMI (kg/m²)	26.09 ± 5.26	25.61 ± 3.79	*0.42*
SBP (mmHg)	123 ± 18	120 ± 14	*0.17*
DBP (mmHg)	71 ± 11	71 ± 10	*0.92*
HR (bpm)	67 ± 10	66 ± 9	*0.53*
Glucose (mmol/L)	5.17 ± 0.5	5.20 ± 0.45	*0.59*
TC (mmol/L)	5.84 ± 1.07	5.76 ± 0.99	*0.57*
HDL (mmol/L)	1.72 ± 0.52	1.68 ± 0.45	*0.68*
Trigs (mmol/L)	1.23 ± 1.07	1.10 ± 0.7	*0.52*
LDL (mmol/L)	3.54 ± 0.88	3.57 ± 0.91	*0.82*
NonHDL (mmol/L)	4.12 ± 1.10	4.08 ± 1.06	*0.76*
CRP (mg/L)	1.82 ± 2.14	1.61 ± 2.38	*0.25*
IL-6 (pg/ml)	2.16 ± 1.64	1.72 ± 1.52	***0.024***
BL dia (mm)	3.70 ± 0.69	3.62 ± 0.64	*0.36*
$FMD_{\%}$ (%)	5.07 ± 2.79	5.60 ± 3.26	*0.23*
FMD_{ABS} (mm)	0.18 ± 0.09	0.19 ± 0.10	*0.29*
RH % (%)	656 ± 277	646 ± 256	*0.79*

Table 3.22: Participant characteristics for those with and without depression. BL dia = baseline diameter; RH% = reactive hyperaemia.

When looking at correlations between measures of endothelial function and cardiovascular risk factors it was shown that $FMD_{\%}$ was positively associated with heart rate whilst, FMD_{ABS} had borderline associations with both diastolic blood pressure and heart rate in the depressed group (table 3.23). For those without depression $FMD_{\%}$ was negatively

associated with age, triglycerides and nonHDL and positively associated with heart rate. FMD_{ABS} was only negatively associated with age in this group.

	$FMD_{\%}$				FMD_{ABS}			
	Depression		No depression		Depression		No depression	
	r	p	r	p	r	p	r	p
Age (yrs)	-0.11	*0.39*	-0.19	***0.004***	-0.06	*0.63*	-0.15	***0.024***
Waist (cm)								
Men	0.08	*0.68*	0.00	*0.96*	0.13	*0.49*	0.06	*0.51*
Women	-0.33	*0.05*	-0.03	*0.79*	-0.24	*0.17*	0.01	*0.93*
Waist/hip ratio								
Men	0.04	*0.85*	-0.04	*0.69*	0.03	*0.88*	0.00	*0.98*
Women	-0.13	*0.46*	0.02	*0.88*	-0.08	*0.64*	0.04	*0.74*
BMI (kg/m^2)	-0.09	*0.47*	-0.08	*0.26*	0.00	*0.99*	0.01	*0.85*
SBP (mmHg)	0.16	*0.21*	-0.09	*0.18*	0.20	*0.10*	-0.06	*0.34*
DBP (mmHg)	0.22	*0.08*	0.04	*0.54*	0.24	*0.05*	0.04	*0.56*
HR (bpm)	0.24	***0.049***	0.16	***0.015***	0.24	*0.05*	0.10	*0.14*
Glucose (mmol/L)	0.05	*0.69*	-0.01	*0.87*	0.10	*0.42*	0.01	*0.87*
TC (mmol/L)	-0.06	*0.64*	-0.12	*0.08*	-0.12	*0.35*	-0.09	*0.20*
HDL (mmol/L)	0.13	*0.28*	0.09	*0.18*	0.02	*0.89*	-0.01	*0.92*
Trigs (mmol/L)	-0.17	*0.18*	-0.16	***0.018***	-0.11	*0.39*	-0.09	*0.16*
LDL (mmol/L)	-0.03	*0.81*	-0.12	*0.07*	-0.07	*0.60*	-0.06	*0.39*
NonHDL (mmol/L)	-0.12	*0.34*	-0.14	***0.031***	-0.12	*0.33*	-0.07	*0.27*
CRP (mg/L)	-0.13	*0.30*	0.05	*0.46*	-0.08	*0.50*	0.07	*0.28*
IL-6 (pg/ml)	-0.05	*0.70*	0.02	*0.82*	-0.02	*0.88*	0.06	*0.39*
BL dia (mm)	-0.46	***<0.001***	-0.43	***<0.001***	-0.12	*0.32*	-0.18	***0.006***
FMD_{ABS} (mm)	0.92	***<0.001***	0.95	***<0.001***	0.94	***<0.001***	0.96	***<0.001***
RH % (%)	0.08	*0.55*	0.05	*0.44*	0.12	*0.36*	0.01	*0.88*

Table 3.23: Correlations between $FMD_{\%}$ and FMD_{ABS} with cardiovascular risk factors for depressed and non-depressed.

There were no relationships between endothelial function and depression having adjusted for baseline brachial artery diameter and reactive hyperaemia. Adding age and gender to this model did not change the results nor did adding each cardiovascular risk factor individually to the model (table 3.24).

	$FMD_{\%}$		FMD_{ABS}	
	F	**p**	**F**	**p**
BL dia & RH%*	0.52	*0.47*	0.55	*0.46*
BL dia, RH%, age & gender	0.01	*0.91*	0.04	*0.84*
Waist (cm)	0.02	*0.90*	0.02	*0.88*
Waist/hip ratio	0.01	*0.91*	0.02	*0.90*
BMI (kg/m²)	0.01	*0.92*	0.02	*0.90*
SBP (mmHg)	0.00	*0.97*	0.01	*0.90*
DBP (mmHg)	0.01	*0.94*	0.03	*0.87*
HR (bpm)	0.00	*0.98*	0.01	*0.91*
Glucose (mmol/L)	0.04	*0.83*	0.08	*0.78*
TC (mmol/L)	0.01	*0.90*	0.04	*0.83*
HDL (mmol/L)	0.01	*0.92*	0.04	*0.84*
Trigs (mmol/L)	0.02	*0.90*	0.05	*0.83*
LDL (mmol/L)	0.04	*0.84*	0.11	*0.74*
NonHDL (mmol/L)	0.01	*0.91*	0.04	*0.84*
CRP (mg/L)	0.00	*0.96*	0.01	*0.91*
IL-6 (pg/ml)	0.01	*0.94*	0.02	*0.90*

Table 3.24: Association between depression and $FMD_{\%}$ and depression and FMD_{ABS} adjusted for baseline brachial artery diameter, reactive hyperaemia, age, gender and each individual risk factor. *= adjusted for baseline diameter and reactive hyperaemia.

When $FMD_{\%}$ and FMD_{ABS} were adjusted for Framingham risk factor variables there were no relationships with depression. Adding markers for obesity, inflammation and SES to the model did not improve the lack of association (table 3.25).

	$FMD_{\%}$		FMD_{ABS}	
	F	**p**	**F**	**p**
Model 1	0.03	*0.87*	0.05	*0.82*
Model 2	0.06	*0.81*	0.09	*0.76*
Model 3	0.07	*0.80*	0.09	*0.76*
Model 4	0.07	*0.79*	0.08	*0.78*

Table 3.25: Relationship between depression and $FMD_{\%}$ and depression and FMD_{ABS} with adjustments for cardiovascular risk factors.

Model 1 = Age, gender, SBP, TC, HDL & Glucose;

Model 2 =Age, gender , baseline diameter, reactive hyperaemia, SBP, TC, HDL, Trigs, Glucose, waist/hip ratio & waist; Model 3 = Age, gender, baseline diameter, reactive hyperaemia, SBP, TC, HDL, Trigs, Glucose, waist/hip ratio, waist, CRP & IL-6;

Model 4 = Age, gender, baseline diameter, reactive hyperaemia, SBP, TC, HDL, Trigs, Glucose, waist/hip ratio, waist, CRP, IL-6 & SES.

3.4.2.2 Anxiety

In this section it was found that there was no association between anxiety and endothelial function.

88 participants were classified as having anxiety. Table 3.26 shows that there were no differences in the risk factor profile for those with and without anxiety.

	Anxiety (88)	No anxiety (200)	p
Age (yrs)	58 ± 5	59 ± 5	*0.18*
Women (%)	43 (49)	77 (39)	*0.10*
Waist (cm)			
Men	94.38 ± 11.61	92.29 ± 10.69	*0.28*
Women	85.24 ± 12.88	84.58 ± 13.26	*0.79*
Waist/hip ratio			
Men	0.95 ± 0.06	0.94 ± 0.06	*0.36*
Women	0.85 ± 0.09	0.85 ± 0.08	*0.99*
BMI (kg/m²)	25.92 ± 4.71	25.64 ± 3.92	*0.60*
SBP (mmHg)	121 ± 17	120 ± 14	*0.58*
DBP (mmHg)	71 ± 11	71 ± 10	*0.76*
HR (bpm)	67 ± 9	66 ± 10	*0.50*
Glucose (mmol/L)	5.18 ± 0.46	5.21 ± 0.46	*0.58*
TC (mmol/L)	5.76 ± 1.02	5.78 ± 1.00	*0.88*
HDL (mmol/L)	1.76 ± 0.49	1.66 ± 0.46	*0.08*
Trigs (mmol/L)	1.06 ± 0.61	1.16 ± 0.87	*0.31*
LDL (mmol/L)	3.51 ± 0.92	3.59 ± 0.9	*0.50*
NonHDL (mmol/L)	4.00 ± 1.02	4.13 ± 1.08	*0.36*
CRP (mg/L)	1.76 ± 2.45	1.62 ± 2.27	*0.77*
IL-6 (pg/ml)	1.95 ± 1.5	1.76 ± 1.57	*0.21*
BL dia (mm)	3.61 ± 0.69	3.65 ± 0.63	*0.67*
$FMD_{\%}$ (%)	5.44 ± 2.71	5.50 ± 3.34	*0.88*
FMD_{ABS} (mm)	0.19 ± 0.09	0.19 ± 0.10	*0.77*
$RH_{\%}$ (%)	647 ± 264	650 ± 259	*0.94*

Table 3.26: Participant characteristics for those with and without anxiety.

Correlations between the two measures of endothelial function and cardiovascular risk factors within the anxious and non-anxious groups showed that $FMD_{\%}$ was negatively correlated with CRP in the group with anxiety whilst FMD_{ABS} was positively correlated with diastolic blood pressure. In the group without anxiety $FMD_{\%}$ was negatively correlated with age and all lipid variables except HDL and positively associated with heart rate. FMD_{ABS} was not correlated with any risk factors in this group (table 3.27).

	$FMD_{\%}$ (%)				FMD_{ABS} (mm)			
	Anxiety		No anxiety		Anxiety		No anxiety	
Anxiety	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Age (yrs)	-0.18	*0.09*	-0.18	***0.010***	-0.13	*0.23*	-0.14	*0.05*
Waist (cm)								
Men	-0.09	*0.55*	0.03	*0.75*	-0.02	*0.90*	0.09	*0.33*
Women	-0.04	*0.82*	-0.12	*0.30*	-0.01	*0.93*	-0.06	*0.60*
Waist/hip ratio								
Men	-0.14	*0.35*	0.00	*0.97*	-0.13	*0.40*	0.04	*0.70*
Women	0.08	*0.60*	-0.05	*0.65*	0.07	*0.67*	-0.01	*0.92*
BMI (kg/m^2)	-0.11	*0.29*	-0.07	*0.33*	-0.04	*0.74*	0.00	*0.99*
SBP (mmHg)	0.04	*0.69*	-0.07	*0.32*	0.10	*0.36*	-0.04	*0.55*
DBP (mmHg)	0.16	*0.13*	0.05	*0.48*	0.22	***0.044***	0.04	*0.62*
HR (bpm)	0.20	*0.06*	0.17	***0.017***	0.18	*0.09*	0.11	*0.12*
Glucose (mmol/L)	0.11	*0.29*	-0.04	*0.61*	0.20	*0.06*	-0.03	*0.68*
TC (mmol/L)	0.04	*0.74*	-0.16	***0.026***	0.01	*0.94*	-0.13	*0.06*
HDL (mmol/L)	0.14	*0.20*	0.09	*0.23*	0.01	*0.92*	-0.01	*0.93*
Trigs (mmol/L)	-0.14	*0.18*	-0.17	***0.018***	-0.10	*0.37*	-0.10	*0.15*
LDL (mmol/L)	0.02	*0.88*	-0.15	***0.038***	0.03	*0.75*	-0.10	*0.18*
NonHDL (mmol/L)	-0.02	*0.85*	-0.18	***0.010***	0.01	*0.92*	-0.12	*0.09*
CRP (mg/L)	-0.21	*0.045*	0.10	*0.18*	-0.15	*0.15*	0.11	*0.12*
IL-6 (pg/ml)	-0.05	*0.66*	0.01	*0.92*	-0.02	*0.89*	0.05	*0.48*
BL dia (mm)	-0.47	***<0.001***	-0.43	***<0.001***	-0.15	*0.16*	-0.18	***0.010***
FMD_{ABS} (mm)	0.93	***<0.001***	0.95	***<0.001***	0.93	***<0.001***	0.95	***<0.001***
$RH_{\%}$ (%)	-0.04	*0.71*	0.09	*0.22*	-0.02	*0.82*	0.05	*0.46*

Table 3.27: Correlations between $FMD_{\%}$ and cardiovascular risk factors and FMD_{ABS} and cardiovascular risk factors for anxiety and non-anxiety.

Following adjustment for age, gender, baseline diameter and reactive hyperaemia there were no relationships between measures of endothelial function and anxiety. Adding cardiovascular risk factors to the model individually did not result in any significant association between endothelial function and anxiety being found (table 3.28).

	$FMD_{\%}$		FMD_{ABS}	
	F	**p**	**F**	**p**
age & gender*	0.35	*0.56*	0.13	*0.72*
BL dia & RH%$	0.29	*0.59*	0.25	*0.61*
BL dia, RH%, age & gender	0.11	*0.74*	0.05	*0.82*
Waist (cm)	0.19	*0.67*	0.11	*0.74*
Waist/hip ratio	0.14	*0.71*	0.07	*0.80*
BMI (kg/m²)	0.14	*0.71*	0.08	*0.78*
SBP (mmHg)	0.17	*0.68*	0.09	*0.76*
DBP (mmHg)	0.18	*0.67*	0.09	*0.77*
HR (bpm)	0.20	*0.66*	0.10	*0.75*
Glucose (mmol/L)	0.07	*0.79*	0.03	*0.85*
TC (mmol/L)	0.14	*0.71*	0.06	*0.80*
HDL (mmol/L)	0.08	*0.77*	0.04	*0.85*
Trigs (mmol/L)	0.13	*0.72*	0.06	*0.80*
LDL (mmol/L)	0.16	*0.69*	0.08	*0.78*
NonHDL (mmol/L)	0.15	*0.70*	0.07	*0.79*
CRP (mg/L)	0.14	*0.71*	0.09	*0.76*
IL-6 (pg/ml)	0.22	*0.64*	0.17	*0.68*

Table 3.28: Association between $FMD_{\%}$ and FMD_{ABS} and anxiety adjusted for baseline brachial artery diameter, reactive hyperaemia, age, gender and each individual risk factor. *= adjusted for age and gender $= adjusted for baseline diameter and reactive hyperaemia

Anxiety was not associated with either $FMD_{\%}$ or FMD_{ABS} following adjustment for Framingham risk factor variables. Adding markers for obesity, inflammation and SES did not change the lack of association between anxiety and endothelial function (table 3.29).

	$FMD_{\%}$		FMD_{ABS}	
	F	**p**	**F**	**p**
Model 1	0.11	*0.74*	0.08	*0.78*
Model 2	0.25	*0.62*	0.23	*0.63*
Model 3	0.44	*0.51*	0.44	*0.51*
Model 4	0.33	*0.56*	0.35	*0.55*

Table 3.29: Relationship between anxiety and $FMD_{\%}$ and FMD_{ABS} with adjustments for cardiovascular risk factors.

Model 1 = Age, gender, SBP, TC, HDL & Glucose;

Model 2 =Age, gender , baseline diameter, reactive hyperaemia, SBP, TC, HDL, Trigs, Glucose, waist/hip ratio & waist; Model 3 = Age, gender, baseline diameter, reactive hyperaemia, SBP, TC, HDL, Trigs, Glucose, waist/hip ratio, waist, CRP & IL-6;

Model 4 = Age, gender, baseline diameter, reactive hyperaemia, SBP, TC, HDL, Trigs, Glucose, waist/hip ratio, waist, CRP, IL-6 & SES.

3.4.2.3 Depression and anxiety

In this section it was shown that there was no association between measures of chronic stress and endothelial function.

Participants who had depression and/ or anxiety were grouped as described for the IMT cohort to create 4 groups. Table 3.30 shows the number in each group and the mean $FMD_{\%}$ and FMD_{ABS} for each group. There were no significant differences in endothelial function measures between the groups. Adjustment for age, gender baseline diameter and reactive hyperaemia did not show any relationship between these measures of chronic stress and endothelial function (table 3.31).

	n	$FMD_{\%}$	FMD_{ABS}
D+A+	42	4.80 ± 2.49	0.17 ± 0.09
D+A-	24	5.54 ± 3.24	0.20 ± 0.10
D-A+	46	6.02 ± 2.80	0.20 ± 0.09
D-A-	176	5.49 ± 3.37	0.19 ± 0.10

Table 3.30: Combined depression and analysis categorisation with mean ± SD for $FMD_{\%}$ and FMD_{ABS}. D+A+ = depression and anxiety, D+A- = depression only; D-A+ = anxiety only; D-A- neither depression nor anxiety.

	$FMD_{\%}$		FMD_{ABS}	
	F	**p**	**F**	**p**
Anova unadj	1.11	*0.35*	0.92	*0.43*
Age & gender	1.26	*0.29*	0.91	*0.43*
BL dia & $RH_{\%}$	0.71	*0.55*	0.59	*0.63*
BL dia, $RH_{\%}$, age & gender	0.86	*0.46*	0.69	*0.56*

Table 3.31: Association between groupings of depression and anxiety with $FMD_{\%}$ and FMD_{ABS}, with adjustments.

3.4.2.4 Dyslipidaemia

In this section it has been shown that there remained no relationship between chronic stress and endothelial dysfunction in the presence of dyslipidaemia.

Participants were divided into those with and without dyslipidaemia if they met at least one of the criteria as set out in table 3.32. Of the 288 participants, 141were categorised as having dyslipidaemia with most participants having high total cholesterol and meeting at least two of the qualifying criteria.

	Dyslipidaemia categories				Nº of categories met				
	TC ≥6	Trigs ≥1.7	HDL <1	LDL ≥4	1	2	3	4	Dyslipidaemia
Yes	119	45	6	97	42	75	17	3	141
No	169	243	282	189					

Table 3.32: Number of participants who met each lipid category and the number of categories they met. Units are mmol/l.

Participants with and without dyslipidaemia were then grouped as to whether they had depression and/or anxiety or not, creating four groups. Table 3.33 shows the risk factor profiles for each group. Those with dyslipidaemia and depression and/ or anxiety had higher heart rates and the males had greater waist circumference and waist hip ratio than those with dyslipidaemia but without depression and/ or anxiety. Within the normal lipid groups there were a greater proportion of females to males in the depressed and/ or anxious group than in the non-depressed/anxious group. Additionally, those with depression and/or anxiety had lower Trigs than those without chronic stress (table 3.33).

	Dyslipidaemia			Normal		
	Dep/Anx n=59	No dep/anx n=82	p	Dep/Anx n=53	No dep/anx n=94	p
Age (yrs)	59 ± 5	58 ± 5	*0.20*	57 ± 5	58 ± 5	*0.23*
Women (%)	27 (46)	33 (40)	*0.51*	28 (53)	32 (34)	***0.026***
Waist (cm)						
Men	98.29 ± 10.96	93.06 ± 10.3	***0.032***	88.20 ± 8.91	91.74 ± 11.26	*0.17*
Women	91.60 ± 10.56	88.67 ± 14.31	*0.38*	78.65 ± 11.09	80.53 ± 11.61	*0.52*
Waist/hip ratio						
Men	0.98 ± 0.06	0.94 ± 0.05	***0.004***	0.91 ± 0.05	0.93 ± 0.06	*0.09*
Women	0.89 ± 0.07	0.86 ± 0.09	*0.26*	0.81 ± 0.08	0.83 ± 0.07	*0.33*
BMI (kg/m²)	27.30 ± 4.51	26.26 ± 3.92	*0.15*	24.23 ± 3.85	25.10 ± 3.98	*0.20*
SBP (mmHg)	126 ± 16	121 ± 16	*0.10*	117 ± 14	119 ± 13	*0.25*
DBP (mmHg)	73 ± 10	71 ± 10	*0.19*	68 ± 10	70 ± 10	*0.23*
HR (bpm)	69 ± 9	65 ± 9	***0.005***	64 ± 9	66 ± 10	*0.19*
Glucose (mmol/L)	5.17 ± 0.45	5.27 ± 0.47	*0.20*	5.16 ± 0.48	5.16 ± 0.46	*0.98*
TC (mmol/L)	6.45 ± 0.93	6.54 ± 0.73	*0.55*	5.08 ± 0.61	5.08 ± 0.59	*0.99*
HDL (mmol/L)	1.67 ± 0.51	1.62 ± 0.51	*0.52*	1.80 ± 0.44	1.70 ± 0.41	*0.16*
Trigs (mmol/L)	1.51 ± 1.08	1.43 ± 0.94	*0.66*	0.74 ± 0.27	0.84 ± 0.30	***0.039***
LDL (mmol/L)	4.08 ± 0.8	4.27 ± 0.71	*0.14*	2.93 ± 0.59	2.99 ± 0.57	*0.52*
NonHDL (mmol/L)	4.78 ± 0.9	4.92 ± 0.8	*0.33*	3.28 ± 0.64	3.38 ± 0.65	*0.34*
CRP (mg/L)	2.07 ± 2.29	1.73 ± 2.22	*0.23*	1.34 ± 2.16	1.51 ± 2.51	*0.58*
IL-6 (pg/ml)	2.03 ± 1.33	1.72 ± 1.28	*0.07*	1.74 ± 1.46	1.82 ± 1.92	*0.84*
BL dia (mm)	3.76 ± 0.74	3.66 ± 0.62	*0.37*	3.51 ± 0.61	3.61 ± 0.64	*0.34*
$FMD_{\%}$ (%)	5.52 ± 2.93	5.24 ± 3.14	*0.59*	5.39 ± 2.72	5.71 ± 3.56	*0.57*
FMD_{ABS} (mm)	0.20 ± 0.09	0.19 ± 0.1	*0.52*	0.18 ± 0.09	0.2 ± 0.11	*0.40*
RH % (%)	587 ± 269	594 ± 221	*0.87*	742 ± 260	681 ± 269	*0.19*

Table 3.33: Risk factor for participants with and without depression or anxiety by presence or absence of dyslipidaemia.

Comparisons were then made to see whether there were differences in FMD between the groups adjusting for age and gender and brachial artery baseline diameter and RH%. There were no relationships between endothelial function and dyslipidaemia and chronic stress. Further adjustments for each individual risk factor in addition to age, gender, baseline diameter and RH% did not demonstrate any significant relationships (table3.34).

	$FMD_{\%}$		FMD_{ABS}	
	F	**p**	**F**	**p**
age & gender	0.62	*0.60*	0.52	*0.67*
bldia & RH%	0.55	*0.65*	0.38	*0.77*
bldia, RH%, age & gender	0.83	*0.48*	0.67	*0.57*
Waist (cm)	0.58	*0.63*	0.38	*0.77*
Waist Hip	0.62	*0.60*	0.46	*0.71*
BMI (kg/m²)	0.69	*0.56*	0.48	*0.69*
SBP (mmHg)	0.74	*0.53*	0.56	*0.64*
DBP (mmHg)	0.62	*0.60*	0.48	*0.70*
HR (bpm)	0.47	*0.71*	0.36	*0.78*
Glucose (mmol/L)	0.87	*0.46*	0.70	*0.55*
CRP (mg/L)	0.66	*0.57*	0.48	*0.69*
IL-6 (pg/ml)	0.67	*0.57*	0.50	*0.68*

Table 3.34: Association between depression and or anxiety and $FMD_{\%}$ and depression and or anxiety and FMD_{ABS} with and without dyslipidaemia, adjusted for baseline brachial artery diameter, reactive hyperaemia, age, gender and each individual risk factor.

3.5 Discussion

The novel finding of this chapter was that dyslipidaemia influenced the association between chronic stress and IMT in women. Those women with dyslipidaemia and depression or anxiety had the greatest IMT even after adjustment for other potential confounding risk factors. It was also found that women who had both depression and anxiety had greater IMT than those with neither depression nor anxiety.

In addition, DC was found to be greater in women with regard to both individual measures of chronic stress and those with both depression and anxiety but the relationship was not unchanged by dyslipidaemia.

Men with anxiety had lower DC than those men without anxiety, however once heart rate was included in the adjustments this relationship was no longer significant. Those men with dyslipidaemia had greater IMT than those with normal lipids but this was irrespective of their chronic stress status.

Interestingly, there were no significant differences in endothelial function in those with depression and or anxiety, regardless of the presence or absence of dyslipidaemia.

3.5.1 Gender differences in the associations of depression and anxiety with IMT and DC

The initial finding within the whole cohort, whereby no difference was found in IMT between those with and without depression, agrees with previous work; however, other studies have found increased IMT in those with depression[262, 263, 274, 275]. On dividing the cohort by gender, the finding of increased IMT in women with depression does fit with some previous studies. Lee et al found in a middle aged Korean cohort that females with depressive symptoms had greater IMT than those without symptoms but there was no difference between males with and without symptoms of depression[271]. In contrast, Chirinos et al only found an association between IMT and depression in South American Hispanic males but not females[270]. The reasons for these different findings are not fully understood. It may be due to women losing the protective effect of oestrogen as they become postmenopausal. Methodological differences between studies may also be partially responsible as there is substantial heterogeneity within the literature which can make comparisons difficult. For example, Lee et al and Chirinos et al used different questionnaires to assess depression symptoms and Lee et al included both the common carotid and the bifurcation in their cIMT measurements, whilst Chirinos et al used just the common carotid and did not avoid plaque if it was in the area of measurement[270, 271].

The finding of greater arterial stiffness in the control women in comparison to those with anxiety or depression was surprising. For the men, the finding of decreased DC in those with anxiety was more expected, although this relationship was no longer apparent when dividing the groups to look at co morbidity and the individual traits. There is limited work looking at the relationship between arterial stiffness and depression or anxiety. Tiemeier et al found that participants with lower carotid DC and greater carotid to femoral pulse wave velocity were more likely to have depressive symptoms[286]. Seldenrijk et al found an association between increased heart rate normalised central augmentation index and current depressive and /or anxiety disorder, but did not find any relationship with carotid DC[287]. These findings both differ from my study, although neither looked at differences within genders and Seldenrijk et al's participants were part of a cohort specifically selected

for having depressive or anxiety disorders rather than a cohort of the general population as in my study[287].

3.5.2 Dyslipidaemia

The novel finding that those women with dyslipidaemia and depression or anxiety had greater IMT was interesting. It has previously been shown that people with depression and/or anxiety have increased lipid levels and have increased risk of cardiovascular disease and events, in both the healthy population and those with pre-existing coronary disease[204, 211, 252, 253]. The finding in this study, of increased IMT in older women (largely post-menopausal) with high lipid levels and depression or anxiety symptoms, highlights a group that are potentially at greater risk of future cardiovascular events. This finding may have an impact on cardiovascular risk stratification of these women. In addition it may help inform whether or not lipid lowering therapy should be initiated which can reduce the progression of atherosclerotic disease or whether they should be given greater encouragement to make lifestyle changes which are beneficial to cardiovascular health. The finding that DC was greatest in those with depression and or anxiety irrespective of their lipid profile is interesting but does potentially reflect the lesser effect of lipid levels on DC rather than IMT.

There is little previous work looking at the impact of specific cardiovascular risk factors on the relationship between depression and anxiety and measures of subclinical vascular disease. In a study of police officers, Violanti et al found a positive association between depressive symptoms and IMT but only in those officers without hypertension following adjustment for other cardiovascular risk factors[268]. Wagner et al found that the presence of type 2 diabetes had no impact on the association between lifetime history of depression and impaired endothelial function[359]. The finding by Wagner et al does fit with the lack of association between dyslipidaemia and endothelial function within this analysis, but neither were any relationships between depression or anxiety symptoms found in my study. However, Wagner's study only included those who had a life time history of major depression disorder and excluded subjects with a depressive disorder within the previous 6 months of the study, whereas the participants in my study mostly had current symptoms of depression or anxiety; this may account in part, for the difference in findings.

3.5.3 Endothelial function

It was surprising that the previous finding from the Whitehall II study of a difference in FMD in those with depressive symptoms and endothelial function was not replicated in this analysis, although the lack of difference in endothelial function between those with and without anxiety did agree with the previous study[284]. This difference in the study findings, may, in part be due to the different approaches to categorisation of presence of depression. Hemingway et al took the top quartile of the general GHQ, whereas I took those with either a result of 4 or more on the GHQ, 16 plus on the CES-D questionnaire, those on antidepressant medication or had previously been diagnosed with depression. The participants in this analysis were now older and those on blood pressure and cholesterol lowering medications (which can improve endothelial function) were excluded. This may have excluded some participants with higher depression scores who may have accounted, at least in part, for differences in the previous study. A number of other studies have identified an association between depression/anxiety and endothelial function[293, 294, 296-298, 300]. Many of these studies have been in patients who already have cardiovascular disease and depression/depressive symptoms or in people with major depression[293, 294, 297, 300] . Some studies have shown no association between endothelial function and depression and/or anxiety[302-304, 360]. Schott et al, in a study population not dissimilar in age and gender to the Whitehall II cohort, found that FMD was lower in males with higher hostility and females with greater anger suppression, both traits that can be viewed as negative affect, but there was no association between FMD and depression or anxiety symptoms in their cohort[303].

3.5.4 Potential mechanisms

A number of potential mechanisms have been suggested for the relationship between the increased risk of cardiovascular disease and depression/anxiety. These include increased hypothalamic-pituitary-adrenal axis activity (HPA), autonomic nervous system dysfunction, inflammatory processes and altered platelet function.

HPA axis dysregulation has been associated with depression and anxiety and can lead to elevated levels of cortisol and impaired regulatory feedback. Increased cortisol, through short-term oral supplementation, impairs endothelial function; inhibition of adrenal 11-hydroxylase, and consequently cortisol synthesis, by metyrapone improves flow-mediated

dilatation in patients with treated depression, suggesting a role in the development of cardiovascular disease[361, 362].

Inflammatory processes play a major role in all stages of cardiovascular disease from initiation through to the destabilisation of plaques triggering an event. Endothelial dysfunction, increased IMT and arterial stiffness are all associated with inflammation as are depression and anxiety [363-367]. This could therefore provide one potential pathway for the increased IMT in women with depression shown in this study. However, the effects of CRP and IL-6 were adjusted for in this analysis so other mechanisms are likely to be more prominent.

Lifestyle behaviours may provide an additional factor in the relationship between increased IMT and depression. Cigarette smoking, lower physical activity and poor diet with increased fat intake are all associated with depression, anxiety and increased cardiovascular risk [260, 351]. These circumstances may help create a favourable environment for the development/exacerbation of atherosclerosis.

Dyslipidaemia is associated with worse cardiovascular outcome, increased IMT and endothelial dysfunction[89, 90, 352]. It has been shown that patients with depression or anxiety do have increased lipid levels and increased risk of cardiovascular disease[204, 211, 253]. Van Reedt Dortland et al demonstrated that patients with more severe symptoms of depression and anxiety were more prone to dyslipidaemia alongside obesity[252]. It is worth noting that in my analysis those men and women with dyslipidaemia and depression and/or anxiety had the greatest waist/hip ratios, but the relationship between dyslipidaemia and IMT remained after adjustment for obesity measures. The combination of depression and dyslipidaemia resulting in the greatest IMT in this cohort may be due to a mixture of lifestyle factors, and biological mechanisms such as inflammation.

3.5.5 Limitations

All the measures of subclinical vascular disease have their limitations. A lack of standardization of methodology for both FMD and IMT in particular, can make comparisons between studies difficult. In the WHII study IMT was assessed using callipers, however, edge detection software has now become the preferred method of for measuring IMT as it is less operator dependent. Although those participants on blood

pressure and lipid lowering medications were excluded from the analysis other drugs may influence the measurements as can environmental factors, exercise, food and stress. Although actions were taken to limit and adjust for these influences where possible, alongside the intrinsic biological variability they may have an influence on the findings. DC only provides a measure of local arterial stiffness and not large artery stiffness for which carotid to femoral pulse wave velocity is considered the gold standard method. Unfortunately, these data were not available at this phase of the WHII study. However, the two measures are related and carotid artery DC can still give valuable information about subclinical vascular disease and its relationship with chronic stress.

The depression group is a composite of participants who have been classified as having clinically significant depressive symptoms by meeting the cut off point on either one of two validated questionnaires investigating depressive symptoms, have previously been diagnosed as depressed or taking anti-depressant medication. Therefore, there is likely to be a wide range of severity of symptoms from mild depression to severe clinical depression which may have altered some of the findings with the vascular measures. Additionally, some of the participants may not have been currently undergoing a depressive episode. This study also did not look at whether participants have had recurrent or just single episodes of depression. These divisions may have identified more relationships within the data.

This composite group may also explain why the number of participants identified with depressive symptoms was relatively high in comparison to other studies such as Lee et al who used just the CES-D who identified 18.7% of Korean women and 7.8% of Korean men as having depressive symptoms[271]. However, in another study in the Whitehall II population, 27% of participants were identified as having a common mental disorder identified using the GHQ, which is similar to the 25% of participants having been identified with depressive symptoms in my analysis[368]. Additionally the prevalence of depression in the FMD subgroup was the same as an analysis from an earlier phase of the Whitehall II study[284].

Similarly using the top quartile of the GHQ for identifying anxiety means that those identified as having anxiety symptoms would not have them as severely as if the cut off of 8 plus had been used. This would have reduced the number of participants which may have had implications for the power of the analyses. Prevalence of anxiety in FMD study is

greater than Whitehall II previously but participants who were taking anxiolytic medication were included in the anxiety group in this analysis[284].

Similarly due to issues surrounding power of the analysis particularly in the FMD cohort, anxiety disorder was taken as those within the top quartile rather than achieving 8 or more on the GHQ anxiety scale. This may mean that those classified with anxiety within the cohort were not as severely anxious as if the normal cut off point had been used.

As this was a relatively healthy population the dyslipidaemia definition was quite broad, therefore participants meeting the criteria did not necessarily have severe dyslipidaemia which may have impacted on the findings. However, despite this, dyslipidaemia did still influence the association between chronic stress and IMT in women. Replication of this analysis in a population with more severe dyslipidaemia would be useful to help confirm whether this finding is true or due to chance and may also identify differences with the other measures of subclinical vascular disease. Also the classification did not distinguish between whether participants had raised LDL or high triglycerides with low HDL. It was therefore not possible to investigate whether these different pathologies/classifications exerted different influences on the relationships between the sub clinical vascular measures and depression and/or anxiety.

Due to a lack of numbers it was not possible to look at dyslipidaemia and the different influences of depression and anxiety separately within both cohorts. Nor was it possible to investigate the influence of gender within the FMD cohort. The smaller numbers with FMD data may have reduced the power of the analysis which therefore may mean that it was not possible to see differences that would be present in a larger population.

Finally, the statistically significant differences in IMT that were seen between the groups in women are small and therefore may have limited clinical significance although they potentially indicate an acceleration of the atherosclerotic process within the artery wall. Follow up of these women would be useful to see if they are more likely to develop CVD than women without dyslipidaemia and chronic stress. Furthermore this was a cross-sectional analysis to investigate the biological relationships intervention studies and prospective studies looking at prognostic outcomes would be required.

3.5.6 Conclusion

In conclusion, it has been shown that women with depression and anxiety have increased IMT, which was driven by the presence of dyslipidaemia. Neither depression nor anxiety had a significant effect on subclinical vascular measures in men.

Chapter 4. Acute mental stress, lipids and endothelial function in an epidemiological cohort

4.1 Introduction

In the previous chapter I have looked at the impact of chronic stress on endothelial function and found that there were no differences between those with and without depression and /or anxiety and indices of FMD. In this chapter I wish to explore the effects of acute mental stress on future vascular function to see whether these may have a greater impact than chronic stress.

As previously discussed, stress has been shown to be an important major risk factor for cardiovascular disease[26]. In addition to chronic stress, acute stressors, such as earthquakes, major sporting events or high pressure deadlines have been shown to trigger cardiovascular events, although there has been some inconsistency in the results of these studies[216-218, 220-222, 225]. Laboratory studies have demonstrated increases in haemodynamic and inflammatory markers in response to acute mental stress challenges[369-371]. Inflammatory processes play a key role in the development of atherosclerotic lesions in the vessel wall for which decreased endothelial function is an early signal[25]. In keeping with this potential link, acute inflammation induced by injection of the typhoid vaccination and acute mental stress both cause transient endothelial dysfunction[325, 364]. Therefore, the nature and magnitude of the inflammatory response to acute mental stress could be a potential determinant of future endothelial dysfunction and cardiovascular events.

Dyslipidaemia is associated with a worse cardiovascular outcome, impaired endothelial function and an increased inflammatory profile[67, 89, 372]. The relationship between lipids and stress responses both within the context of changes in response to a mental stress stimuli and the influence of an unfavourable lipid profile are not yet fully understood. It is not yet clear whether the stress response contributes to dyslipidaemia or if an unfavourable lipid profile increases the inflammatory and vascular responses to stress. Lipid levels have been demonstrated to increase in response to a stress challenge[373]. Additionally, Steptoe et al have also shown that 56% of participants whose total cholesterol during stress was in the

top tertile had a clinically elevated total cholesterol of greater than or equal to 6.2 mmol/L three years later compared with just 16% from the lowest tertile[373].

There are a limited number of small studies looking at the immediate response to an acute mental stress challenge on endothelial function in cardiovascular risk factor groups and the results have been mixed. Ghiadoni et al found no change in FMD following a stress task in type 2 diabetics, whilst Cardillo et al saw no change in NO-dependent dilatation as assessed by forearm plethysmography in subjects with hypercholesterolaemia but did see a reduction in participants with hypertension[325, 374]. However, the longer term effects of the inflammatory responses to mental stress both alone and in combination with a cardiovascular risk factor on endothelial function are unknown.

In the Whitehall II psychobiology substudy, inflammatory responses to acute mental stress have been associated with both increased local arterial stiffness (a measure of structural arteriosclerotic/atherosclerotic changes) and larger increases in ambulatory systolic blood pressure when these were assessed three years later[324, 369]. Thus raising the question of whether there are relationships between inflammatory responses to stress and endothelial function, as assessed by flow-mediated dilatation, a more dynamic measure of arterial pathophysiology than arterial stiffness. This would therefore add additional valuable information to the current knowledge about the relationship between acute mental stress and sub clinical vascular disease. Although the participants in this cohort were purposefully selected from among those without overt major cardiovascular risk factors, it provides the opportunity to investigate the influences of high normal lipids, which have been shown to infer increased lifetime risk of cardiovascular disease, on the inflammatory responses to stress and future endothelial function[375].

4.2 Aims

Aim: To investigate the effects of the inflammatory response to acute mental stress on endothelial function and the influence of cardiovascular risk factors

Primary Hypothesis: Those participants with a greater inflammatory response to an acute mental stress challenge have lower FMD when assessed 3 years later

Secondary hypothesis 1: Having a cardiovascular risk factor influences the inflammatory response to acute mental stress.

Secondary hypothesis 2: The size of the inflammatory response to an acute mental stress in the context of dyslipidaemia is associated with a lower FMD 3 years later

4.3 Methods

4.3.1 Participants

Participants were from the Whitehall II epidemiological cohort who took part in a psychobiology substudy in 1999-2000 as described in Chapter 2. Participants were aged 45-59 years, of white European origin, lived in the London area, were in full time employment and did not have coronary heart disease or hypertension. Grade of employment was used to identify socio-economic status (SES) and participants were selected on the basis of this to ensure wide variation in status within the cohort. Three years after the acute mental stress testing 158 participants underwent an assessment of endothelial function at Phase 7 of the Whitehall study.

4.3.2 Acute mental stress testing

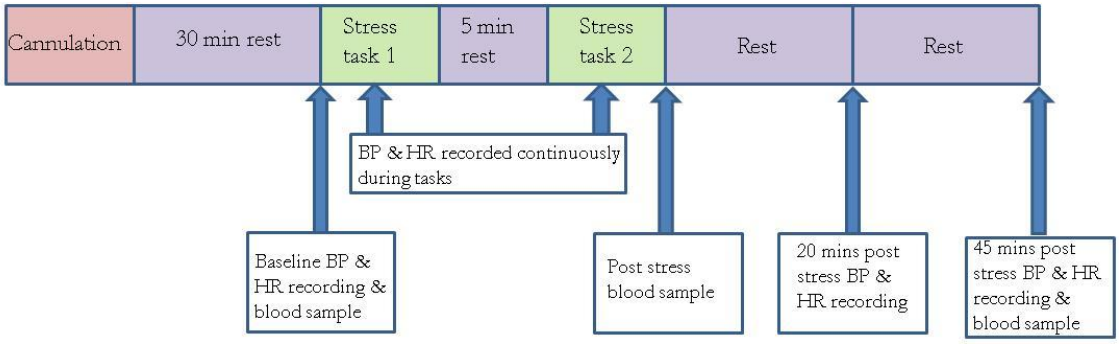

Figure 4.1: Protocol for acute mental stress testing

Studies took place in either the morning or afternoon in a light and temperature controlled laboratory. Participants were requested to have not drunk alcohol or exercised on the evening before or on the day of testing and to have not consumed caffeine or smoked for the 2 hours prior to the study. Anthropometric measurements were made as described in Chapter 2. Blood pressure and heart rate were continuously monitored using a Portapress-2 (Finapress Medical Systems, Amsterdam, NL). After the insertion of a venous cannula for blood sample collection, participants rested for 30 minutes. Baseline blood pressure and heart rate were recorded for the last 5 minutes of this rest period and a baseline blood sample was drawn. Following this two stress tasks (computerized colour-word interference task and mirror tracing) were administered in random order with a 5 minute inter-task interval. The tasks each lasted for 5 minutes during which blood pressure and heart rate were continuously monitored. A second blood sample was taken immediately after the second task. Participants then rested quietly reading or watching wildlife videos. Two 5

minute post-stress blood pressure and heart rate recordings were made at 15-20 minutes and 40-45 minutes. A third blood sample was taken after 45 minutes (figure 4.1).

Blood samples were processed as described in Chapter 2. Lipids, glucose and C-reactive protein (CRP) were assessed at baseline. Fibrinogen (Fbg) was measured at all three time points whilst IL-6 and TNFα were assessed at baseline and 45 minutes post-stress.

4.3.3 Flow-mediated dilatation

Having rested supine for 10 mins, participants' right brachial artery was imaged using ultrasound; a Doppler cursor was positioned in the centre of the artery to assess blood flow. Baseline diameter and blood flow measures were recorded for 1 minute. Following this a blood pressure cuff that had previously been positioned around the forearm was inflated to 300 mmHg for 5 minutes to occlude the artery and induce reactive hyperaemia on release of the cuff. Recording carried on for a further 5 minutes. Images were saved to a computer for later offline analysis as described in Chapter 2.

4.3.4 Phase 7 measurements

A fasting blood sample was taken at the clinic visit for phase 7. From this sample glucose, total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (Trigs) were measured. Samples were assessed as described in Chapter 2. Height, weight, hip and waist measurements were also taken at this visit as described in Chapter 2.

4.3.5 Statistical analysis

The two task systolic blood pressure (SBP) recordings were averaged to give one stress response. Diastolic blood pressure (DBP) and heart rate (HR) task recordings were treated in the same way. Stress reactivity was calculated as the change between baseline level and stress sample. Data was checked for normality and non-normally distributed variables were transformed using ln transformation.

4.3.5.1 Acute mental stress and endothelial function

Analysis was carried out on the whole cohort and then separated by gender. Five participants were excluded from the analyses as they had started taking lipid or blood

pressure medications since participating in the stress testing study. Differences in baseline characteristics between the two genders were analysed using either an independent samples t-test or the independent samples Mann Whitney U test. Chi square was used to assess for differences in smoking and SES status between the two groups. Associations between endothelial function and baseline haemodynamic, inflammatory and lipid variables were investigated using Pearson correlations, partial correlations with adjustment for age, gender, reactive hyperaemia ($RH_{\%}$) and baseline brachial artery diameter, were used to account for differences between males and females.

Responses to stress testing were assessed using repeated measures analysis of variance with trial as the within-subject factor and Greenhouse-Geisser adjustments employed where appropriate. Post hoc comparisons were made with Tukey's least significant differences (LSD) test. To see whether there was a difference in stress responses by gender, repeated measures analysis of variance was used with the addition of gender as a between-subject factor. Results are presented as F (degrees of freedom of the effect of the model, degrees of freedom for the residuals of the model). Correlations between responses to stress and endothelial function were assessed using Pearson correlations.

To compare endothelial function between those with a high response to stress versus those with a low or no response to stress for haemodynamic and inflammatory variables, the cohort were divided into the top and bottom 40% responders for each variable as per Kunz-Ebrecht et al[376]. This division meant those participants who bordered the two categories were not included allowing for a comparison of those with a more distinct difference in their respective responses between the two groups. Differences in $FMD_{\%}$ and FMD_{ABS} between the high and low stress responders for each variable were assessed using one-way analysis of variance (ANOVA) and analysis of co-variance (ANCOVA) with adjustments for baseline stress variable, baseline brachial artery diameter and $RH_{\%}$.

Presence of associations between endothelial function and inflammatory responses were tested for using multiple linear regression, regressing $FMD_{\%}$ or FMD_{ABS} on stress-induced changes in Fbg, IL-6 and TNFα. Additional models incorporating other relevant co-variables were run. These included baseline level of the inflammatory marker, baseline brachial artery diameter, $RH_{\%}$, age, gender, BMI, waist/hip ratio, systolic and diastolic blood pressure, HDL and LDL cholesterol, glucose, CRP socio-economic status (employment grade) and smoking. Two sets of models were run using these co-variables measured firstly at the time of mental stress testing and secondly at phase 7 of the

Whitehall II study. Results are presented as unstandardised regression coefficients (β) with standard error of the mean (SEM).

4.3.5.2 Dyslipidaemia and responses to acute mental stress:

Participants were classified as having dyslipidaemia if they met any of the following lipid parameters: total cholesterol≥6; triglycerides ≥1.7; HDL <1; LDL≥ 4 (mmol/L).

Analyses were carried out as for the whole cohort. To look at the influence of dyslipidaemia on the responses to mental stress testing, the variable presence of dyslipidaemia replaced gender as the between subject factor in the repeated measures ANOVA.

To further investigate whether the inflammatory response to stress might influence endothelial function for participants with and without dyslipidaemia the stress change for each inflammatory variable was divided into high and low inflammatory responses to stress by the median. These two groups were each further divided by the presence or absence of dyslipidaemia creating four groups; dyslipidaemia & high inflammatory response; dyslipidaemia & low inflammatory response; normal lipids & high inflammatory response and normal lipids & low inflammatory response. These groups were created for each inflammatory variable. ANOVA and ANCOVA were used with adjustments for baseline diameter, $RH_{\%}$ and baseline inflammatory variable to test whether there were differences within the groups with Bonferroni post hoc analyses.

4.3.5.3 Reactive hyperaemia and responses to acute mental stress

Associations between $RH_{\%}$ and cardiovascular risk factors and the haemodynamic and inflammatory responses to stress were investigated using Pearson correlations. Comparisons with $RH_{\%}$ were made between low and high stress responders using ANOVA and ANCOVA as described above. Adjustments were made for baseline velocity time integral (VTI) and baseline stress variable. Finally the influence of dyslipidaemia and inflammatory response on $RH_{\%}$ was investigated having used the same four groupings as described in 4.3.5.2 using ANCOVA with the same adjustments as for the low and high stress response analysis.

4.4 Results

4.4.1 Acute mental stress and endothelial function

The key finding in this section was that a larger fibrinogen response 45 minutes post stress was associated with poorer endothelial function assessed 3 years later. There was no association between IL-6 and TNFα responses to stress and endothelial function.

Table 4.1 shows the participant characteristics of the acute mental stress cohort with endothelial function data as a whole and divided by gender. It can be seen that males were older with higher blood pressure and lipid profile except for HDL which was lower than females. $FMD_{\%}$ was significantly lower in males but they had a larger baseline brachial artery diameter. FMD_{ABS} was higher in women but not significantly.

Characteristic	All (n=153)	Male (n=84)	Female (n=69)	p
Age (yrs)	52 ± 3	53 ± 3	52 ± 3	***0.021***
Smoker (Y)	9 (5.9%)	6 (7.1%)	3 (4.2%)	*0.47*
SES				
Higher	60 (39.2%)	34 (40.5%)	26 (37.7%)	*0.84*
Intermediate	50 (32.7%)	28 (33.3%)	22 (31.9%)	
Lower	43 (28.1%)	22 (26.2%)	21 (30.4%)	
BMI (kg/m²)	25.23 ± 3.52	25.36 ± 3.25	25.08 ± 3.85	*0.63*
Waist/hip ratio	0.84 ± 0.09	0.90 ± 0.07	0.78 ±0.06	***<0.001***
SBP(mmHg)	114 ± 12	118 ± 11	109 ± 12	***<0.001***
DBP(mmHg)	69 ± 9	71 ± 9	67 ± 9	***0.005***
HR(bpm)	64 ± 8	64 ± 9	66 ± 8	*0.14*
TC(mmol/L)	5.38 ± 0.87	5.42 ± 0.84	5.32 ± 0.91	*0.47*
Trigs (mmol/L)	1.34 ± 0.71	1.45 ± 0.65	1.21 ± 0.76	***0.003***
HDL (mmol/L)	1.57 ± 0.39	1.43 ± 0.30	1.74 ± 0.41	***<0.001***
LDL (mmol/L)	3.20 ± 0.81	3.34 ± 0.79	3.02 ± 0.82	***0.018***
Total/HDL	3.65 ± 1.13	3.96 ± 1.01	3.24 ± 1.13	***<0.001***
Glucose (mmol/L)	5.30 ± 0.79	5.34 ± 0.72	5.25 ± 0.88	*0.54*
C-reactive protein (mg/L)	1.02 ± 1.29	0.92 ± 0.97	1.13 ± 1.59	*0.8*
BL dia (mm)	3.60 ± 0.71	4.02 ± 0.61	3.08 ± 0.41	***<0.001***
FMD_{ABS} (mm)	0.19 ± 0.10	0.19 ± 0.10	0.20 ± 0.10	*0.81*
$FMD_{\%}$ (%)	5.70 ± 3.39	5.03 ± 2.96	6.51 ± 3.69	***0.012***
RH% (%)	658 ± 260	648 ± 249	665 ± 270	*0.61*

Table 4.1: Participant characteristics for the whole acute mental stress and endothelial function cohort and by gender. P value is for differences between genders. Mean ± SD or n (%) Data is untransformed

	Pearson Correlation						Partial#	
	All		Male		Female		All	
Risk factor	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Age (yrs)	-0.17	***0.032***	-0.10	*0.36*	-0.19	*0.12*		
BMI (kg/m²)	-0.05	*0.55*	0.04	*0.72*	-0.12	*0.33*	0.06	*0.46*
Waist/hip ratio	-0.14	*0.09*	0.05	*0.66*	-0.09	*0.48*	0.06	*0.49*
SBP (mmHg)	0.04	*0.61*	0.17	*0.12*	0.07	*0.56*	0.12	*0.15*
DBP (mmHg)	0.12	*0.14*	0.19	*0.08*	0.15	*0.22*	0.22	***0.011***
HR (bpm)	0.22	***0.006***	0.29	***0.007***	0.09	*0.47*	0.24	***0.006***
TC (mmol/L)	-0.06	*0.47*	0.03	*0.78*	-0.13	*0.28*	0.06	*0.48*
Trigs (mmol/L)	-0.04	*0.59*	-0.01	*0.90*	0.00	*0.97*	-0.01	*0.90*
HDL (mmol/L)	0.08	*0.34*	0.09	*0.44*	-0.07	*0.57*	0.04	*0.66*
LDL (mmol/L)	-0.09	*0.28*	0.00	*1.00*	-0.11	*0.36*	0.05	*0.53*
Total/HDL	-0.10	*0.21*	-0.05	*0.67*	-0.03	*0.78*	0.01	*0.91*
Glucose (mmol/L)	-0.05	*0.57*	-0.05	*0.68*	-0.02	*0.88*	0.01	*0.93*
CRP (mg/L)	-0.04	*0.61*	0.17	*0.13*	-0.24	*0.05*	-0.04	*0.64*
BL dia (mm)	-0.45	***<0.001***	-0.50	***<0.001***	-0.36	***0.002***		
FMD_{ABS} (mm)	0.95	***<0.001***	0.96	***<0.001***	0.94	***<0.001***	0.94	***<0.001***
RH % (%)	0.05	*0.55*	-0.05	*0.64*	0.14	*0.27*		

Table 4.2: Pearson correlations between $FMD_{\%}$ and participant characteristics/risk factors in the whole cohort and separately by gender plus partial correlations in the whole cohort adjusted for age, gender, reactive hyperaemia and brachial artery baseline diameter.

$FMD_{\%}$ was positively associated with heart rate and negatively associated with age and brachial artery baseline diameter in the whole cohort (table 4.2). When the population was split by gender there was no association with age and the relationship with heart rate was only present in males. The same associations were present with FMD_{ABS} (table 4.3), there was a borderline negative association with waist hip ratio but this was not present when the cohort was divided by gender.

Partial correlations between FMD variables and cardiovascular risk factors with adjustment for age, gender, reactive hyperaemia and brachial artery diameter showed there to be significant positive associations with diastolic blood pressure and heart rate for both $FMD_{\%}$ and FMS_{ABS} (tables 4.2 & 4.3).

	Pearson Correlation						Partial#	
	All		Male		Female		All	
Risk factor	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Age (yrs)	-0.18	***0.029***	-0.04	*0.69*	-0.24	*0.05*		
BMI (kg/m²)	-0.06	*0.48*	0.00	*1.00*	-0.09	*0.45*	0.04	*0.64*
Waist/hip ratio	-0.16	*0.05*	0.02	*0.84*	-0.05	*0.66*	0.05	*0.60*
SBP (mmHg)	0.02	*0.77*	0.14	*0.20*	0.08	*0.50*	0.11	*0.19*
DBP (mmHg)	0.08	*0.35*	0.15	*0.17*	0.12	*0.34*	0.18	***0.037***
HR (bpm)	0.23	***0.006***	0.27	***0.012***	0.13	*0.28*	0.23	***0.007***
TC (mmol/L)	-0.07	*0.41*	0.03	*0.76*	-0.14	*0.26*	0.05	*0.56*
Trigs (mmol/L)	-0.07	*0.37*	-0.05	*0.64*	-0.01	*0.93*	-0.04	*0.65*
HDL (mmol/L)	0.07	*0.38*	0.10	*0.36*	-0.10	*0.40*	0.01	*0.91*
LDL (mmol/L)	-0.08	*0.32*	0.01	*0.90*	-0.10	*0.43*	0.07	*0.45*
Total/HDL	-0.10	*0.20*	-0.06	*0.60*	-0.01	*0.92*	0.02	*0.79*
Glucose (mmol/L)	-0.08	*0.33*	-0.09	*0.41*	-0.04	*0.73*	-0.03	*0.73*
CRP (mg/L)	-0.05	*0.59*	0.15	*0.17*	-0.21	*0.10*	-0.05	*0.59*
BL dia (mm)	-0.45	***<0.001***	-0.49	***<0.001***	-0.35	***0.003***		
$FMD_{\%}$ (%)	0.95	***<0.001***	0.96	***<0.001***	0.94	***<0.001***	0.94	***<0.001***
RH % (%)	0.02	*0.81*	-0.08	*0.48*	0.09	*0.48*		

Table 4.3: Pearson correlations between FMD_{ABS} and participant characteristics/ risk factors in the whole cohort and separately by gender, plus partial correlations in the whole cohort adjusted for age, gender, reactive hyperaemia and brachial artery baseline diameter.

Cardiovascular and inflammatory responses to acute mental stress challenges are shown in table 4.4. There were significant responses to the stress challenge for all variables except TNFα. Post hoc tests showed there to be significant increases in blood pressure, heart rate and Fbg with the stress tasks.

	Baseline	Task	PS_1	PS_2	F	p
SBP (mmHg)	114	138*	119*≠	119*≠	250.3	***<0.001***
DBP (mmHg)	69	83*	73*≠	74*≠	268.3	***<0.001***
HR (bpm)	65	72*	62*≠	63*≠°	234.7	***<0.001***
Fbg (g/L)	2.81	2.87*		2.85*	13.4	***<0.001***
IL-6 (pg/ml)	1.12			1.20*	9.2	***0.003***
TNFα (pg/ml)	2.12			2.18	2.9	*0.09*

Table 4.4: Cardiovascular and biochemical responses to an acute mental stress challenge. Geometric means are shown for IL-6 and TNFα and arithmetic means for all other measures. PS =post stress; * = sig diff from baseline ($p<0.05$); ≠ = sig diff from task mean ($p<0.05$); ° = sig diff from post stress 1($p<0.05$).

Table 4.5 shows the responses to acute mental stress by gender. Fbg was the only inflammatory marker with a significant difference between baseline levels for males and

females (p=0.006). There were significant responses in all variables except for TNFα. However, there was a significantly different response to the stress challenge between the two genders for TNFα (F(1,140) =4.0 p=0.047) with a borderline effect for heart rate (F(2,296) =3.0 p=0.05). TNFα increased in response to the stress challenge in males and decreased in females.

						Trial		Gender interaction	
		Baseline	Task	PS_1	PS_2	F	p	F	p
SBP (mmHg)	M	118	142	122	122	247.4	***<0.001***	2.1	*0.10*
	F	109	132	116	116				
DBP (mmHg)	M	71	84	75	75	267.6	***<0.001***	0.9	*0.41*
	F	67	82	72	72				
HR (bpm)	M	63	70	61	61	240.3	***<0.001***	3.0	*0.05*
	F	66	74	63	64				
Fbg (g/l)	M	2.69	2.75		2.72	13.7	***<0.001***	1.1	*0.33*
	F	2.95	3.02		3.01				
IL-6 (pg/ml)	M	1.08			1.14	9.6	***0.002***	0.6	*0.44*
	F	1.17			1.28				
TNFα (pg/ml)	M	2.19			2.31	2.4	*0.12*	4.0	***0.047***
	F	2.04			2.02				

Table 4.5: Cardiovascular and biochemical responses to an acute mental stress challenge by gender. Geometric means are shown for IL-6 and TNFα with arithmetic means for all other measures. PS= post stress

Table 4.6 shows the correlations between $FMD_{\%}$ and FMD_{ABS} and stress variables for the whole cohort and when divided by gender. Both $FMD_{\%}$ and FMD_{ABS} were positively correlated with baseline heart rate (r= 0.22 p=0.006 & r= 0.23 p=0.006 respectively) and task heart rate (r= 0.21 p=0.009 & r=0.22 p=0.006 respectively). However, once the population were divided by gender only the correlation between baseline heart rate remained for both $FMD_{\%}$ and FMD_{ABS} in males. In addition, there was a positive correlation between FMD_{ABS} and IL-6 response (r=0.24 p=0.035) in males.

	$FMD_{\%}$						FMD_{ABS}					
	All		Male		Female		All		Male		Female	
	r	p	r	p	r	p	r	p	r	p	r	p
SBP bl (mmHg)	0.04	*0.61*	0.17	*0.12*	0.07	*0.56*	0.02	*0.77*	0.14	*0.20*	0.08	*0.50*
SBP tk (mmHg)	0.03	*0.74*	0.07	*0.55*	0.09	*0.45*	0.00	*0.98*	0.07	*0.54*	0.05	*0.67*
SBP ch (mmHg)	-0.01	*0.94*	-0.05	*0.64*	0.07	*0.59*	-0.02	*0.77*	-0.03	*0.81*	0.00	*1.00*
DBP bl (mmHg)	0.12	*0.14*	0.19	*0.08*	0.15	*0.22*	0.08	*0.35*	0.15	*0.17*	0.12	*0.34*
DBP tk (mmHg)	0.12	*0.13*	0.19	*0.09*	0.10	*0.41*	0.08	*0.34*	0.16	*0.14*	0.05	*0.69*
DBP ch (mmHg)	0.03	*0.71*	0.04	*0.69*	-0.03	*0.83*	0.01	*0.86*	0.06	*0.60*	-0.07	*0.58*
HR bl (bpm)	0.22	***0.006***	0.29	***0.007***	0.09	*0.47*	0.23	***0.006***	0.27	***0.012***	0.13	*0.28*
HR tk (bpm)	0.21	***0.009***	0.20	*0.08*	0.16	*0.18*	0.22	***0.006***	0.21	*0.06*	0.17	*0.16*
HR ch (bpm)	0.04	*0.67*	-0.13	*0.25*	0.16	*0.21*	0.05	*0.53*	-0.08	*0.47*	0.12	*0.35*
Fbg bl (g/L)	-0.01	*0.93*	0.09	*0.39*	-0.17	*0.17*	0.02	*0.83*	0.13	*0.26*	-0.14	*0.25*
Fbg tk (g/L)	0.00	*0.95*	0.10	*0.39*	-0.17	*0.16*	0.00	*0.97*	0.13	*0.23*	-0.18	*0.15*
Fbg 45 (g/L)	-0.01	*0.89*	0.09	*0.44*	-0.18	*0.14*	0.00	*0.99*	0.11	*0.31*	-0.17	*0.17*
Fbg ch (g/L)	-0.02	*0.84*	0.00	*0.99*	-0.05	*0.71*	-0.08	*0.35*	0.03	*0.81*	-0.17	*0.18*
Fbg 45 ch (g/L)	-0.01	*0.86*	0.02	*0.85*	-0.10	*0.44*	-0.06	*0.46*	0.01	*0.91*	-0.17	*0.16*
IL-6 bl (pg/ml)	-0.07	*0.41*	-0.05	*0.65*	-0.12	*0.35*	-0.07	*0.39*	-0.10	*0.37*	-0.09	*0.50*
IL-6 45 (pg/ml)	-0.03	*0.77*	0.07	*0.56*	-0.14	*0.28*	-0.01	*0.94*	0.06	*0.60*	-0.10	*0.45*
IL-6 ch (pg/ml)	0.08	*0.32*	0.17	*0.14*	-0.08	*0.54*	0.13	*0.13*	0.24	***0.035***	-0.05	*0.71*
TNFα bl (pg/ml)	0.10	*0.22*	0.09	*0.42*	0.16	*0.20*	0.10	*0.26*	0.12	*0.29*	0.12	*0.35*
TNFα 45 (pg/ml)	0.10	*0.24*	0.08	*0.49*	0.21	*0.10*	0.10	*0.26*	0.11	*0.34*	0.17	*0.17*
TNFα ch (pg/ml)	0.00	*0.99*	-0.02	*0.84*	0.08	*0.51*	0.01	*0.90*	-0.02	*0.90*	0.10	*0.43*

Table 4.6: Pearson correlations for $FMD_{\%}$ and FMD_{ABS} with the cardiovascular and biochemical responses to stress. bl = measurement taken at baseline; tk = measurement taken during or immediately after task; 45 = measurement taken 45 minutes post task & ch = difference between either task or 45 measurement and baseline measurement

When haemodynamic and inflammatory responses to acute mental stress were divided into high and low responders by taking the top and bottom 40% there were no differences between the two groups in $FMD_{\%}$ and FMD_{ABS} (table 4.7). Following adjustment for baseline Fbg, baseline brachial artery diameter and $RH_{\%}$, FMD_{ABS} was significantly lower in those with a high Fbg response at 45 minutes in relation to those with a low response (figure 4.2). There were no differences between high or low responders for the other variables following adjustments.

Stress change variable	Model	$FMD_{\%}$		FMD_{ABS}	
		F	p	F	p
SBP change	Unadjusted	0.37	*0.54*	0.14	*0.71*
	BL SBP	0.28	*0.60*	0.12	*0.73*
	BL dia & RH%	0.37	*0.54*	0.69	*0.41*
	BL dia, RH% & BL SBP	1.28	*0.26*	1.66	*0.20*
DBP change	Unadjusted	0.49	*0.48*	0.24	*0.62*
	BL DBP	0.29	*0.59*	0.12	*0.73*
	BL dia & RH%	0.01	*0.93*	0.04	*0.85*
	BL dia, RH% & BL DBP	0.13	*0.72*	0.21	*0.65*
HR change	Unadjusted	0.01	*0.91*	0.02	*0.89*
	BL HR	0.02	*0.88*	0.17	*0.68*
	BL dia & RH%	0.44	*0.51*	0.22	*0.64*
	BL dia, RH% & BL HR	0.22	*0.64*	0.07	*0.80*
Fbg change	Unadjusted	1.60	*0.21*	1.12	*0.29*
	BL Fbg	1.55	*0.22*	1.11	*0.29*
	BL dia & RH%	0.32	*0.57*	0.08	*0.78*
	BL dia, RH% & BL Fbg	0.29	*0.59*	0.07	*0.79*
Fbg 45 change	Unadjusted	0.81	*0.37*	2.92	*0.09*
	BL Fbg	0.83	*0.36*	2.91	*0.09*
	BL dia & RH%	3.35	*0.07*	7.69	***0.007***
	BL dia, RH% & BL Fbg	3.53	*0.06*	7.85	***0.006***
IL-6 change	Unadjusted	0.02	*0.89*	0.03	*0.86*
	BL IL-6	0.00	*0.98*	0.00	*0.98*
	BL dia & RH%	0.01	*0.91*	0.00	*0.95*
	BL dia, RH% & BL IL-6	0.02	*0.89*	0.09	*0.76*
TNFα change	Unadjusted	0.04	*0.85*	0.02	*0.90*
	BL TNFα	0.08	*0.78*	0.00	*0.95*
	BL dia & RH%	0.04	*0.85*	0.04	*0.85*
	BL dia, RH% & BL TNFα	0.08	*0.78*	0.01	*0.91*

Table 4.7: Associations between endothelial function ($FMD_{\%}$ & FMD_{ABS}) and haemodynamic and inflammatory responses to acute mental stress grouped into the highest and lowest 40% of responders.

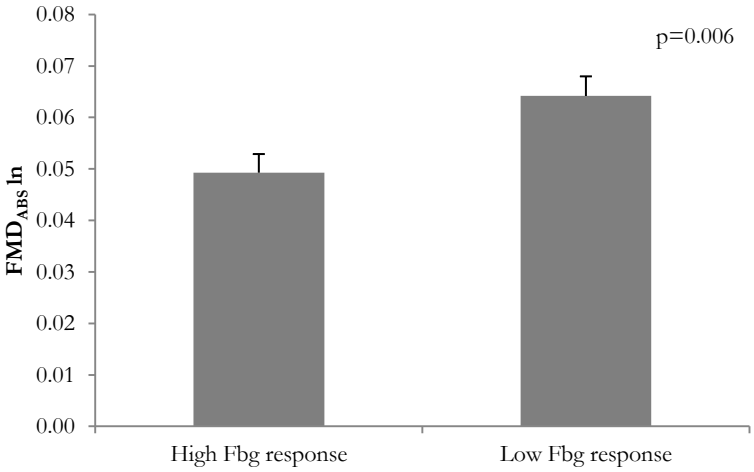

Figure 4.2: FMD_{ABS} calculated from ln transformed baseline and peak brachial artery diameters with SEM bars, by high and low Fbg response to a mental stress challenge at 45 minutes, adjusted for baseline arterial diameter, reactive hyperaemia and baseline Fbg. Data presented as mean±SEM.

Following multiple linear regression, where change in Fbg at 45 minutes was a continuous variable, those participants whose Fbg response remained elevated 45 minutes after the stress task had a lower FMD_{ABS} after controlling for age, gender, baseline diameter, $RH_{\%}$ and baseline Fbg level (table 4.8). This relationship remained following additional adjustment for waist hip ratio, SBP, DBP, HDL, LDL, glucose, BMI, SES and smoking status (table 4.10). There were no relationships between $FMD_{\%}$ and the inflammatory responses to the stress challenge, even after adjustment for risk factors assessed contemporaneously to the mental stress testing (tables 4.8 & 4.9).

When the analyses were repeated using risk factors measured at phase 7 of the Whitehall II study there were no associations with IL-6 or TNFα (data not shown) or Fbg and $FMD_{\%}$ (table 4.11). The association between the Fbg response 45 minutes after stress and FMD_{ABS} was retained having controlled for risk factors assessed at phase 7 (table 4.11).

	Fbg stress change		Fbg 45 change	
Adjustment	**β (SE)**	**p**	**β (SE)**	**p**
$FMD_{\%}$				
Unadjusted	-0.07 (0.36)	*0.84*	-0.07 (0.39)	*0.86*
BL sv	-0.07 (0.37)	*0.84*	-0.07 (0.39)	*0.86*
BL dia & RH%	-0.15 (0.33)	*0.65*	-0.52 (0.37)	*0.16*
BL dia, RH% & BL sv	-0.16 (0.33)	*0.64*	-0.52 (0.37)	*0.16*
BL dia, RH%, BL sv, age & gender	-0.15 (0.32)	*0.64*	-0.48 (0.36)	*0.19*
FMD_{ABS}				
Unadjusted	-0.017 (0.018)	*0.35*	-0.015 (0.02)	*0.46*
BL sv	-0.017 (0.018)	*0.35*	-0.015 (0.02)	*0.45*
BL dia & RH%	-0.022 (0.017)	*0.19*	-0.038 (0.018)	***0.041***
BL dia, RH% & BL sv	-0.022 (0.017)	*0.19*	-0.038 (0.019)	***0.042***
BL dia, RH%, BL sv, age & gender	-0.022 (0.016)	*0.18*	-0.037 (0.018)	***0.048***
	IL-6 change		**TNFα change**	
$FMD_{\%}$				
Unadjusted	0.34 (0.34)	*0.32*	-0.003 (0.4)	*0.99*
BL sv	0.28 (0.36)	*0.43*	0.07 (0.4)	*0.87*
BL dia & RH%	0.18 (0.31)	*0.56*	0.082 (0.35)	*0.82*
BL dia, RH% & BL sv	0.07 (0.32)	*0.83*	0.14 (0.36)	*0.70*
BL dia, RH%, BL sv, age & gender	-0.06 (0.32)	*0.85*	0.01 (0.35)	*0.97*
FMD_{ABS}				
Unadjusted	0.025 (0.017)	*0.13*	0.003 (0.02)	*0.90*
BL sv	0.023 (0.018)	*0.19*	0.006 (0.02)	*0.78*
BL dia & RH%	0.017 (0.015)	*0.27*	0.007 (0.018)	*0.71*
BL dia, RH% & BL sv	0.012 (0.016)	*0.47*	0.009 (0.018)	*0.61*
BL dia, RH%, BL sv, age & gender	0.005 (0.016)	*0.75*	0.006 (0.018)	*0.75*

Table 4.8: Regression analyses examining relationships between endothelial function ($FMD_{\%}$ and FMD_{ABS}) and the inflammatory responses to mental stress. Analyses are adjusted for key predictors of the inflammatory response to mental stress and endothelial function. BL sv = baseline stress variable

	Fbg change		Fbg 45 change		IL-6 change		TNFα change	
Adjustment	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**
SBP (mmHg)	-0.22 (0.33)	*0.51*	-0.5 (0.37)	*0.18*	-0.08 (0.33)	*0.81*	0.013 (0.36)	*0.97*
DBP (mmHg)	-0.27 (0.33)	*0.40*	-0.58 (0.36)	*0.11*	-0.11 (0.32)	*0.74*	-0.024 (0.36)	*0.95*
HR (bpm)	-0.3 (0.33)	*0.36*	-0.41 (0.36)	*0.25*	-0.19 (0.32)	*0.55*	-0.023 (0.36)	*0.95*
TC (mmol/L)	-0.14 (0.33)	*0.66*	-0.46 (0.37)	*0.21*	-0.09 (0.33)	*0.78*	0.007 (0.35)	*0.98*
TG (mmol/L)	-0.14 (0.33)	*0.66*	-0.48 (0.36)	*0.19*	-0.07 (0.33)	*0.83*	0.011 (0.36)	*0.97*
HDL (mmol/L)	-0.14 (0.33)	*0.66*	-0.47 (0.36)	*0.20*	-0.05 (0.33)	*0.88*	0.019 (0.36)	*0.96*
LDL (mmol/L)	-0.14 (0.33)	*0.66*	-0.47 (0.37)	*0.21*	-0.08 (0.33)	*0.81*	0.004 (0.36)	*0.99*
TC/HDL	-0.15 (0.33)	*0.65*	-0.48 (0.36)	*0.19*	-0.07 (0.33)	*0.84*	0.012 (0.36)	*0.97*
Glucose (mmol/L)	-0.22 (0.32)	*0.51*	-0.59 (0.36)	*0.11*	0.03 (0.33)	*0.93*	0.014 (0.36)	*0.97*
CRP (mg/L)	-0.12 (0.33)	*0.73*	-0.5 (0.37)	*0.17*	-0.05 (0.33)	*0.88*	0.081 (0.38)	*0.83*
BMI (kg/m²)	-0.19 (0.33)	*0.57*	-0.47 (0.36)	*0.19*	-0.12 (0.33)	*0.71*	-0.002 (0.36)	*0.99*
Waist/hip ratio	-0.17 (0.33)	*0.60*	-0.47 (0.36)	*0.20*	-0.1 (0.33)	*0.76*	0.013 (0.36)	*0.97*
Model 1	-0.34 (0.33)	*0.30*	-0.69 (0.37)	*0.07*	-0.03 (0.33)	*0.94*	-0.068 (0.37)	*0.85*
Model 2	-0.19 (0.34)	*0.57*	-0.59 (0.38)	*0.12*	-0.07 (0.33)	*0.83*	-0.038 (0.36)	*0.92*
Model 3	-0.26 (0.34)	*0.45*	-0.68 (0.38)	*0.07*	0 .00 (0.34)	*0.99*	-0.08 (0.37)	*0.83*

Table 4.9: Stress responses vs $FMD_{\%}$: Multiple linear regression analyses examining the relationship between changes in inflammatory variables after mental stress with $FMD_{\%}$ adjusted by each risk factor measured contemporaneously to stress testing. In addition to each risk factor each analysis was additionally adjusted for baseline diameter, $RH_{\%}$, baseline inflammatory variable, age & gender. Three models were then created including the following variables; model 1 = baseline inflammatory variable, baseline diameter, $RH_{\%}$, age, gender, waist/hip ratio, SBP, DBP, Glucose, HDL & LDL; model 2= as for model 1 excluding glucose and with the addition of socio-economic status, smoking and BMI; model 3 = as for model 2 including glucose.

	Fbg change		Fbg 45 change		IL-6 change		TNFα change	
	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p
SBP (mmHg)	-0.026 (0.017)	*0.12*	-0.038 (0.019)	***0.044***	0.004 (0.016)	*0.79*	0.005 (0.019)	*0.79*
DBP (mmHg)	-0.028 (0.017)	*0.09*	-0.042 (0.018)	***0.026***	0.004 (0.016)	*0.82*	0.004 (0.019)	*0.85*
HR (bpm)	-0.031 (0.017)	*0.06*	-0.034 (0.018)	*0.07*	-0.001 (0.016)	*0.95*	0.003 (0.018)	*0.88*
TC (mmol/L)	-0.022 (0.016)	*0.19*	-0.036 (0.019)	*0.052*	0.004 (0.016)	*0.80*	0.006 (0.018)	*0.76*
TG (mmol/L)	-0.021 (0.017)	*0.20*	-0.036 (0.018)	*0.052*	0.006 (0.016)	*0.73*	0.006 (0.018)	*0.74*
HDL (mmol/L)	-0.022 (0.016)	*0.18*	-0.036 (0.018)	*0.050*	0.005 (0.016)	*0.74*	0.006 (0.018)	*0.75*
LDL (mmol/L)	-0.022 (0.017)	*0.19*	-0.036 (0.019)	*0.054*	0.004 (0.016)	*0.80*	0.005 (0.018)	*0.77*
TC/HDL	-0.022 (0.016)	*0.18*	-0.037 (0.018)	***0.048***	0.005 (0.016)	*0.76*	0.006 (0.018)	*0.75*
Glucose (mmol/L)	-0.024 (0.017)	*0.15*	-0.04 (0.018)	***0.030***	0.009 (0.017)	*0.60*	0.008 (0.019)	*0.67*
CRP (mg/L)	-0.02 (0.017)	*0.23*	-0.037 (0.019)	***0.046***	0.007 (0.016)	*0.69*	0.005 (0.02)	*0.79*
BMI (kg/m²)	-0.024 (0.017)	*0.16*	-0.036 (0.018)	*0.050*	0.003 (0.017)	*0.84*	0.005 (0.018)	*0.77*
Waist/hip ratio	-0.023 (0.017)	*0.17*	-0.036 (0.018)	*0.050*	0.004 (0.017)	*0.82*	0.006 (0.018)	*0.75*
Model 1	-0.03 (0.017)	*0.08*	-0.046 (0.019)	***0.017***	0.006 (0.017)	*0.71*	0.004 (0.019)	*0.84*
Model 2	-0.023 (0.018)	*0.20*	-0.044 (0.019)	***0.024***	0.006 (0.017)	*0.72*	0.002 (0.019)	*0.90*
Model 3	-0.025 (0.018)	*0.16*	-0.047 (0.019)	***0.016***	0.008 (0.017)	*0.64*	0.002 (0.019)	*0.90*

Table 4.10: Stress responses vs FMD_{ABS}: Multiple linear regression analyses examining the relationship between changes in inflammatory variables after mental stress with FMD_{ABS} adjusted by each risk factor measured contemporaneously to stress testing. In addition to each risk factor, each analysis was additionally adjusted for baseline diameter, $RH_{\%}$, baseline inflammatory variable, age & gender. Three models were then created including the following variables; model 1 = baseline inflammatory variable, baseline diameter, $RH_{\%}$, age, gender, waist/hip ratio, SBP, DBP, Glucose, HDL & LDL; model 2= as for model 1 excluding glucose and with the addition of socio-economic status, smoking and BMI; model 3 = as for model 2 including glucose.

	$FMD_{\%}$				FMD_{ABS}			
	Fbg change		Fbg 45 change		Fbg change		Fbg 45 change	
	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p
SBP (mmHg)	-0.22 (0.33)	*0.50*	-0.47 (0.36)	*0.19*	-0.025 (0.017)	*0.14*	-0.036 (0.018)	*0.053*
DBP (mmHg)	-0.18 (0.32)	*0.57*	-0.5 (0.36)	*0.17*	-0.024 (0.016)	*0.15*	-0.038 (0.018)	***0.040***
HR (bpm)	-0.21 (0.32)	*0.52*	-0.6 (0.36)	*0.10*	-0.026 (0.016)	*0.12*	-0.043 (0.018)	***0.018***
TC (mmol/L)	-0.09 (0.33)	*0.78*	-0.45 (0.37)	*0.22*	-0.018 (0.017)	*0.28*	-0.034 (0.018)	*0.06*
Trigs (mmol/L)	-0.06 (0.34)	*0.86*	-0.4 (0.38)	*0.29*	-0.016 (0.017)	*0.34*	-0.03 (0.019)	*0.11*
HDL (mmol/L)	-0.14 (0.33)	*0.68*	-0.48 (0.37)	*0.20*	-0.022 (0.017)	*0.20*	-0.036 (0.019)	*0.053*
LDL (mmol/L)	-0.1 (0.33)	*0.75*	-0.46 (0.37)	*0.22*	-0.019 (0.017)	*0.26*	-0.035 (0.018)	*0.06*
Glucose (mmol/L)	-0.1 (0.33)	*0.76*	-0.5 (0.36)	*0.17*	-0.02 (0.017)	*0.23*	-0.037 (0.018)	***0.046***
BMI (kg/m^2)	-0.2 (0.33)	*0.54*	-0.51 (0.36)	*0.17*	-0.024 (0.017)	*0.16*	-0.037 (0.018)	***0.048***
Waist/hip ratio	-0.15 (0.33)	*0.65*	-0.45 (0.37)	*0.22*	-0.022 (0.017)	*0.19*	-0.035 (0.019)	*0.06*
Model 1	-0.08 (0.33)	*0.80*	-0.54 (0.37)	*0.14*	-0.016 (0.017)	*0.33*	-0.04 (0.018)	***0.029***
Model 2	-0.09 (0.34)	*0.79*	-0.54 (0.37)	*0.15*	-0.015 (0.017)	*0.37*	-0.041 (0.018)	***0.028***
Model 3	-0.05 (0.34)	*0.87*	-0.56 (0.37)	*0.13*	-0.014 (0.017)	*0.42*	-0.042 (0.018)	***0.023***

Table 4.11: Stress responses vs $FMD_{\%}$ & FMD_{ABS}: Multiple linear regression analyses examining the relationship between changes in inflammatory variables after mental stress challenge with $FMD_{\%}$ adjusted by each risk factor measured at Phase 7 of the WHII study. In addition to each risk factor each analysis was additionally adjusted for baseline diameter, $RH_{\%}$, baseline inflammatory variable, age & gender. Three models were then created including the following variables; model 1 = baseline inflammatory variable, baseline diameter, $RH_{\%}$, age, gender, waist/hip ratio, SBP, DBP, Glucose, HDL & LDL; model 2= as for model 1 excluding glucose and with the addition of socio-economic status, smoking and BMI; model 3 = as for model 2 including glucose.

4.4.2 Acute mental stress, endothelial function and the influence of dyslipidaemia

The key findings in this section were that the presence of dyslipidaemia did not influence the inflammatory responses to a stress challenge and did not further affect the relationships between inflammatory responses and FMD.

A total of 62 participants were categorised as having dyslipidaemia. Table 4.12 shows the number of participants who met each individual category for dyslipidaemia.

	Dyslipidaemia categories				Nº of categories met		
	TC≥6	Trigs ≥1.7	HDL <1	LDL ≥4	1	2	3
Yes	40	36	2	33	27	21	14
No	112	116	150	119			

Table 4.12: Number of participants who met each category for dyslipidaemia and the number of participants who met one or more of the categories (units= mmol/L)

Participant characteristics for the two groups are shown in table 4.13. Those participants with dyslipidaemia had a significantly greater BMI, heart rate, lipid profile and CRP level. Reactive hyperaemia was significantly lower in the participants with dyslipidaemia but there was no difference in FMD between the two groups.

Characteristic	Dyslipidaemia (n=62)	Normal (n=90)	p
Age (yrs)	52 ± 3	52 ± 3	*0.72*
Gender (F)	21 (33.9%)	47 (52.2%)	***0.025***
Smoker (Y)	4 (6.5%)	4 (4.4%)	*0.59*
SES			*0.83*
Higher	23 (37.1%)	37 (41.1%)	
Intermediate	22 (35.5%)	28 (31.1%)	
Lower	17 (27.4%)	25 (27.8%)	
BMI (kg/m^2)	26.74 ± 3.02	24.26 ± 3.46	***<0.001***
Waist/hip ratio			
M	0.93 ± 0.07	0.88 ± 0.06	***0.001***
F	0.82 ± 0.06	0.76 ± 0.06	***0.001***
SBP (mmHg)	116 ± 12	113 ± 12	*0.12*
DBP (mmHg)	71 ± 9	68 ± 9	*0.08*
HR (bpm)	67 ± 8	63 ± 8	***0.005***
TC (mmol/L)	6.11 ± 0.62	4.87 ± 0.62	***<0.001***
Trigs (mmol/L)	1.89 ± 0.77	0.97 ± 0.32	***<0.001***
HDL (mmol/L)	1.41 ± 0.31	1.68 ± 0.39	***<0.001***
LDL (mmol/L)	3.84 ± 0.61	2.75 ± 0.6	***<0.001***
Total/HDL	4.52 ± 0.98	3.04 ± 0.79	***<0.001***
Glucose (mmol/L)	5.40 ± 0.90	5.24 ± 0.70	*0.38*
CRP (mg/L?)	1.40 ± 1.34	0.73 ± 1.18	***<0.001***
BL dia (mm)	3.71 ± 0.68	3.53 ± 0.71	*0.09*
FMD_{ABS} (mm)	0.20 ± 0.11	0.19 ± 0.1	*0.75*
$FMD_{\%}$ (%)	5.79 ± 3.49	5.59 ± 3.34	*0.31*
RH% (%)	596 ± 241	700 ± 266	***0.016***

Table 4.13: Participant characteristics for those with and without dyslipidaemia. Mean±SD or n (%). Data is untransformed.

Associations between FMD and baseline risk factors were the same as shown previously within the whole cohort (data not shown). As within the whole cohort there were significant changes in the cardiovascular and biochemical variables following acute mental stress (table 4.14). The dyslipidaemia group had higher baseline levels for all inflammatory markers except IL-6. However, there were no significant differences in the response to the mental stress challenge between those with and without dyslipidaemia.

						Trial		Lipid interaction	
		Baseline	**Task**	**PS_1**	**PS_2**	**F**	**p**	**F**	**p**
SBP (mmHg)	Normal	112	135	118	117	246.09	***<0.001***	0.62	*0.53*
	Dyslipid	116	141	121	122				
DBP (mmHg)	Normal	68	81	72	72	266.35	***<0.001***	1.58	*0.20*
	Dyslipid	71	86	76	77				
HR (bpm)	Normal	63	70	61	61	226.40	***<0.001***	0.05	*0.96*
	Dyslipid	67	74	65	65				
Fbg (g/L)	Normal	2.73	2.79		2.79	12.90	***<0.001***	1.85	*0.16*
	Dyslipid	2.92	2.98		2.94				
IL-6 (pg/ml)	Normal	1.18			1.22	10.69	***0.001***	2.87	*0.09*
	Dyslipid	1.04			1.18				
TNFα (pg/ml)	Normal	2.03			2.09	2.12	*0.15*	0.00	*0.95*
	Dyslipid	2.24			2.30				

Table 4.14: Acute mental stress responses by dyslipidaemia. Geometric means presented for IL-6 and TNFα with arithmetic means for all other measures. Dyslipid = dyslipidaemia; PS= post-stress

Both baseline and task heart rate were positively correlated with $FMD_{\%}$ and FMD_{ABS} in the normal lipid group (table 4.15). The change in Fbg 20 minutes after stress was positively correlated with FMD_{ABS} in the normal lipid group (r=0.23 p=0.027) the correlation with $FMD_{\%}$ was borderline (r=0.21 p=0.05). However, in the dyslipidaemia group the correlation between the change in Fbg immediately post task and FMD_{ABS} was negative (r=-0.36 p=0.004). There were no correlations between endothelial function and the other inflammatory variables.

	$FMD_{\%}$				FMD_{ABS}			
	Dyslipidaemia		Normal		Dyslipidaemia		Normal	
	r	p	r	p	r	p	r	p
SBP bl (mmHg)	0.01	*0.97*	0.06	*0.57*	-0.03	*0.80*	0.06	*0.59*
SBP tk (mmHg)	0.03	*0.81*	0.01	*0.91*	0.00	*0.98*	0.00	*0.97*
SBP ch (mmHg)	0.04	*0.74*	-0.05	*0.67*	0.03	*0.83*	-0.07	*0.53*
DBP bl (mmHg)	0.17	*0.20*	0.08	*0.48*	0.10	*0.47*	0.05	*0.64*
DBP tk (mmHg)	0.14	*0.29*	0.10	*0.35*	0.07	*0.61*	0.07	*0.50*
DBP ch (mmHg)	0.01	*0.94*	0.03	*0.76*	-0.02	*0.89*	0.02	*0.82*
HR bl (bpm)	0.15	*0.24*	0.28	***0.009***	0.08	*0.52*	0.33	***0.001***
HR tk (bpm)	0.09	*0.51*	0.29	***0.006***	0.04	*0.77*	0.35	***0.001***
HR ch (bpm)	-0.07	*0.59*	0.09	*0.42*	-0.06	*0.68*	0.11	*0.31*
Fbg bl (g/L)	0.02	*0.90*	-0.03	*0.76*	-0.01	*0.91*	0.03	*0.76*
Fbg tk (g/L)	-0.06	*0.66*	0.02	*0.84*	-0.12	*0.34*	0.09	*0.40*
Fbg 45 (g/L)	-0.01	*0.94*	-0.02	*0.86*	-0.05	*0.71*	0.03	*0.76*
Fbg ch (g/L)	-0.24	*0.07*	0.21	*0.05*	-0.36	***0.004***	0.23	***0.027***
Fbg 45 ch (g/L)	-0.11	*0.39*	0.06	*0.59*	-0.16	*0.23*	0.01	*0.92*
IL-6 bl (pg/ml)	-0.05	*0.69*	-0.08	*0.50*	-0.12	*0.34*	-0.04	*0.72*
IL-6 45 (pg/ml)	-0.02	*0.90*	-0.03	*0.80*	-0.04	*0.79*	0.01	*0.94*
IL-6 ch (pg/ml)	0.06	*0.67*	0.12	*0.29*	0.13	*0.31*	0.13	*0.26*
TNFα bl (pg/ml)	0.11	*0.42*	0.09	*0.39*	0.12	*0.38*	0.08	*0.48*
TNFα 45 (pg/ml)	0.13	*0.32*	0.08	*0.50*	0.14	*0.30*	0.07	*0.55*
TNFα ch (pg/ml)	0.07	*0.60*	-0.04	*0.73*	0.06	*0.65*	-0.02	*0.86*

Table 4.15: Pearson correlations for $FMD_{\%}$ and FMD_{ABS} with the cardiovascular and biochemical responses to stress by presence or absence of dyslipidaemia bl = measurement taken at baseline; tk = measurement taken during or immediately after task; 45 = measurement take 45 minutes post task & ch = difference between either task or 45 measurement and baseline measurement

To investigate whether the inflammatory response to the acute mental stress alongside the presence or absence of dyslipidaemia influenced future endothelial function, the cohort were divided into four groups based on their inflammatory responses to stress and lipid profile. Table 4.16 shows that there were initially no differences in endothelial function between the four groups. Following adjustment for baseline arterial diameter, $RH_{\%}$ and baseline Fbg level there was a significant difference in FMD_{ABS} between the groups for Fbg response at 45 minutes post stress. Post hoc analyses identified that those without dyslipidaemia but with a high Fbg response had a lower FMD_{ABS} than those with

dyslipidaemia and low Fbg response and (p=0.022) (figure 4.3). There was also a borderline difference in FMD_{ABS} between those without dyslipidaemia with a high Fbg response and those without dyslipidaemia with a low Fbg response (p=0.056).

Inflammatory marker*	Model	$FMD_{\%}$		FMD_{ABS}	
		F	p	F	p
Fbg change	Unadjusted	0.48	*0.70*	0.99	*0.40*
	BL Fbg	0.48	*0.70*	0.97	*0.41*
	BL dia & RH%	0.62	*0.61*	0.73	*0.54*
	BL dia, RH% & BL Fbg	0.72	*0.54*	0.77	*0.51*
Fbg 45 change	Unadjusted	0.34	*0.80*	1.10	*0.35*
	BL Fbg	0.33	*0.80*	1.09	*0.35*
	BL dia & RH%	1.98	*0.12*	3.66	***0.014***
	BL dia, RH% & BL Fbg	2.10	*0.10*	3.71	***0.013***
IL-6 change	Unadjusted	0.15	*0.93*	0.25	*0.86*
	BL IL-6	0.26	*0.85*	0.37	*0.78*
	BL dia & RH%	0.71	*0.54*	0.65	*0.58*
	BL dia, RH% & BL IL-6	0.76	*0.52*	0.76	*0.52*
TNFα change	Unadjusted	0.19	*0.90*	0.26	*0.86*
	BL TNFα	0.13	*0.94*	0.21	*0.21*
	BL dia & RH%	1.15	*0.33*	1.20	*0.31*
	BL dia, RH% & BL TNFα	1.00	*0.39*	1.09	*0.36*

Table 4.16: The effect of dyslipidaemia and inflammatory responses to mental stress on endothelial function. *Participants were divided into presence or absence of dyslipidaemia and (for each inflammatory marker) into those with low or high inflammatory response. Thus, for analysis of each inflammatory variable, participants were allocated into four groups; i, dyslipidaemia plus high inflammatory response; ii, dyslipidaemia and low inflammatory response; iii, normal lipids plus high inflammatory response and iv, normal lipids with a low inflammatory response. F and p are for the ANOVA and ANCOVA with Bonferroni post hoc analyses to examine for differences in $FMD_{\%}$ and FMD_{ABS} between the groups. ANCOVAs were adjusted individually for baseline inflammatory variable, baseline diameter and $RH_{\%}$ were put in together and then all 3 variables were put in the model together.

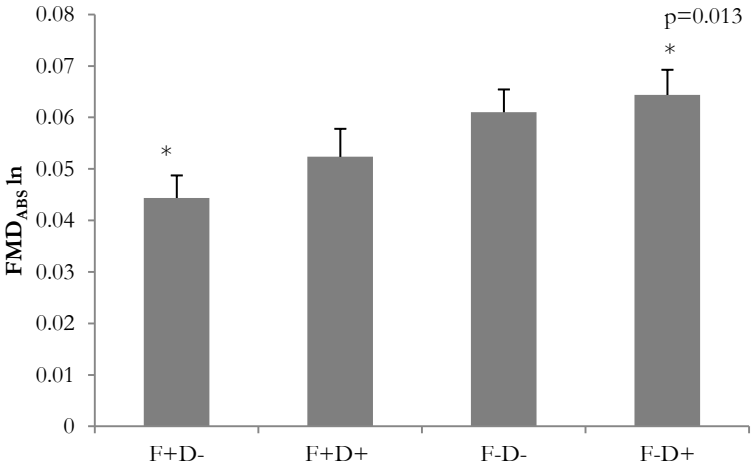

Figure 4.3: Mean FMD_{ABS} calculated from ln transformed baseline and peak brachial artery diameters with SEM bars, adjusted for baseline arterial diameter, $RH_{\%}$ and baseline Fbg by grouping of presence or absence of dyslipidaemia and low or high Fbg response to acute mental stress at 45 minutes. D+ F + = dyslipidaemia and high Fbg at 45min response; D+F- = dyslipidaemia and low Fbg at 45min response; D- F+= normal lipids and high Fbg at 45min response; D-F- = normal lipids and low Fbg/ at 45min response * = significantly different by Bonferroni p=0.022.

4.4.3 Acute mental stress and reactive hyperaemia

The key findings in this section were that there was no association between inflammatory responses to stress and $RH_{\%}$. Although the presence of dyslipidaemia did affect $RH_{\%}$, this was independent of the inflammatory responses to stress.

	BL VTI		$RH_{\%}$	
Risk factor	**r**	**p**	**r**	**p**
Age (yrs)	0.01	*0.89*	0.01	*0.91*
BMI (kg/m²)	-0.09	*0.25*	-0.09	*0.29*
Waist/hip ratio	-0.05	*0.56*	-0.09	*0.25*
SBP (mmHg)	-0.08	*0.33*	0.00	*0.98*
DBP (mmHg)	-0.07	*0.38*	0.01	*0.91*
HR (bpm)	-0.04	*0.59*	-0.22	***0.008***
TC (mmol/L)	-0.05	*0.52*	-0.13	*0.13*
Trigs (mmol/L)	-0.01	*0.94*	-0.19	***0.021***
HDL (mmol/L)	-0.03	*0.67*	0.12	*0.14*
LDL (mmol/L)	-0.04	*0.62*	-0.12	*0.15*
Total/HDL	0.01	*0.89*	-0.17	***0.038***
Glucose (mmol/L)	0.06	*0.50*	-0.10	*0.24*
CRP (mg/L)	0.06	*0.49*	-0.10	*0.24*
BL VTI (cm)			-0.53	***<0.001***

Table 4.17: Pearson correlations between baseline VTI, $RH_{\%}$ and cardiovascular risk factors

As can be seen in table 4.17 there were no associations between baseline velocity time integral (VTI) and any cardiovascular risk factors. $RH_{\%}$ was negatively associated with heart rate, triglycerides and total/HDL cholesterol. When baseline VTI and $RH_{\%}$ were correlated with the haemodynamic and inflammatory stress variables baseline VTI was negatively associated with task SBP and change in SBP from baseline (r=-0.19 p=0.023 & r=-0.20 p= 0.017 respectively). It was also positively associated with change in IL-6. $RH_{\%}$ was negatively associated with baseline HR and task HR (r= -0.22 p= 0.008 & r= -0.20 p= 0.017) and positively associated with change in Fbg at 45 minutes post stress (r= 0.16 p=0.048). There were no further associations with the other stress variables.

Stress change variable	Model	$RH_{\%}$	
		F	p
SBP change	Unadjusted	0.97	*0.33*
	BL SBP	0.93	*0.34*
	BL VTI	0.10	*0.75*
	BL VTI & BL SBP	0.14	*0.71*
DBP change	Unadjusted	0.07	*0.79*
	BL DBP	0.05	*0.82*
	BL VTI	0.28	*0.60*
	BL VTI & BL DBP	0.32	*0.57*
HR change	Unadjusted	0.12	*0.72*
	BL HR	0.03	*0.86*
	BL VTI	0.55	*0.46*
	BL VTI & BL HR	1.09	*0.30*
Fbg change	Unadjusted	1.76	*0.19*
	BL Fbg	1.79	*0.18*
	BL VTI	0.67	*0.41*
	BL VTI & BL Fbg	0.71	*0.40*
Fbg 45 change	Unadjusted	4.22	***0.042***
	BL Fbg	4.13	***0.044***
	BL VTI	2.97	*0.09*
	BL VTI & BL Fbg	2.86	*0.09*
IL-6 change	Unadjusted	1.85	*0.18*
	BL IL-6	1.95	*0.17*
	BL VTI	0.43	*0.52*
	BL VTI & BL IL-6	0.33	*0.57*
TNFα change	Unadjusted	0.24	*0.63*
	BL TNFα	0.23	*0.63*
	BL VTI	1.26	*0.26*
	BL VTI & BL TNFα	1.27	*0.26*

Table 4.18: Associations between reactive hyperaemia and haemodynamic and inflammatory responses to acute mental stress grouped into the highest and lowest 40% of responders

Having grouped the cohort by dividing the stress change variables into the highest and lowest 40% responders, comparisons were made between $RH_{\%}$ for the two groups. $RH_{\%}$ was significantly different between high and low change in Fbg at 45 minutes unadjusted and when adjusting for baseline Fbg. However, when baseline VTI was added to the model this relationship was lost (table 4.18).

Inflammatory marker*	Model	$RH_{\%}$	
		F	p
Fbg change	Unadjusted	3.90	***0.010***
	BL Fbg	3.82	***0.011***
	BL VTI	4.00	***0.009***
	BL VTI & BL Fbg	3.66	***0.014***
Fbg 45 change	Unadjusted	4.26	***0.006***
	BL Fbg	4.14	***0.008***
	BL VTI	4.47	***0.005***
	BL VTI & BL Fbg	4.21	***0.007***
IL-6 change	Unadjusted	2.68	*0.050*
	BL IL-6	2.75	***0.045***
	BL VTI	2.19	*0.09*
	BL VTI & BL IL-6	2.35	*0.08*
TNFα change	Unadjusted	1.69	*0.17*
	BL TNFα	1.65	*0.18*
	BL VTI	2.22	*0.09*
	BL VTI & BL TNFα	2.20	*0.09*

Table 4.19: The effect of dyslipidaemia and inflammatory responses to mental stress on $RH_{\%}$. Participants were divided into presence or absence of dyslipidaemia and (for each inflammatory marker) into those with high or low inflammatory response. Thus, for analysis of each inflammatory variable, participants were allocated into four groups: 1, dyslipidaemia plus high inflammatory response; ii, dyslipidaemia and low inflammatory response; iii, normal lipids plus high inflammatory response and iv, normal lipids with a low inflammatory response. F and p are for the ANOVA and ANCOVA with Bonferroni post hoc analyses to examine for differences in RH% between the groups. ANCOVAs were adjusted individually for baseline inflammatory variable and baseline VTI, both variables were then added to the model together.

As $RH_{\%}$ was found to be significantly lower in the participants with dyslipidaemia (table 4.13) the effect of presence of dyslipidaemia and inflammatory responses to stress on $RH_{\%}$ was investigated. There were significant differences in $RH_{\%}$ within the groups for Fbg stress change, Fbg 45 change and change in IL-6. Once adjustments were made for baseline VTI (which was not different between those with and without dyslipidaemia [5.57 ± 2.43 & 5.62 ± 3.77cm p=0.91]) and inflammatory variable the differences within the IL-

6 groups were lost (table 4.19). The differences in $RH_{\%}$ remained for both Fbg change and Fbg 45 change. Figures 4.4 A and B illustrate that for both Fbg variables the differences were between those with and without dyslipidaemia rather than high and low inflammatory responders.

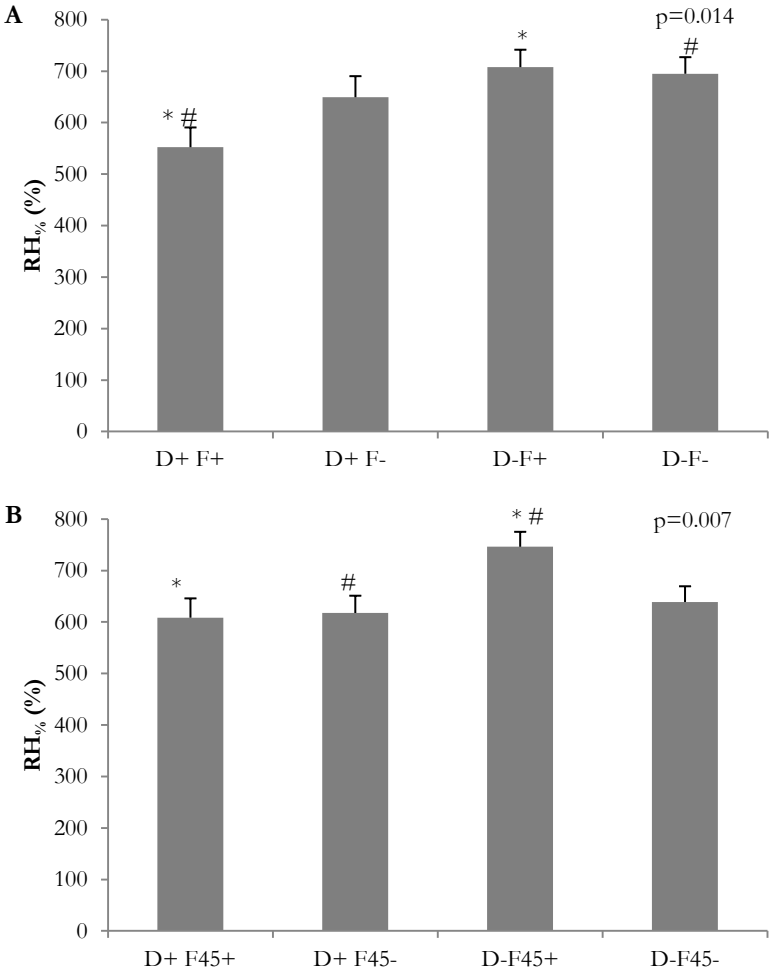

Figure 4.4: Mean $RH_{\%}$ with SEM bars, adjusted for baseline VTI and baseline Fbg by grouping of presence or absence of dyslipidaemia and low or high Fbg response to acute mental stress **A**: immediately and **B** 45 minutes post stress. D+ F + = dyslipidaemia and high Fbg response; D+F- = dyslipidaemia and low Fbg response; D- F+= normal lipids and high Fbg response; D-F- = normal lipids and low Fbg response * and # = significantly different by Bonferroni: **A** * p=0.017 # p=0.032; **B** * p=0.027 # p=0.028

4.5 Discussion

The major finding of this chapter is that participants with an elevated Fbg response to stress, 45 minutes post task had lower FMD, reflecting a relative impairment in endothelial function, when assessed 3 years later. This association remained regardless of the presence of mild to moderate dyslipidaemia. It was also shown that microvascular function,

expressed as $RH_{\%}$, is lower in those with mild dyslipidaemia irrespective of their Fbg response to stress. For both FMD and $RH_{\%}$ there were no associations with either IL-6 or TNFα. Finally, the presence of a cardiovascular risk factor (dyslipidaemia) did not affect the inflammatory responses to an acute mental stress challenge.

The main finding of a significantly lower FMD_{ABS} in those with a high Fbg response at 45 minutes post stress challenge, was seen, when the data was compared both as categorical and continuous data and following adjustment for factors that may affect endothelial function. This finding complements previous analyses from this cohort, which found increased carotid artery stiffness and ambulatory blood pressure in those participants who had increased inflammatory responses (including Fbg) to a stress challenge[324, 369]. Therefore, the finding from this analysis adds to the evidence of a potential role for inflammation in the relationship between psychological stress and adverse cardiovascular outcome.

Endothelial dysfunction is an early indicator of atherogenesis and can be assessed by FMD which is associated with cardiovascular risk[377]. Acute mental stress causes endothelial dysfunction both during a stress challenge and immediately afterwards, but the mechanisms for this are not yet fully understod[325, 378]. Inhibition of cortisol and the endothelin-A receptor, have both prevented the impairment of FMD induced by mental stress suggesting potential roles for both of these factors[326, 327].

Inflammation plays a key role in the initiation, development and progression of atherosclerosis and is associated with endothelial dysfunction[25]. Induced acute systemic inflammation and acute mental stress have both been shown to cause immediate endothelial dysfunction[325, 364, 379]. However, there is limited other work investigating whether and to what extent the acute inflammatory response to mental stress is implicated in the associated endothelial dysfunction. Ghiadoni et al measured the inflammatory markers IL-6, IL-1 & TNFα at baseline and 60 mins post stress, but did not find a significant change in the cytokine levels or any association between cytokine levels and FMD or change in FMD[325]. Therefore the finding of an association between Fbg response at 45 minutes and FMD in this chapter is potentially the first to show an association between the inflammatory response to acute mental stress and future development of endothelial dysfunction.

Fbg, an acute phase protein induced by IL-6 in the inflammatory pathway and a major component of the coagulation cascade, is associated with increased risk of coronary heart disease and stroke[235]. It is implicated in the development of atherosclerosis and vascular reactivity, through its effects on plaque composition, blood viscosity, endothelial and smooth muscle cell activation, platelet aggregation and activation, and immune cell recruitment[380, 381].

Elevated Fbg may affect endothelial function both by mechanical and biochemical processes. Raised Fbg levels increase blood viscosity, which, in turn, augments shear stress within the artery, activating endothelial cells[238, 380, 382]. This stimulates increased expression and activation of adhesion markers and integrins, resulting in attraction and adherence of monocytes to endothelial cells and the greater production of vasoconstricting agents which may further affect endothelial function and vascular tone[237, 383, 384].

Vascular injury triggers the coagulation cascade which results in the conversion of Fbg to fibrin which then forms a thin monolayer covering the damaged area. This layer attracts platelets which are also activated by Fbg causing platelet aggregation, inflammatory responses and endothelial dysfunction[385, 386]. As the injury heals the platelets can also become part of the developing lesion/plaque. Therefore sustained elevation of Fbg levels through reactions to acute stressors could further exacerbate this process. The lack of an association between IL-6 and future FMD may indicate that it is these haemostatic/prothrombotic properties of Fbg that could be more important in this setting than its inflammatory properties.

These findings suggest not only might the elevated fibrinogen response triggered by acute stress contribute to the development of ED and clinical CVD, it might also have a role in identification of those at greatest future vascular risk and selection of appropriate preventive therapy in due course. Further work would be required to address these questions more specifically.

4.5.1 Reactive hyperaemia

Although there was a significant difference in $RH_{\%}$ between participants with low and high Fbg responses at 45 minutes post stress, the relationship was lost following adjustment for baseline VTI. However, when the impact of the inflammatory responses in the presence or absence of dyslipidaemia on $RH_{\%}$ was investigated, further differences were found with

change in both Fbg immediately and 45 minutes post task following adjustment for baseline Fbg and VTI. Post hoc analyses indicated that for both the change in Fbg immediately and 45 minutes post stress, the differences appeared to be driven more by presence of dyslipidaemia than the size of the inflammatory response.

$RH_{\%}$ is an indicator of microvascular function reflecting vasodilatation of the resistance vessels in response to ischaemia-induced vasoactive stimuli[387, 388]. Microvascular function is associated with cardiovascular risk factors and predictive of cardiovascular events[388-390]. The finding of impaired $RH_{\%}$ alongside an unchanged FMD in those with dyslipidaemia has been shown previously in a study which demonstrated decreased $RH_{\%}$ following cuff occlusion in untreated hypercholesterolaemia patients compared with treated hypercholesterolaemia patients and control subjects[391]. As with my study there was no difference in the FMD between the untreated hypercholesterolaemia patients and control subjects. The diminished $RH_{\%}$ alongside maintained FMD in those with dyslipidaemia may in part be due to differences in the vasodilators responsible for driving the change in tone which results in $RH_{\%}$ and FMD. Whilst nitric oxide is the main mediator of FMD, it has a less significant role at peak flow and during the flow debt repayment[149, 392]. In addition, other vasodilators such as adenosine have a greater role in resistance vessel vasodilatation during ischaemia than NO[393-395].

4.5.2 Inflammatory responses to stress

Fibrinogen and IL-6 both significantly increased in response to the mental stress challenge and although TNFα increased, the change was not significant. However, when the effect of gender was taken into account in the analysis, there was a significant difference in the responses to stress for TNFα. Women showed a decrease in TNFα, whereas there was an increase in men. This finding does complement a previous analysis by Steptoe et al in the Whitehall II psychobiology sub study where they found that women had larger IL-6 responses and smaller TNFα responses to stress than men[396]. This may partly account for the lack of a significant increase in TNFα within the whole cohort. In addition, a meta-analysis of the effects of mental stress challenges on inflammatory markers by Steptoe et al, found that some markers (e.g. IL-6) may be more sensitive than others (e.g. TNFα) to stress[350].

4.5.3 Dyslipidaemia

The presence of mild dyslipidaemia did not appear to have any influence on the haemodynamic or inflammatory responses to an acute mental stress challenge. Neither did the presence of mild dyslipidaemia have an impact on the relationship between Fbg responses to stress and future endothelial function. Indeed, somewhat surprisingly, those with mild dyslipidaemia had a tendency towards better FMD_{ABS} than those without. The finding of a lack of influence of dyslipidaemia, together with that of Steptoe and Brydon, that a greater increase in total cholesterol in response to stress is predictive of having higher total cholesterol 3 years later, could suggest that the stress response may influence future incidence of dyslipidaemia rather than current dyslipidaemia affecting the stress response[373]. However, it is also worth noting that the participants in this study typically only had mild dyslipidaemia which may account for why differences were not found between groups. Another study in males with cholesterol >4.1 mmol/L and triglycerides >2.8 mmol/L (greater than the ≥1.7mmol/L I used) had similar haemodynamic and lipid responses to stress to those with normal lipid levels, further indicating that dyslipidaemia may well not influence these stress responses[397].

4.5.4 Limitations

There are several limitations to this study. Endothelial function was not assessed at the time of stress testing so it is unknown whether those participants with poorer FMD 3 years later already had endothelial dysfunction at the time of psychophysiological stress testing. In addition endothelial function is influenced by medications, environmental factors, exercise, infection and food and although these factors were controlled for as much as is possible in a cohort study during FMD assessment and the analysis they may have influenced the measurements. The population was selected for being free of cardiovascular disease, so the dyslipidaemia group consists of those participants with lipids at the adverse end of the normal range or who have mild dyslipidaemia rather than including those with more severe dyslipidaemia. Therefore, the findings may differ in those with more severe dyslipidaemia. Additionally, blood samples for this study were not taken in a fasted state. This may mean that some participants were classified as having dyslipidaemia due to elevated non-fasting triglycerides, but may have been categorised as normal from a fasted measurement. Although this may influence the findings, an important association between non-fasting triglycerides and cardiovascular outcomes is well recognised and suggests it is not

inappropriate to select participants on the basis of non-fasting triglycerides[398]. These results are from just one day and do not take into account changes within individual's circumstances in the 3 years between the stress challenge and endothelial function assessment which may influence their stress response or cardiovascular health. Finally, this is a post-hoc analysis of the WHII psychobiology study and although the results are consistent with previously published analyses from this study showing a relationship between fibrinogen stress responses and large artery stiffness, replication in another cohort would be beneficial to confirm whether the findings are due to chance or not. Further prospective studies would also be beneficial to gain greater understanding of the clinical significance of these results.

4.5.5 Conclusion

In conclusion, in this chapter I have shown that those participants with an elevated fibrinogen response at 45 minutes post stress have reduced endothelial function three years later. The presence of high levels of lipids did not influence the relationship between fibrinogen and endothelial function.

Chapter 5. Summary of section 1: associations between chronic and acute stress and subclinical vascular measures

5.1 Summary and review of findings

In recent years awareness of the impact of the psychosocial environment on health has been increasing. Numerous studies have shown the impact of psychosocial factors on cardiovascular mortality and risk in both those who already have cardiovascular (CVD) and the general population[204, 206]. My aim in the first two chapters was to further investigate the influences of long term/ chronic stress and acute stress on subclinical vascular disease.

In Chapter 3 I investigated whether there were any cross-sectional associations between depression and anxiety as chronic stressors and subclinical vascular disease, in a large prospective cohort. In addition I also looked at whether the presence of a known cardiovascular risk factor influenced any relationships between depression and anxiety symptoms and subclinical vascular disease. I found that women with symptoms of depression had greater intima-media thickness (IMT) than those without significant depression symptoms, which was also seen in women with both depression and anxiety symptoms. This would suggest that these women were potentially at greater risk of developing cardiovascular disease than women without depression or anxiety symptoms further adding to the body of literature in this area[262, 263, 271]. The presence of dyslipidaemia further increased the potential risk of cardiovascular disease, as women with depression and or anxiety symptoms and dyslipidaemia were found to have the greatest IMT. However, it was not possible in this analysis to decipher the causal pathway of whether in these women the depression and or anxiety came first or the dyslipidaemia, which in this cohort is only mild dyslipidaemia. It is worth noting that an increase in IMT in men was influenced by the presence of dyslipidaemia rather than depression and or anxiety.

It was surprising to see that the association between anxiety symptoms and distensibility coefficient (DC) in women went in the opposite direction than expected; those with anxiety

symptoms having more compliant arteries. Further work could investigate the relationships with other measures of arterial stiffness such as aortic pulse wave velocity and central blood pressure.

The association between chronic stress and FMD was not significant, this may have been due to the relatively small sample size, and if there may have been a very small influence on FMD, this study may have had insufficient power to detect such a difference. Additionally, although the participants included in the depression group had scores greater than the questionnaire threshold for significant symptoms, they may have been clustered at the threshold and therefore did not have severe enough psychological "distress" to influence vascular pathophysiology more than those with lower scores. Equally, as those participants taking lipid lowering medications had been excluded, participants with dyslipidaemia in this analysis only had mildly raised lipids. Including participants with a more severe lipid abnormality may have shown different results.

In Chapter 4 the main purpose was to investigate the effect of the inflammatory response to a mental stress challenge on future endothelial function to try and further understand the associations between stress and increased cardiovascular risk. Additionally, I also looked at whether the presence of a cardiovascular risk factor (dyslipidaemia) had any bearing on the inflammatory response to a stress challenge and future endothelial function. It was found that those participants with an elevated fibrinogen response at 45 minutes had poorer endothelial function when assessed 3 years later. When the influence of dyslipidaemia was investigated it was shown to have no impact on the haemodynamic or inflammatory responses to the mental stress challenge. However, those who had dyslipidaemia and greater fibrinogen responses to the stress challenge had poorer conduit and micro-vascular function, thereby potentially putting these individuals at greater cardiovascular risk.

Fibrinogen has various potential influences on atherogenesis; for example through its effects activating endothelial and smooth muscle cells, inflammatory responses, blood viscosity, platelet activation and aggregation and through the coagulation cascade contributing to the development of potentially occlusive thrombi[380, 381]. Therefore, prolonged elevation of fibrinogen levels in response to acute emotional triggers may help to create a sustained proatherogenic environment, encouraging development of potentially vulnerable atherosclerotic plaques. If through the haemodynamic responses to acute stress the plaque ruptures, increased levels of fibrinogen, which activates platelets, can lead to

larger thrombi which are more prone to occluding arteries and causing a cardiovascular event[385]. The addition of a cardiovascular risk factor such as an adverse lipid profile increases the propensity for arterial wall lipoprotein uptake, retention and oxidation, further exacerbating inflammatory response and atherogenic disease processes.

5.2 Future directions for research and overcoming technical challenges

5.2.1 Chronic stress

To gain further insights into the associations between depression and anxiety symptoms and subclinical vascular disease it would be interesting to take advantage of the longitudinal data available from the Whitehall II cohort. The GHQ was administered at phases 3 and 5 of the study. It would therefore be possible to investigate whether those with long-term depressive or anxiety symptoms were more likely to develop subclinical vascular disease or other cardiovascular risk factors. Equally it would be interesting to investigate whether those participants with raised (higher end of normal) lipid levels at phase 3 were more likely to develop depression/anxiety symptoms to try and further understand the finding of increased IMT in those women with dyslipidaemia and depression and/or anxiety symptoms.

In addition it would be interesting to follow-up those participants identified with depressive/anxiety symptoms at phase 7. Further data on depression and anxiety symptoms and cardiovascular risk factors have been collected at phase 9, and although subclinical vascular measures from phase 7 were not repeated, central pulse wave velocity data was collected. This would enable further investigation of the relationship between anxiety and arterial stiffness. In addition, this data would allow investigation into whether those participants with depression and/or anxiety developed cardiovascular risk factors or disease or had experienced a cardiovascular event. It would also be possible to investigate whether there were differences between those who continued to have depressive/anxiety symptoms and those who had recovered. Finally, to further understand the associations between dyslipidaemia, depression/anxiety and IMT it would be helpful to investigate this in a group with severe dyslipidaemia such as familial hypercholesterolaemia. This would potentially add to the evidence of chronic stressors, such as depression and anxiety, increasing the risk and exacerbating the development of cardiovascular disease.

5.2.2 Acute stress

Although there were no associations with IL-6 and TNFα within this analysis it would be interesting to look at changes in these variables over a longer time frame post stress, such as 2 hours. Greater changes in inflammatory cytokines have been seen over a longer time course of measurement and it may be that relationships may be seen with flow-mediated dilatation (FMD) measured both concurrently and in the future[350, 399]. Other work could further investigate the association between fibrinogen response, dyslipidaemia and FMD. It would be beneficial to look at this in a group of participants with a more distinct lipid profile such as familial hypercholesterolaemia who have very high LDL levels and also patients with metabolic syndrome that have high triglycerides with low HDL. Using two groups, who have more severe dyslipidaemia than in my analysis but also qualitatively different atherogenic lipid profiles, would potentially give further insights into possible relationships and help identify those at increased risk of cardiovascular disease from acute emotional triggers.

Future work could also look at the data collected on these participants from later phases of the Whitehall II study. This would enable us to investigate whether those participants who had elevated fibrinogen at 45 minutes post stress challenge and poorer endothelial function, were more likely to develop progressive arterial disease or suffer a cardiovascular event than those participants with a lower response.

5.2.3 Endothelial function assessment

Within both chapters analyses were limited to some extent by numbers of participants who had undergone endothelial function testing especially when compared with those who had IMT & DC assessment. Ultrasound assessment of IMT is easier and quicker to carry out than FMD. Although FMD has been measured in large studies such as ALSPAC and Framingham, it is not a simple technique to carry out and can be time consuming to set up in a time limited protocol[151, 389]. A technique for assessing endothelial function that is quick to set up, easy to use and largely operator independent would be beneficial not only to large population studies such as the Whitehall II study but also for studies in specialist vascular laboratories. The dataset for mental stress and endothelial function is one of the largest to date. A lot of the studies looking at acute mental stress testing and concurrently assessed FMD are on relatively small numbers of participants. Therefore, a relatively simple

technique for assessing endothelial function that could fit easily within large vascular epidemiology protocols would be very useful for assessing changes occurring acutely and over longer-term follow up.

Chapter 6. Development of protocol and reproducibility of flow-mediated slowing

6.1 Introduction

Endothelial dysfunction is one of the earliest clinically measurable indicators of atherogenesis within an artery. Assessment of endothelial function is a useful tool for understanding the disease process and has been demonstrated to be related to increased cardiovascular risk[174, 176]. The current gold standard technique for the non-invasive assessment of endothelial function is flow-mediated dilatation (FMD), which is highly reproducible in a controlled specialist vascular laboratory, but requires a high level of operator skill and relatively expensive equipment. It can be used in large population studies but this can be logistically challenging and expensive[151]. As previously discussed in Chapter 1, there are alternative methods available but their reproducibility and clinical relationships to endothelial function are less well defined and validated[150].

Pulse wave velocity (PWV) is a measure of arterial stiffness. Increased central PWV is associated with the presence of cardiovascular risk factors, coronary artery disease and higher cardiovascular event rates[33, 93, 400, 401]. This technique has recently been harnessed as a potential method for assessing endothelial function. First described by Naka et al, it utilises a two cuff oscillometric method of measuring PWV in the brachio-radial tract and uses a hyperaemic stimulus to cause smooth muscle relaxation in the artery leading to a decrease in the speed of the pressure waveform[164]. Its use as a method for measuring endothelial function is based on the Moens–Korteweg equation (equation 6.1) which demonstrates that PWV is directly proportional to arterial wall width and Young's modulus (a measurement of elastin and collagen concentration or arterial stiffness) and inversely proportional to vessel diameter and blood viscosity. Therefore, increased arterial wall width and stiffness leads to higher PWV and smooth muscle tone, whilst a larger lumen diameter and greater blood viscosity decreases PWV[402]. Additionally, the release of NO and vascular tone also influence PWV[403, 404]. This would suggest that the reactive hyperaemic stimulus increases the shear stress, releasing NO from the endothelium, leading

to smooth muscle relaxation and vasodilatation of the blood vessel, thereby decreasing PWV.

Equation 6.1:[402]

$$PWV = \sqrt{Eh/2R\rho}$$

PWV =Pulse wave velocity; E= Young's modulus; h =wall thickness; R= radius;ϱ = blood density

Since this method was first published other groups have applied this theory and tested it using other techniques for assessing PWV (such as applanation tonometry), most commonly in the carotid – radial tract[165]. Different occlusion cuff positions have also been used with some favouring an upper arm occlusion and whilst others place it just below the medial epicondyle[165, 166]. The equipment first used in the original study by Naka et al, has since been transformed and is now a small portable device run by a laptop which is very simple to use. This development means that this technique has the potential to be a simpler, more affordable alternative method for the assessment of endothelial function which may be ideally placed for use in non-specialist vascular laboratories and large population studies.

Naka et al have already demonstrated that PWV slows in response to a hyperaemic stimulus in healthy subjects and that this response is reduced in patients with chronic heart failure[164]. However, the reproducibility of this change in PWV has not been demonstrated and neither has a direct comparison with FMD (as the gold standard method) been carried out. Therefore, it is important to carry out studies to validate this potential method of non-invasive endothelial function assessment in order to justify its wider use.

6.2 Study Aims

Aims:

to investigate whether a change (slowing) in PWV could be seen in response to an increase in flow following a standard hyperaemic stimulus (flow-mediated slowing [FMS]) and to validate and/or refine the protocol as developed by Naka et al[164].

to investigate the technical and logistical issues and determine the reproducibility of FMS.

Hypothesis: FMS is a reproducible method of assessing changes in conduit vessel stiffness in response to reactive hyperaemia and can therefore serve as a measure of endothelium dependent vasomotor function.

6.3 Method

6.3.1 Flow-mediated dilatation

All measurements were carried out in a temperature controlled room. Blood pressure was assessed before and after endothelial function testing using an automatic sphygmomanometer (Omron 750) on the left arm. Skin temperature was measured and if less than 29^{O}C the hand was warmed with a heated wheat bag.

Using a high-resolution ultrasound probe (7.5MHz, ALOKA Prosound 5500) held in a stereotactic clamp (ALOKA), the right brachial artery was imaged, 5 to 10 cm above the antecubital fossa. A Doppler cursor was positioned in the centre of the vessel for continuous assessment of blood flow using pulsed-wave Doppler. Baseline measures of diameter and flow were recorded for 1 minute. An automatic air regulator (Logan Research;Rochester, Kent, United Kingdom) was used to inflate a pneumatic cuff (Hokanson SC5) positioned around the forearm as specified in each protocol to 250 mmHg for 5 minutes to induce brachial artery FMD. Following rapid deflation of the cuff brachial artery diameter and blood flow was recorded for a further 5 minutes. Images triggered to the R-wave of the ECG were captured every 3 seconds directly to a computer for later offline analysis and recorded onto video for backup.

Brachial artery diameter was measured with edge detection software (Brachial Tools, Iowa City, Iowa) (see figure 6.1). Baseline diameter was an average of the first 20 frames; peak diameter was an average of 3 consecutive frames. Absolute change in diameter was calculated as in equation 6.2a and FMD percentage change was calculated as in equation 6.2b.

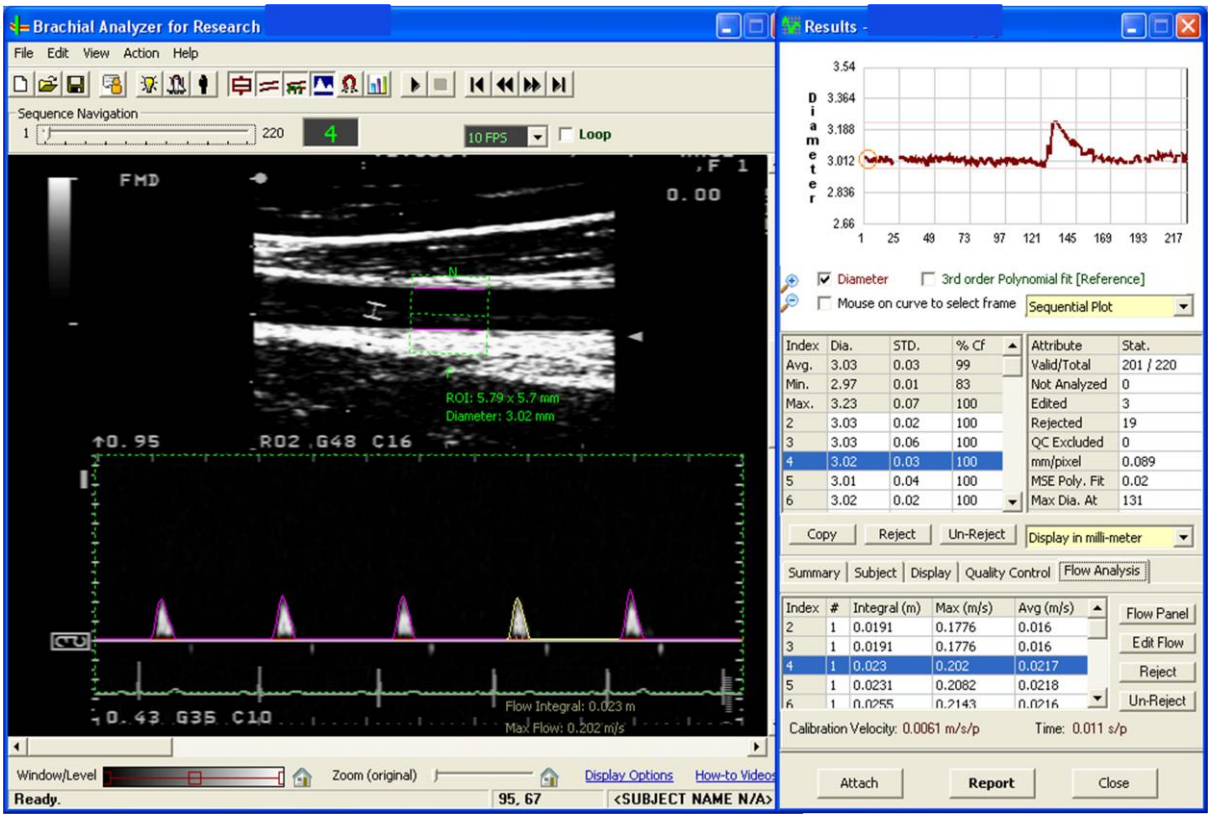

Figure 6.1: Demonstration of an ultrasound image with region of interest for analysis of change in diameter following a 5 minute occlusion of the forearm. On the right of the figure is the change of diameter over the course of the study.

Equation 6.2a

$$Absolute\ FMD = Peak\ Diameter - Baseline\ Diameter$$

6.2b

$$FMD\% = \frac{(Peak\ Diameter - Baseline\ Diameter)}{Baseline\ Diameter} x\ 100$$

Blood flow was analysed using the flow analyser within the Brachial Tools software. Velocity time integral (VTI) was used to represent blood flow. Baseline VTI was an average of the first 20 frames. Peak VTI was the maximum VTI within the first 15 seconds following deflation of the occlusion cuff. Absolute change in VTI was the difference between peak VTI and baseline VTI. Reactive hyperaemia ($RH_{\%}$)was calculated as in equation 6.3.

Equation 6.3

$$RH\% = \frac{Peak\ VTI}{Baseline\ VTI} x\ 100$$

6.4 Protocol Development of flow-mediated slowing method

6.4.1 Flow-mediated slowing, general method

FMS of the brachial artery was measured using the Vicorder (Skidmore Medical, UK) on the right arm unless otherwise specified. Two narrow Hokanson SC5 blood pressure cuffs were positioned (one on the upper arm just below the armpit; the second on the lower arm) in order to assess PWV. A blood pressure cuff attached to a rapid cuff inflator was placed on the lower arm for occlusion of the forearm (figure 6.2). The positioning and order of the cuffs on the lower arm varied according to study.

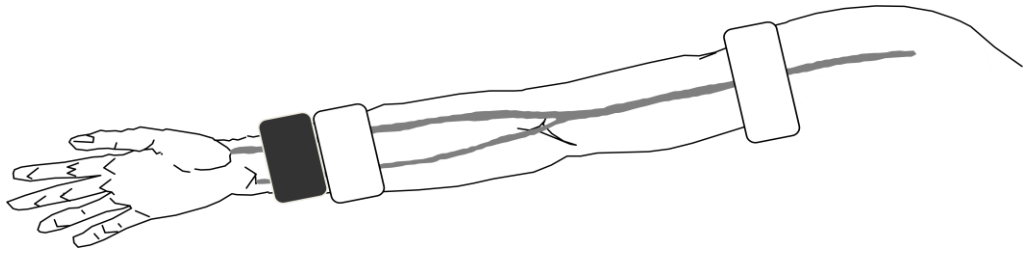

Figure 6.2: Example of cuff positions for assessing FMS. ■ = occlusion cuff □ = PWV cuff.

Baseline PWV recordings were made for 5-10 minutes. The occlusion cuff was then inflated to 250mmHg for 5 minutes. Changes in PWV following deflation of the occlusion cuff were recorded for a further 10 minutes. Positioning of the lower PWV cuff and occlusion cuff varied according to protocol. Baseline PWV was the average of all readings recorded over the 5 or 10 minute period prior to forearm occlusion. Using the lowest PWV value following occlusion cuff release unless otherwise specified, FMS% was calculated using equation 6.4.

Equation 6.4.

$$FMS\% = \frac{(Min\ PWV - BL\ PWV)}{BL\ PWV} x\ 100$$

FMS = Flow-mediated slowing; PWV = Pulse wave velocity; Min = Minimum PWV post occlusion cuff release; BL = Baseline

6.4.2 Statistical analysis

Although none of the preliminary studies were powered for statistical analysis, data was tested for normality using Shapiro-Wilk. Paired sample t-test or Wilcoxon signed rank test was used to test for differences between methods when comparing two groups. For larger comparisons, one-way ANOVA with Bonferroni was used to explore differences between

groups or for non-parametric data, Friedmans two-way analysis of variance by rank was used. A p-value of <0.05 was considered as statistically significant. Statistical analysis was carried out using SPSS version 18.

6.4.3 Preliminary scoping studies

6.4.3.1 Aim

The aim of the preliminary study was to repeat the published technique by Naka et al and evaluate concurrent measurement of FMS and FMD[164].

6.4.3.2 Methods

FMS, FMD and blood flow were evaluated as described above. The cuffs were positioned with the upper cuff just below the armpit; the occlusion cuff was proximal to the wrist with the second PWV cuff proximal to the occluding cuff (figure 6.3). PWV data was captured every minute for 10 minutes before and after cuff inflation. Assessments of FMS and FMD were made both individually and simultaneously on the right arm see figure 6.4. Changes in brachial artery diameter, blood flow and PWV were assessed in one subject.

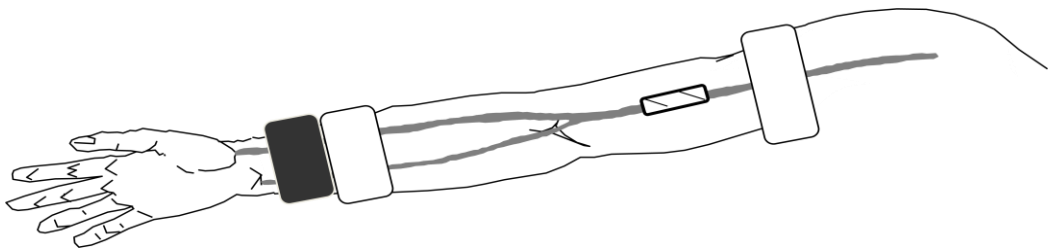

Figure 6.3: Set up for simultaneous assessment of FMS and FMD. Cuff positions remained the same for separate assessment of both FMS and FMD. ■ = occlusion cuff □ = PWV cuff ▭ = ultrasound probe.

Figure 6.4: Protocol for the initial testing of the FMS method for comparison with FMD

6.4.3.3 Results and implications

When FMS was assessed on its own and simultaneously with FMD, PWV did not decrease following release of the occlusion cuff (see figure 6.5). When taking change in PWV at 1 minute post cuff release as undertaken by Naka et al, PWV had increased by 4.50% when assessed individually and by 2.14% when assessed concurrently with FMD[164]. Brachial artery diameter increased following release of the occlusion cuff on both occasions indicating that there was vasodilatation (FMD alone 3.8% and FMD with concurrent FMS assessment 4.84%).

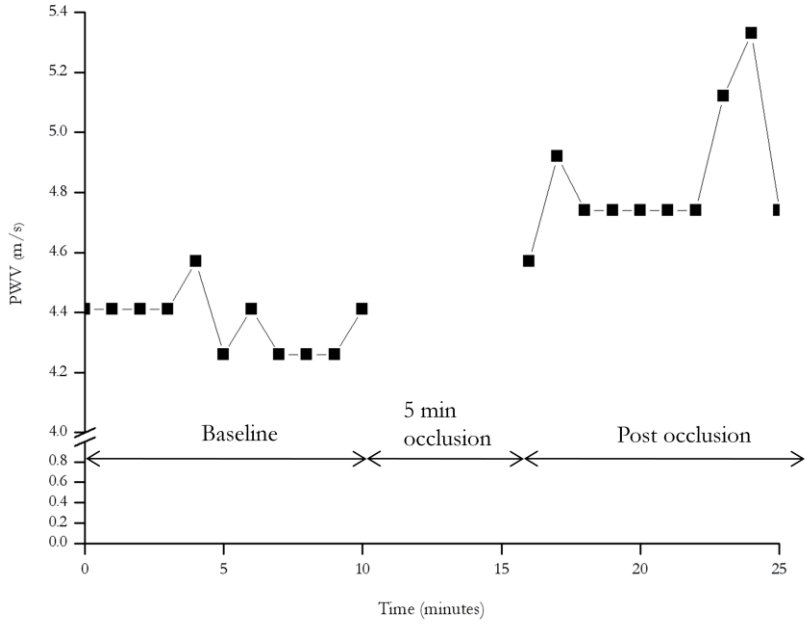

Figure 6.5: Results for the initial FMS study in one subject. Pulse wave velocity (PWV) was recorded at one minute intervals for 10 minutes as baseline and 10 minutes post occlusion cuff release. PWV was not recorded during the occlusion period.

A measurable change in blood flow was also seen (reactive hyperaemia FMD alone 351% and with simultaneous FMS assessment 254%). This was low when compared to that of published data with the standard forearm cuff position (RH% 642±350)[405].

These observations raised two important questions:

- How does cuff position affect the magnitude of the hyperaemic stimulus and the quality of the FMD image;

- What is the optimal protocol for acquisition of the PWV measurement to capture flow-mediated changes?

6.4.4 Study 1 Assessment of forearm cuff positions

6.4.4.1 Aim

To assess the impact of varying the hyperaemic stimulus on FMS and FMD responses.

6.4.4.2 Method

The flow stimulus was increased by positioning the occlusion cuff in the middle of the forearm to increase the amount of arm being occluded (figure 6.6B). This was compared with the original cuff position just proximal to the wrist (figure 6.6A) in 5 subjects. FMS and FMD measurements were obtained simultaneously for both positions. Tests were carried out 30 minutes apart with the order alternated (figure 6.7). FMS and FMD were assessed as previously described but PWV was captured approximately every 20 seconds.

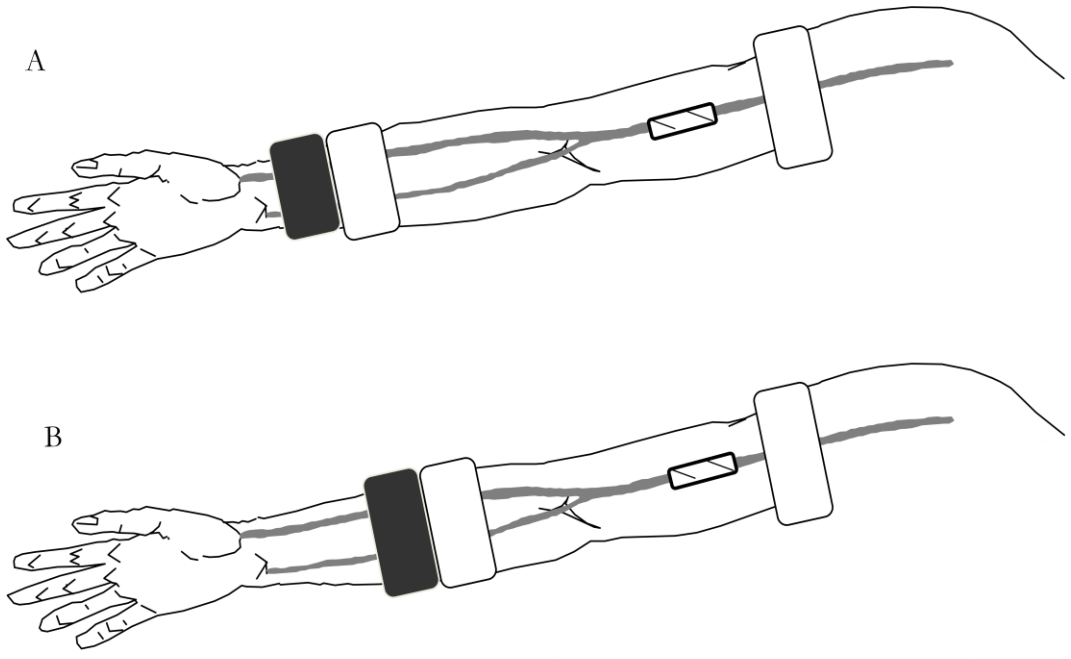

Figure 6.6: The different cuff positions for the occluding cuff and lower PWV cuff. A. Wrist occlusion cuff. B. Mid forearm occlusion cuff. ■ = occlusion cuff □ = PWV cuff = ultrasound probe.

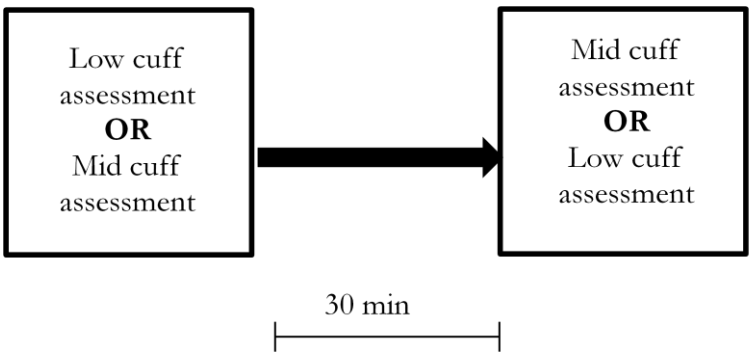

Figure 6.7: Protocol for assessment of FMS and FMD with different occlusion cuff positions. Subjects underwent either low cuff or mid cuff assessment first. Following a 30 minute break they then underwent repeat FMS and FMD assessment with the alternative occlusion cuff position.

6.4.4.3 Results

n=5	**Subjects**
Age (years)	27 ± 7
Gender (M:F)	4:1
SBP (mmHg)	126 ± 19
DBP (mmHg)	72 ± 16

Table 6.1: Subject characteristics

	Mid cuff placement				**Low cuff placement**			
Subject	**BL PWV (m/s)**	**Min PWV (m/s)**	**FMS_{ABS} (m/s)**	**$FMS_{\%}$ (%)**	**BL PWV (m/s)**	**Min PWV (m/s)**	**FMS_{ABS} (m/s)**	**$FMS_{\%}$ (%)**
1	6.15	6.07	-0.08	-1.23	7.98	7.49	-0.49	-6.18
2	5.37	5.45	0.08	1.48	*			
3	5.15	4.79	-0.36	-7.06	5.88	5.56	-0.32	-5.43
4	7.62	6.81	-0.81	-10.61	9.44	9.18	-0.26	-2.78
5	5.34	5.10	-0.24	-4.53	7.23	7.01	-0.22	-3.04

Table 6.2: PWV data for both occlusion cuff positions. * = data missing for technical reasons. BL PWV = baseline pulse wave velocity; Min PWV = minimum pulse wave velocity; FMS_{ABS} = absolute change in pulse wave velocity; $FMS_{\%}$ = flow-mediated slowing as a percentage.

Five subjects were studied who were all non-smokers, age and blood pressure can be seen in table 6.1. There was variable amount of slowing of PWV in response to reactive hyperaemia with both the mid and wrist occluding cuff positions (table 6.2). Mean $FMS_{\%}$ was similar for both cuff positions (mid -4.39± 4.75 % and low -4.36±1.7 %, p= 0.61). Table 6.3 shows the individual results for FMD. The mean $FMD_{\%}$ was lower when the occlusion cuff was positioned low on the arm (mid cuff $FMD_{\%}$ 3.05 ± 1.18 % vs low cuff

$FMD_{\%}$ 2.30 ± 1.74 %), however, this was not a significant reduction (p=0.52), although this study was not powered for statistical comparison between groups.

Subject	Mid cuff placement BL dia (mm)	PK dia (mm)	FMD_{ABS} (mm)	$FMD_{\%}$ (%)	Low cuff placement BL dia (mm)	PK dia (mm)	FMD_{ABS} (mm)	$FMD_{\%}$ (%)
1	3.24	3.40	0.16	4.95	3.49	3.51	0.02	0.68
2	4.21	4.31	0.11	2.50	*			
3	3.79	3.89	0.09	2.50	3.77	3.89	0.12	3.14
4	4.06	4.20	0.14	3.38	4.18	4.37	0.18	4.41
5	2.85	2.90	0.05	1.92	2.88	2.91	0.03	0.98

Table 6.3 FMD data for both occlusion cuff positions. *Data missing due to technical problem. BL dia= baseline diameter; PK dia = peak diameter; FMD_{ABS} = absolute change in diameter; $FMD_{\%}$= flow-mediated dilatation expressed as a percentage

Subject	Mid cuff placement BL VTI (m)	Pk VTI (m)	Abs VTI (m)	RH (%)	Low cuff placement BL VTI (m)	Pk VTI (m)	Abs VTI (m)	$RH_{\%}$ (%)
1	0.039	0.268	0.229	689	0.049	0.193	0.144	394
2	0.088	0.354	0.267	404	*			
3	0.052	0.188	0.136	363	0.063	0.137	0.074	219
4	0.039	0.183	0.144	464	0.039	0.136	0.097	347
5	0.055	0.185	0.130	338	0.034	0.112	0.078	329

Table 6.4: Flow data for both occluding cuff positions.*Data missing due to technical problem. BL VTI = baseline velocity time integral; PK VTI = peak velocity time integral; ABS VTI = absolute change in velocity change integral; $RH_{\%}$ = Reactive hyperaemia as a percentage

In the majority of subjects $RH_{\%}$ was measurably greater with the mid arm occlusion cuff than the wrist cuff (table 6.4). The mean $RH_{\%}$ for the mid cuff position was 452 ± 141 % and for the low cuff position it was 322 ± 74 % but the difference between the two was not significant (p=0.10). However, absolute VTI was significantly reduced with the lower occlusion cuff position (mid cuff 0.160 ± 0.047 m & low cuff 0.098 ± 0.032 m, p = 0.005).

6.4.4.4 Implications

Changing the position of the occluding cuff to the mid-forearm did not increase the change in PWV following a 5 minute period of ischaemia. There was a slight increase in the size of the reactive hyperaemic stimulus but not enough to achieve similar results as Naka et al who found a reduction in PWV of 14% (absolute 0.7 m/s) when the occlusion

cuff was positioned at the wrist[164]. These results suggest that a more proximal position for the occlusion cuff is likely to be required in order to generate consistently sufficient reactive hyperaemia to be able to test the potential of this method.

6.4.5 Study 2A Intermittent or continuous PWV recording

6.4.5.1 Aim

Two methods are available on the Vicorder to record PWV. The method used in the previous studies was to inflate the cuffs to 65mmHg, take a measurement and then deflate the cuffs, a process that takes approximately 15-20 seconds. Alternatively the cuffs are inflated and left whilst a reading is saved automatically every 3.5 seconds (continuous). A continuous method might technically seem preferable as the cuffs are inflated to sub-diastolic pressure and left for the duration of the baseline and reinflated for post release of the occlusion cuff. However, there is potential that a continuous venous occlusion may have an effect on arterial stiffness which may have an impact on the FMS and blood flow responses following reactive hyperaemia. Therefore, the aim of the study was to assess the effect of continuous versus intermittent PWV recording on the hyperaemic stimulus.

6.4.5.2 Methods

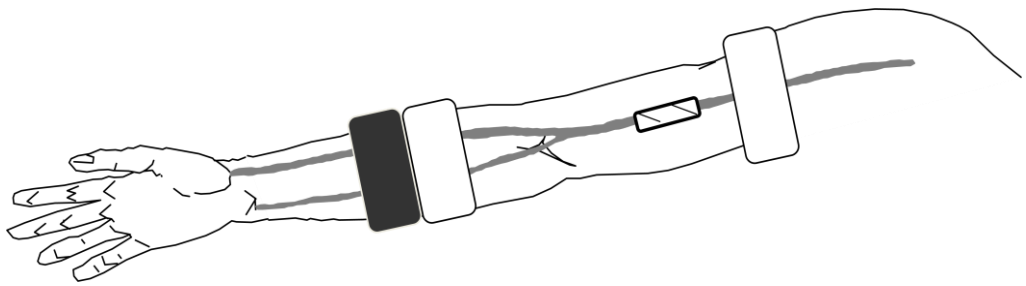

Figure 6.8: Set up of the cuffs and ultrasound probe for FMS and FMD assessment. ■ = occlusion cuff □ = PWV cuff ▭= ultrasound probe.

Three subjects were studied who had previously had FMS and FMD assessed with the intermittent PWV recording method. FMS and FMD were set up as previously described with the occluding cuff positioned in the mid-forearm (figure 6.8). Changes in flow were measured using pulse-waved Doppler during vascular studies in the three healthy volunteers under the following conditions (figure 6.9).

- During intermittent assessments of PWV at 20 second intervals with simultaneous FMD acquisition

- During continuous acquisition of PWV (measurement recorded every 3 seconds) with simultaneous FMD assessment
- During FMD without PWV assessment

Figure 6.9: Protocol for assessment of FMS and FMD to compare recording of the PWV signal either continuously or intermittently.

6.4.5.3 Results

Despite the PWV trace being displayed on the screen during the study the data failed to register in the system due to a malfunction within the software (repaired after consultation with the manufacturers). Therefore there was no PWV data available for all 3 subjects for the continuous PWV recording method. However, the intermittent recording PWV data capture was successful but as previously shown there was limited detectable FMS (mean $FMS_{\%}$ -1.42 ± 3.01 %) (table 6.5)

Subject	BL PWV (m/s)	Min PWV (m/s)	FMS_{ABS} (m/s)	$FMS_{\%}$ (%)
1	6.15	6.07	-0.08	-1.23
2	5.37	5.45	0.08	1.48
3	5.34	5.10	-0.24	-4.53

Table 6.5: Data for the intermittent recording of PWV. BL PWV = baseline pulse wave velocity; Min PWV = minimum pulse wave velocity; FMS_{ABS} = absolute change in pulse wave velocity; $FMS_{\%}$ = flow-mediated slowing as a percentage.

Flow data showed that the reactive hyperaemic response was lower for continuous PWV recording than intermittent PWV recording and FMD by itself (mean responses for $RH_{\%}$ were 357 ± 88 %, 471 ± 183 % and 467 ± 138 % respectively p=0.26). This difference was not statistically significant, but the study was not powered for statistical analysis. The hyperaemic responses for intermittent PWV recording and FMD were similar (table 6.6).

	Continuous				Intermittent				FMD only			
Subject	BL VTI (m)	PK VTI (m)	ABS RH (m)	RH (%)	BL VTI (m)	PK VTI (m)	ABS RH (m)	RH (%)	BL VTI (m)	PK VTI (m)	ABS RH (m)	RH (%)
1	0.075	0.232	0.157	311	0.039	0.268	0.229	682	0.052	0.167	0.115	324
2	0.068	0.310	0.242	458	0.087	0.332	0.245	382	0.070	0.334	0.264	478
3	0.068	0.204	0.136	301	0.053	0.185	0.132	350	0.038	0.23	0.192	600

Table 6.6: Flow data from the brachial artery during continuous and intermittent PWV recording and FMD only. BL VTI = baseline velocity time integral; PK VTI = peak velocity time integral; ABS RH = Absolute change in VTI; RH% = Reactive hyperaemia

Individual FMD results are presented in table 6.7. The mean results for FMD suggested that it was attenuated during continuously recorded PWV in comparison to both intermittent recording and FMD only (2.16 ± 2.17 %, 3.12 ± 1.61 % and 3.71± 2.74 % respectively).

	Continuous PWV recording				Intermittent PWV recording				FMD only			
Subject	BL dia (mm)	PK dia (mm)	FMD_{ABS} (mm)	$FMD_{\%}$ (%)	BL dia (mm)	PK dia (mm)	FMD_{ABS} (mm)	$FMD_{\%}$ (%)	BL dia (mm)	PK dia (mm)	FMD_{ABS} (mm)	$FMD_{\%}$ (%)
1	3.33	3.34	0.01	0.33	3.24	3.40	0.16	4.95	3.74	3.86	0.12	3.25
2	4.21	4.40	0.19	4.55	4.21	4.31	0.11	2.50	4.10	4.37	0.27	6.65
3	2.91	2.95	0.05	1.59	2.85	2.90	0.05	1.92	2.90	2.94	0.04	1.22

Table 6.7: Subject data for FMD assessed during continuous and intermittent PWV recording and FMD only. BL dia= baseline diameter; PK dia = peak diameter; FMD_{ABS} = absolute change in diameter; $FMD_{\%}$= flow-mediated dilatation as a percentage.

6.4.5.4 Implications

With the limited data available the continuous recording of PWV appeared to decrease the flow stimulus and FMD response suggesting that this may not be the most appropriate method for assessing the two techniques simultaneously. As the change in PWV following a period of forearm ischaemia was still very low with the mid-forearm cuff position, moving the occlusion cuff to just below the medial epicondyle may stimulate a greater slowing in PWV. The additional stimulus may also make it easier to investigate the impact of continuous PWV recording on the flow stimulus in comparison to intermittent PWV recording.

6.4.6 Study 2B: Further investigations of cuff position and PWV recording

6.4.6.1 Aim

Due to the limited changes in PWV with the occlusion cuff at the wrist or mid-forearm, it was decided to test the effect of positioning the occlusion cuff in the traditional position for an FMD study, just below the medial epicondyle. This would increase the area of arm being made ischaemic and which in turn would increase the flow stimulus and therefore increase the slowing of PWV. Therefore, the aim of this study was to assess the effect on hyperaemia and FMS of changing the occluding cuff position to just below the medial epicondyle. The second aim was to further investigate the most appropriate method for recording the PWV data by comparing continuous and intermittent recordings of PWV in a larger number of subjects.

6.4.6.2 Methods

Ten subjects were studied with the PWV cuffs positioned as previously described and the occlusion cuff placed just below the medial epicondyle as in figure 6.10. The order of the studies varied for each subject (figure 6.11). Area under the curve (AUC) of the flow response to the 5 minute ischaemic period was assessed in addition to the other measures. VTI was calculated from each Doppler waveform using proprietary software on the ALOKA for the 2 minute period following release of the occlusion cuff. The time of each screen of waveforms was noted and AUC was then calculated from these data.

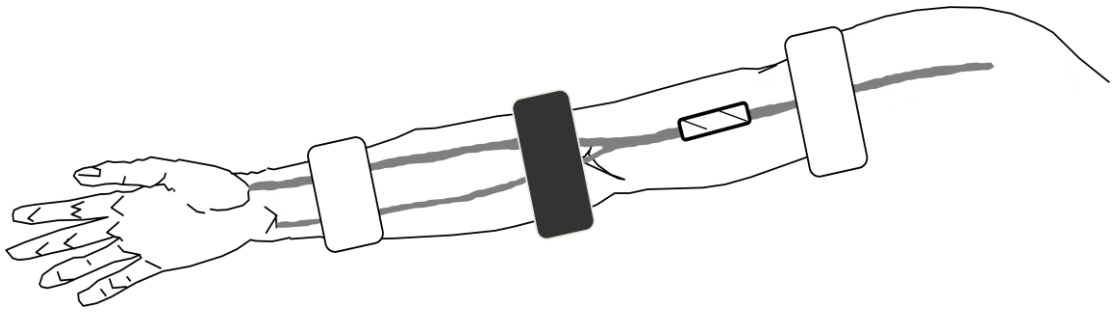

Figure 6.10: Occluding cuff positioned up to 1cm below the medial epicondyle. ■ = occlusion cuff □ = PWV cuff =ultrasound probe

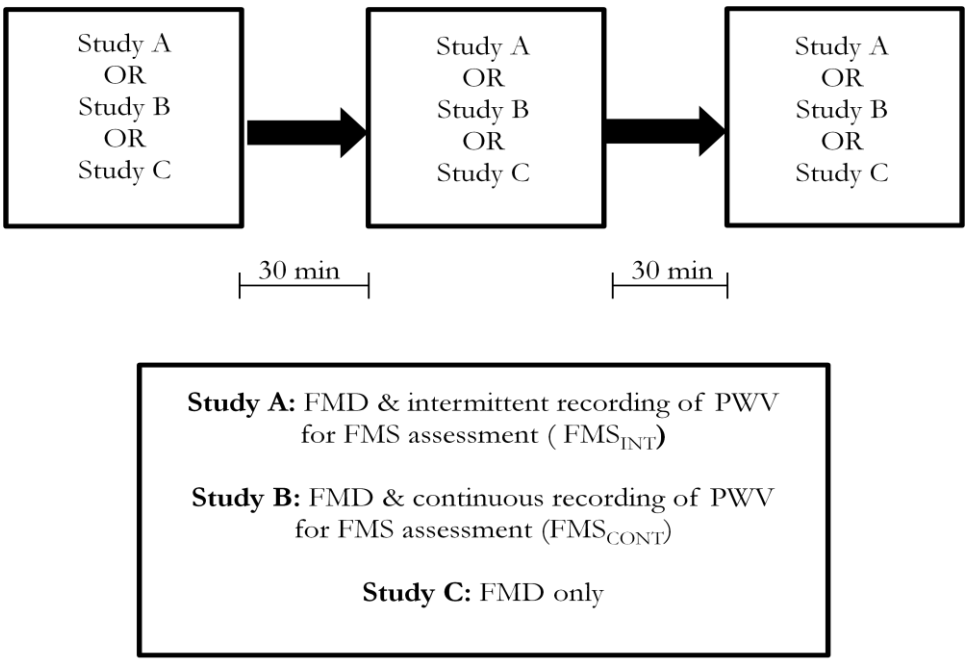

Figure 6.11: Protocol for comparing intermittent and continuous recording of PWV for FMS assessment with the occlusion cuff at the forearm. Each subject completed all 3 studies in varying order

6.4.6.3 Results

Brief characteristics of the ten subjects studied are included in table 6.8. Baseline PWV was similar when measured with both the intermittent or continuous PWV recording methods (table 6.9). Absolute change in PWV (FMS$_{ABS}$) and FMS$_{\%}$ were numerically higher with the intermittent method of recording PWV than the continuous method but this was not statistically significant (figure 6.12).

n=10	**Subjects**
Age (years)	28 ± 6
Gender (M:F)	5:5
Systolic blood pressure (mmHg)	113 ± 9
Diastolic blood pressure (mmHg)	69 ± 7

Table 6.8: Subject characteristics

n=10	**Study A (FMS$_{INT}$)**	**Study B (FMS$_{CONT}$)**	**p-value**
BL PWV(m/s)	7.29 ± 0.91	7.23 ± 0.73	*0.70*
Min PWV (m/s)	5.95 ± 0.66	6.09 ± 0.75	*0.46*
FMS$_{ABS}$ (m/s)	-1.35 ± 0.41	-1.13 ± 0.40	*0.24*
FMS$_{\%}$ (%)	-19.69 ± 4.15	-15.72 ± 5.40	*0.12*

Table 6.9: Comparison of intermittent (study A) and continuous (study B) recording of PWV for FMS studies (mean ± SD). BL PWV = baseline pulse wave velocity; Min PWV = minimum pulse wave velocity; FMS$_{ABS}$ = absolute change in pulse wave velocity; FMS$_{\%}$ = flow-mediated slowing as a percentage.

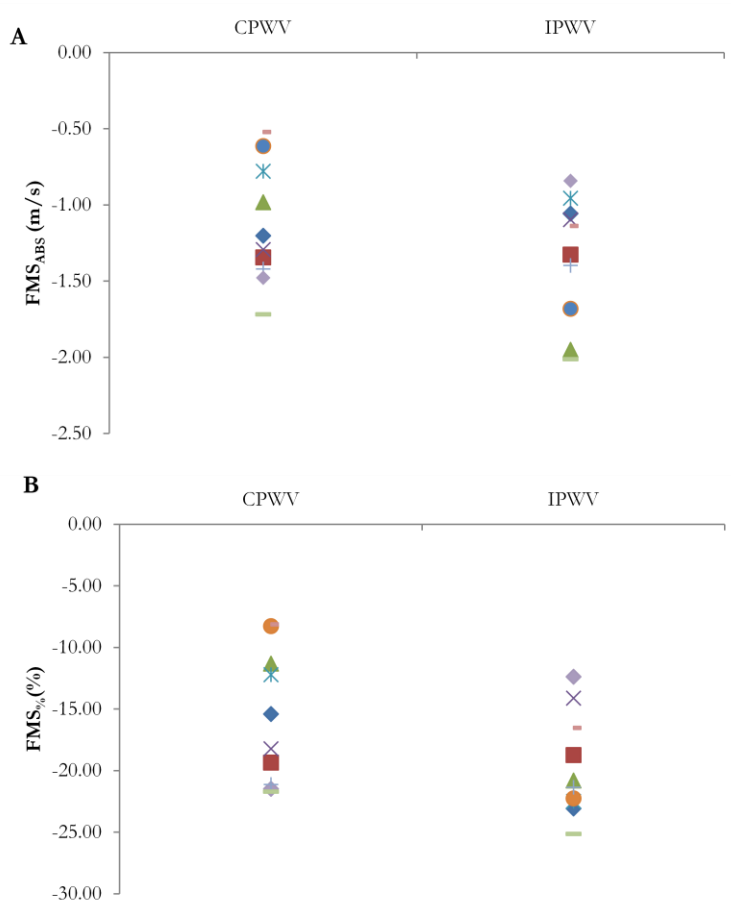

Figure 6.12: Figures showing the individual results for comparison of continuous and intermittent recording of PWV for A absolute change in PWV and B percentage change from baseline –FMS%. CPWV = continuous recording of pulse wave velocity; IPWV = intermittent recording of pulse wave velocity.

Flow and FMD responses during intermittent and continuous recording of PWV

Both $RH_{\%}$ and the AUC of the hyperaemic response were significantly lower with continuous PWV recording than intermediate PWV recording (p=0.013 & 0.001 respectively) suggesting a decreased flow stimulus for FMD and FMS. Peak diameter measured during continuous PWV recording was significantly lower than when assessed during intermittent PWV recording (p=0.035). Both absolute and percentage change in diameter measured during continuous PWV recording were lower than those recorded during intermittent PWV recording but neither quite reached significance (p=0.050 & 0.054 respectively) (table 6.10).

There was no difference between the 3 groups for baseline brachial artery diameter. (p=0.95) Analysis of the data including FMD alone with oneway-ANOVA, revealed differences between the groups for the flow response (PKVTI, RH and RH AUC), peak diameter, absolute change in diameter and $FMD_{\%}$. Post-hoc analyses (Bonferroni) identified that in all cases the differences were between continuous PWV recording and FMD only (table 6.10). Reactive hyperaemia was lower during continuous PWV recording in comparison to both intermittent recording and FMD only but this did not reach significance in post hoc analysis(p=0.06 & 0.09 respectively).

N=10	**Study A (FMS_{INT})**	**Study B (FMS_{CONT})**	**p-value∫**	**Study C (FMD)**	**p-value§**
BL dia (mm)	3.59 ± 0.56	3.57 ± 0.57	*0.51*	3.51 ± 0.58	*0.95*
PK dia (mm)	3.77 ± 0.55	3.67 ± 0.60	***0.035***	3.77 ± 0.60	*0.90*
FMD_{ABS} (mm)	0.18 ± 0.09	0.10 ± 0.06*	*0.050*	0.26 ± 0.11*	***0.002***
$FMD_{\%}$ (%)	5.19 ± 3.10	2.70 ± 1.36*	*0.054*	7.62 ± 3.99*	***0.005***
BL VTI(m)	0.047 ± 0.111	0.049 ± 0.009	*0.36*	0.055 ± 0.013	*0.30*
PK VTI(m)	0.349 ± 0.135	0.228 ± 0.090*	***0.013***	0.357 ± 0.089*	***0.021***
ABS RH (m)	0.302 ± 0.130	0.179 ± 0.090*	***0.011***	0.302 ± 0.089*	***0.019***
$RH_{\%}$ (%)	756 ± 266	471 ± 174	***0.013***	687 ± 224	***0.022***
RH AUC	1461 ± 341	1105 ± 344*	***0.001***	1675 ± 261*	***0.002***

Table 6.10: Comparison of the impact of intermittent (study A) and continuous (study B) recording of PWV for FMS studies on FMD and flow data (mean ± SD). ∫= p-values for paired t-test between the two PWV recording methods. §= p-value for ANOVA between FMD and flow data for all groups. * identifies significance between the groups with bonferroni post hoc test. BL dia = baseline diameter; PK dia = peak diameter; FMD_{ABS} = absolute change in diameter; $FMD_{\%}$ = Flow-mediated dilatation as a percentage; BL VTI = baseline velocity time integral; PK VTI = peak velocity time integral; ABS RH = Absolute reactive hyperaemia; $RH_{\%}$ = Reactive hyperaemia; RH AUC = reactive hyperaemia area under the curve during reactive hyperaemia.

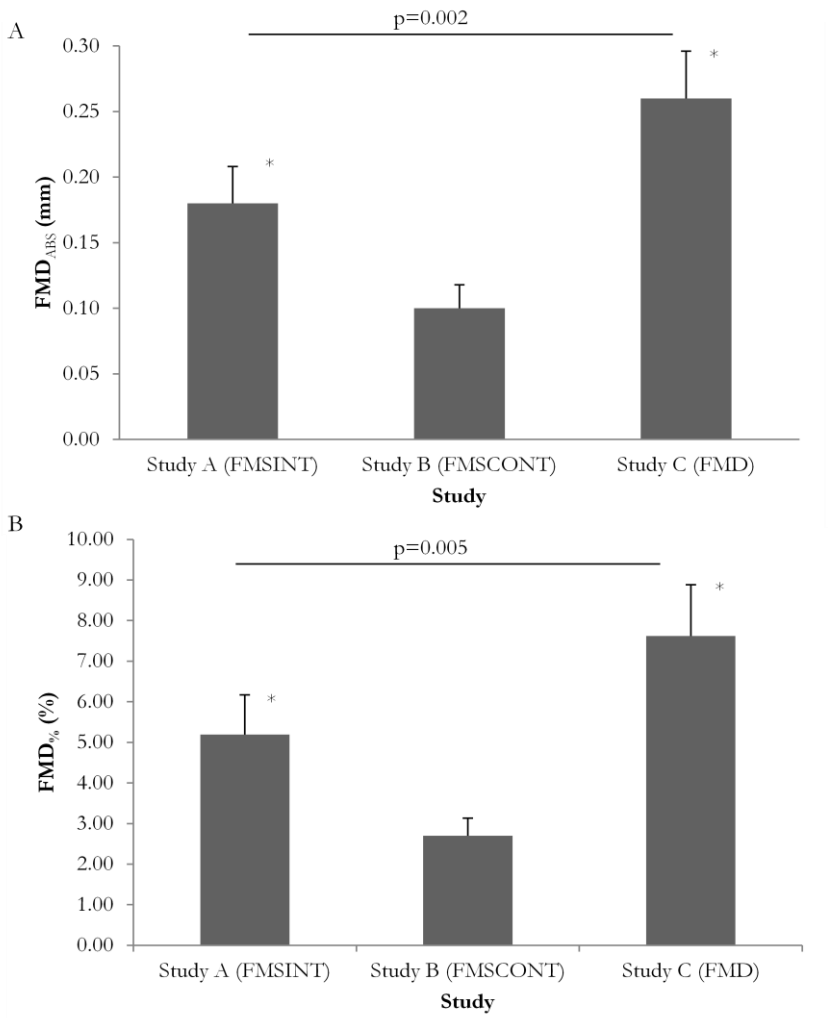

Figure 6.13: Comparison of absolute and percentage change FMD assessed during intermittent and continuous methods of recording PWV with FMD measured by itself. p-values are for comparison by ANOVA, * = significant difference between the two variables by post hoc Bonferroni.

6.4.6.4 Implications

Positioning of the occlusion cuff just below the medial epicondyle provided enough of a stimulus to drive detectable slowing in PWV in addition the FMD response was also increased. Comparison of the two different methods for recording the PWV measurements demonstrated that continuous assessment of PWV had a significant impact on reactive hyperaemia and other blood flow measures. Additionally, there was a trend for FMS to be lower with this method although this was not significant. FMD responses were also lower with the continuous PWV recording method. These results suggest that placing the occlusion cuff positioned just below the medial epicondyle, with the two PWV cuffs positioned on the upper arm and above the wrist appears to be the most technically suitable protocol for assessment of FMS. PWV should be recorded using the intermittent method as this has the least impact on the flow stimulus.

6.4.7 Study 3 Assessment of FMS on the same or contralateral arm to FMD

6.4.7.1 Aim

The aim was to assess the impact of measuring both techniques on the one arm, on the FMS and FMD responses when compared with measurements made on separate arms. This would also provide the opportunity to consider the practicality of studying both arms simultaneously.

6.4.7.2 Methods

Ten subjects were studied on two occasions. FMS and FMD were assessed simultaneously on the right arm or with FMS on the left arm and FMD on the right (figure 6.14). FMD and FMS were measured as previously described. PWV was captured using the intermittent method (figure 6.15).

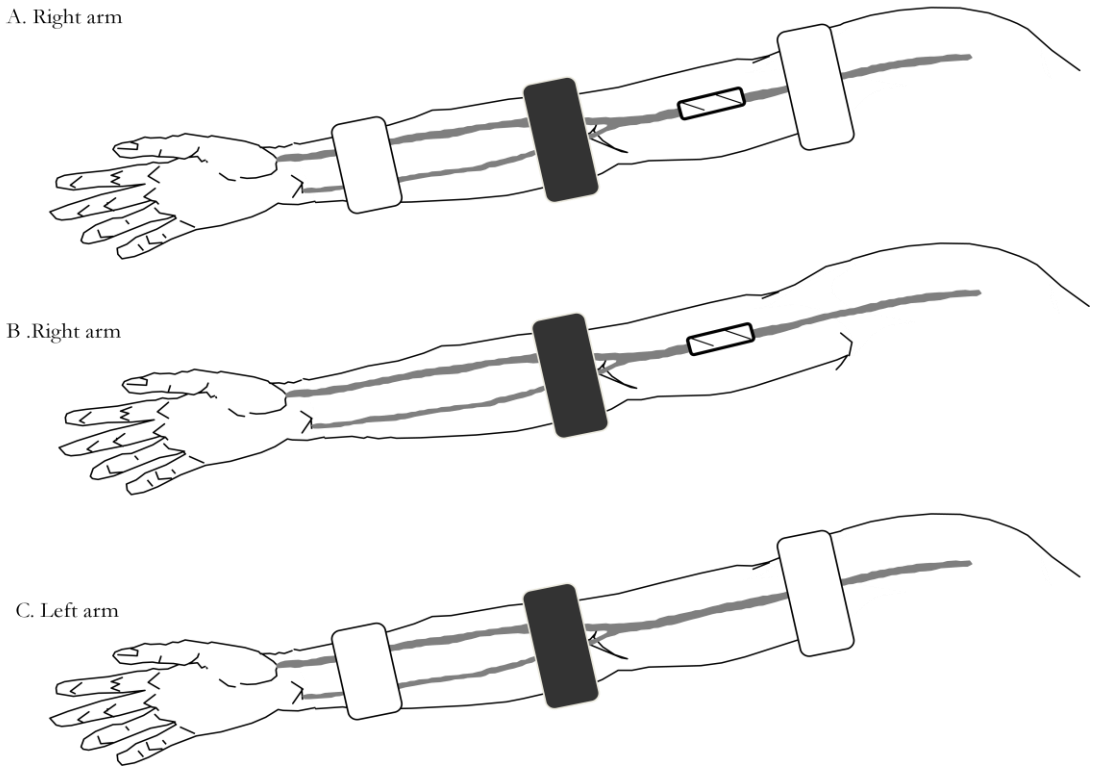

Figure 6.14: Set up of cuffs and ultrasound probe for assessment of FMS and FMD for A simultaneous assessment of FMS and FMD on the right arm. B assessment of FMD on the right arm with C assessment of FMS on the left arm. . ■ = occlusion cuff □ = PWV cuff =ultrasound probe

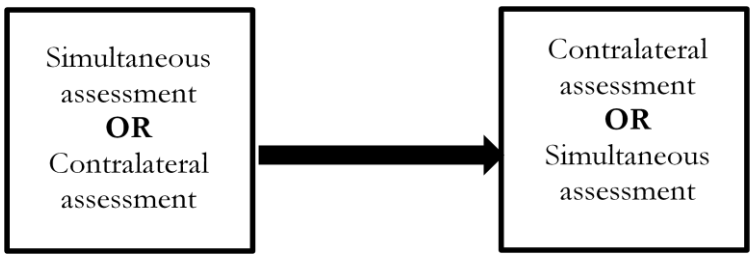

Figure 6.15: Protocol for comparison of FMS and FMD assessed simultaneously on the same arm or on with FMD measured on the right arm with FMS on the left arm

6.4.7.3 Results

As shown in table 6.11 $FMD_{\%}$ was greater when assessed on its own but this did not reach significance (p=0.11) (figure 6.16). The flow responses were similar between FMD assessed on the same arm as FMS or on separate arms.

N=10	Right arm FMD	Right arm both	p-value
BL dia(mm)	3.43 ± 0.61	3.59 ± 0.59	*0.16*
PK dia (mm)	3.67 ± 0.62	3.76 ± 0.60	*0.24*
FMD_{ABS} (mm)	0.24 ± 0.16	0.17 ± 0.07	*0.17*
$FMD_{\%}$ (%)	7.21 ± 5.07	4.79 ± 2.11	*0.11*
BL VTI(m)	0.049 ± 0.019	0.044 ± 0.009	*0.51*
PK VTI(m)	0.350 ± 0.104	0.364 ± 0.125	*0.61*
ABS RH (m)	0.301 ± 0.103	0.319 ± 0.117	*0.49*
RH (%)	785 ± 229	802 ± 163	*0.96*

Table 6.11: Comparison of data when assessing both FMS and FMD on the right arm or FMS on the left arm and FMD on the right arm (mean ± SD). BL dia= baseline diameter; PK dia = peak diameter; FMD_{ABS} = absolute change in diameter; $FMD_{\%}$ = flow-mediated dilatation as a percentage; BL VTI = baseline velocity time integral; PK VTI = peak velocity time integral; ABS RH = absolute change in velocity time integral; RH = Reactive hyperaemia.

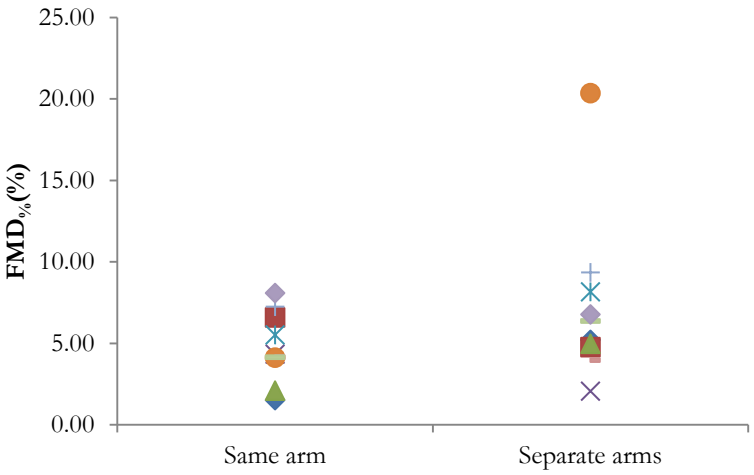

Figure 6.16: Comparison of FMD % when assessed on the same arm as FMS assessment or on the contralateral arm to FMS measurement.

Baseline PWV was significantly higher in the left arm than the right (8.87 ± 0.56 m/s vs 7.49 ± 0.29 m/s, p=0.012). The difference in FMS between the two arms was numerically lower but not statistically significant (left arm -24.42 ± 1.96 % vs right arm -19.90 ± 1.35 %, p=0.14) (figure 6.17).

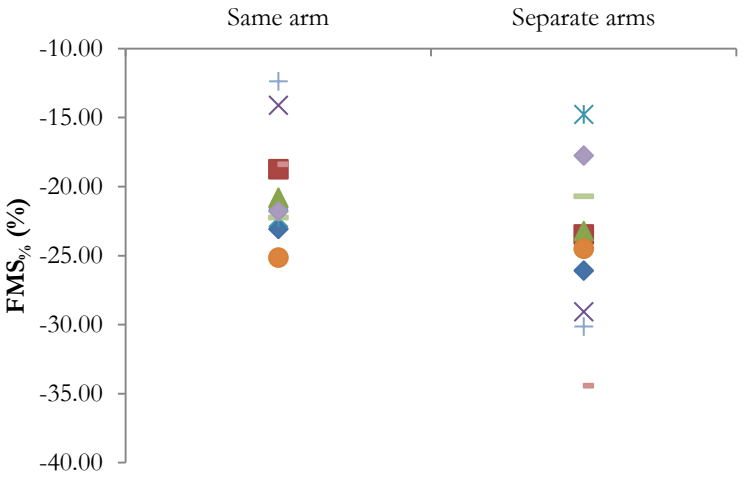

Figure 6.17: Figure showing individual results for comparison of FMS % when assessed on the same arm as FMD assessment and on the contralateral arm to FMD measurement.

6.4.7.4 Implications

From the results it can be seen that using one arm for simultaneous comparison of the two techniques did not have a significant effect on the measurements. Participants had also reported that one arm was more comfortable for them, which could have an impact on

their results. Additionally using just one arm allows other measures to be assessed during the study such as blood pressure.

6.4.8 Summary and brief discussion of protocol development

The aim of this section was to ensure that a change in PWV could be seen following a standard hyperaemic stimulus and to refine the protocol as required. The key initial finding was that when first testing the method as specified in Naka et al, using a 5 minute occlusion just above the wrist, a reduction in PWV was not seen[164].However, after adaptation and rigorous refinement of the protocol, a reduction in PWV post-hyperaemia could be induced.

The unexpected observation of a lack of slowing of PWV in the first participant evaluated raised questions about the most appropriate position for the occlusion cuff and the best method of recording the PWV waveforms to achieve the most accurate and therefore repeatable results. The next study therefore looked at whether moving the occlusion cuff position to mid-forearm led to a reduction in PWV following reactive hyperaemia and if this change was greater than that achieved with the occlusion cuff at the wrist. The initial studies in 5 participants comparing the cuff position just above the wrist and the cuff on the mid-forearm showed limited reductions in PWV following reactive hyperaemia. There was however, no difference in $FMS_{\%}$ between the two cuff positions. FMD did appear to be marginally greater with the mid forearm cuff position.

We then compared two methods for acquiring the PWV data, intermittent recording and continuous recording. The initial comparison study on three participants was carried out with the occlusion cuff positioned around the mid forearm. We became concerned that the persistent cuff inflation for the continuous recording of PWV was having an adverse impact on reactive hyperaemia through venule occlusion. As the change in PWV was also still rather low in these studies it was decided to repeat the experiment in a larger number of subjects but with the occlusion cuff positioned just below the medial epicondyle as most commonly used in FMD studies. This led to an increase in the flow stimulus and consequently a larger reduction in PWV, along with a greater change in brachial artery diameter. Although continuous recording of PWV did not significantly affect the change in PWV following reactive hyperaemia, there was a decrease in the flow stimulus.

Finally, we looked at whether FMS acquisition had an impact on FMD when assessed simultaneously on the same arm. The data appeared to show that there was no significant effect on FMD. However, it was noted that there was a significant difference in baseline PWV between the two arms. This may be due to differences in how the arteries originate from the aorta. On the right side the brachocephalic artery branches off the aorta before dividing into the common carotid and the subclavian arteries, whereas on the left the subclavian artery comes directly off the aorta.

It is worth noting that the number of participants tested in these preliminary studies was small and therefore they may be underpowered for statistical analysis, which would therefore put the statistical findings at risk of type I and type II errors.

In conclusion, from these preliminary studies it was found that the most appropriate protocol for FMS assessment is for the occlusion cuff to be positioned just below the medial epicondyle, with the PWV cuffs positioned just above the wrist and immediately below the arm pit. The PWV waveforms should be acquired following regular inflation and deflation of the cuffs at approximately 20 second intervals. It is worth noting, that with this method, part of the arterial tract being studied (approximately one third) is being made ischaemic. This does have implications regarding whether the changes in PWV following reactive hyperaemia will be purely mediated by NO or whether other vasodilators may play a role. The next stage in the validation process of this technique would be to assess the reproducibility of this protocol.

6.5 Reproducibility study

6.5.1 Aim

To demonstrate the reproducibility of the FMS technique

6.5.2 Methods

6.5.2.1 FMS

Flow-mediated slowing was assessed using the Vicorder. Two Hokanson SC5 cuffs were positioned around the upper and lower right arm to assess PWV, a third cuff was placed just below the medial epicondyle to occlude the forearm for 5 minutes (figure 6.18). PWV was assessed using intermittent inflation and deflation for 5 minutes prior to the occlusion

to assess baseline PWV. Following the release of the occluding cuff PWV data were acquired for a further 10 minutes.

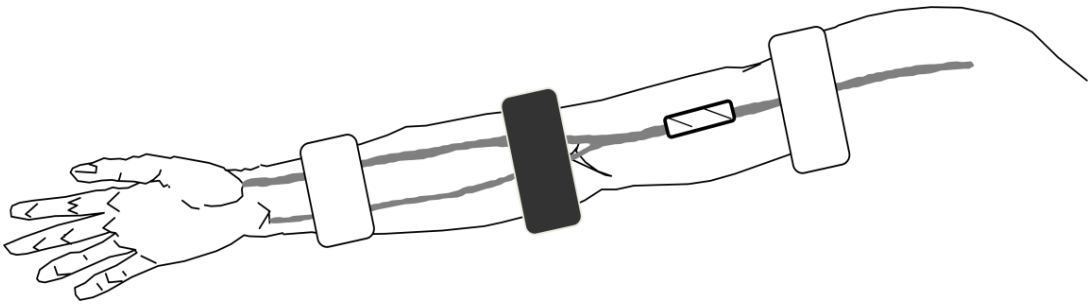

Figure 6.18: Set up of cuffs and ultrasound probe for assessment of FMS and FMD. ■ = occlusion cuff □= PWV cuff ▭ =ultrasound probe

6.5.2.2 FMD

FMD was assessed as described in the protocol development section.

6.5.2.3 Arterial stiffness

Pulse wave analysis was assessed using the Vicorder. Participants were seated and a Hokanson SC10 was positioned around the upper portion of the right arm. A blood pressure measurement was taken using the Vicorder, the cuff was then reinflated to diastolic blood pressure to record brachial artery waveforms which were saved to the Vicorder. This was repeated a second time and the results were averaged. Measurements of augmentation pressure and central blood pressure were recorded.

Central pulse wave velocity was assessed using the Vicorder. Subjects were rested semi-supine at an angle of approximately 30º. A narrow cuff was positioned around the neck with the bladder over the right carotid artery; a Hokanson SC10 was then position around the upper thigh of the right leg. The distance from the suprasternal notch to the centre of the thigh cuff was measured with a tape measure and entered into the Vicorder. Both cuffs were then inflated to 65 mmHg and the waveforms were recorded. Two readings within 0.5 m/s of each other were accepted and the results averaged.

6.5.2.4 Protocol

25 apparently healthy volunteers aged between 21 and 37 were recruited locally. Subjects were asked on visit one to arrive having previously fasted and avoided caffeine and heavy exercise for 8 hours. Consent was obtained and a medical history taken to ensure subjects met the inclusion criteria. Anthropometric measurements of height, weight, hip and waist

were taken. Seated blood pressure was taken using the Vicorder. Blood samples were taken from the finger tip of the non dominant hand and analysed using the Alere Cholestech LDX (Alere LTD, Cheshire, UK) to check fasting lipid and glucose status. Subjects then rested supine on a bed for 10 minutes before their vascular function was assessed.

6.5.2.5 Analysis

Baseline PWV was calculated as the average of all PWV measurements recorded in the first 5 minutes. Minimum PWV was the lowest PWV value recorded within 2 and a half minutes following the release of the occlusion cuff. As previously described FMS was expressed as absolute change in PWV (minimum PWV – baseline PWV) and percentage change ([absolute change/ baseline PWV]x100)). In addition, FMS at 1 min post cuff release was also calculated as absolute change in PWV and percentage change) for comparison with previously published work and to see if this was more reproducible than maximum change.

Data are presented as mean ± SD. Data were checked for normality using the Shapiro-Wilk test. Within method reproducibility was demonstrated using percentage of the coefficient of variation calculated as ((SD of the differences/overall mean)/√2)x100. Bland-Altman plots were also used to assess agreement between the repeated data. The relationship between FMD and FMS was examined using Pearson and Spearman correlations. Paired t-tests and related-samples Wilcoxon signed rank tests were used to look for differences between variables from visit 1 and visit 2. A p-value of <0.05 was considered statistically significant. Statistical analysis was carried out using SPSS version 18.

6.5.3 Results

The key finding in this section was that this new FMS protocol produced reproducible results.

n=25	Healthy controls
Age (years)	27 ± 5
Gender (M:F)	18:7
Body mass index (kg/m2)	25.35 ± 2.41
SBP(mmHg)	122 ± 10
DBP (mmHg)	72 ± 7
Central SBP (mmHg)	111 ± 8
Augmentation pressure (mmHg)	3 ± 3
Aortic PWV (m/s)	6.42 ± 0.76
TC (mmol/L)	3.92 ± 0.79
Glucose (mmol/L)	4.54 ± 0.58

Table 6.12: Subject characteristics

Table 6.12 shows the characteristics of the 25 participants recruited for the study. Room temperature, skin temperature and blood pressure pre and post endothelial function testing were not significantly different between visits (table 6.13).

	Visit 1	Visit 2	p-value
Room temperature (°C)	22.87 ± 1.27	22.86 ± 1.09	*0.98*
Skin temperature (°C)	31.45 ± 2.09	31.57 ± 1.82	*0.82*
Pre systolic blood pressure (mmHg)	113 ± 10	114 ± 12	*0.65*
Pre diastolic blood pressure (mmHg)	65 ±7	64 ± 6	*0.67*
Pre heart rate (bpm)	60 ± 11	59 ±7	*0.27*
Post systolic blood pressure (mmHg)	113 ± 10	112 ± 12	*0.42*
Post diastolic blood pressure (mmHg)	64 ± 6	64 ± 6	*0.89*
Post heart rate (bpm)	60 ± 9	60 ± 8	*0.72*
Distance between FMS PWV cuffs (cm)	32.5 ± 3.48	32.4 ± 3.21	*0.94*

Table 6.13: Factors that may influence vascular measurements for visit 1 and 2. Supine blood pressure results pre and post assessment of endothelial function are also shown.

	Visit 1	Visit 2	p-value	CV%
Baseline PWV (m/s)	7.64 ± 1.05	7.53 ± 0.91	*0.37*	3.3
Min PWV (m/s)	6.04 ± 0.78*	5.93 ± 0.72*	*0.40*	4.1
Max FMS_{ABS} (m/s)	-1.60 ± 0.58	-1.60 ± 0.42	*0.99*	8.2
Max $FMS_{\%}$ (%)	-20.66 ± 6.02	-21.12 ± 4.32	*0.55*	7.3
1 min PWV (m/s)	6.45 ± 0.82*	6.27 ± 0.83*	*0.14*	4.8
1 min FMS_{ABS} (m/s)	-1.19 ± 0.59	-1.27 ± 0.43	*0.47*	18.4
1 min $FMS_{\%}$ (%)	-15.22 ± 6.33	-16.95 ± 5.35	*0.25*	17.2
Baseline Diameter (mm)	3.85 ± 0.72	3.84 ± 0.71	*0.71*	2.2
Peak Diameter (mm)	4.01 ± 0.71	4.00 ± 0.70	*0.86*	2.3
FMD_{ABS} (mm)	0.16 ± 0.12	0.17 ± 0.11	*0.58*	28.1
$FMD_{\%}$ (%)	4.31 ± 3.36	4.55 ± 3.14	*0.55*	26.6
Baseline VTI (m)	0.045 ± 0.016	0.042 ± 0.016	*0.34*	15.5
Peak VTI (m)	0.299 ± 0.071	0.292 ± 0.076	*0.57*	8.1
Absolute VTI (m)	0.254 ± 0.063	0.25 ± 0.072	*0.73*	10.3
$RH_{\%}$ (%)	715 ± 187	752 ± 229	*0.42*	14.9

Table 6.14: Results for both FMS and FMD studies for visits 1 and 2. P-values are for comparison between visit 1 and visit 2. Reproducibility is demonstrated by CV% = coefficient of variation expressed as a percentage. * = significantly different from baseline pulse wave velocity (PWV), P<0.001. For FMS studies two analyses of results were completed, maximum change post cuff release (max) and change in PWV at 1 minute post cuff release (1min). Min PWV = minimum PWV post release of occlusion cuff; FMS_{ABS} = absolute change in PWV; $FMS_{\%}$ = percentage change in PWV from baseline; FMD_{ABS} = absolute change in brachial artery diameter; $FMD_{\%}$ = change in brachial artery diameter from baseline diameter expressed as a percentage; VTI = velocity time integral.

The results for both methods of vasomotor function assessment are shown in table 6.14. There was no significant difference between visits for either technique. Following reactive hyperaemia both minimum PWV values for the two methods of analysing FMS (maximum change and PWV at 1 minute) were significantly decreased from baseline PWV. From table 6.14 it can be seen that for both absolute and $FMS_{\%}$, a larger response was seen with taking the maximum change rather than change at 1 minute. Maximum change also had a lower CV% (Max $FMS_{\%}$ = 7.3 % vs 1 min $FMS_{\%}$ = 17.2 %) demonstrating better reproducibility. Overall the technique was very reproducible with a good CV% for the baseline PWV and a lower CV% than FMD in this study. Bland-Altman plots also show good agreement for the measures (figure 6.19).

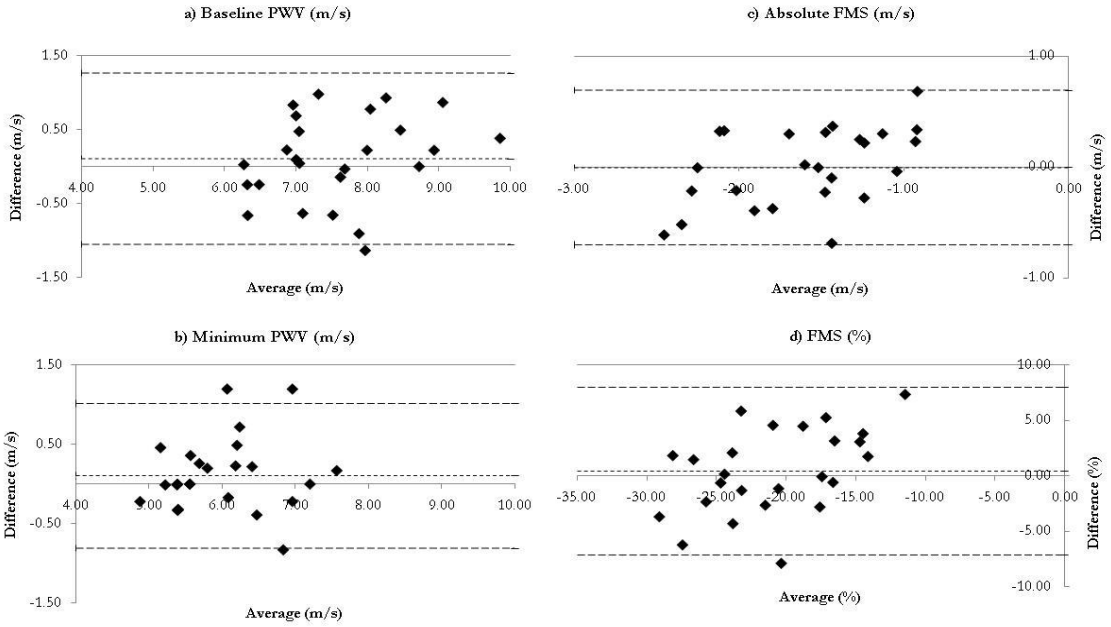

Figure 6.19: Bland-Altman plots for a) baseline PWV, b) minimum change PWV, c) absolute change in PWV d) FMS as a percentage. _____= 1.96 standard deviation; --------=mean difference between the two measures

Interestingly, there was no correlation between the two methods of vasomotor function assessment except for between max FMS_{ABS} and FMD_{ABS} for visit 2 (max $FMS_{\%}$/ $FMD_{\%}$ V1 r = 0.27, p=0.19, max $FMS_{\%}$/$FMD_{\%}$ V2 r = -0.39, p=0.05, max FMS_{ABS}/FMD_{ABS} V1 r = 0.14 p=0.52 & max FMS_{ABS}/FMD_{ABS} V2 r = -0.45, p = 0.022). The only association between FMS assessed at 1 minute and FMD was again between 1min FMS_{ABS} and FMD_{ABS} for visit 2 (1 min $FMS_{\%}$/$FMD_{\%}$ V1 r = 0.18 p = 0.39, 1 min $FMS_{\%}$/$FMD_{\%}$ V2 r =0.28 p =0.18, 1 min FMS_{ABS}/FMD_{ABS} V1 r = 0.08, p= 0.72 & 1 min FMS_{ABS}/FMD_{ABS} V2 r = -0.45 p = 0.024) (figure 6.20).

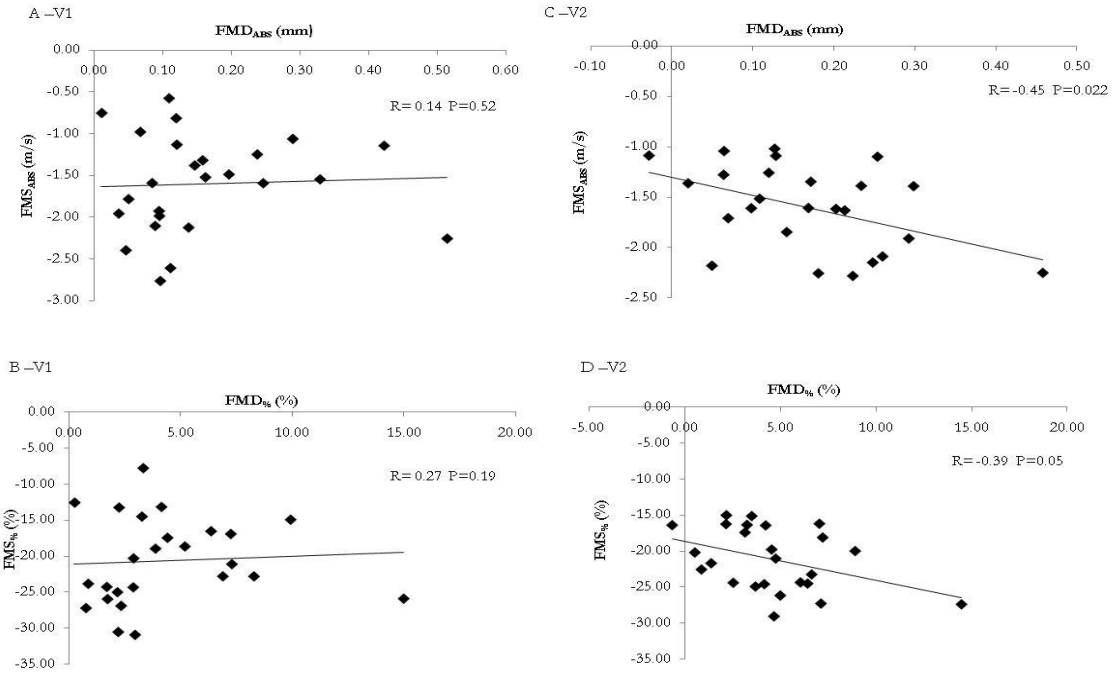

Figure 6.20: Relationship between max FMS_{ABS} and FMD_{ABS} and max $FMS_{\%}$ and $FMD_{\%}$ for both visit 1 (A &B) and visit 2 (C&D).

6.5.4 Discussion

The novel finding in this section was that the oscillometric method for assessing FMS was very reproducible. Both maximal percentage and absolute changes in PWV were more reproducible than readings taken at the one-minute time point post-cuff release. The change in PWV at 1 minute was also lower than maximal FMS, suggesting that taking readings at 1 minute intervals misses the maximum change in PWV, therefore potentially underestimating the result. It would also partially explain why my results using maximal FMS are greater than those seen by Naka et al who assessed PWV 1 minute post occlusion cuff release, in addition to any influence from the difference in occlusion cuff position.

FMS also had superior reproducibility to that of FMD acquired simultaneously using ultrasound on the same arm in this study. Despite a very good CV% for measurement of baseline diameter (CV 2.2%), FMD had relatively poor reproducibility when expressed as both absolute and percentage change in diameter (CV 28.1% and 26.6% respectively). Although in the preliminary studies concurrent assessment of FMS on the same arm did not appear to have a great effect on FMD, the results of the reproducibility study suggest there may have been more of an impact than expected. The repeated inflation and deflation of the PWV cuffs with the resultant movement of the artery and surrounding muscles may well have limited the accuracy of measurement of arterial diameter by

ultrasound during reactive hyperaemia. A CV% of 7.1% for FMD has been shown in a study by Donald et al which is comparable to that found for FMS in this chapter[150]. In the study by Donald et al, FMD had superior reproducibility to other non-invasive methods of assessing endothelial function (changes in pulse contour analysis and pulse wave analysis following inhalation of salbutamol). The CV% for FMS were also smaller than these alternative methods (pulse wave analysis CV% 11.5%) and pulse contour analysis CV% 18.2%), demonstrating its potential for use as a measure of vasomotor function[150].

Other studies have investigated the change in PWV following a reactive hyperaemic stimulus as a method for assessing vasomotor function and shown good reproducibility[165, 166]. However, these studies have assessed carotid to radial PWV and used different techniques such as applanation tonometry and mechanotransducers, to measure PWV. In addition, different occlusion cuff positions have been used. For example, Kamran et al placed the occlusion cuff around the upper arm, whilst Graf et al. placed the occluding cuff at the forearm, as we have done in our reproducibility study[165, 166].

The mixed results for associations between FMD and FMS are surprising. The lack of association between FMD and FMS parameters for visit one does agree with previous findings by Dhindsa et al.[406]. Donald et al also found no association between FMD and other measures of endothelial function and therefore this could indicate different influences of mechanical and physiological changes on diameter and stiffness of the vessels walls for the different methods[150]. It is unclear why visit 2 should then show an association between the FMS_{ABS} and FMD_{ABS}. Although Kamran et al did find a similar relationship between FMD and change in PWV expressed as a percentage[167]. The numbers in this study are relatively small and therefore the findings may be due to chance and would therefore comparisons of these two methods would benefit from being carried out in larger populations.

6.5.4.1 Limitations

One limitation of this study is due to the repositioning of the occlusion cuff to just below the medial epicondyle and therefore above the lower PWV measurement cuff. This repositioning may impact on the mechanisms which underpin the changes in vascular tone. It has been shown in FMD studies that when the cuff is positioned just below the elbow, the resultant dilatation is mediated by NO. However, when an upper arm occlusion cuff

position is used, the change in diameter is not purely mediated by NO, and as such other factors may also be contributing to the reduction in PWV in response to hyperaemia[149]. This limitation could be overcome if the software on the Vicorder was developed to allow the lower PWV measurement cuff to also be used as the occlusion cuff.

6.5.4.2 Conclusion

In conclusion, it has been shown in this chapter that FMS is a highly reproducible technique and has potential to become a useful tool for the assessment of vasomotor function. Further validation studies are needed to explore its ability to detect differences in vasomotor function in those with a cardiovascular risk factors and following interventions which are known to alter endothelial function.

Chapter 7. Flow-mediated slowing and familial hypercholesterolaemia

7.1 Introduction

In the previous chapter I have developed the protocol for FMS and demonstrated that it is a reproducible technique. To further validate this method it now needs to be tested in a population of patients that have previously been shown to have diminished endothelial function.

Familial hypercholesterolaemia (FH) is a common autosomal dominant disorder of lipoprotein metabolism. There are a number of causes; most commonly it is due to mutations in the LDL receptor gene, which decrease the function or numbers of the receptors. Genetic abnormalities have also been found in the apolipoprotein B gene, reducing interactions between the receptor and LDL, and in the PCSK9 gene, which increases the rate of turnover of the LDL receptor[75]. In response to these mutations, the liver's capacity to catabolise LDL in a regulated manner is impaired, prolonging the length of time these atherogenic lipoproteins remain in the plasma, and increasing their propensity to undergo oxidation and other chemical modification. These LDL particles pass through the endothelium into the subendothelial space, where they are retained, or are taken up by macrophages leading to the formation of cholesterol-laden foam cells. This therefore promotes the rapid development of atherosclerotic disease.

Patients can either have two defective alleles (homozygous) or just one (heterozygous). Homozygous FH is relatively rare, but patients tend to have dramatically elevated high total and LDL cholesterol levels, resulting in accelerated atherosclerosis, supra valvular aortic stenosis and are at very high risk of dying prematurely[76]. Heterozygous FH is relatively milder and far more common affecting 1 in 500 of the population, although more recent analyses have found this to be as high as 1 in 250[77]. Patients are still at a greater risk of rapid development of atherosclerosis, typically presenting clinically at a much earlier stage in life than those without FH[76].

Previous work has shown the presence of endothelial dysfunction in conduit arteries (including by assessment of flow-mediated dilatation [FMD]) and the microcirculation in both adults and children with FH[92, 139, 405, 407, 408]. The effects of increased LDL are both complex and multifactorial, but, may in part, be due to direct effects of oxidised and modified LDL on endothelial nitric oxide synthase and nitric oxide production[409, 410]. In addition, other changes that occur in the vessel wall, such as lipoprotein-endothelial activation and the uptake of lipoproteins by macrophages, causes the activation of inflammatory processes and increased oxidative stress, which also lead to endothelial dysfunction[9, 411, 412]. Therefore, diminished endothelial function as assessed by FMD has previously been shown in those with FH, this would be an interesting group to study to test whether differences in vasomotor function could be seen in FMS and compared with FMD.

7.2 Aims

Aim: To investigate whether FMS is able to detect vasomotor dysfunction in a group of patients with a cardiovascular risk factor known to cause endothelial dysfunction.

Hypothesis: FMS is able to detect vasomotor dysfunction in those with FH when compared with a group of healthy matched control subjects.

7.3 Methods

7.3.1 Subjects

FH patients who met the Simon Broome criteria (as described in Chapter 1) were recruited through the FH cascade database or when attending lipid clinics at the University Hospital of Wales and University Hospital Llandough[78]. Healthy controls were recruited from staff and the local area. Those FH patients on vasoactive medication were asked to not take them on the morning of the study. Patients were not requested to stop lipid lowering therapy.

7.3.2 Pulse wave analysis

Pulse wave analysis was assessed with the Vicorder (software version 5.04) with the participants seated. A Hokanson SC10 cuff was positioned on the study arm and blood

pressure was assessed. The cuff was then inflated to diastolic pressure and augmentation index and pressure were measured. Two readings were accepted which had differences in augmentation pressure (AP) of ≤5 mmHg and augmentation index (AIx) of ≤5%, the mean of the two readings was then taken as the result.

7.3.3 Pulse wave velocity

Central PWV was assessed using the Vicorder; with the patients lying semi supine at an angle of approximately 30º to reduce venous interference. A narrow cuff was positioned around the neck with the bladder placed over the site of the right carotid artery. A second cuff (Hokanson SC10) was wrapped tightly around the upper right thigh over the femoral artery. Path length was measured from the supra sternal notch to the middle of the thigh cuff using a tape measure, care was taken not to follow the contour of the body. Having entered the path length into the Vicorder both cuffs were then inflated to 65mmHg. Once good quality waveforms were acquired the signal was saved and a repeated measurement made. Two readings with a difference of in PWV ≤0.5m/s were averaged for the final result.

7.3.4 Flow-mediated slowing

FMS was assessed as previously described in the reproducibility study in Chapter 6. Three cuffs were positioned around the study arm as shown in figure 7.1. The outer two cuffs were to assess PWV whilst the middle cuff occluded the vessel for 5 minutes to induce reactive hyperaemia. The distance between the two outer cuffs was measured using a tape measure from mid point to mid point and entered into the Vicorder. PWV was assessed for 5 minutes for a baseline reading prior to occlusion and recorded for a further 10 minutes following cuff deflation. Data was analysed as previously described.

7.3.5 Flow-mediated dilatation

The right brachial artery was imaged using an ultrasound probe; a Doppler cursor was positioned in the centre of the artery image to assess changes in flow. The same cuff was used for the 5 minute occlusion period as for FMS (see figure 7.1). Baseline images were recorded 1 minute before inflation of the occluding cuff continuously until 5 minutes post cuff release. Images were analysed and changes calculated as described in Chapter 6.

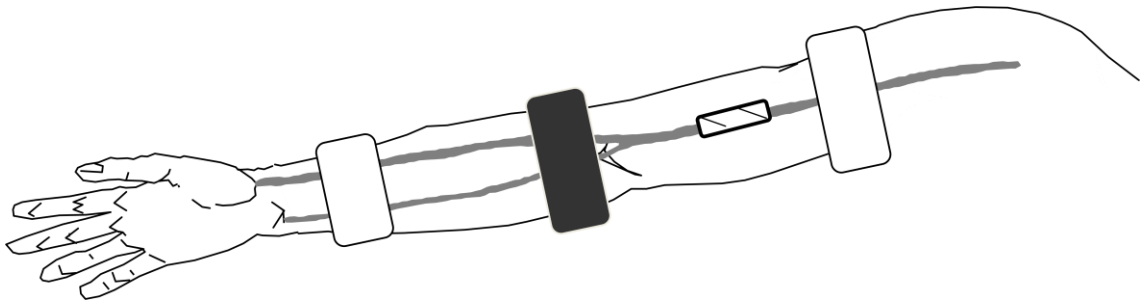

Figure 7.1: Diagram of cuff and ultrasound probe placement for assessment of endothelial function by FMS and FMD. ■ = occlusion cuff □ = PWV cuffs =ultrasound probe

7.3.6 Bloods

Blood samples were collected for assessment of total cholesterol, high density lipoprotein (HDL), Triglycerides, low density lipoprotien (LDL), total-HDL ratio and fasting glucose. All samples were analysed at the Clinical Biochemistry laboratory at University Hospital Wales.

Serum was prepared by centrifugation of blood at 4000 rpm for 8 minutes and stored at -30°C prior to analysis. Total cholesterol, HDL, and triglycerides were assayed using an Aeroset automated analyser (Abbott Diagnostics, Berkshire, UK); LDL was calculated using Friedewald's formula and glucose was measured using the Aeroset chemistry system [Abbott Diagnostics, Berkshire, UK].The intra- and inter-assay coefficients of variation were all less than 9%.

7.3.7 Protocol

Subjects attended the Wales Heart Research Institute having fasted overnight. Participants were consented and completed a medical history form to check they were eligible for the study. A blood sample was taken from the left arm to test lipids and fasting glucose levels. Height and weight were measured.

Participants then underwent arterial stiffness assessment. Subjects then rested seated for 5 minutes before blood pressure was assessed followed by PWA using the Vicorder. This was followed by assessment of aortic PWV.

Vasomotor function was then assessed. Participants rested supine for 10 minutes and two blood pressure measurements were taken using an automatic sphygmomanometer (Omron 705IT). Blood pressure was repeated before and after both vasomotor function assessments. FMD and FMS were simultaneously assessed on the right arm as described above.

7.3.8 Analysis

Power calculations had suggested that with α=0.05 and a required Power of 0.8 (80%) and σ=5%, a sample of at least 20 was required to detect a difference in FMS between the two groups.

Variables were checked for normality using the Shapiro-Wilk test. Relationships between healthy controls and patients with FH were tested using either an independent samples t-test or the independent samples Mann-Whitney U test. Spearman correlations were used to look at associations between FMS and FMD and risk factor variables. SPSS version 20 was used for the analysis. Data is presented as mean ± SD unless otherwise stated.

7.4 Results

The key finding in this section was that FMS was lower in those patients with FH compared with controls. There were no differences in FMD (obtained concurrently with FMS) observed between the two groups.

Characteristics	FH	Control	p
Age (years)	49 ± 11	48 ± 11	*0.75*
Gender (M:F)	10:12	10:12	
BMI (kg/m²)	28.11 ± 3.69	25.34 ± 2.76	***0.007***
TC (mmol/L)	5.72 ± 1.54	5.01 ± 0.83	*0.06*
LDL (mmol/L)	3.83 ± 1.53	3.07 ± 0.77	*0.08*
Trigs (mmol/L)	1.36 ± 0.97	0.96 ± 0.44	***0.037***
non-HDL (mmol/L)	4.46 ± 1.62	3.52 ± 0.8	***0.021***
HDL (mmol/L)	1.26 ± 0.32	1.49 ± 0.50	*0.07*
TC/HDL	4.9 ± 2.23	3.78 ± 1.61	***0.023***
TG/HDL	1.22 ± 1.08	0.89 ± 1.05	***0.016***
FGluc (mmol/L)	4.96 ± 0.53	5.04 ± 0.39	*0.37*

Table 7.1: Patient characteristics and lipid profiles for the two groups. TG/HDL = triglycerides – high density lipoprotein ratio

Table 7.1 shows the patient characteristics for the two groups. The healthy control group had significantly lower BMI, non-HDL, total cholesterol to HDL ratio and triglycerides to HDL ratio than FH patients. HDL was higher in the control group and the remaining lipid parameters were lower but these did not reach significance. All but two FH patients were on lipid lowering therapy. Of the treated patients all were on statin therapy, with 8 also taking ezetimibe, 1 on a fibrate and 3 patients taking ezetimibe and a resin. Three patients had evidence of cardiovascular disease, either from an event or having had bypass surgery.

	FH	Control	p
SBP (mmHg)	138 ± 17	123 ± 14	***0.007***
DBP (mmHg)	80 ± 10	75 ± 8	*0.07*
PP (mmHg)	57 ± 14	48 ± 10	***0.009***
HR (bpm)	57 ± 7	57 ± 8	*0.99*
Central SBP (mmHg)	132 ± 17	117 ± 13	***0.006***
Central PP (mmHg)	52 ± 14	42 ± 10	***0.013***
AP (mmHg)	11.86 ± 7.62	7.89 ± 4.73	*0.06*
AIx (%)	21.50 ± 7.63	18.00 ± 8.78	*0.17*
Aortic PWV (m/s)	7.4 ± 0.77	6.85 ± 0.82	*0.06*

Table 7.2: Seated blood pressure and measurements of arterial stiffness.

Three FH patients were on blood pressure medications but had not taken them on the morning of the study. All blood pressure and measurements of arterial stiffness were lower in the healthy control subjects when compared with the FH patients. However, only peripheral systolic pressure, pulse pressure and central systolic and pulse pressure reached significance as can be seen in table 7.2.

	FH	HC	p
Room temp (°C)	23.1 ± 1.2	22.4 ± 1.4	*0.12*
Skin temp (°C)	31.4 ± 1.8	31.4 ± 1.9	*0.98*
SBP (mmHg)	127 ± 17	116 ± 12	***0.016***
DBP (mmHg)	74 ± 10	70 ± 9	*0.12*
HR (bpm)	59 ± 7	56 ± 9	*0.30*

Table 7.3: Room and skin temperature and supine blood pressure assessed prior to vasomotor function assessment.

Room and skin temperature did not differ significantly between the two groups (table 7.3). Only supine systolic blood pressure was significantly higher in the FH group than the control group.

	FH	Control	p
BL dia (mm)	3.59 ± 0.60	3.56 ± 0.61	*0.86*
Pk dia (mm)	3.75 ± 0.63	3.72 ± 0.63	*0.89*
FMD_{ABS} (mm)	0.16 ± 0.07	0.16 ± 0.11	*0.98*
$FMD_{\%}$ (%)	4.33 ± 1.64	4.68 ± 3.04	*0.90*
BL VTI (m)	0.045 ± 0.010	0.042 ± 0.015	*0.54*
Pk VTI (m)	0.349 ± 0.075	0.328 ± 0.101	*0.43*
Absolute RH (m)	0.304 ± 0.069	0.286 ± 0.091	*0.45*
$RH_{\%}$ (%)	796 ± 159	816 ± 234	*0.74*

Table 7.4: FMD and blood flow measurements for FH and healthy control participants. FMD =Flow-mediated dilatation.

There was no difference in brachial artery baseline diameter between the two groups (FH 3.59 ± 0.60 vs HC 3.56 ± 0.61 mm p=0.86). Both baseline velocity time integral and reactive hyperaemia were similar between FH and control subjects (table 7.4). FMD did not differ significantly between FH patients and the age and gender matched controls (see figure 7.2).

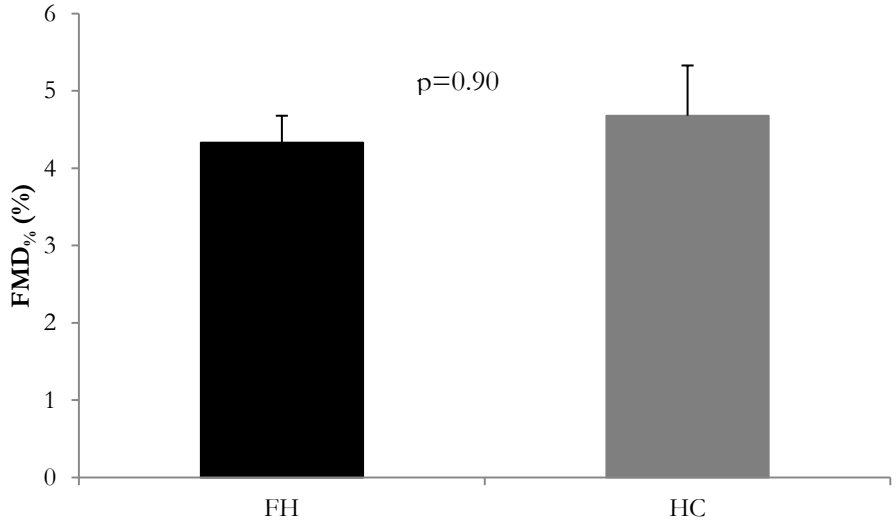

Figure 7.2: FMD results for FH and healthy controls mean ± SEM

	FH	Control	p
BL PWV (m/s)	7.74 ± 0.79	8.15 ± 0.93	*0.12*
Min PWV (m/s)	6.55 ± 0.54	6.63 ± 0.71	*0.66*
FMS_{ABS} (m/s)	-1.20 ± 0.49	-1.52 ± 0.53	***0.023***
$FMS_{\%}$ (%)	-15.13 ± 5.04	-18.41 ± 5.15	***0.023***

Table 7.5: FMS results for FH and healthy controls.

Baseline PWV was similar between the two groups (table 7.5). FMS_{ABS} was greater in the healthy controls compared to the FH patients (-1.52 ± 0.53 vs -1.20 ± 0.49 p=0.023). $FMS_{\%}$ was also lower in the FH group than the healthy controls as shown in figures 7.3 and 7.4.

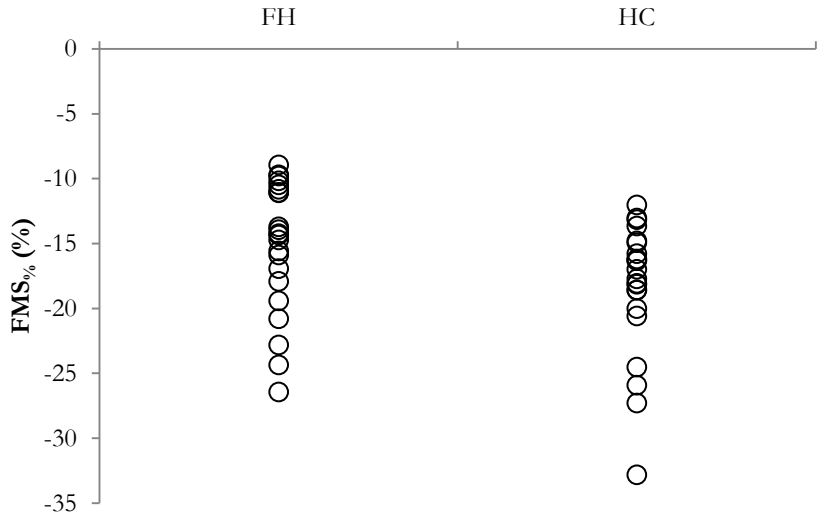

Figure 7.3: Individual results for $FMS_{\%}$ for FH patients and matched controls

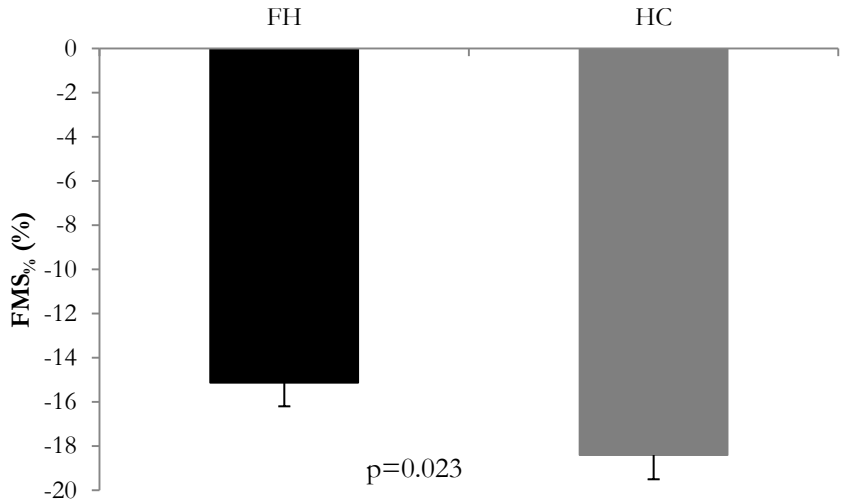

Figure 7.4: $FMS_{\%}$ results for FH patients and matched controls mean ± SEM

There were no correlations between FMD and FMS variables within FH or control groups or when both groups were combined. Within the FH group, FMD_{ABS} was positively correlated with BMI and TG/HDL ratio (r=0.45 p=0.034 & r=0.46 p=0.033 respectively) and negatively correlated with HDL (r=-0.55 p=0.008) as was $FMD_{\%}$ (r=-0.49 p=0.048). There were no associations with FMS variables in this group. In the healthy control both FMS_{ABS} and $FMS_{\%}$ were negatively correlated with TG/HDL ratio (r=-0.49 p=0.021 & r=-

0.43 p=0.046 respectively) and positively correlated with fasting glucose (r=0.47 p=0.029 & r=0.48 p=0.023 respectively). FMS% was also negatively associated with HR (r=-0.45 p=0.035). FMD_{ABS} and $FMD_{\%}$ were not correlated with any risk factor variable in the healthy control group.

7.5 Discussion

The aim of this chapter was to investigate whether it is possible to detect a difference in vasomotor function using the FMS method in a group of patients with FH, a condition in which endothelial dysfunction is well recognised. The novel finding of this study was that vasomotor function, as determined by FMS, was diminished in patients with FH. Both FMS_{ABS} and $FMS_{\%}$ were significantly lower in the FH group than the control group, in this study. However, there was no difference in FMD between the two groups. The finding of poorer vasomotor function in patients with FH does agree with previous findings[139, 405, 407]. This work also adds to that of Naka et al and Pegge et al who found that the reduction in PWV following reactive hyperaemia was diminished in chronic heart failure and diabetes patients[164, 171]. Furthermore, other groups who have used a similar approach, but with different assessment techniques, have shown reduced slowing of PWV following reactive hyperaemia, in patients with hypertension and other risk factors [165, 167, 170, 413]. The finding of diniminshed vasomotor function in this study therefore demonstrates the potential of this method for identifying changes/differences in vasomotor function in people with cardiovascular risk factors. Whether or not the differences seen in this study are clinical significant requires further studies with hard clinical outcomes. In addition, clinical cut off values of vasomotor dysfunction, which may help identify those at increased cardiovascular risk, have not been determined for this method and would be of interest to determine in future prospective studies.

The lack of difference in FMD between the two groups may be due to the impact of concurrent assessment of FMS. This does impact on the reproducibility of FMD, as reported in the previous chapter, so could be expected to decrease the sensitivity and power of the measure. It may also be that the two methods assess different vasomotor pathways. Additionally, the FH patients in this study are an actively managed group and the majority were on statin therapy, which is known to improve endothelial function[98]. This may account for the lack of difference in FMD between the FH patients and controls. However, we were still able to detect a decrease in FMS, indicating that either alternative

pathways are involved, or the concurrent assessment of the two techniques diminished the power of FMD in this study.

Peripheral blood pressure and central pressure were significantly greater in the FH group. Although aortic PWV and augmentation pressure and index were all greater in magnitude in the FH group, these differences only trended towards statistical significance. Both aortic PWV and augmentation index have been shown to be increased in people with hypercholesterolaemia but statin therapy does decrease both aortic PWV and augmentation index which may account for the borderline significance of these two variables in my study[93, 414, 415]. In addition, these measures were not the primary outcome of the study and as such the study may have been underpowered to see a significant difference in these measures.

A limitation of this study is the lack of comparison of the two techniques with endothelium independent vasodilatation through the administration of the NO donor glyceryl trinitrate (GTN). Naka et al had previously demonstrated that the reduction in PWV following administration of GTN was similar in chronic heart failure patients and controls (9.0% and 8.8% p=0.95), demonstrating a maintained smooth muscle function in these patients[164]. Also, the main aim of this study was the comparison of FMS with FMD as a practical measure of vasomotor function in response to reactive hyperaemia, which is more consistently associated with cardiovascular physiology than endothelium independent vasodilatation responses.

Both FMS and FMD are likely to be influenced by external environmental factors and other factors such as infection and medications, although we tried to control for these as far as possible, using a range of accepted recommendations, there may have been some influence from external factors on the study results.

It would also have been interesting to use multiple regression to identify factors independently associated with FMS. However, theses analyses were not carried out in this study as it is a relatively small cohort, with well treated FH patients, and therefore would have had limited power and be less likely to demonstrate clinically meaningful relationships.

It is also worth noting that although the FH group did have higher TC and LDL, they were not significantly greater than the control group. This indicates that the participants with FH were clinically well managed and this may have had an impact on the results seen in this

chapter, although vasomotor dysfunction was still identifiable as indicated by the reduced FMS response.

7.5.1 Conclusion

In conclusion, differences in vasomotor function as assessed by FMS were detectable in a stable group of patients with FH, when compared with age and gender matched controls.

Chapter 8. Lipoprotein apheresis and flow-mediated slowing in patients with familial hypercholesterolaemia

8.1 Introduction

In the preceding chapter, it was demonstrated that flow-mediated slowing (FMS) was reduced in patients with familial hypercholesterolaemia (FH), when compared with healthy matched controls. To further test FMS as a method for assessing vasomotor function, I wanted to investigate whether this technique was also sensitive enough to detect a change in vasomotor function following lipoprotein apheresis (LA); an acute lipid lowering intervention, which has previously been shown to improve endothelial function.

Statin therapy has been very effective at reducing and controlling LDL levels in patients with FH[96]. However, some patients are unable to tolerate the treatment, are resistant to multi-modality treatment and maintain persistently high LDL levels or have progressive coronary artery disease despite maximal therapy[104]. LA is an extracorporeal treatment akin to renal dialysis, which is used to remove atherogenic lipoprotein particles from the circulation, providing an alternative treatment for lowering LDL levels. There are a number methods used for extracting LDL using an extracorporeal circulatory process; these include the use of a dextran sulphate-cellulose column and a non-haemolytic column (DALI).

In addition to reducing LDL cholesterol levels, long term use of lipoprotein aphereis treatment has been demonstrated to reduce the progression of atherosclerosis and to improve outcome and survival[111]. Koziolek et al carried out a retrospective analysis of 10, 906 lipoprotein apheresis sessions and found that major cardiovascular events (death, cerebrovascular accident, myocardial infarction, limb amputation and end-stage renal failure) reduced from 7.02% events per patient per year at the beginning of treatment to 1.17% during treatment. Myocardial revascularisation rates also decreased from 22.8% to 3.8% per patient per year[111].

One possible reason for these reductions in cardiovascular events may be the improvement and preservation of endothelial function. Studies in patients with FH or high cholesterol have shown diminished endothelial function in both the peripheral and coronary circulation, as we have also found and reported on in the previous chapter[89-91]. This may in part, be due to inflammation, increased oxidative stress and inhibition of nitric oxide synthase[379, 410, 412]. Vasodilatory function can be improved acutely in both the microvasculature and the coronary circulation following a single session of lipoprotein apheresis treatment in FH patients[113, 114, 116]. However, only one study has looked at the effect of LA treatment on FMD in FH patients[117]. They found that FMD was similar in patients, who had been on LA treatment for 3 to 5 years, and age and gender matched controls, however, there was no change in FMD immediately following treatment. It is worth noting that the study only consisted of 6 patients and so may have been underpowered to detect a difference. This finding also contrasts with Mellwig et al, who found an acute improvement in coronary vasodilatory responses to dipyridamole post-HELP apheresis (as assessed using cardiac positron emission tomography) in both patients on long term apheresis and those undergoing their initial treatment[115]. Acute LA treatment could therefore be a useful clinical model to investigate whether FMS is able to detect an immediate improvement in vasomotor function.

8.2 Aim

Aim: to investigate the effect of lipoprotein apheresis treatment on vasomotor function as assessed by flow-mediated slowing.

Hypothesis: an improvement in vasomotor function will be detectable by flow-mediated slowing.

8.3 Methods

8.3.1 Subjects

12 patients who attend the Lipid Apheresis Unit at University Hospital Llandough were recruited. Patients attend the unit once a fortnight for their treatment, so the study was fitted around their normal treatment day. Patients were asked to attend having fasted overnight, and to refrain from taking any vasoactive medications. All patients had heterozygous FH, 10 had been shown to have genetic mutations.

8.3.2 Pulse wave analysis and aortic pulse wave velocity

These were assessed as described in Chapter 7.

8.3.3 FMS

FMS was assessed as previously described. Three cuffs were positioned around the study arm as shown in figure 8.1. The outer two cuffs were to assess PWV whilst the middle cuff occluded the vessel for 5 minutes to induce reactive hyperaemia. The distance between the two outer cuffs was measured using a tape measure from mid point to mid point and entered into the Vicorder. PWV was assessed for 5 minutes for a baseline reading prior to occlusion and recorded for a further 10 minutes following cuff deflation. Data was analysed as previously described.

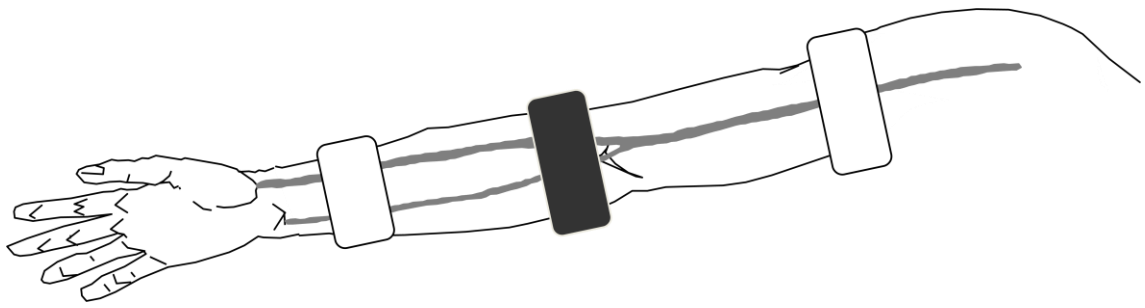

Figure 8.1: Diagram of cuff placement for FMS assessment of endothelial function. ■ = occlusion cuff □ = PWV cuff

8.3.4 Blood samples

Blood samples were collected for assessment of total cholesterol (TC), high density lipoprotein (HDL), Triglycerides (Trigs), low density lipoprotein (LDL), TC-HDL ratio and high sensitivity C-reactive protein (hsCRP). Lipid parameters were measured by the Clinical Biochemistry laboratory at University Hospital Llandough and hsCRP was analysed at the Clinical Biochemistry laboratory University Hospital Wales. The lipid samples were analysed as described in Chapter 7. hsCRP was assayed by nephelometry (BN™ II system, Dade Behring, Milton Keynes, UK).

8.3.5 Lipoprotein apheresis treatment

Direct adsorption of lipoproteins (DALI)

The DALI system, (Fresenius Medical Care, Germany) adsorbs LDL directly from whole blood. Blood was perfused through an adsorber containing polyacrylate beads which consist of polyanions with negative charges which attract the cationic groups of apolioprotein B-rich lipoproteins. A 15-17 gauge backeye needle was inserted into the patient's vein. The machine was primed with 20000 units of heparin for anticoagulation; acid citrate dextrose solution was then continuously infused into the blood line as it exited the patient's vein. Two sizes of adsorber were used for the treatment, 750 and 1000ml. Blood flow was in the range of 60 -75ml/min and 5.5-7.5L of whole blood were treated per session.

Liposorber LA-15

The LA-15 (Kaneka, Japan) separates the plasma from the whole blood. It consists of two columns of cellulose beads covalently bound with dextran sulphate to selectively adsorb ApoB-rich lipoproteins from plasma. The two columns were used alternately, as one was operated for apheresis the other was regenerated with a solution of hypertonic sodium chloride. Heparin was used to prime the system (5000 units) and then infused at 1.3ml - 1.5ml/hr for anticoagulation. Plasma flow rate ranged from 50-75ml/min with 3-4L of plasma processed. The blood was returned to the patient through a second needle in either the same or other arm.

LiposorberD

The LiposorberD system (Kaneka, Japan) takes the whole blood from the patient and uses heparin (5000 units) for priming and acid citrate solution as an anticoagulant. Whole blood was pumped through the dextran sulphate column where the ApoB-rich lipoproteins are removed. Once the lipids were removed the blood was returned to the patient. Blood flow was up to 110ml/min with 12L of whole blood treated in the session.

8.3.6 Protocol

Patients were consented on arrival at the unit, and height and weight were measured. Patients were asked which arm was normally used for their apheresis treatment and the non-treatment arm was used for measurements. Where both arms were used for treatment,

the right arm was used unless there were old fistulas present. Vascular assessment was carried out in a temperature controlled room prior to apheresis. Subjects then had two 15 to 17 gauge backeye needles positioned, as per their usual clinical care. Blood samples were taken immediately from one of the needles for clinical purposes, including lipid parameters. An additional sample was taken for assessment of hsCRP. Subjects then underwent their standard apheresis treatment. Blood samples were repeated at the end of treatment. A second vascular assessment was undertaken following the treatment after a period of acclimatisation (see figure 8.2).

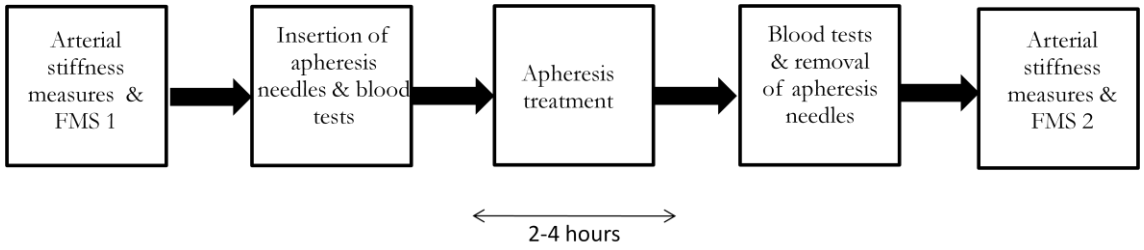

Figure 8.2: Protocol for the investigation of the effect of lipoprotein apheresis on vasomotor function assessed using flow-mediated slowing.

8.3.7 Analysis

A power calculation estimated that a sample size of 12 (two-sample t-test, α=0.05 and a required power of 0.8 (80%) would be needed to see a significant change in FMS following apheresis treatment.

Data were checked for normality using the Shapiro-Wilk test. Differences between variables before and after LA were tested using either a paired t-test or a related samples Wilcoxon-signed rank test depending on whether the data was normally distributed or not. Pearson and Spearman correlations were used to look at the associations between lipid and inflammatory parameters with FMS responses. Data are presented as mean ± SD unless otherwise stated.

8.4 Results

The novel finding in this section was that vasomotor function as assessed by FMS in the peripheral circulation was improved by a single LA treatment.

Table 8.1 shows the patient characteristics. As can be seen ten patients were on statins and three were on nitrate medications. Further clinical information on the participants is in appendix 2. Participants had been asked to fast and if possible to avoid taking vasoactive medications on the morning of the study. Two patients had a light breakfast at least 4 hours prior to testing, whilst two participants had taken their blood pressure medications on the morning of the study.

Characteristics	n=12
Age (years)	58 ± 10
Gender (M:F)	9:3
BMI (kg/m2)	30 ± 4
SBP (mmHg)	140 ± 18
DBP (mmHg)	82 ± 10
Statins	
Rosuvastatin	6/12
Atorvastatin	4/12
Nitrates	3/12
Diabetes	6/12
Presence of coronary disease	.10/12

Table 8.1: Patient characteristics and medications

The patients were being treated using three different LA systems (DALI n=8, LA-15 n=3 & LiposorberD n=1). Average treatment time was 2 hrs 37 mins, ranging from 1 hour 55 mins to 3 hours 40 mins.

	Pre LA	Post LA	p-value
TC (mmol/L)	6.05 ± 1.57	2.72 ± 0.84	***<0.001***
LDL (mmol/L)	4.08 ± 1.44	1.37 ± 0.65	***0.002***
Trigs (mmol/L)	1.77 ± 0.57	0.89 ± 0.34	***<0.001***
NonHDL (mmol/L)	4.90 ± 1.40	1.78 ±0.56	***0.002***
HDL (mmol/L)	1.15 ± 0.47	0.94 ± 0.46	***0.002***
TC/HDL	5.82 ± 2.09	3.38 ± 1.66	***0.002***
Trigs/HDL	1.95 ± 1.68	1.47 ± 1.87	***0.003***
hsCRP (mg/L)	2.31 ± 4.65	1.76 ± 3.83	***0.002***

Table 8.2: Lipid and hsCRP measures before and after LA treatment. Trig/HDL = triglycerides – high density lipoprotein ratio.

Table 8.2 shows the changes in the lipid profile of the participants. All lipid parameters and hsCRP decreased significantly following treatment.

	Pre LA	Post LA	p-value
AIx(%)	23.9 ± 6.0	23.0 ± 8.7	*0.82*
AP (mmHg)	13± 5	14 ± 7	*0.59*
Central SBP (mmHg)	137 ± 17	143 ± 17	*0.06*
Central PP (mmHg)	54 ± 12	60 ± 12	*0.09*
Aortic PWV (m/s)	9.2 ± 2.5	8.7 ± 3.0	*0.30*
FMS SBP (mmHg)	143 ± 25	143 ± 23	*0.93*
FMS DBP (mmHg)	79 ± 9	78 ± 9	*0.91*
FMS HR (bpm)	58 ± 10	63 ± 13	***0.027***

Table 8.3: Results for measures of arterial stiffness, blood pressure and heart rate pre and post LA treatment

There was no difference in room and skin temperature before the pre and post apheresis FMS studies (room temperature 24.5 ± 0.8 ºC vs 24.9 ± 0.6 ºC p=0.07 and skin temperature 31.3 ± 2.1 ºC vs 30.3 ± 1.4 ºC p=0.17) Augmentation index and pressure were unaffected by LA treatment as was aortic PWV (table 8.3). Central blood pressure increased slightly post-treatment, but this did not reach significance. Heart rate rose following apheresis treatment (58 ± 10 bpm vs 63 ± 13 bpm, p=0.027).

	BL PWV (m/s)	Min PWV (m/s)	FMS_{ABS} (m/s)	$FMS_{\%}$ (%)
Pre LA	8.75 ± 1.18	7.06 ± 1.00	-1.69 ± 0.92	-18.81 ± 9.84
Post LA	9.16 ± 1.18	6.92 ± 0.91	-2.24 ± 0.91	-24.09 ± 7.61
Difference	0.41	-0.14	-0.55	-5.28
Percentage change (%)			32.54	28.07
p-value	*0.19*	*0.53*	***0.015***	***0.016***

Table 8.4: FMS results for pre and post LA treatment. P-value is for the difference between the pre and post values. .

Baseline PWV did not change significantly between the two measurements (pre LA 8.75 ± 1.18 m/s & post LA 9.16 ± 1.18 m/s, p=0.19). FMS_{ABS} did improve after apheresis treatment (table 8.4). As can be seen in figure 8.3, $FMS_{\%}$ increased significantly by 28% following LA treatment (p=0.016). Two participants did not withhold their vasoactive medication on the morning of the study. When the analysis was repeated with these two participants excluded the improvement in FMS remained significant (FMS_{ABS} p=0.028 & $FMS_{\%}$ p=0.028).

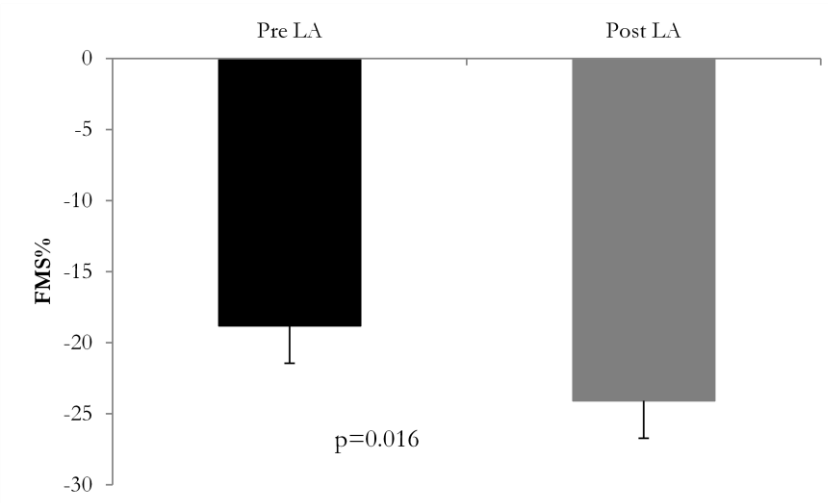

Figure 8.3: FMS before and after LA treatment (mean ± SEM)

In figure 8.4 it can be seen that $FMS_{\%}$ increased in the majority of participants following LA treatment. A slight decrease in $FMS_{\%}$ response was seen in three participants.

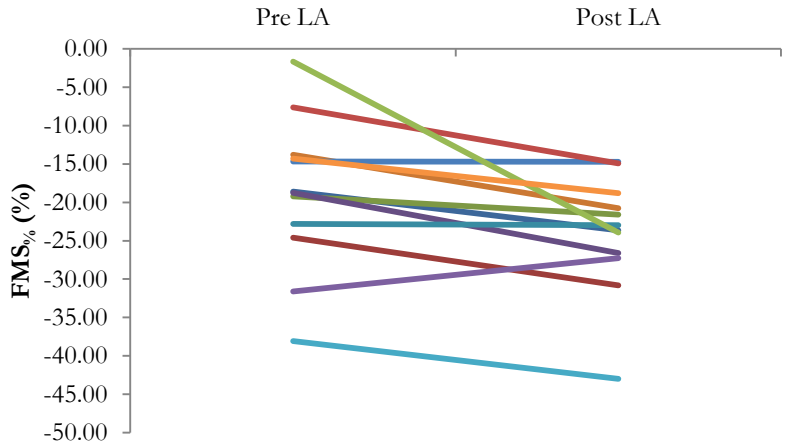

Figure 8.4: Individual FMS responses before and after LA treatment

	BL PWV (m/s)		Min PWV (m/s)		FMS_{ABS} (m/s)		$FMS_{\%}$ (%)	
	r	p	r	p	r	p	r	p
TC (mmol/L)	-0.15	*0.65*	0.04	*0.91*	0.23	*0.47*	0.25	*0.43*
LDL (mmol/L)	-0.120	*0.54*	-0.10	*0.76*	0.15	*0.65*	0.15	*0.63*
Trigs (mmol/L)	-0.39	*0.21*	-0.22	*0.49*	0.26	*0.42*	0.23	*0.48*
NonHDL (mmol/L)	-0.28	*0.38*	-0.14	*0.67*	0.21	*0.52*	0.20	*0.52*
HDL (mmol/L)	0.29	*0.35*	0.35	*0.26*	0.15	*0.63*	0.30	*0.34*
TC/HDL	-0.36	*0.25*	-0.42	*0.18*	0.02	*0.96*	-0.03	*0.92*
Trig/HDL	-0.57	*0.05*	-0.52	*0.09*	0.23	*0.47*	0.11	*0.73*
hsCRP (mg/L)	0.35	*0.27*	0.09	*0.79*	-0.39	*0.22*	-0.37	*0.24*

Table 8.5: Pearson and Spearman correlations between pre apheresis FMS responses and lipids and inflammatory parameters.

Correlations were used to investigate whether there were any associations between baseline lipid and inflammatory parameters and pre apheresis FMS responses and also between the change in $FMS_{\%}$ and FMS_{ABS} and changes in lipid and inflammatory parameters. As can be seen in table 8.5, there were no significant correlations between baseline measures.

	Change in FMS_{ABS}		**Change in $FMS_{\%}$**	
	r	**p**	**r**	**p**
TC (mmol/L)	-0.08	*0.80*	-0.06	*0.86*
LDL (mmol/L)	0.02	*0.95*	0.03	*0.92*
Trigs (mmol/L)	-0.41	*0.19*	-0.58	*0.05*
Non-HDL (mmol/L)	-0.06	*0.87*	-0.10	*0.76*
HDL (mmol/L)	-0.12	*0.72*	-0.22	*0.50*
TC/HDL	0.03	*0.92*	-0.06	*0.85*
Trig/HDL	-0.19	*0.56*	-0.19	*0.56*
hsCRP (mg/L)	0.11	*0.75*	0.15	*0.63*
Change in Total C (mmol/L)	0.04	*0.91*	0.05	*0.89*
Change in LDL (mmol/L)	-0.08	*0.81*	-0.06	*0.85*
Change in Trigs (mmol/L)	0.90	***<0.001***	0.78	***0.003***
Change in nonHDL (mmol/L)	0.03	*0.93*	0.08	*0.81*
Change in HDL (mmol/L)	0.16	*0.61*	0.34	*0.27*
Change in TC/HDL	-0.18	*0.57*	-0.09	*0.78*
Change in Trigs/HDL	0.38	*0.23*	0.26	*0.41*
Change in hsCRP (mg/L)	0.32	*0.31*	0.01	*0.97*

Table 8.6: Correlations between change in pre and post FMS_{ABS} and $FMS_{\%}$ and lipid and inflammatory parameters

As can be seen in table 8.6, there was an association between change in FMS_{ABS} and change in triglycerides (r=0.90 p=<0.001). This association was also seen between change in $FMS_{\%}$ and change in triglycerides (r=0.78 p=0.003). There was a negative correlation between baseline triglycerides and change in $FMS_{\%}$ but this did not quite reach significance (r=-0.58 p=0.05).

	Diabetic n=6	**Non-diabetic n=6**	**p**
BL PWV (m/s)	8.54 ± 1.48	8.96 ± 0.9	*0.56*
Min PWV (m/s)	6.9 ± 1.39	7.22 ± 0.46	*0.61*
FMS_{ABS} (m/s)	-1.64 ± 1.05	-1.75 ± 0.88	*0.85*
$FMS_{\%}$ (%)	-18.68 ± 12.12	-18.94 ± 8.11	*0.97*
Change FMS_{ABS} (m/s)	-0.84 ± 0.55	-0.26 ± 0.58	*0.22*
Change in $FMS_{\%}$ (%)	-7.63 ± 7.63	-2.93 ± 4.48	*0.11*

Table 8.7: Comparison of baseline FMS responses and change in absolute and percentage change FMS following LA in diabetic and non-diabetic patients.

As there were equal numbers of those with and without diabetes an exploratory analysis between the two groups was carried out. There were no differences in baseline or post apheresis FMS between the two groups (table 8.7).

n=7	LA	Healthy controls	p
BL PWV (m/s)	8.68 ± 1.11	8.00 ± 0.59	*0.10*
Min PWV (m/s)	6.73 ± 0.8	6.64 ± 0.49	*0.95*
FMS_{ABS} (m/s)	-1.95 ± 1.11	-1.36 ± 0.25	*0.14*
$FMS_{\%}$ (%)	-21.59 ± 11.89	-16.96 ± 2.53	*0.23*

Table 8.8: Comparison of baseline FMS results between 7 LA patients and 7 age and gender matched controls.

In a further exploratory analysis comparing a small group of the LA patients to age and gender matched healthy controls there were no significant differences between baseline PWV or FMS variables (table 8.8).

8.5 Discussion

The major finding in this chapter was that a single treatment of LA treatment improved vasomotor function as assessed by flow-mediated slowing in the brachio-radio arterial tract in patients with FH on chronic apheresis treatment. In agreement with previous studies a single session of LA resulted in significant reductions in LDL and other ApoB containing lipoproteins [113, 114]. A significant decrease in HDL was also found in this study in contrast to some studies[113, 416].

The finding of improved vasomotor function is in contrast with Stadler et al, who found that patients on long term LA treatment had similar FMD to age and gender matched healthy controls and saw no additional improvement in FMD immediately after LA treatment[117]. However, their study was in a smaller number of patients compared to ours (6 versus 12) and therefore may not have had enough power to detect a difference in FMD before and after apheresis treatment. My findings do agree with a greater body of work that has shown an improvement in vasomotor function in the microvasculature and coronary circulation[113-116]. A comparison of some of my LA patients with age and gender matched controls did show that baseline endothelial function was maintained and in fact was higher than the controls, although this did not reach significance. Furthermore, the study was not powered to address this comparison. Nevertheless, this does agree with Stadler et al, who also found no difference in baseline FMD between apheresis patients and

matched controls[117]. However, it does suggest that long term apheresis treatment may improve baseline endothelial function. In another study of 11 patients with end stage renal disease and peripheral arterial disease who underwent 10 sessions of LA treatment FMD was improved 4 weeks after the final treatment[118]. This could suggest a longer term beneficial effect of apheresis treatment on vasomotor function and may be one pathway through which LA treatment leads to a decrease in CVD in FH.

The improvement in vasomotor function following LA treatment may be due to a number of factors. These include the significant reduction of LDL and oxidised LDL, increased expression of eNOS mRNA in endothelial cells and the reduction of inflammatory cytokines such as CRP and TNFα [116, 417 418-420].

Unlike Igarashi et al who found an association between LDL and change in coronary endothelial function here there was no relationship between FMS and LDL but there was an association between change in triglycerides and change in FMS [113]. Those participants who showed the greatest decrease in triglycerides had the most improved vasomotor function. Decreased endothelial function has been shown to be associated with high levels of triglycerides, although the results of studies are varied[421, 422]. One study that did demonstrate lower FMD in those with mild to moderate hypertriglyceridaemia compared to matched controls also found that they had higher levels of the eNOS inhibitor asymmetric dimethylarginine (ADMA)[421]. Treatment of hypertriglyceridaemia with drug therapy (fenofibrate) has been shown to improve endothelial function and reduce levels of ADMA[423]. The more specific reduction of hypertriglyceridaemia by LA could have a beneficial effect on vasomotor function.

None of the measures of arterial stiffness (aortic PWV, augmentation pressure and augmentation index) were significantly changed by LA treatment. This agrees with a previous study by Passauer et al who found no change in aortic PWV or augmentation index following a single LA treatment in 20 hypercholesterolaemia patients undergoing regular treatment for a minimum of 3 months[120]. Similarly, Reimann et al, found no change in augmentation index and central blood pressure one year after baseline measurement, in patients with a history of cardiovascular disease, who previously had been undergoing regular apheresis treatment[121]. PWV is predominantly a measure of the mechanical properties of the vessel wall and as such was unlikely to be changed by apheresis treatment. Augmentation pressure and index reflect changes in wave reflection and AIx in particular is dependent on the duration of the cardiac cycle, the speed and intensity of the pulse wave.

However, despite a significant increase in heart rate post apheresis treatment, there was no change in AIx. It is possible, that the change in heart rate was not great enough to cause changes in AIx, or the study had insufficient power to see a difference.

There are a number of limitations with this study. Due to time and space constraints it was not possible to assess flow-mediated dilatation alongside FMS as had been undertaken in previous chapters. Assessment of endothelium-independent function with glyceryl trinitrate would have been useful to test whether LA treatment also had beneficial effects on the underlying smooth muscle responsiveness to NO as well as the flow-mediated response. In addition, it was not possible to check whether changes in baseline blood flow or reactive hyperaemia were in part responsible for the improvement of vasomotor function. This is one limitation of the FMS method in comparison with FMD as it is not possible to assess the flow stimulus which is driving the change in PWV.

Participants were asked not to take vasodilatory medications on the morning of the study to limit the effect of these medications on their vascular function. However, two patients forgot to withhold this medication, so this may influence the results, although exclusion of these two participants did not affect these results. In addition, due to the size of this study, it was also not possible to adjust for the effects of other medications that the participants were taking.

It would have been interesting to investigate whether the system used for LA, influenced the change in vasomotor responses. However, to investigate this, a larger, multicentre (ideally international) trial would be required, as within the UK, the number of patients on LA treatment at each centre is very small, due to the nature of the treatment and its expense, and is therefore limited to those in most need.

The analyses exploring differences between diabetic and non-diabetic FH patients within the cohort, and the comparisons with age and gender matched controls, were unpowered. Therefore, they were very likely to be underpowered, increasing the likelihood of type I and type II errors within the analysis. These analyses are only speculative and any findings need to be confirmed in a properly powered study in patients with diabetes and age and gender matched controls. In addition, it is worth noting that this is a small study and although powered to detect a difference in FMS other findings may be due to chance. A larger study that replicated these findings would add greater confidence that these results were not due to chance. It would also have enabled multiple regression analyses to be

carried out allowing for adjustment of other clinically relevant variables which may have influenced vasomotor function. Finally, additional studies with prognostic outcomes would also enable determination of whether the statistically significant improvement in FMS was of clinical significance.

8.5.1 Conclusion

In conclusion, it was found that a single treatment of LA treatment improves vasomotor function, as assessed by FMS, in patients with FH. This finding indicates that the technique is sensitive enough to be able to detect acute changes in vasomotor function.

Chapter 9. Summary of section 2: development and validation of flow-mediated slowing

9.1 Summary of development and validation of FMS protocol

The aim of the second half of this thesis was to demonstrate the reproducibility and further validate a method, flow-mediated slowing (FMS) for the assessment of vasomotor function.

In Chapter 6 the original protocol, described by Naka et al, was tested and found not to be repeatable[164]. I therefore developed and refined the technique. Initial studies first looked at the most appropriate position for the occlusion cuff (wrist, mid forearm or just below the elbow) for inducing the reactive hyperaemia. It was found, that positioning the occlusion cuff just below the elbow gave the best stimulus for assessing both FMS and flow-mediated dilatation (FMD). Studies looking at the most appropriate method for acquiring pulse wave velocity (PWV), whether using continuous cuff inflation or intermittent cuff inflation, were also carried out. Intermittent cuff inflation, which provided a reading at approximately 15 second intervals, was identified as being the most suitable method. Finally, I looked at whether concomitant assessment of FMS and FMD on the same arm was possible, and concluded that it had limited impact on the resultant FMD. This initial body of work resulted in identifying the most appropriate protocol for the assessment of FMS which included the occlusion cuff being positioned just below the elbow, PWV assessed intermittently and FMD and FMS being concomitantly assessed on the same arm.

Having determined the most appropriate protocol for the method, I then demonstrated the reproducibility of the technique in a group of healthy volunteers. It was shown that FMS was highly reproducible, when expressed either as an absolute, or percentage change derived from the maximal change in PWV, following the release of the occlusion cuff. Naka et al, in their original study had assessed PWV at 1 minute intervals; however in our study this was found to be less comprehensive and less reproducible than the maximum

change in PWV[164]. The reproducibility shown for this technique was similar to other studies using the same principles but different techniques for assessing PWV. Kamran et al, used applanation tonometry to measure carotid to radial PWV and demonstrated a coefficient of variation (CV) of 12%, whilst Graf et al, who used mechanotransducers for carotid to radial PWV assessment, had a CV of 9.7%[165, 166]. Our method was more reproducible than some other potential methods of endothelial function assessment such as pulse contour analysis[150].

In Chapters 7 and 8, the aim was to further validate the technique for assessing FMS by investigating whether the method was sensitive enough to detect a) differences vasomotor responses in those with and without a cardiovascular risk factor, and b) changes in vasomotor responses following an acute intervention. For this, two studies were carried out in patients with familial hypercholesterolaemia (FH), a population at very high risk of developing cardiovascular disease. The first study was a case-control to investigate whether differences in vasomotor function could be detected between patients with FH and age and gender matched controls. Patients with FH were indeed found to have lower FMS responses than the control group. In Chapter 8 we then assessed FMS before and immediately after a single lipoprotein apheresis treatment, an acute non-pharmacological lipid lowering therapy. An improvement in FMS was demonstrated post apheresis treatment in these patients. These two chapters show that this technique is sensitive enough to detect an impact on vasomotor function, as has been demonstrated with other established methods for assessing vascular physiology [91, 113, 116, 139].

There are limitations with this method. Moving the occluding cuff to just below the elbow, means that approximately a third of the arterial tract being studied is made ischaemic during occlusion. In FMD studies, it has been shown that when the occlusion cuff is proximal to the imaged artery, the resultant vasodilatation is not entirely NO mediated. This is demonstrated by the arterial response not being completely inhibited by the administration of the nitric oxide synthase inhibitor L-NMMA. However, when a distal occlusion cuff is used the FMD response is abolished[89, 149]. It is therefore possible that the observed slowing in PWV following reactive hyperaemia is not fully mediated by NO. Thus, although this specific protocol is likely to reflect endothelium dependent vasomotor responses to increased shear stress, the specific mechanisms and whether it is NO-dependent is unclear at this time. Future work would ideally look at combining the lower PWV cuff and the occlusion cuff into one to remove this problem. In addition, studies comparing FMS responses prior to and post administration of L-NMMA and other

endothelial vasodilator pathway inhibitors such as tetraethylammonium would help to answer this question. It is also worth noting, that whilst Naka et al demonstrated the effects of acetylcholine and L-NMMA on PWV in the arm, they did not evaluate its effect on changes in PWV with reactive hyperaemia[164].

Another limitation of the method is that it does not provide detailed information regarding the flow stimulus which can be measured in FMD studies. Differences in reactive hyperaemia between groups with risk factors for cardiovascular disease are not common as was shown in those with and without FH Chapter 7, although differences may be seen at more advanced stages of disease. This may potentially limit the methods used for detailed mechanistic studies of vascular function, particularly in groups with advanced diseases. However, it is well placed, due to its ease of use and relatively low cost and consequent scalability, to be used as a global indicator of vasomotor function for use in large population studies, early stage disease groups and, potentially, clinical trials.

Together the studies in these three chapters demonstrate that FMS is a highly reproducible method for the assessment of vasomotor function and is sensitive enough to detect differences in vascular function in response to disease and following an acute intervention. This simple and reliable technique is an accessible method for assessing vascular function which can provide clinically relevant and complementary information in addition to other non-invasive methods.

Future work would potentially involve incorporating the protocol into the Vicorder system software allowing it to be run directly from the computer. An advantage of this would be the ability to use the lower cuff for both PWV assessment and occlusion of the arm to induce reactive hyperaemia. By decreasing the number of cuffs, and removing the need for an external cuff inflator, this would streamline the study process reducing the cost and complexity of the technique and importantly, it would mean that part of the arterial tract being studied was not being made ischaemic. Work has begun on this in partnership with Skidmore Medical and it is intended to carry out trials of this software. Further studies also need to be carried out in different disease groups, larger populations, and to explore the ability to detect changes with other interventions known to affect endothelial function such as ischaemia reperfusion, acute mental stress and a high fat meal. To extend the work within the FH population it would also be interesting to see whether other lipid lowering therapy such as statins and emerging lipid lowering drugs also improve FMS.

Chapter 10. General summary and conclusions

The aims of this thesis were: Firstly, to investigate the associations between both chronic and acute stress and endothelial function, as well as between chronic stress and structural vascular disease; Secondly to develop, refine and validate a new technique for the assessment of vascular endothelial function that could be more suitable for large scale use by researchers (and potentially clinicians) outside of the setting of a specialist vascular laboratory.

The first half of this thesis explored the relationships between both chronic and acute mental stress and vascular structure and function and the influence of dyslipidaemia on these relationships. In Chapter 3 the key findings were that women with depression and/or anxiety had the greatest IMT which was further exacerbated by the presence of dyslipidaemia, suggesting an important progression of early vascular disease and potentially increased long-term risk. The implication of this for women is that those with depression and/or anxiety aside from improving their mental health should also have their other cardiovascular risk factors carefully monitored to further reduce their risk of a cardiovascular event. Equally, chronic stressors such as depression or anxiety should also be potentially taken into account when assessing and treating an individual's cardiovascular risk, particularly in women.

In Chapter 4 it was found that those participants who had the greatest increase in the inflammatory marker fibrinogen 45 minutes after the acute mental stress challenge were subsequently found to have poorer endothelial function, suggesting they may be at increased cardiovascular risk in the long term. It also builds on previous work showing that participants with high inflammatory responses (IL-6, TNFα & fibrinogen) have greater arterial stiffness and blood pressure 3 years later[324, 369]. Further work is required to investigate whether and how this might lead to future development of structural and functional changes and cardiovascular events, as well as exploring these responses as a potential means of improving long-term risk prediction and treatment selection.

This work also identified the need for a straight forward method of vascular function assessment that would be provide reliable data as well as be easy to use in large population

studies and outside the specialist vascular laboratory. The second half of this thesis therefore focussed on demonstrating the reproducibility and validation of such a potential technique.

In Chapter 6 the key finding was that FMS was a highly reproducible method for assessing vasomotor function. This demonstrated the potential of this method to become an important tool for assessing vasomotor function. This was highlighted in Chapter 7 in which I found that vasomotor responses in FH patients, as assessed by FMS, were impaired compred with age and gender matched controls, in keeping with studies using established techniques. This showed that this new method could potentially be used to address scientific questions requiring evaluation of vasomotor dysfunction, for example the influence of novel pathways and treatments on the development vascular pathology in teh clinical setting. Finally the sensitivity of the technique was shown in Chapter 8 where the key finding was that a single treatment of an acute lipid lowering intervention (lipoprotein apheresis) improved vasomotor function which was detectable by FMS.

Together these novel findings shown in Chapters 6 to 8 demonstrate the potential of FMS as a valid method for assessing vasomotor (dys)function with potential capability for being used in large epidemiological studies and, importantly, by non-vascular specialists. This technique could be used in larger studies to look at associations with other cardiovascular risk factors as well as the impact of interventions which may alter vasomotor function. For example, studies could look at the implications of chronic stress, such as depression and anxiety, on the cardiovascular system and also the physiological responses to acute mental stressors, to further understand how these responses lead to acute cardiovascular events and disease formation and progression.

10.1 Future research

Due to its ease of use and operator independence, this cuff based method of assessing vasomotor function by FMS could be used in a variety studies in many different areas and not just by vascular specialists. Following on from investigating associations between vascular function and acute and chronic stress, FMS could be used to investigate the role of vasomotor function in psychopathology, for example I plan to undertake a study with colleagues investigating the influence of vasomotor function on the relationship between hydration status and cognitive function of older adults. Furthering the LA study, FMS

could be used to investigate the effects of new drugs, such as PCSK-9 inhibitors, on vasomotor function, to assess how their effect on vascular function compares with that of LA in patients with FH. Also based on the model of the LA study, renal physicians could use FMS to investigate the effects of renal dialysis on vasomotor function looking at both the acute and short term effects of a single dialysis session as well as the impact of longer term therapy.

Appendix 1

Whitehall II Phase 7 questionnaire

https://www.ucl.ac.uk/whitehallII/pdf/s7-questionnaire

Appendix 2

Subject	Gender	Cardiovascular history	LA system	Lipid lowering medications	Cardiac medication
1	M		Kaneka D100	Rosuvastatin	AP
2	M	MI	Dali 750	Rosuvastatin, Ezetimibe + Omacor	βB + AP
3	M	MI, stroke	Dali 1000	Rosuvastatin, Ezetimibe + Eicosapentaenoic acid	βB , AIIRA, DIUR + AP
4	M	CABG	Dali 750	Omacor	βB, DIUR, RI + AP
5	M	MI	Dali 1000	Atorvastatin	AP
6	F		Kaneka LA-15		
7	M	MI, CABG, Stents	Dali 1000	Atorvastatin, Ezetimibe + Eicosapentaenoic acid Docoshexaenoic acid + Fenofibrate	βB ,DIUR ,AP + NI
8	M	MI, CABG , PCI	Dali 750	Rosuvastatin, Ezetimibe, Omacor + Fenofibrate	AP
9	F	CABG, chronic stable angina	Kaneka LA-15	Rosuvastatin + Ezetimibe	βB, AIIRA, DIUR, CCB, AP +NI
10	M	CABG, MI	Dali 1000	Atorvastatin, Ezetimibe + Tredaptive	AP
11	F	MI, stents	Dali 750	Rosuvastatin + Ezetimibe	βB + AP
12	M	CABG, PCI, MI , PVD aortic-bifemoral graft, TIA & AF, AV fistula	Kaneka LA-15	Atorvastatin, Ezetimibe, Omacor + Tredaptive	βB, AIIRA, DIUR, AP, NI, AC + CG

Table 1. Cardiovascular medical history and medications of the patients undergoing lipoprotein apheresis treatement. LA = lipoprotein apheresis; M= male; F=female; MI=myocardial infarction; CABG= coronary artery bypass graft; PCI=percutaneous intervention; PVD= peripheral vascular disease; TIA = transient ischaemic attack; AF= atrial fibrillation; AV fistula = arteriovenous fistula; AP = Antiplatelet; βB= beta blocker; AIIRA = angiotensin II receptor agonist; DIUR = diuretic; RI = renin inhibitor; NI= nitrate; CCB – calcium channel blocker; AC = anticoagulant; CG= cardiac glycoside.

Publications

Published papers

1. Ellins EA, New KJ, Datta DB, Watkins S, Haralambos K, Rees A, Rees DA, Halcox JP. Validation of a new method for non-invasive assessment of vasomotor function. European Journal of Preventive Cardiology. 2016;6:577-83. doi: 10.1177/2047487315597210.
2. Ellins EA, Halcox JP. Where are we heading with noninvasive clinical vascular physiology? Why and how should we assess endothelial function? Cardiology Research and Practice. 2011;2011:870132. doi: 10.4061/2011/870132.

Publications related to thesis

1. Connolly KD, Willis GR, Datta DB, Ellins EA, Ladell K, Price DA, Guschina IA, Rees DA, James PE.Lipoprotein-apheresis reduces circulating microparticles in individuals with familial hypercholesterolemia.Journal of Lipid Research. 2014;55(10):2064-72. doi: 10.1194/jlr.M049726.
2. Ellins E, Halcox J, Donald A, Field B, Brydon L, Deanfield J, Steptoe A. Arterial stiffness and inflammatory response to psychophysiological stress. Brain, Behavior, and Immunity. 2008;22:941-948. doi: 10.1016/j.bbi.2008.01.009.

Accepted for publication

1. Ellins EA, Rees, DA, Deanfield JE, Steptoe A, Halcox JP. Increased fibrinogen responses to psychophysiological stress predict future endothelial dysfunction implications for cardiovascular disease? Accepted by *Brain, Behavior, and Immunity.*

Conference presentations

1. Ellins EA, Rees DA, Deanfield JE, Brunner EJ, Halcox JP. Gender, depression, anxiety and carotid intima media thickness and the influence of lipids. *Journal of the American College of Cardiology* 2016;67:2311-2311 doi:10.1016/S0735-1097(16)32312-9. Presented as a poster presentation at the ACC Scientific sessions, Chicago, April 2016. Oral presentation at HEART UK, Edinburgh, July 2016.
   Winner of the Bill Richmond Best Medical & Scientific Oral presentation Award.
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