Computer aided diagnosis of early vascular disease from ultrasound images

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MD

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Computer aided diagnosis of early vascular disease from ultrasound images

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I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree of Doctor of Medicine, is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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<td>Abdominal aortic aneurysm</td>
</tr>
<tr>
<td>ABI</td>
<td>Ankle brachial index</td>
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<tr>
<td>AI</td>
<td>Augmentation index</td>
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<td>AMS</td>
<td>Arterial measurement system</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>AP</td>
<td>Augmentation pressure</td>
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<td>ARIC</td>
<td>The Atherosclerosis in Communities at Risk Study</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>ASE</td>
<td>American society for echocardiography</td>
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<td>BaPWV</td>
<td>Brachial ankle pulse wave velocity</td>
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<tr>
<td>BIF</td>
<td>Bifurcation</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>CABG</td>
<td>Coronary artery bypass graft</td>
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<td>CAC</td>
<td>Coronary artery calcium score</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<tr>
<td>CB</td>
<td>Carotid bifurcation</td>
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<tr>
<td>CC</td>
<td>Compliance coefficient</td>
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<td>CCA</td>
<td>Common carotid artery</td>
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<td>CFI</td>
<td>Colour flow imaging</td>
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<tr>
<td>C-F PWV</td>
<td>Carotid to femoral pulse wave velocity</td>
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<td>CFR</td>
<td>Coronary flow reserve</td>
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<td>CHD</td>
<td>Coronary heart disease</td>
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<td>CHS</td>
<td>The Cardiovascular Health Study</td>
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<td>CIMT</td>
<td>Carotid intima-media thickness</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DC</td>
<td>Distensibility coefficient</td>
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<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>DICOM</td>
<td>Digital imaging and communications in medicine</td>
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<td>DM</td>
<td>Diabetes mellitus</td>
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<td>ECA</td>
<td>External carotid artery</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>ED</td>
<td>Endothelial dysfunction</td>
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<tr>
<td>EDHF</td>
<td>Endothelium-derived hyperpolarizing factor</td>
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<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
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<tr>
<td>ESS</td>
<td>Endothelial shear stress</td>
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<tr>
<td>ET-1</td>
<td>Endothelin 1</td>
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<td>FBN1</td>
<td>Fibrillin 1</td>
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<td>FMD</td>
<td>Flow mediated dilatation</td>
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<tr>
<td>GH</td>
<td>Gestational hypertension</td>
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<tr>
<td>GTN</td>
<td>Glyceryl trinitrate</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>HR</td>
<td>Hazard ratio</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>ICA</td>
<td>Internal carotid artery</td>
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<tr>
<td>ICAM</td>
<td>Intercellular adhesion molecule 1</td>
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<td>IDDM</td>
<td>Insulin dependent diabetes mellitus</td>
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<tr>
<td>IMT</td>
<td>Intima-media thickness</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>MESA</td>
<td>Multi-ethnic study of atherosclerosis</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<tr>
<td>NIDDM</td>
<td>Non-insulin dependent diabetes mellitus</td>
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<tr>
<td>NID</td>
<td>Nitrate induced dilatation</td>
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<tr>
<td>kHz</td>
<td>Kilohertz</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetres</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<td>OPAL</td>
<td>Osteoporosis prevention and arterial effects of tibolone study</td>
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<tr>
<td>PET</td>
<td>Pre-eclampsia</td>
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<tr>
<td>PI</td>
<td>Prostacyclin</td>
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<td>PP</td>
<td>Pulse pressure</td>
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<td>PRF</td>
<td>Pulse repetition frequency</td>
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<td>PWI</td>
<td>Pulse wave imaging</td>
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<td>PWV</td>
<td>Pulse wave velocity</td>
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<td>PXE</td>
<td>Pseudoxanthoma elasticum</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SE</td>
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<td>STI</td>
<td>Speckle tracking imaging</td>
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<td>SV</td>
<td>Stroke volume</td>
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<td>Triglyceride</td>
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<td>THI</td>
<td>Tissue harmonic imaging</td>
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<tr>
<td>tLoD</td>
<td>Total longitudinal displacements</td>
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<td>US</td>
<td>Ultrasound</td>
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<td>USPSTF</td>
<td>United States preventative services task force</td>
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<td>VCAM1</td>
<td>Vascular cell-adhesion molecule 1</td>
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Summary. This thesis consists of 2 separate ultrasound (US) based studies, performed with the common aim of improving the diagnosis of early vascular disease from US images.

Study 1

Introduction: Flow mediated dilatation (FMD) is an endothelium-dependent process reflecting the dilatation of a conduit artery when it is exposed to increased blood flow and therefore increased shear stress. FMD requires a healthy endothelium and is depressed in those with cardiovascular risk factors. Current 2D US assessment is limited as a research tool only secondary to variable reproducibility, technical difficulties and difficulties determining true diameter measurement. To our knowledge this is the first study comparing 2D and 3D US assessment of FMD.

Methods: This was a cross sectional reproducibility study with 27 male patients. 2D and 3D FMD were performed on both study visits. Nitrate induced dilatation (NID) was performed as a control. We hypothesised that 3D US would eliminate the systematic underestimation of diameter that we believe occurs using 2D US. We believe this is secondary to probe malalignment errors occurring in 2D US that are eliminated using 3D US. Furthermore, we tested if 3D FMD is more reproducible than 2D FMD.

Results: We discovered 3D diameter to be greater than 2D diameter with between visit FMD correlation and reproducibility being similar in both 3D and 2D.

Conclusion: Findings suggest 3D US gives a greater and more accurate measurement of diameter, however this should be confirmed with an arterial phantom bench study comparing 2D and 3D US diameter measurements. With real-time high resolution 4D US likely to provide better temporal resolution, the advent of 4D FMD is only around the corner. This is likely to be more accurate, reproducible and user friendly than 2D and may soon find its way into clinical practice. We believe by identifying 3D US as a useful and comparable tool to 2D US in the assessment of FMD, this will provide a stepping stone for this to happen, thereby facilitating better quantification of endothelial function.
Study 2

Introduction: Pre-eclampsia (PET) results in hypertension and proteinuria in pregnancy. It is associated with increased prevalence of cardiovascular risk factors and future cardiovascular risk, including increased intima-media thickness (IMT) and arterial stiffness. We used 2D US to assess for subtle alterations in vascular structure and function in young women with and without a history of gestational hypertension (GH) or PET.

Methods: This was a phase 2 cohort study of 40 women with at least 1 pregnancy in the last 5 years. Alterations in IMT distribution and compression patterns between the 3 groups were assessed according to multiple angles of insonation in the distal common carotid artery (CCA), and along the vascular tree (proximal versus distal CCA versus bifurcation (BIF) versus internal carotid artery (ICA)). Arterial stiffness within the proximal and distal CCA was also assessed. Using ANOVA we tested the hypotheses that the PET group would illustrate different values to the other groups.

Results: In women with a history of pre-eclampsia, IMT was greater in areas of the vascular tree with a predilection for atherosclerosis i.e. the medial wall of the common carotid artery and within the ICA. IMT compression in PET differed according to vascular tree and angle. Arterial stiffness was increased in the GH and PET groups with less compliant and distensible arteries in the distal CCA when compared to normotensives.

Conclusion: Women with PET have greater IMT than those without such a history. The pattern of IMT distribution by angle and along the vascular tree has been seen in previous studies, however to our knowledge never in such a group of asymptomatic women. A stepwise increase of IMT along the vascular tree was observed in the normal and GH groups with a subsequent decrease in IMT in the ICA, however, there was a further increase in IMT in the ICA in the PET group, suggesting an accelerated atherosclerotic process. Increased CCA stiffness in the PET and GH groups further supports this statement. Our results warrant further evaluation in other pre-eclampsia sufferers and perhaps similar asymptomatic groups using more novel non-invasive ultrasound techniques studying vascular wall structure and mechanics.
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Chapter 1. Introduction
1.1 Overview

In recent years there has been an increasing emphasis on the early identification of those at an increased risk of developing disease so that we may provide them with early treatment options thereby arresting or delaying the onset of morbidity and mortality. Preventative medicine is a massive field and there has been and continues to be an enormous amount of research into identifying those at risk of developing cardiovascular disease which continues to be the leading cause of death in western society.

This thesis consists of two separate ultrasound based studies. The first uses ultrasound as a tool to assess endothelial function and aims to improve on existing and well established methods by comparing 3 dimensional and 2 dimensional ultrasound approaches. The second study uses ultrasound to assess the vascular structure and function in young women with and without a history of gestational hypertension or preeclampsia.

This introduction chapter will consist of a literature review of atherosclerosis and endothelial function and in particular the use of ultrasound in its assessment. It will also examine ultrasonic assessment of carotid intima media thickness and its use as a marker of cardiovascular disease. The concept of vascular stiffness will be described in addition to its value as a cardiovascular risk assessment tool. An appreciation of the physics of ultrasound was required in order to complete the studies, therefore this is also explained in detail. Finally the hypotheses and aims of the two studies are discussed.

1.2 Introduction to atherosclerosis, its pathogenesis and important consequences

Large vessel atherosclerosis leads to heart attacks and strokes, which account for 45% of all deaths in the western world. The earliest detectable indications of atherosclerotic changes are alterations in two important vascular properties,
namely impaired flow mediated dilatation and increased vascular wall stiffness. Atherosclerotic plaque rupture followed by intravascular thrombosis is the final common pathway leading to heart attacks and strokes.

1.2.1 Definition of atherosclerosis

Atherosclerosis is a progressive chronic inflammatory disorder of large arteries. It results in focal intimal disease and atherosclerotic plaques characterized by accumulation of both extracellular and intracellular lipid derived from oxidized low density lipoprotein (LDL), macrophages derived from circulating monocytes, and vascular smooth muscle proliferation and migration (Webb DJ et al, 1997).

Its prevalence in premenopausal women is less than half that in men of equal age but between sex differences disappear within five years post menopause (Kiechl S et al, 1999).

1.2.2 Aetiology/risk factors

The risk factors for the development of atherosclerosis are numerous and include a variable combination of genetic, and environmental or traditional risk factors such as smoking and the development of free radicals, hypertension, diabetes, dyslipidaemia, age and hyperhomocysteinaemia. Certain infections such as Chlamydia pneumoniae and herpesviruses are also thought to play an uncertain role (Stassen FR et al, 2008).
1.2.3 Pathophysiology of atherosclerosis

The earliest signs of atherosclerosis are seen in the first decades of life. At this stage it is purely an inflammatory lesion containing macrophages and T-lymphocytes. Initially, atherogenic LDL’s enter the tunica intima where they are oxidized and deposited in the extracellular intimal space and phagocytosed by macrophages. This in turn, leads to the formation of lipid rich foam cells that progress to form fatty streaks, which are the first pre-atherosclerotic lesion seen in life. They are observed in the aorta of children in the first decade, progress to the coronary arteries of adolescents in the second decade and to the cerebral arteries of young adults in the third decade (Rader et al, 2008).

Vascular smooth muscle cells secrete extracellular matrix components such as collagen and proteoglycans into the intima and in turn increase the retention and aggregation of LDL. A chronic inflammatory process results with further monocyte and T-cell recruitment. As the plaque expands, compensatory vascular remodelling occurs resulting in a preserved overall lumen size, however the overall diameter increases. Foam cells die and release crystalline cholesterol and debris. A fibrous cap formed by smooth muscle cells walls off the plaque and by doing so, separates it from the blood. In time, a necrotic core forms in the plaque and promotes further inflammation via recruitment of inflammatory cells. At this stage the endothelium may erode or the plaque may rupture leading to formation of a thrombus in the arterial lumen. This may result in acute coronary syndrome or stroke. In the event that the plaque remains intact and continues to narrow the lumen, and therapeutic interventions are not initiated, clinically obstructive disease is inevitable. Figure 1.1 below illustrates the progression of atherosclerosis.
Figure 1.1 The progression of an atherosclerotic lesion is shown in a simplified form. Plaque develops from a normal blood vessel (far left) to a vessel with an atherosclerotic plaque and superimposed thrombus (far right). Potential targets for molecular imaging at each stage are also listed. AHA, American Heart Association; ICAM1, intercellular adhesion molecule 1; LDL, low-density lipoprotein; MMP, matrix metalloproteinase; VCAM1, vascular cell-adhesion molecule 1 (Figure and legend from Sanz et al, 2008).

Despite the systemic nature of the major cardiovascular risk factors atherosclerosis is a geometrically focal disease with a propensity to develop on the outer walls of arterial bifurcations and on the inner walls of arterial curves (Caro CG et al, 1971 and Zarins CK et al, 1983). The carotid bifurcation, the coronary arteries, the infrarenal abdominal aorta, and the vessels supplying the lower extremities are at greatest risk (Glagov S et al, 1988). These are areas of low shear and high tensile stress, and there is evidence that these haemodynamic alterations contribute to atherogenesis in these regions (figure 1.2).
Figure 1.2 Localization of Atherosclerosis Lesions.

A. Illustration of focal nature of atherosclerosis and its tendency to affect outer walls of vascular bifurcations. B. Carotid angiogram demonstrating focal narrowing at the outer walls of the carotid bifurcation involving the internal and external carotid arteries (arrowheads). C. Velocity map of the carotid artery in end-systole illustrates the lower velocities at the outer lateral edges (blue) where the shear stress is lower. Flow velocity and shear stress is higher at the inner edge of the bifurcation (green). (Figure taken and legend adapted from Malek AM et al, 1999).

Blood flow and the blood viscosity exert a frictional force per unit area on the endothelial cells and lumen wall that is known as shear stress. Low shear stress leads to leukocyte adhesion, accumulation of subendothelial macrophages and lymphocytes, and irregular endothelial morphology with platelet aggregation at damaged endothelial sites. It also results in dilated intercellular clefts in the outer walls of flow dividers such as the carotid bifurcation (Malek et al, 1999). Shear stress is inversely related to intima-media thickness (IMT), age, systolic BP, and BMI (Gnasso et al, 1996, Carallo et al, 1999). Tensile stress is circumferential wall tension divided by the wall thickness. It acts perpendicularly to the arterial wall and results from the dilating effect of blood pressure on the vessel. Increased tensile stress is directly associated with increasing wall thickness, age and BMI (Carallo et al, 1999). Plaques have a greater propensity to develop when arterial remodelling reduces flow velocity and shear stress resulting in turbulent blood flow. This
allows more time for offending particles such as circulating LDL for example, to congregate at the outer walls of bifurcations and exert their damaging effects. Conversely, areas of laminar unidirectional blood flow with high shear stress rates clear these particles more rapidly resulting in less atherosclerotic lesions at these sites.

1.3 Endothelial dysfunction and its relationship with early atherosclerosis

The following section will describe the normal endothelium, the process of endothelial dysfunction and the methods by which it is quantified with particular emphasis on flow mediated dilatation (FMD).

1.3.1 The normal endothelium

A normal healthy conduit artery is comprised of an endothelium that consists of a monolayer of endothelial cells lining the arterial lumen each anchored to an underlying basal lamina. This is surrounded by the media, containing concentric layers of smooth muscle cells, elastin fibres and extracellular matrix. The outermost connective tissue layer is the adventitia. A normal artery is seen below in Figure 1.3.
Figure 1.3 The structure of the normal human artery.

This illustrates a single layer of endothelial cells, the innermost intima, the media and adventitia (Figure taken from Lusis AJ, 2000).

Endothelial cells are able to sense changes in blood flow, pressure, and oxygen tension. In response to changes in local conditions, they secrete a range of bioactive substances that have powerful regulatory effects on vascular smooth muscle tone, platelet and leukocyte interactions, coagulation, fibrinolysis and vascular growth (Pohl U et al, 1986). Important vasodilator mediators, secreted by the endothelium in response to increased blood flow and shear stress, include nitric oxide (NO) and prostacyclin (Pi), whilst angiotensin II and endothelin are the most important vasoconstrictor substances. In addition to effects on vascular tone, the endothelium also influences coagulation, platelet aggregation and adhesion, leukocyte activation, adhesion and migration, and smooth muscle proliferation. It does this through release of interleukins, endothelial growth factors, adhesion molecules, plasminogen inhibitors and von Willebrand factor.
1.3.2 Endothelial dysfunction

Under physiological conditions, in the vasculature of healthy young humans, a balance exists between vasodilators and vasoconstrictors. However, endothelial dysfunction (a deficit of anti-atherosclerotic vasodilating growth inhibitors and an excess of pro-atherosclerotic vasoconstricting growth promoters) has been described with age, hypertension, dyslipidaemia, diabetes mellitus, and smoking and in patients with coronary artery disease. Recent models have emphasised the key role played by endothelial dysfunction in the initiation and propagation of macrovascular and microvascular disease (Ross R, 1993) (Dzau VJ, 2001). In addition, endothelial dysfunction is detectable long before any structural atherosclerotic disease is present (Celermajer DS et al, 1992). The effects of ED and its combination with traditional and non-traditional cardiovascular risk factors and their consequences are illustrated below in Figure 1.4.
Figure 1.4 Endothelial dysfunction as the “risk of the risk factors.”

The endothelium represents a mechanical and biological barrier between the blood and the vascular wall. Traditional and non-traditional risk factors, local factors (e.g., shear stress), genetic factors, and yet-unknown factors (protective or harmful) determine the status of endothelial function, which may be regarded as an integrated index of both the overall cardiovascular risk factor burden and the vasculoprotective factors in any given individual. The presence of endothelial dysfunction reflects a specific atherogenic vascular milieu, which is associated with perfusion abnormalities and cardiovascular events. (Figure and legend taken from Bonetti PO et al, 2003).

1.3.3 Nitric oxide and endothelial dysfunction

Endothelial nitric oxide is a free radical that is generated from L-Arginine via endothelial nitric oxide synthase (eNOS). It plays a pivotal role in vascular homeostasis and tone. NO decreases the expression and activity of endothelin-1 (ET-1), a potent and opposing vasoconstrictor, and has been demonstrated to oppose vascular smooth muscle cell proliferation and extracellular matrix production (Kolpakov V et al, 1995). In addition, NO has an
inhibitory effect on platelet adhesion via stimulation of cyclic adenosine monophosphate (cAMP) pathways (Giannarelli et al, 2007). With ED there is diminished availability and production of NO leading to increased endogenous oxidative stress and an increase in the production of superoxide anions (Herrmann J et al, 2008). These reactive oxygen species (ROS) interact with NO to form peroxynitrite, further decreasing production of NO and decreasing the availability of eNOS via the reduction of its important co-factor BH4. This process is known as eNOS uncoupling. Xanthine oxidase and NADH/NADPH oxidase in conjunction with uncoupled eNOS are the 3 main enzymatic substances that lead to an increase in ROS in those with ED. Their relationship to cardiovascular risk factors, oxidant stress and ED is illustrated in Figure 1.5 below.

Figure 1.5 Endothelial dysfunction and the relationship between cardiovascular risk factors and oxidant stress (Figure taken from Cai H et al, 2000).
1.3.4 Quantification of endothelial dysfunction

As endothelial dysfunction acts as an early indicator of future risk of atherosclerotic events, there is considerable interest in its accurate quantification. An indication of dysfunction may be provided through measurement of biochemical markers such as plasma levels of endothelin, CD40 ligand, cell adhesion molecules, C reactive protein, or urinary NO metabolites (nitrates and nitrites). However most investigators favour methods, which directly assess endothelial regulation of vascular tone, such as:

- Coronary angiography with measurement of blood flow and artery diameter during selective infusion of acetylcholine and nitric oxide synthase inhibitors (Ludmer PL et al, 1986).
- Measurement of changes in vascular diameter with varying flow rates in cannulated arterioles from tissue biopsies (Paniagua OA et al, 2001).
- Ultrasonic measurements of brachial artery diameter and flow velocities post-ischaemia (Celermajer DS et al, 1992).

The first three methods are well-established, accurate reproducible methods for assessment of endothelial dysfunction in a variety of vascular beds. Furthermore, coronary endothelial vasodilator dysfunction when assessed using quantitative coronary angiography and infusion of acetylcholine, has been shown to be a strong independent predictor of long-term atherosclerotic disease progression and cardiovascular event rates (Schächinger V et al, 2000). However, because of the invasive nature of these three methods they are unsuitable as instruments to be applied to large asymptomatic populations or to be used repeatedly in order to evaluate responses to therapeutic interventions. Assessment of endothelial dysfunction by the fourth method, brachial flow mediated vasodilation (FMD), is non-invasive, and is therefore the current most commonly used method. However this method currently utilises 2-dimensional (2D) ultrasound and therefore has disadvantages.
including operator dependency and poor reproducibility (both intra-observer and inter-observer).

1.4 Introduction to flow mediated dilatation

Flow mediated dilatation is defined as an endothelium-dependent process reflecting the relaxation of a conduit artery when it is exposed to increased blood flow and therefore increased shear stress (Stout M, 2009). This requires an intact or healthy endothelium and can be demonstrated in femoral, radial or brachial arteries. It is widely used in clinical research to assess for endothelial dysfunction.

1.4.1 Physiology of endothelial dependant flow mediated dilatation

The technique was first described by Celermajer in 1992, and involves measurement of the change in diameter of a conduit artery in response to increased blood flow that is induced by a period of ischaemia in the distal circulatory bed (Deanfield J et al, 2005). The ischaemia is created artificially by inflation of a blood pressure cuff to supra-systolic pressure for a period of 4-5 minutes. Following abrupt deflation of the cuff there is a reactive hyperaemia and increase in shear stress causing a release of NO from the endothelium and a dilatation of the artery. The degree of FMD is representative of endothelial function. As this is an endothelium dependent process and is predominantly mediated by NO, it is depressed in those with endothelial dysfunction, atherosclerosis and cardiovascular risk factors (Celermajer DS et al, 1992). It is well known to correlate with coronary artery vasodilator function (Anderson TJ et al, 1995). Methodology will be described in detail elsewhere.

As stated above, NO is thought to be the major mediator in the FMD process (Joannides R et al, 1995), with a lesser role played by prostaglandins (Okahara et al, 1998) and endothelium-derived hyperpolarizing factor (EDHF).
FMD cannot be fully explained by NO activation. Recently, mice were genetically engineered to lack the eNOS gene. Subsequently, their arteries were shown to respond to shear stress by dilating, proving that NO is not the only mediator involved (Sun D et al, 1999). The precise mechanism of FMD is not fully understood but is thought to involve specialized ion channels in the endothelial cell membrane called calcium-activated potassium channels. These channels open in response to shear stress and hyperpolarize the endothelial cell thus providing a mechanism for calcium entry (Cooke JP et al, 1991). Calcium activates eNOS resulting in generation of NO causing FMD.

Figure 1.6 Flow mediated dilation schema: in response to different changes in shear stress. * = very short-term changes; ** = changes taking place over minutes; *** = changes taking place over many minutes or hours

PGI2 = prostacycline; EDHF = endothelium-derived hyperpolarizing factor; Kc = calcium-activated potassium.

(Figure and legend taken from http://www.balgrist.ch/en/Home/Forschung_und_Lehre/Anaesthesiology/Laboratory_Research/Effects_on_endothelial_function.aspx).
1.4.2 Flow mediated dilatation and early endothelial dysfunction

Early signs of ED in the younger population can be demonstrated via impaired FMD. Low birth weight babies have demonstrated decreased FMD as early as 9 years of age (Leeson CP et al, 1997), and this can also be seen in children with familial hypercholesterolaemia (Sorensen KE et al, 1994).

1.4.3 Flow mediated dilatation and relationship to cardiovascular risk factors

FMD has been shown to correlate with risk factors for coronary artery disease in asymptomatic individuals (Celermajer DS et al, 1994). It is also impaired in hypertension, type II diabetes, active and passive smoking and hypercholesterolaemia (Kelm M et al, 1996; Williams SB et al, 1996; Celermajer DS et al, 1993; Raitakari et al, 1999; Vogel RA et al, 1998).

1.4.4 Flow mediated dilatation and the prediction of cardiovascular events

Impaired FMD has been shown to accurately predict future cardiovascular disease risk. In 2000, Suwadi et al demonstrated an absence of cardiac events in those with absent or mild coronary artery dysfunction. However, they noted a 14% cardiac event (death, MI or revascularisation) rate in those with impaired FMD/severe ED (Suwaidi et al, 2000). Another study carried out in 2008, assessed FMD in 435 healthy middle-aged subjects. A median FMD of 10.7% was demonstrated and those with dilatation less than this value were found to have significantly higher risk of cardiovascular end-points including MI, heart failure, stroke and coronary intervention in addition to traditional

1.4.5 Limitations of flow mediated dilatation

FMD is not carried out in routine clinical practice due to the following reasons:

- Variable reproducibility: One research group found large inter-individual variability of measurements of FMD when assessing 18 young healthy volunteers. Measurements made by 2 experienced physicians on the same day were repeated after 1 week and were found to be significantly different; 5.95 +/- 2.93% versus 4.23 +/- 1.6% (P=0.03) and 7.63 +/- 4.3% versus 4.94 +/- 2.69% (P=0.003). However, when they compared FMD at 1st and 2nd measurements taken by one physician there were no significant differences; Physician 1: 5.95 +/- 2.93% versus 7.63 +/- 4.3% (P=0.21); Physician 2: 4.23 +/- 1.6% versus 4.94 +/- 2.69% (P=0.22) (Sejda T et al, 2005). In 1997, Hardie KL et al conducted a similar reproducibility study using 19 subjects and found poor reproducibility with the greatest variability occurring between studies; mean difference in FMD of 0.57% with a SD of difference of 6.83 (Hardie KL et al, 1997). There are significant individual skill differences and operator dependency issues with 2D US and because FMD is a percentage-ratio measure, small inter-observer differences will appear large. Other research groups have found this technique to be accurate and reproducible (Sorensen KE et al, 1995; Uehata A et al, 1997).

- To date, FMD has been assessed using 2D US, therefore it can be difficult to determine exactly when the probe is accurately bisecting the artery in the longitudinal plane giving a true diameter measurement. A transverse approach is not reliable as it only measures a single point along the length of the artery and is difficult to reproduce.

- Lack of standardised protocols: For example, in healthy volunteers FMD can vary from a few percent to 22% depending on cuff occlusion site (Agewall S et al, 2001) and greater dilatation is seen when the blood pressure cuff is
placed on the upper arm versus the forearm with differing physiological mechanisms for each (Betik AC et al, 2003; Peretz A et al, 2007).

- It is technically challenging with a significant learning curve requiring several months of training.

- Artery size: Brachial artery diameters of between 2.5-5mm are suitable for FMD. However, arteries less than 2.5mm are difficult to image accurately and those greater than 5mm do not vasodilate significantly even in those with normal endothelial function (Patel S et al, 2006). 10% of the adult population will therefore fall outside this category.

- FMD is affected by the following:
  1. Biological factors such as blood viscosity and changes in haematocrit (Giannattasio et al, 2002), mental stress (Gottdeiner JS et al, 2003), exercise (Hwang IC et al, 2012), glucose levels and high fat meals (Keogh JB et al, 2005), menstrual cycle and circulating levels of oestrogen and progesterone (Hashimoto M et al, 1995).
  2. Drugs especially vasoactive substances, caffeine (Papamichael CM et al, 2005) and smoking (Amato M et al, 2013).
  3. Age and gender. There is a progressive decrease in FMD with age occurring earlier in men, however at the time of the menopause there is a steep decline in dilatation in females (Celermajer DS et al, 1994).
  5. Environmental factors such as room temperature and weather (Widlansky ME et al, 2007).

In summary, it can be said that FMD has enormous potential in preventative medicine, diagnostics and monitoring responses to therapy. Due to the afore
mentioned factors and variables surrounding FMD it is currently a research tool only and therefore there remains great interest in improving methodology, in particular with a view to greater accuracy and reproducibility.
1.5 Carotid intima-media thickness

The following section will describe Carotid intima-media thickness (CIMT) in detail including its value as a cardiovascular risk assessment tool in evaluating subclinical atherosclerosis, its relationship to cardiovascular risk factors and its accuracy in detecting the prevalence of cardiovascular disease. Measurement of CIMT and the need for standardized protocols will also be described as will its dynamic properties throughout the cardiac cycle.

1.5.1 Introduction

Carotid US is the most commonly performed non-invasive assessment used to detect the presence, progression or treatment response of atherosclerotic plaques. Carotid plaque can be defined as a “focal wall thickening that is at least 50% greater than that of the surrounding vessel wall or as a focal region with CIMT >1.5mm that protrudes into the lumen that is distinct from the adjacent boundary” (Stein JH et al, 2008). Carotid intima-media thickness is a measure of the thickness of arterial walls. When imaged via conventional B-mode US the wall of the artery can be seen as two echogenic lines (representing the intima and the media) separated by a hypoechoic space (See Figure 1.7 below). It is well known to have good correlation with histologic specimens (Pignoli et al, 2006) and therefore is widely used as a tool in clinical research and less so in clinical practice to detect subclinical atherosclerotic disease. CIMT varies with age, ethnicity and is generally thicker in men than women (Stein JH et al, 2008).
Figure 1.7 Ultrasound image illustrating the intima-media thickness.

Longitudinal carotid ultrasound image of the distal common carotid artery on the right and the carotid bifurcation on the left. Note the blown up image of the far wall at the bottom of the figure which shows the intima-media thickness representing the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface (Figure taken from Wald DS et al, 2009).

1.5.2 Carotid intima-media thickness and its relationship to age

The measurement of CIMT may be used to evaluate signs of early or subclinical atherosclerosis in younger individuals. CIMT in subjects under the age of 18 years is extremely difficult to measure due to the elastic nature of the artery and the fact that CIMT at this age consists mainly of media. The carotid arterial wall of a normal healthy individual appears to be unaffected by age and gender until after 18 years of age. However, following this period, there is
diffuse progressive intimal thickening (O’Leary et al, 2010). This occurs in a uniform manner in linear segments of arteries with advancing age, and at an accelerated rate with increasing levels of cardiovascular risk. Hypertension and inherited genetic factors result in greater acceleration of this process (Touboul PJ et al, 2007).

1.5.3 Carotid intima-media thickness and its relationship to cardiovascular risk factors

Several studies have highlighted increased CIMT in younger individuals and their relationship to traditional cardiovascular risk factors. The Bogalusa Heart Study in Louisiana (Urbina EM et al, 2002) examined the impact of multiple risk factors on the IMT of different segments of the carotid artery in 518 young, biracial (71% white) asymptomatic healthy adults with a mean age of 32. Gender distribution was 39% male and 61% female. The results demonstrated increased IMT in the common carotid artery (CCA) and the carotid bifurcation (CB), with age, race (black more than white), systolic BP, LDL and high-density lipoprotein (HDL) (inverse relationship), and insulin (inverse relationship in CB only) being independent predictors of IMT in the CCA and CB. Males, more so than females, and BMI were found to be predictors of IMT in the internal carotid artery (ICA). The CB was found to have the greatest IMT. In 1980, the Cardiovascular Risk in Young Finns Study (Raitakari OT et al, 2003) set out to determine if childhood or adolescent cardiovascular risk factors were associated with increased IMT in adulthood in a cohort of 2229 young Finnish subjects followed over a period of 21 years. They found that when adjusted for age and sex, adult IMT (33-39 years) was associated with levels of childhood (12-18 years) LDL, BP, BMI and smoking. The Muscatine study (Davis PH et al, 2001) also found increased CIMT in young and middle aged adults to be associated with both childhood and current cardiovascular risk factors and risk factor load.
1.5.4 Carotid intima-media thickness in the prediction of cardiovascular disease

Numerous studies have demonstrated that CIMT can predict future cardiovascular events. The Atherosclerosis Risk in Communities Study (ARIC) found that a 0.1mm difference in CIMT adjusted for age and gender was associated with an increased incidence of myocardial infarction (MI), hazard ratio (HR 1.13) (Chamberless LE et al, 1997). In a similar analysis, the Rotterdam Study reported a HR of 1.19 for MI (Bots ML et al, 1997). The Cardiovascular Health Study (CHS) (O’Leary DH et al, 1992) reported on 4476 individuals without clinical cardiovascular disease (CVD) followed over a median period of 6.2 years. Using combined CCA and internal carotid artery (ICA) IMT measurements they discovered a HR of 1.15 for MI. In 2007 a meta-analysis examining over 35,000 participants including those in the above studies found that a 0.1mm absolute difference in CIMT increased risk of MI by 13-15% and stroke by 13-18% (Lorenz MW et al, 2007). CIMT can also reclassify patients at intermediate cardiovascular risk into a higher or lower risk category, and as such can play a role in the management options for patients (Stein JH et al, 2004).

1.5.5 Carotid intima-media thickness and the prevalence of cardiovascular disease

The presence of true atherosclerotic lesions is less than 1% in men under the age of 40 and premenopausal women (Kiechl S et al, 1999). A CIMT of between 0.6-0.7mm is considered normal in healthy middle aged adults and varies according to specific measurement sites (see section on measurement of CIMT below). An IMT of >0.9mm is estimated to be indicative of existing abnormalities and plaque can be identified with an IMT of >1.3mm, or a focal increase in thickness of 0.5mm or 50% of the surrounding IMT value (Mancia et al, 2007). CIMT is associated with the prevalence of CVD. In the CHS study
mentioned above, coronary artery disease and stroke were significantly associated with CIMT (O’Leary DH et al, 1992). Amato M et al, evaluated thickness in the coronary arteries, as assessed by coronary angiography and intravascular US, and CIMT of the carotid arteries using traditional non-invasive US. They found significant correlation between CIMT and coronary atherosclerosis (Amato M et al, 2007).

1.5.6 Measurement and variations of carotid intima-media thickness

The use of carotid intima-media thickness to non-invasively quantify atherosclerotic disease is used extensively in clinical trials as previously eluded to, however consensus regarding the most accurate and reliable method of doing so is divided.

The carotid artery is typically separated into three 1cm segments for the purposes of quantifying IMT. These consist of the following:

- CCA – 1cm segment of distal common carotid artery (CCA) immediately prior to the carotid bifurcation or bulb (CB).
- CB – 1cm segment of the CB, beginning at the divergence of the near and far walls and ending at the start of the flow divider (representing the beginning of both the internal carotid artery (ICA) and the external carotid artery (ECA)).
- ICA – 1cm proximal segment of ICA.
Figure 1.8 Illustration depicting the segments of the distal common carotid artery, the carotid bulb and the internal carotid artery.

Figure demonstrates from right to left; distal common carotid artery immediately prior to the bulb, the bifurcation between the start of the bulb and prior to the flow divider, and lastly the internal carotid artery (Figure taken from Polak JL et al, 2010).

There is evidence that different risk factors and cardiovascular outcomes are associated with different CIMT segments. For example, diastolic BP and fasting glucose are more closely associated with the CCA, whilst others are related to the CB (hypertension, current smoking and diabetes) and ICA (LDL) (Polak JF et al, 2010). IMT segment differences are also due in part to the linear nature of the proximal CCA (linear CCA measured in the proximal neck above the clavicle some distance prior to the bifurcation) and the high shear stress and laminar flow occurring in this region. This is contrasted to the complex nature of both the CB and the ICA, and the haemodynamic differences occurring at these sites namely low shear stress and turbulent blood flow as mentioned previously.
1.5.7 Variation of the carotid intima-media thickness throughout the cardiac cycle

CIMT varies throughout the cardiac cycle and is thickest in end-diastole and thinnest during peak systole with variations of up to 0.03mm (O’Leary DH et al, 2010). Other studies demonstrated changes in IMT (ΔIMT) of 5.3% or 0.039mm (Selzer et al, 2001). A smaller study examining the ΔIMT in a healthy cohort of 50 men and 50 women aged between 18 and 25 years found a difference of 7.1% or 0.026mm between IMT values in end-diastole and end-systole and also found IMT values to be up to 3.1% greater in the right CCA (Gonzalez et al, 2007).

Numerous groups have attempted to explain the change in IMT over the cardiac cycle.

Polak et al examined the ΔIMT in 5633 patients with an average age of 62 in the Multi-ethnic Study of Atherosclerosis (MESA) study. They found ΔIMT to be 0.041mm between end-diastole and end-systole and found the difference to be significantly related to both pulse pressure and ethnicity and none of the traditional cardiovascular risk factors with pulse pressure the most important determinant. They found that an increase of 10mmHg in pulse pressure resulted in a 0.0022mm decrease in IMT. Ethnicity and its relationship to ΔIMT was felt possibly to relate to inherent differences in the structural properties of arteries between ethnic groups. They also found the association of traditional risk factors with both end-diastolic and end-systolic IMT was similar (Polak and Johnson et al, 2012).

Another study by Polak et al examined 2930 subjects from the Framingham Offspring Study with an average age of 60 years and found an average difference of 0.037mm in IMT through the cardiac cycle. Once again they found the ΔIMT to be associated with pulse pressure, but in addition they discovered a weaker correlation with LDL and age. The decrease in IMT with increased pulse pressure is felt to be due increased arterial expansion and consequent wall thinning. It was difficult to explain the relationship between
LDL and age with the ΔIMT. One theory is that they alter the biochemical and structural characteristics of the wall resulting in greater arterial expansion in the radial direction hence causing a thinner IMT. This theory however, conflicts with the concept of increasing arterial stiffness with increasing age and so the association is not fully explained (Polak and Meisner et al, 2012).

In 2013 Zahnd et al examined 57 healthy and 25 high cardiovascular risk patients and found that the amplitude of ΔIMT was greatest in the at risk group; 0.079mm +/- 0.036mm versus 0.064mm +/- 0.026mm in the healthy group (P=0.032). Again a possible explanation was that increased radial stress on the lumen alters endothelial function during the atherosclerotic process resulting in greater IMT compression (Zahnd et al, 2013). An important limitation of the study is that analysis was performed over longitudinal segments of CCA measuring just 3mm possibly discounting the heterogeneous nature of CCA, especially in the at risk group.

A study of arterial wall compressibility in 2001 found the ΔIMT in 19 female patients with Pseudoxanthoma Elasticum (PXE), a disease characterized by proteoglycan accumulation in connective tissues leading to large artery calcification and stenosis, to be greater in these patients versus 15 healthy controls. Proteoglycans play a pivotal role in sustaining compression and shaping the arterial wall. In PXE they accumulate and cause increased compressibility and can increase the transmural transport of lipids and their subsequent retention in the arterial wall. They found that the ΔIMT was greater earlier in the disease process in younger patients and less so in those over 40 suggesting that proteoglycans play an important role in compressibility (Boutouyrie et al, 2001).

Meinders et al found IMT compression to be less in younger groups of healthy subjects versus their elderly counterparts, possibly due in part to the surrounding tissue being more compressed than the actual IMT in the younger group with the IMT being more compressed than the surrounding tissues with increasing age (Meinders et al, 2003).
There are also variations in near versus far wall measurements and in the way in which IMT is measured; in an offline semi-automated fashion or using manual tracings (Bots ML et al, 2003).

1.5.8 Strategy to standardize measurement protocols

Since 2000, there have been 7 consensus statements or guidelines drawn up, recommending CIMT measurement and detection of carotid plaque as useful tools in the assessment of vascular disease. In 2008 the American Society of Echocardiography (ASE) addressed standardization of imaging protocols to aid in the interpretation and variability of findings from the numerous trials that are carried out in this field (Stein JH et al, 2008). These guidelines are highlighted below.

American Society of Echocardiography 2008 Consensus Statement guidelines for carotid intima-media thickness (CIMT) measurement.

Instrumentation and Image Display:

- The carotid arteries should be interrogated using a state-of-the-art ultrasound system with a linear-array transducer operating at fundamental frequency of at least 7 MHz. The typical pixel size with imaging at 4cm depth is approximately 0.11 mm.

- B-mode imaging is preferred over M-mode imaging (M-mode provides measurement of only a single point of thickness, rather than a segmental measurement).

- Digital images should be stored directly from the ultrasound system, rather than digitized video captures.
CIMT Imaging Protocol:

• Ultrasound images of the distal 1 cm of the far wall of each common carotid artery should be obtained and compared with values from a normative data set.

• These measurements should be supplemented by a thorough scan of the extracranial carotid arteries for presence of carotid plaques.

• Transverse B-mode scan (3–5 beat cine-loop in each segment) from proximal common carotid artery (CCA) through middle of the internal carotid artery.

• Internal and external carotid artery Doppler recordings (one frame of each) at proximal 1 cm of each branch.

• Longitudinal plaque screen scan (3–5 beat cine-loop from at least 3 different angles in each segment) at near and far walls of CCA, bulb, and internal carotid artery (ICA) segments.

• CIMT imaging (3–5 beat cine-loop and optimized R-wave gated still frames at each angle) at distal 1 cm of each CCA.

• Mean CIMT values from the far walls of the right and left CCA’s (mean-mean) should be reported.

In 2009 the United States Preventative services Task Force (USPSTF) criticized the ASE guidelines and recommended against measuring CIMT as a marker of atherosclerosis (Helfand M et al, 2009). Some of the reasons behind these recommendations were as follows (Stein JH et al, 2010):

• The independent predictive value of CIMT for those at intermediate coronary heart disease (CHD) risk was questionable due to lack of specific data in this area.

• Concerns regarding the use of CIMT to reclassify these patients into lower or
higher risk categories.

- They highlighted that some studies used to support CIMT as a predictive tool were of short duration, and included subjects with pre-existing CHD or risk equivalent conditions.

A recent study in 2010 carried out by Nambi et al heavily criticized the USPSTF’s statements and proved that measurement of carotid plaque and CIMT could accurately predict CHD and stroke. They examined 13,145 patients who were free of CHD or stroke at the start of the study. Following over 15 years follow up they were found to have 1,812 CHD events that included MI or death. The area under the receiver operating characteristic curve (AUC) for traditional risk factor prediction of CHD events was 0.742. This increased significantly to 0.750 and 0.751 when CIMT and carotid plaque were added respectively. When all three parameters were included this further increased to 0.755. They also noted that 37.5% of subjects in the 5-10% risk category (based on risk factors) and 38.3% in the 10-20% category were reclassified when plaque and CIMT data were added, with plaque presence being more important than increased CIMT among women (Nambi et al, 2010). It is important to mention that the majority of those whose risk was reclassified were placed into a lower risk category meaning less intensive treatment implications for this group. This study definitively proves the value of measuring CIMT or detecting carotid plaque in cardiovascular risk prediction in those at intermediate cardiovascular risk.

### 1.5.9 Near versus far wall CIMT measurement

Near wall CIMT measurements can present certain technical difficulties, and are believed by some to be less reproducible whilst not improving risk prediction when compared to far wall CIMT measurement (Roman MJ et al, 2006). The reasons for this are laid out below:

- At the adventitia-media and intima-lumen interfaces the US beam is travelling from a more echogenic to less echogenic layer.
• The precise location of the boundaries between tissues with different echogenic properties is represented by the leading edge of an ultrasound echo reflective surface. This is the edge closest to the US probe.

• The edge furthest from the probe or trailing edge of the echo line is thought by some to be an acoustic shadow cast by the tissue interface and may give inaccurate readings when measured (Wikstrand J, 2007).

• Studies have shown that CIMT measurement at the near wall was increased by 20% when using US contrast agents (as these agents provide a “true” acoustic delineation between the intima-media interface by enhancement of the arterial lumen) and these findings have been observed in previous histological examination (Coll et al, 2008).

However, other studies report that by using stricter protocols and taking measurements from multiple predefined segments, at consistent angles of acquisition and by using improved technology such as automated edge detection algorithms, that near and far wall measurement can be combined giving more reproducibility and accuracy. The following studies have recommended a combined approach:

• In 2009 Dogan et al performed a post-hoc analysis of the “Osteoporosis Prevention and Arterial effects of tibolone” (OPAL) study, a 3-year randomized controlled trial among healthy postmenopausal women. They compared 66 different US protocols based on combinations of 60 CIMT measurements (2 sides, 2 walls, 3 segments and 5 angles) that were used to measure CIMT progression rate in 675 women. They found that mean common CIMT protocols that included both near and far walls at ≥2 angles give the greatest reproducibility combined with the greatest estimates of CIMT progression and recommend usage of this protocol in such a population (Dogan et al, 2009). The group conducted a similar trial in 2010 that confirmed these results amongst patients with mixed dyslipidaemia and familial hypercholesterolaemia (Dogan et al, 2010).

In summary, CIMT can be used to detect early atherosclerosis, is related to traditional cardiovascular risk factors and can predict future cardiovascular
events and prevalence of coronary artery disease and stroke. Technical difficulties associated with its measurement have led to varying opinions in the field and several recommendations on best practice. However, semi-automated edge detection software that analyses multiple arterial segments at multiple angles appears to be the most accurate and reproducible method at present in the assessment of CIMT. CIMT varies throughout the cardiac cycle being greatest in diastole and thinnest in diastole. The degree of compression has been found to be positively and significantly related to pulse pressure, increasing age, LDL and ethnicity.

1.6 Arterial stiffness

1.6.1 Introduction

This section will describe arterial stiffness in detail including the arterial waveform, its pathophysiology, factors contributing to stiffness, its value in detecting the prevalence of cardiovascular disease and also its predictive power. There are numerous methods used in its detection and these will also be described.

1.6.2 Functions of the arterial system

The arterial system acts as a conduit in transporting blood from the heart to the peripheries and also as a reservoir dampening the pressures received from the contracting heart, thereby providing a steady flow of blood throughout the arterial system. There are 3 separate anatomical regions in the body used to explain the functions of the arterial system. The initial dampening of oscillation comes from the large elastic aorta which functions as a reservoir. Next the large muscular arteries or branches of the aorta act as the conduit and modify wave propagation via regulating smooth muscle tone. Lastly, the distal arterioles alter the peripheral vascular resistance resulting in the maintenance of mean arterial pressure and the delivery of constant flow to the capillary beds (O’Rourke MF et al, 2005). With age and the development of cardiovascular
disease the composition of the arterial wall changes resulting in an imbalance between the ratio of elastin and collagen fibres. This causes the arteries to become stiffer and more resistant to wall deformation resulting in hypertension.

1.6.3 The arterial pressure waveform

The propagative / distensible tube model (Laurent S et al, 2006) is felt to be the most accurate model illustrating the arterial pressure waveform. The model consists of a single distensible tube with two ends. The first end represents peripheral resistance with the other end receiving blood in pulses via the heart. The contraction of the left ventricle results in a pressure wave that travels down the tube and is subsequently propagated to all of the distal arteries and cushioned by the viscoelastic properties of the vessel wall. The pulse becomes amplified as it propagates down the arterial tree due to the increasing stiffness of arteries in the peripheries. As the pressure wave arrives at sites of impedance mismatch or branch points the pressure wave is reflected back to the heart. These reflected pressure waves arrive during diastole thus merging with the diastolic pressure wave. The returning pressure wave from the peripheries results in an amplification of the pressure signal. This is greater in the peripheral vasculature than in the central arteries due to the numerous bifurcations and branching points. This is referred to as the amplification phenomenon (Laurent S et al, 2006). The final pressure wave is a summation of both the incident and reflected pressure waves and is illustrated in figure 1.9 below.
Illustration depicting the summation of the incident and reflected waves (left and middle) resulting in the resultant wave on the (right). (Figure taken from Koelwyn GJ et al, 2012).

1.6.4 Pathophysiology of arterial stiffness

In normal healthy arteries the velocity of the pressure pulse is relatively slow, however when arterial stiffness increases the resultant velocity of the pressure wave also increases. This results in the reflected waves arriving at the heart and aorta earlier, thereby augmenting central systolic blood pressure that in turn, increases the afterload within the left ventricle and compromises blood flow within the coronary arteries.

The properties of the arterial wall are heterogeneous throughout the arterial system and there are differences in the elastin and collagen content depending on the central or peripheral nature of the vessel. In the proximal aorta the ratio of elastin to collagen fibres is greatest with an equal amount of both noted in the abdominal aorta. More peripherally, the collagen content predominates (Harkness et al, 1957).

1.6.5 Factors contributing to arterial stiffness

The process of arterial stiffening occurs secondary to a series of complicated interactions involving cellular and structural components of the arterial wall as
depicted in figure 1.10 below. Haemodynamic factors and external factors such as glucose regulation, hormones and salt all play a role in increasing vascular stiffness (Zieman SJ et al, 2005). Diabetes and hypertension (Tedesco MA et al, 2004) and normal ageing also result in arterial stiffness (Kelly RP et al, 1989). Obviously, the arterial system will also degenerate secondary to the other traditional cardiovascular risk factors such as smoking (Mahmud A et al, 2003) and dyslipidaemia (Urbina EM et al, 2012). Healthy ageing demonstrates increased stiffness that is seen predominantly within the aorta and its proximal elastic branches and to a lesser degree the peripheral arterial system (O'Rourke MF et al, 2002). The primary mechanism associated with ageing and disease processes causing stiffening of the arteries is degeneration of the media within the central arteries. This begins with fracturing and degeneration of the elastin and collagen fibres. The elastin becomes fragmented and thinned and the collagen content increases resulting in a more inelastic artery (Laurent S et al, 2007). Ageing of the arterial media is coupled with increased expression of matrix metalloproteinases (MMP) that result in degradation of elastin and collagen fibres. Two enzymes linked with vascular stiffness in this regard are MMP-2 and MMP-9. Animal studies have shown that elastin fragmentation and thinning is associated with increased expression of MMP-2 in the arterial intima and media (Wang et al, 2002). Levels of MMP-2 and MMP-9 and associated genetic polymorphisms correlate with arterial stiffness in humans (Yasmin et al, 2009) and MMP-2 correlates with increased pulse wave velocity (PWV) and calcium deposits in renal transplants (Chung et al, 2009). Changes in the structure of collagen fibres and collagen cross linking by advanced glycation end products also has a link to arterial stiffness. In animal models, suppression of these end products prevents vascular stiffness without altering the structural content of elastin or collagen fibres (Corman B et al, 1998). In a subsequent human trial, treatment of hypertensive patients with a non-enzymatic breaker of collagen cross-links led to significant reduction in BP and PWV versus placebo (Kass DA et al, 2001).
Endothelial dysfunction has already been discussed in detail, however the decreased levels of nitric oxide that is observed in ED, leads to an increase in vascular tone of the small arterioles that are involved in the major changes of total peripheral vascular resistance. This in turn results in functional and structural changes of the larger more central arteries causing stiffening, and vascular remodelling, atherosclerosis and an elevated blood pressure (Koelwyn GJ et al, 2012).

Several genetic links have been made to arterial stiffness. Marfan’s syndrome is associated with mutation of the FBN1 gene that encodes fibrillin-1. This gene is involved in regulation of the elastin fibres and is associated with increased arterial stiffness (Jondeau G et al, 1999). Fibrillin-1 is also associated with increased severity of coronary artery disease and aortic stiffness (Medley T L et al, 2002). William’s syndrome, a connective-tissue disorder caused by deletion of chromosome 7q, results in disruption of the elastin gene resulting in
decreased arterial stiffness (Lacolley P et al, 2002). Endothelin receptor and angiotensin 2 type-1 receptor genes have been shown to relate to increased arterial stiffness in hypertensive patients (Lajemi M et al, 2001).

1.6.6 Arterial stiffness and the prevalence of cardiovascular disease

Several studies have shown that arterial stiffness can detect the prevalence of cardiovascular disease. In 2012 Wang JW et al measured brachial-ankle pulse wave velocity (BaPWV), a marker of stiffness in both central and peripheral muscular arteries, in 2,852 Chinese subjects. They found increased BaPWV in 22.3% of men and 26.4% of women. Heart rate, systolic blood pressure, smoking and fasting glucose were significantly associated with arterial stiffness in men. Serum cholesterol, diabetes, heart rate and systolic blood pressure were significant associations in women (Wang JW et al, 2012).

In 2007, Maple-Brown LJ et al investigated the incidence of increased PWV in both indigenous Australians (162) and Australians of European decent (121), of similar age and sex who were at a high risk of CVD. 60 of the indigenous group had type 2 diabetes and a corresponding 38 in the European group. The group assessed stiffness via applanation tonometry to assess PWV and the augmentation index (AI). Using these measures they found the indigenous Australians to have higher indices of arterial stiffness than their European counterparts. Factors likely contributing to the difference were smoking, metabolic syndrome variables, homocysteine levels, CRP and heart rate (Maple-Brown LJ et al, 2007).

In 2011, Liu CS et al examined the relationship between BaPWV and coronary artery disease. They examined 654 asymptomatic patients including 296 men and 358 women with a mean age of 55. Coronary artery disease was assessed using CT coronary artery calcium scoring (CAC) to detect stenotic vessels and arterial stiffness was assessed using BaPWV. 127 patients or 19.4% of the group had at least one stenotic coronary artery assessed by
means of CT coronary angiography, with the mean BaPWV and mean CAC being significantly higher in the stenotic group than in controls. They found arterial stiffness to correlate well with coronary artery atherosclerosis and to be a useful additional tool in the detection of cardiovascular disease (Liu CS et al, 2011).

The relationship between PWV and type 1 diabetes was evaluated by Prince CT et al, in 2010. They examined 144 subjects with a diagnosis of childhood onset type 1 diabetes and looked at various arterial stiffness parameters including Augmentation index (AI) and Augmentation pressure (AP). They also examined myocardial perfusion, CAC and low ankle brachial index (ABI). The results demonstrated greater AP (but not AI) to be independently associated with prevalent coronary artery disease, decreased myocardial perfusion and low ABI’s in type 1 diabetes (Prince CT et al, 2010).

1.6.7 Arterial stiffness and the prediction of cardiovascular events

In the case of uncomplicated hypertension, measurement of arterial stiffness via carotid-femoral pulse wave velocity has been shown to have an independent predictive value for all-cause morbidity and cardiovascular mortality and cerebrovascular and coronary events (Mancia G et al, 2007). In 2001 Laurent S et al, examined aortic stiffness via carotid-femoral PWV in 1890 patients with essential hypertension. The mean age was 50 and patients were followed for approximately 9 years, during which time there were 107 fatal events, 46 of which were cardiovascular in nature. They found that PWV was significantly associated with all-cause and cardiovascular mortality independent of diabetes, prior cardiovascular disease and age (Laurent S et al, 2001).

In 2010 Mitchell et al, examined an asymptomatic population of 2232 individuals from the Framingham heart study using pulse wave velocity, the non-invasive gold standard of measuring arterial stiffness. During a median
follow up of 7.8 years, 6.8% of the study group experienced a cardiovascular event. They found a PWV of >11.8 m/s to be associated with a 48% increased risk of a first major cardiovascular event including stroke, unstable angina, myocardial infarction and heart failure. They also found that aortic PWV when combined with the traditional cardiovascular risk factors improved risk prediction. (Mitchell GL et al, 2010)

In 2001, Barenbrock et al reported on carotid distensibility in 68 renal transplant recipients. Subjects were followed for approximately 8 years post transplant during which time there was 19 cardiovascular events. Carotid distensibility was found to be an independent predictor of cardiovascular events (Barenbrock et al, 2001). In 1998 Blacher J et al, examined carotid elasticity in 79 end-stage renal patients undergoing haemodialysis over a period of approximately 2 and a half years. Over this period there were 8 non-cardiovascular and 10 cardiovascular deaths. They discovered that decreased carotid elasticity as measured by ultrasound and low diastolic blood pressure were predictors of all-cause and cardiovascular mortality in high risk patients (Blacher J et al, 1998).

Aortic stiffness, as opposed to myocardial dysfunction of the left ventricle, via the development of higher pressures in the left ventricle and aorta, has been found to be the predominant factor leading to heart failure (Levy D et al, 2005). In 2011 it was discovered that aortic stiffness was a predictor of cardiovascular events independent of age, conventional cardiovascular risk factors and arterial pressure (Adji A et al, 2011).

Aortic pulse wave velocity has also found to be predictive of cardiovascular outcomes in the general population as highlighted by a Danish study of 1678 participants aged between 40 and 70. Subjects were chosen at random and followed over a median of 9 years and monitored for both cardiovascular morbidity and mortality. Aortic PWV predicted a composite of cardiovascular outcomes above and beyond the traditional cardiovascular risk factors and found that an increase in PWV of 3.4 m/s (an increment of 1 SD) increased the risk of a cardiovascular event from 16% to 20% (Willum-Hansen T et al, 2007).
In 2006, it was reported by Williams et al that pulse pressure and central augmentation index (AI) were independent predictors of cardiovascular events in hypertensive patients (Williams et al, 2006).

Carotid-femoral pulse wave velocity remains the gold standard measurement of vascular stiffness. The other techniques have less predictive power but nonetheless play an important role in the assessment of cardiovascular disease.

1.6.8 Methods of measurement

1.6.9 Pulse pressure

Pulse pressure (PP) is the simplest and one of the first methods developed as a surrogate marker of arterial stiffness (Bramwell JC et al, 1922). It is defined as the difference between systolic and diastolic blood pressure. As it does not take account of blood volume it is not a true measure of arterial stiffness, however its value does depend on arterial stiffness, wave reflection and cardiac output. With increasing age the PP widens as systolic pressure continues to increase after 60 years of age, whilst diastolic pressures do not and may even fall after the 6th decade (Franklin SS et al, 1997). Pressures are derived from the brachial artery using a sphygmomanometer, which may not be a true reflection of central arterial pressures with differences observed of up to 20 mmHg (Wilkinson IB et al, 2000). Regardless of its limitations the Framingham study demonstrated that PP was a better predictor of coronary heart disease in the over 50’s than either systolic or diastolic blood pressure alone (Franklin SS et al, 1997).

1.6.10 Local measurement of arterial stiffness

Ultrasound can be used to determine local arterial stiffness parameters in superficial arteries through measurement of vessel diameters in both systole
and diastole via the change in pressure, leading to the distension of the artery. This is illustrated in figure 1.11 below.

Figure 1.11 Illustration demonstrating the stroke change in lumen cross-sectional area.

(Figure taken from Laurent S et al, 2006).

Vessel wall elasticity is explained by the arterial wall stress and strain relationship. Stress is defined as the force that produces deformation and is applied in a longitudinal, radial or circumferential direction. Strain is the deformation incurred by the artery subjected to stress (Cavalcante JL et al, 2011). Various calculations can be performed on the artery to explain stiffness including the following:

- Young’s elastic modulus

This is the stress/strain ratio and uses the changes in lumen diameter and the arterial wall thickness to measure intrinsic stiffness within the arterial wall. Current techniques used to measure arterial wall thickness cannot distinguish the adventitia from the surrounding soft tissues and therefore the intima-media
thickness is used as a surrogate for wall thickness. This assumes that the arterial wall is homogenous and that the IMT is load bearing. These assumptions can lead to unrealistic and inaccurate measurements (O’Rourke et al, 2002) and therefore caution may be warranted in interpretation.

- **Distensibility**

  This is the relative change in the arterial volume for a given change in pressure. It is the inverse of Young’s elastic modulus.

- **Compliance**

  Arterial compliance is the absolute change in arterial volume (strain) for a given pressure change (stress).

  The above calculations are carried out using the following equations from ultrasound images:

  - Young’s elastic modulus = $(\Delta P \times D) / (\Delta D \times h)$ (mmHg/cm)
  - Distensibility = $\Delta D / (\Delta P \times D)$ (mmHg$^{-1}$)
  - Compliance = $\Delta D / \Delta P$ (cm/mmHg) (or cm$^2$/mmHg)

  Where P=Pressure, D=Diameter, h=Wall thickness.

### 1.6.11 Limitations of local arterial stiffness measurement

Limitations of assessing local arterial stiffness include the operator dependant nature of the measurement. In addition, blood pressure is ideally assessed locally over the artery examined using an applanation tonometer. This is a high fidelity strain gauge transducer that records pressure waveforms in an artery. The artery is flattened, using pressure applied by the operator, against the underlying structures such as ligaments and bone. It is the most accurate non-invasive method to assess local blood pressure (Van Bortel et al, 2001). Difficulties can be encountered in obese patients where it becomes difficult to
flatten the artery due to surrounding structures. When local blood pressure cannot be assessed the brachial pressure measured using a sphygmomanometer can be used as a surrogate for pressures in the carotid artery. The pulse pressure however, is not constant along the arterial tree with the systolic pressures in peripheral muscular arteries being greater than the systolic pressures in the more central elastic arteries such as the aorta and the carotid. Hence, when the brachial artery is used to measure pressure it may have the effect of overestimating arterial stiffness (Laurent S et al, 2006). Brachial pressures may differ with carotid pressures due to wave reflection (Karamanoglu M et al, 1993). However, other studies have demonstrated brachial pressures to be a reasonable approximation of pulse pressure (Van Dijk RA et al, 2000).

1.6.12 Regional measurement of arterial stiffness and Pulse wave velocity

As mentioned previously, when the heart contracts it results in dilatation of the aortic wall and generation of a pulse wave that propagates down the arterial tree at increasing speed depending on the arterial segments and the properties of the wall. When arterial stiffening occurs the velocity of the pulse wave increases. Pulse wave velocity (PWV) involves measuring pulse waves at two different sites over an average of 10 cardiac cycles to ensure measurement over at least one cycle of respiration. Aortic PWV or central PWV is most commonly measured between the carotid and femoral arteries and therefore evaluates stiffness in the large elastic arteries. Muscular medium sized arteries of the upper and lower limb can be assessed using peripheral PWV. It is calculated using the formula:

\[
Pulse \, wave \, velocity = \frac{D}{\Delta t}
\]

Where \( D \)=Distance between measurement sites, \( \Delta t \)=Pulse transit time.

Locations of PWV measurement include:
• Carotid-femoral PWV (the gold standard)
• Carotid-radial PWV
• Femoro-tibial PWV
• Brachial-ankle PWV

Non-invasive recordings are made using sensors or doppler probes. The pulse wave transit time is determined using the foot-to-foot method and is the interval between the onset of the carotid and femoral wave upstroke. This is illustrated in figure 1.12 below.

![Illustration of foot-to-foot method for calculating carotid-femoral pulse wave velocity.](image)

**Figure 1.12** Illustration demonstrating the foot-to-foot method used to calculate carotid-femoral pulse wave velocity.

Pulse waves are obtained using sensors placed over both the carotid and femoral arteries. \( \Delta t \)=Pulse transit time, \( D \)=Distance between measurement sites. (Figure taken from Rhee MY et al, 2008).

The distance between measurement sites is estimated using a measuring tape and therefore is not a true measure. Sensors are applied to the skin over the artery simultaneously measuring the pulse waves. Methods used include doppler, single high fidelity applanation tonometry, plethysmographic sensors incorporated into blood pressure cuffs (for brachial-ankle PWV) and MRI.
1.6.13 Limitations of regional arterial stiffness measurement

Carotid to femoral pulse wave velocity is well recognized as the current gold standard in the assessment of arterial stiffness (Laurent S et al, 2006). As the measurement of the distance between arterial sites is an estimate it can introduce error in the calculation of PWV. It may underestimate distance in those with a tortuous aorta and overestimates distance in obese subjects. MRI is not limited in terms of accurate distance measurement, however its high cost and lack of available equipment limit its widespread use. Physiological parameters such as heart rate and blood pressure can affect PWV, with a sudden rise in heart rate (Latelme P et al, 2002) and BP (Asmar R et al, 1995) causing increased PWV. In patients with peripheral vascular disease, diabetes and in obesity the femoral waveform is difficult to accurately record and therefore assessment of PWV may be limited in these cohorts (Van Bortel LM et al, 2002).

1.6.14 Systemic measurement of arterial stiffness

Systemic arterial compliance can be determined via the measurement of the velocity of aortic blood flow at the level of the suprasternal notch using a velocimeter. The pressure waveform driving the flow is measured using applanation tonometry at the level of proximal right common carotid artery. This is referred to as the ‘area method’ and is expressed as:

Systemic arterial compliance = \( \frac{Ad}{R(Ps-Pd)} \)

Where Ad=area under blood pressure diastolic decay curve from end systole to end diastole, R=total peripheral resistance, Ps=end-systolic blood pressure, Pd=end-diastolic blood pressure (calibrated against brachial artery pressure).

The area method is illustrated in Figure 1.13 below.
Figure 1.13 Measurement of systemic arterial compliance using the 'area method'.

$P_s$ = end-systolic blood pressure, $P_d$ = end-diastolic blood pressure, $A_d$ = area under the diastolic delay portion of the obtained pulse pressure contour.

(Figure taken from Rhee MY et al, 2008).

Another method of calculating systemic stiffness is one based on an electrical circuit and determines a proximal capacitive (large artery) compliance and a distal oscillatory (small artery) compliance. Arterial pulse is recorded at the radial artery using tonometry, and a decaying sinusoidal wave identifies the pulse wave reflections in diastole. This is known as diastolic pulse contour analysis or the Windkessel model. The reliability of this method has been questioned due to the fact that compliance differs within the upper and lower limb suggesting a significant influence of regional circulatory properties such as arterial length, stiffness of individual arteries and the number of reflection sites (Manning TS et al, 2002).

An indirect measure of systemic compliance is obtained by measuring the ratio of stroke volume to pulse pressure (SV/PP). This is based on the crude assumption that the arterial tree can be modelled as an elastic chamber with a
constant compliance in steady-state conditions and therefore is somewhat limited in terms of validity (Laurent S et al, 2006).

### 1.6.15 Central pulse wave analysis

As mentioned previously the arterial pressure waveform consists of a summation of both the forward pressure wave and the reflected wave. When arterial stiffness occurs there is a subsequent rise in PWV causing the reflected wave to arrive at the central arteries sooner, and therefore adding to the forward wave and augmenting the systolic pressure. This process is explained by the augmentation index (Alx) and is defined as the proportion of central pulse pressure resulting from arterial wave reflection. It is the difference between the second and first systolic peaks of the arterial waveform (P2-P1) and is expressed as a percentage of the pulse pressure. This is illustrated in Figure 1.14 below.

![Figure 1.14 Determination of the Augmentation index.](image)

**Figure 1.14** Determination of the Augmentation index.

\[ P_1 = 1^{\text{st}} \text{systolic peak}, \quad P_2 = 2^{\text{nd}} \text{systolic peak}, \quad P_3 = \text{diastolic pressure}. \]

(Figure taken from Antonini-Canterin F et al, 2008).

Augmentation index is thus calculated using the following equation:

\[
\text{Augmentation index} = \frac{P_2 - P_1}{PP}
\]

The augmentation index is influenced by high PWV and changes in reflection sites. Therefore, diastolic BP and height, which are related to reflection sites,
and aortic PWV and age, are the main determinants of Alx. The central arterial waveform is commonly obtained via tonometry of the radial or carotid artery and then, in the case of the radial artery waveform, applying a transfer function, thereby transferring the peripheral pulse wave into a central pulse waveform (Pauca AL et al, 2001). Radial tonometry is perhaps an easier and more accessible approach as the artery is easily planated against the underlying radius, however a transfer function is needed and therefore, whilst used extensively to convert to a central waveform, its accuracy in determining the aortic Alx has been questioned (Millasseau SC et al, 2003). The carotid approach does not require a transfer function as it more closely resembles the pressures in the aorta. However limitations of this method include difficulty in planating the artery in obese individuals or those with extensive atheromatous plaques. Allowing for limitations, the central pulse pressure and central augmentation index are independent predictors for all-cause mortality in end-stage renal disease (Safar ME et al, 2002). They have also been shown to predict re-stenosis and myocardial infarction in patients undergoing cardiac intervention (Weber T et al, 2005).

In summary, there are numerous useful and non-invasive methods of measuring arterial stiffness with some of the more validated and established methods explained above. Each method has its own advantages and limitations. It is important to consider the predictive value, accuracy, cost and level of technical expertise required when deciding on what method to use.
1.7 Novel methods of measuring arterial stiffness using ultrasound

In order to overcome some of the limitations of measuring arterial stiffness there has been recent interest in the development of various novel non-invasive ultrasound based technologies. Some of the more relevant methods are subsequently discussed.

1.7.1 Pulse wave imaging

Pulse wave imaging (PWI) is a novel ultrasound technique developed to visualize the pulse wave propagation and quantitatively estimate regional or local PWV. Due to high pulse wave velocities of approximately 5 m/s in healthy subjects and over 12 m/s in those with established cardiovascular disease, a high frame rate is utilised in PWI. A standard B mode image of the carotid artery will use up to 128 beams giving a maximum frequency of 140 Hz. By reducing the number of beams or the beam density to 16, the frame rate can be increased to 1127 Hz giving a frame rate capable of capturing and estimating the pulse wave propagation (Sorensen GL et al, 2011). The artery wall is displaced during systole, and in a longitudinal scan, this is shown as an upward or towards the probe displacement of the near wall, and a downward or away from the probe displacement of the far wall. Far wall displacement is then subtracted from the near wall motion giving the distension velocity waveform. Local PWV is then estimated from the spatiotemporal variation of the pulse waves. Figure 1.15 below demonstrates the propagation of the pulse wave. This technique has been validated in aortic phantoms (Vappou et al, 2010) and the aortas of healthy subjects using tonometry (Vappou J et al, 2011).
Figure 1.15 Successive PWI images demonstrating the propagation of the pulse wave from left to right.

The white arrows indicate the approximate location of the foot of the wave on both the near and far walls.

(Figure taken from Vappou J et al, 2010).

The technique has also been validated in hypertensive and aneurysmal aortas. In 2013 Li RX et al, used radiofrequency based speckle tracking to estimate the PWV in the aortas of 15 normal, 13 hypertensive and 5 patients with abdominal aortic aneurysms (AAA). They found the aortic PWV in the normal subjects to be 6.03 ± 1.68 m/s, in the hypertensive subjects 6.69 ± 2.80 m/s and in the AAA patients to be 10.53 ± 6.52 m/s. No significant difference was demonstrated between the PWV of the normal and hypertensive patients, however the PWV in the AAA patients was significantly higher compared to the other groups. This study, albeit with a small group of patients, demonstrated the potential of PWI in providing useful information in the characterization and the wall mechanics of abdominal aortic aneurysms (Li RX et al, 2013).

In 2012, Luo et al assessed the feasibility of PWI in the carotid arteries of 8 healthy subjects and found the local PWV to range from 4.0 to 5.2 m/s. These values are felt to be similar to those found using standard PWV measurements, albeit the study number was small (Luo et al, 2012).

PWI attempts to overcome the major limitation of C-F PWV, namely its oversimplification of the arterial tree and its inability to measure the true length of the arterial system. PWI itself however can be limited in its assessment of aneurysms, stenotic lesions, fluctuations in arterial wall properties and arterial morphology and branching (Vappou J et al, 2010). There are also difficulties
imaging obese patients due to beam attenuation. In addition, offline analysis and processing can be time consuming. To date there have been no large clinical trials assessing PWI and therefore, it has yet to be accepted clinically as a tool in the assessment of arterial stiffness.

1.7.2 UltraFast imaging

Recently a technique called UltraFast imaging or Ultra-FastEcho has been developed to measure local arterial stiffness. This is based on a very high frame rate of up to 10,000 images per second or 10 kHz. It is approximately 100 times faster than conventional ultrasound systems and functions by sending a single plane wave in emission (up to 128 are sent by conventional US systems) and focusing on the received signal only. An extremely powerful processing device receives the signals and performs rapid reconstruction of the image. The high temporal resolution is able to capture the propagation of the pulse wave measured locally up to dozens of meters per second (Messas E et al, 2013). The local PWV is calculated from the beginning to the end of systole. It has recently been used to accurately assess the PWV in the carotid arteries of Ehlers-Danlos patients, albeit in a small group of patients (Mirault T et al, 2015). One obvious limitation is that with very high frame rates there is a trade off in image quality. Ultrafast imaging can be used to track the pulse wave in targeted arteries such as the aorta or carotid arteries however, larger trials are needed before it becomes more widely accepted.

1.7.3 Shear wave elastography

The principle of shear wave elastography has recently been adapted to assess carotid artery elasticity. Elastography is a recognized ultrasound technique that evaluates tissue elasticity by generating low frequency vibration in tissues to induce shear stress. The images are subsequently analysed and the shear stress and stiffness parameters calculated. The general principle is that when
organs and tissues are affected by disease processes such as malignancy or cirrhosis for example, they stiffen and become less elastic. The potential benefits of this technology have been evaluated in breast, liver, musculoskeletal, parotid and salivary gland, prostate and thyroid imaging (Cosgrove D et al, 2013). An ultrasonic shear wave is sent into the tissue under examination, and the propagation velocity of the wave is correlated directly with Young’s elastic modulus. The velocity of the shear wave is independent of blood pressure and viscosity. This technique recently demonstrated the ability to quantify carotid plaque elasticity in a group of 81 patients with a mean age of 76. Patients were assessed using both shear wave elastography and the grey scale median. The grey scale median is a technology that analyses the density of carotid plaque and therefore its risk of rupture. This study found that shear wave elastography when combined with the percentage stenosis of the artery, improved diagnostic accuracy (Ramnarine KV et al, 2014). Standard diagnostic transducers are incapable of generating the high intensity ultrasound beams required for shear wave elastography. Therefore, in order for this technology to be more widely accessible and accepted, manufacturers of ultrasound systems will need to integrate plane wave imaging into their transducers and upgrade their beam forming systems (Hopkins PR et al, 2015). Whilst both high temporal resolution imaging and ultrasound elastography show potential in their ability to aid in the early detection of cardiovascular disease, larger multicenter studies need to be performed to further assess their diagnostic and prognostic accuracy.

1.7.4 Speckle tracking imaging

Speckle tracking imaging (STI) is a relatively new, non-invasive approach that was originally developed to assess left ventricular rotation and torsion. It is based on the principle that temporally stable unique acoustic markers, within the tissues under evaluation, are created as a result of constructive and destructive interference of the ultrasound waves from structures smaller than their wavelength (Burns AT et al, 2008). These speckles are ultrasound
reflectors within tissue and are highly reproducible. Algorithms have been developed that filter out random noise yielding small segments of tissue with stable speckle patterns called "kernels" approximately 20-40 pixels in area. These kernels are tracked frame-to-frame and accurate displacement and velocity data can be obtained. Speckle tracking has recently been developed to assess arterial strain and has been validated in arterial phantoms (Hansen HH et al, 2009). Speckle tracking imaging has shown potential in the detection of subclinical arterial disease and lower strain values have been associated with increased cardiovascular risk (Catalano M et al, 2011). The majority of techniques to date have focused on assessment of both radial and circumferential strain patterns. Recently, a study was performed to validate and compare radial, circumferential and longitudinal strain using speckle tracking applied to images obtained using standard ultrasound equipment and on those using high frequency ultrasound. Phantoms mimicking the carotid artery were used and reference strain values were obtained using sonomicrometry. This measures the distance between 2 piezoelectric crystals using the speed of the acoustic signals through the tissues that they are embedded in. The study found good correlation between sonomicrometry and speckle tracking with regards to all strain patterns and found that the speckle tracking performance was not considerably improved with data from the high frequency ultrasound system when compared to the standard clinical ultrasound system (Larsson M et al, 2015). Figure 1.16 below illustrates an example of carotid circumferential strain evaluation using speckle tracking.
Figure 1.16 Speckle tracking evaluation of carotid artery circumferential strain.

Illustration demonstrating a transverse US image of the distal common carotid artery immediately before the carotid bulb. (A) The region of interest (ROI) is manually drawn along the intima–blood interface. (B) Software automatically checks appropriate tracking along segments in which the region of interest is divided. (C) Calculation of the strain appears in colour scale at the level of ROI, according to the degree of distension of the vessel during systole. (D) Curve of average strain along the region of interest during the cardiac cycle, with the possibility to measure the peak systolic value.

(Figure taken and legend adapted from Catalono M et al, 2011).

A study recently performed by Podgórska M et al, in 2015, evaluated 58 patients with a mean age of 61, with the aim of correlating CT calcium score (CS) values with carotid artery function. They performed CT CS and US of the CCA on each participant. 2D speckle tracking (2DST) US assessed both IMT and circumferential strain. They correlated circumferential strain and IMT with CS, age and systolic BP. They concluded that IMT and 2DST used together reflected the development of coronary artery calcification and can be used to evaluate for atherosclerosis, until the 6th decade. It also allowed for a more detailed examination of atherosclerosis risk in those with a normal calcium score (Podgórska M et al, 2015).
STI has also been shown to be superior to the β stiffness index and Young’s elastic modulus in the detection of hypertension (Saito M et al, 2012) and has been used in the detection of subclinical atherosclerosis (Park HE et al, 2012). It has been shown to be more accurate than conventional US in detecting age related differences in the mechanical properties of arteries (Bjällmark A et al, 2010). It is a relatively cheap, quick and non-invasive technique used in the assessment of arterial stiffness. However, it has some limitations, including being unsuitable for use in those with atrial fibrillation and those with near total occlusion of the CCA. It may also be unreliable in looking at aortic stiffness in young people (Oishi Y et al, 2008).

Overall, this technique is extremely promising and may overcome some of the limitations associated with conventional PWV assessment however, larger trials are needed before it is accepted into clinical practice.

### 1.7.5 Velocity vector imaging

Velocity vector imaging (VVI) is a relatively new technology that utilizes both speckle tracking imaging and multiple M-mode measurements. It compares the speckle pattern in a small kernel region of an image to a larger surrounding search region in the previous image and identifies the displacement of the speckle between frames. Algorithms are then used to calculate blood flow velocity vectors. This overcomes the limitations of conventional doppler imaging such as aliasing and angle dependence.

Initially, the technology was developed to assess left ventricular dyssynchrony and predict responses to cardiac resynchronization therapy (Canneson M et al, 2006). This software allows for the simultaneous measurement of radial and longitudinal velocity, strain, strain rate and displacement by detecting the change in tissue position from sequential frames. This is illustrated in Figure 1.17 below.
Figure 1.17 A longitudinal B-mode ultrasound image with Velocity vector imaging software demonstrating the longitudinal and radial wall motion of the common carotid artery at a specific time point of the ECG recording. (Figure taken from Svedlund S et al, 2011).

Velocity vector indexes have recently been found to correlate significantly with histological specimens of the aortas of dogs (Kim SA et al, 2013). In 2015, Fan XJ et al used VVI to examine radial systolic and diastolic velocity, radial and circumferential peak strain, and radial displacement of carotid plaques in 43 patients who recently suffered a large artery atherosclerotic stroke. They enrolled 38 patients who also had carotid plaque as controls. They found radial peak strain to be the best predictor of large artery atherosclerotic stroke with an odds ratio of 1.118, 95% confidence interval, 1.012-1.236 with a P = 0.029. Although a relatively small study, it demonstrates the value of using VVI in the characterisation of the mechanics of carotid plaque and it’s potential to identify those at risk of large artery stroke (Fan XJ et al, 2015).

The mechanics of the longitudinal motion of the arterial wall have not been extensively researched to date, however this has also recently been evaluated using VVI. In 2011 Svedlund S et al, evaluated the longitudinal movement of
the common carotid artery in 16 healthy volunteers and 16 with established coronary artery disease using VVI. In the healthy group total longitudinal displacements (tLoD) was similar in both the right and left common carotid and in the near and far walls of the arteries. In those with coronary artery disease the tLoD was found to be significantly lower (0.543 mm ± 0.394 versus 0.112 mm ± 0.074 with a P<0.0001) (Svedlund S and Gan LM 2011). Using VVI, the same group, investigated the predictive value of tLoD for cardiovascular outcome, by examining the carotid arteries of 441 patients undergoing myocardial perfusion scintigraphy for suspected coronary artery disease. After 1 year follow up, 61 patients suffered a major cardiovascular event. A low tLoD (<0.055 mm) was associated with a greater degree of myocardial ischaemia and following adjustment for IMT, pulse pressure, radial strain, age, gender and percentage reversibility of myocardium, a low tLoD remained a significant independent predictor of a major cardiovascular event. It also gave additional predictive value when combined with IMT. A high tLoD (>0.055 mm) was predictive of 1-year event free survival (Svedlund S et al, 2011).

This technology has shown promise in the evaluation of carotid plaque mechanics and has shown potential in identifying those at risk of stroke. It also can evaluate the longitudinal motion of arteries, of which there is little research data on to date and which is difficult to evaluate using the more established technologies. To date this technology has only been evaluated in small trials.

All of the above novel techniques will no doubt further our understanding of the biomechanics behind arterial wall movement and how this relates to cardiovascular disease. In the near future, they will likely become valuable adjuncts to already well-established non-invasive methods used to measure arterial stiffness. Larger trials are needed however, before they are to be widely accepted within the research community or used in clinical practice.
Chapter 2. The principles of ultrasound
2.1 Introduction

As alluded to in the previous chapter, endothelial dysfunction, carotid intima-media thickness and vascular stiffness are all assessed by way of ultrasound. To accurately capture and interpret ultrasound images it is essential to have a basic knowledge of the properties and principles of how ultrasound works. This chapter will describe the physics of ultrasound and this project will explore improvements in ultrasound assessments and attempt to apply this knowledge to gain further information about vascular structure and function.

2.2 The physics of ultrasound

Ultrasound (US) is mechanical energy that propagates through tissue as an oscillating wave of alternating pressure (Kossoff G, 2000). It is propagated through a medium by alternating between compression and rarefaction (see Figure 2.1 below). Sound waves constitute a mechanical longitudinal wave, which can be described in terms of particle displacement or pressure changes. These waves are reflected off tissues in the body and their echoes are converted into an image called a sonogram. Some of the more important elements of US are subsequently described.
2.1 Sound is a mechanical wave. It is a periodic series of compressions and rarefactions that is represented by a sine wave. (Figure taken from Lieu D, 2010).

2.2.1 Frequency

The frequency of an US wave consists of the number of cycles or pressure changes that occur in 1 second. The units are cycles per second or hertz (Hz). Frequency is determined by the sound source and is unaffected by the medium in which it travels. US has a frequency greater than 20 kHz which is the upper limit of human hearing. Medical US uses frequencies in the range of 2 to 10 MHz.
2.2.2 Wavelength

This is the distance between two corresponding points on a wave. It is the velocity of propagation divided by the frequency. Wavelength is important as it determines the imaging resolution of the equipment and its units are in millimetres (mm). Short wavelengths offer the best resolution but have the disadvantage that they have difficulty penetrating to any great depth in body tissues. Long wavelengths on the other hand penetrate deeply but do not provide good resolution (Figure 2.2).

![Figure 2.2](image)

**Figure 2.2** A comparison of the resolution and penetration of different ultrasound transducer frequencies.

(Figure taken from Lawrence J, 2007).

2.2.3 Velocity of propagation

Propagation speed is the speed at which sound can travel through tissue (Figure 2.3). This is known to be 1540 m/sec through soft tissue. This value does not depend on the frequency, but solely by the characteristics of the medium namely density and stiffness. Velocity of propagation in bone varies between 3000 and 5000 m/sec depending on composition and through air it is
much slower at 440 m/sec. Hence, air containing structures are not amenable to examination by US for this reason, and also due to the fact that air has high attenuation and low acoustic impedance properties. Bone has very high acoustic impedance and therefore it is not possible to view structures lying deep to bone.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Speed (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>330</td>
</tr>
<tr>
<td>Fat</td>
<td>1450</td>
</tr>
<tr>
<td>Water</td>
<td>1480</td>
</tr>
<tr>
<td>Average soft tissue</td>
<td>1540</td>
</tr>
<tr>
<td>Liver</td>
<td>1560</td>
</tr>
<tr>
<td>Blood</td>
<td>1560</td>
</tr>
<tr>
<td>Muscle</td>
<td>1600</td>
</tr>
<tr>
<td>Tendon</td>
<td>1700</td>
</tr>
<tr>
<td>Bone</td>
<td>3500</td>
</tr>
<tr>
<td>Metal</td>
<td>2000–7000</td>
</tr>
</tbody>
</table>

**Figure 2.3** Speed of sound in media.

(Figure taken from Lieu D, 2010).

### 2.2.4 Acoustic impedance

This is a product of the density and the velocity of sound in a material. It is independant of frequency. Air/lung has low acoustic impedance (low density and low velocities of sound). Bone on the other hand has high acoustic impedance. Differences between acoustic impedances at interfaces determine the amount of energy reflected at the interface (Huda JW, 2009).

### 2.2.5 Amplitude

The amplitude is measured in decibels and corresponds to the loudness or intensity of the US wave. Intensity is important as the amount of reflected
energy that is to be sensed by the US transducer is a fraction of the initial strength of the emitted sound wave. This is due to attenuation of the US beam.

2.2.6 Attenuation

This is the loss of intensity of the US beam as a medium is traversed. It occurs due to absorption of US energy by conversion to heat, and other processes such as reflection, refraction and scattering (Figure 2.4). Reflection refers to reflected echoes which form the basis of all US imaging. US waves are reflected at all tissue boundaries and interfaces. Refraction occurs when an US beam encounters media of different velocities and the proportion of the beam that is not reflected but is transmitted undergoes bending or refraction. This can result in artifacts such as a double image. Scattering is the means by which energy is dispersed from its main direction of propagation. The amount of attenuation varies with the frequency of US and the higher the frequency of the US beam the greater the attenuation.

![Figure 2.4 Ultrasound interacting with biological tissue causing scatter, refraction, reflection and attenuation (Figure taken from Lawrence J, 2007).](image)
2.2.7 Interaction of the US beam with tissue

Specular reflectors are structures that are relatively large compared to the US beam. The amount of sound returning to the transducer is a function of the density of the specular reflector and the angle at which the sound wave is reflected. Tissues with different densities reflect sound differently and in general terms the denser the medium the greater the amount of reflected sound. This is portrayed on the display as brighter white. Liquids do not significantly absorb or scatter US waves and are generally considered to be nonattenuating. Soft tissue is a significant absorber but mild scatterer of US and bone is a significant absorber and scatterer. Air containing tissue such as lung has high attenuation with energy being scattered in all directions and therefore lungs cannot be imaged via US.

2.3. Transducers and the US beam

2.3.1 Overview

Ultrasound transducers generate US waves and sense the reflected echoes. The transducers house multiple piezoelectric crystals and when electricity is applied to them they vibrate. This is known as the piezoelectric effect and was first discovered by the Curie brothers in 1880 after they subjected a cut piece of quartz to a mechanical force and found that it developed an electrical charge on its surface. They also found that if electricity was applied to the quartz, it would vibrate (the reverse piezoelectric effect) (Fischetti A et al, 2007). These vibrations cause the alternating rarefaction and compression described above and also seen in Figure 2.1 that allows for propagation of the US wave. The crystals used in modern transducers are synthetic such as lead zirconate titante (PZT) or ceramic. Each crystal vibrates at a certain characteristic frequency. The transducer first excites the crystals to send a short pulse (2-3 cycles) of sound wave into the tissues. Then the transducer becomes a receiving device and waits for the echo to return. Most of the time
the transducer acts as a receiver following the initial short pulse of emitted sound waves. Damping material is used in the housing of the transducer to dampen the initial vibrating crystals so that they can prepare to interpret the returning sound waves (Figure 2.5).

![Components of a typical transducer.](image)

**Figure 2.5** Components of a typical transducer.

When the electrical pulse is applied to the transducer element, it is rather like striking a bell with a hammer: just as the bell produces a sound that is determined by its size, the transducer will resonate (i.e., vibrate at one particular frequency, which is determined by its size). The critical factor here is the width of the transducer. After the pulse is applied, the two opposing faces will move and send out pressure waves (Figure and legend taken from Aldrich J, 2007).

The US beam is initially cylindrical in shape close to the transducer (near field or Fresnel zone) and then it becomes conical and in shape and diverges at a constant angle the further it travels from the transducer (far field or Fraunhofer zone). The near field is proportional to the square of the transducer diameter and inversely proportional to its frequency and the far field is proportional to the diameter and inversely proportional to the frequency (Kosoff G, 2000).

The transducer in its simplest form is the single element circular disc. Frequency is set by the thickness of the disc and in a 5 MHz transducer this would be 0.4mm. The initial beam as stated above is cylindrical and equal to the diameter of the transducer. Focusing of the beam using a spherical lens is done to improve lateral resolution. The point at which the beam is at its
narrowest and greatest intensity is called the focal zone demonstrated in Figure 2.6. Due to diffraction focusing can only be done in the near field. The length of the near field can be lengthened by decreasing the wavelength (increasing the frequency), or by increasing the size of the transducer (Figure 2.7).

**Figure 2.6** Illustration of Ultrasound beam showing the near field (Fresnel zone) and far field (Fraunhofer zone) with the focal zone in the middle. (Figure taken from Lieu D, 2010).
Figure 2.7 The size and frequency of the transducer and their effects on the near zone (Figure taken from Lawrence J, 2007).

2.3.2 Annular array transducers

These devices consist of a disc transducer surrounded by 7 annular or ring shaped transducers. By energising the outer ring first and then progressively the next innermost transducer until the centre transducer is energised, the beam can be focused. Timing delay determines the focusing position. Many different energising sequences can be used to maintain a narrow symmetrical beam over the required examination distance and due to its dynamic focusing capabilities, it provides a greater field of depth than single disc transducers and a much improved focal zone (Reza Chabok H et al, 2011).
2.3.3 Linear array transducers

A linear array transducer is composed of up to 512 elements situated in a linear fashion spaced over 75-120 mm. All of the elements generate an US pulse and receive the returning echo simultaneously and therefore a single still image is generated. By displaying a series of still images the transducer creates the illusion of real-time. The beam pattern of the linear array transducer is narrow in the length plane but similar to that of a single element transducer in the height plane. The beam produced by such a narrow element diverges rapidly after only a few millimetres resulting in poor lateral resolution. In order to overcome this the outer elements are pulsed initially with a subgroup of inner elements pulsed after a time delay, thereby allowing focusing of the beam. Varying the time delays allows for a change in the depth of focus. Typically the image is rectangular and focusing can be accomodated by means of a cocave lens in one direction or electronic “phasing” in the other.

2.3.4 Phased array transducers

Phased arrays work by using a delay in the pulsing of adjacent piezoelectric elements. By varying the timing, for instance by pulsing the elements one by one in sequence along a row, a pattern of constructive interference is set up that results in a beam at a set angle. This means that the beam from an array can be automatically steered in a sweeping or scanning motion. This creates sector scanning or 2D pie shaped images or slices of tissue in real time US machines. The sweeping can be done manually with the operator sweeping the probe back and forth in a single plane. The differences between linear and phased array transducers are shown in Figure 2.8 and 2.9 respectively.
Figure 2.8 Illustration of a linear sequenced array transducer.

A voltage pulse is applied to a small group of elements and then to another group, until the process is repeated over and over again to produce a real-time image. The emitted beams are always parallel to each other.

(Figure and legend taken from Fischetti A et al, 2007).
Figure 2.9 Illustration of a phased array linear transducer.

Note the time delay profile in the first image and then the second. When these delays are applied in rapid succession, the beam is steered to and fro.

(Figure and legend taken from Fischetti A et al, 2007).
2.4 Ultrasound modes

2.4.1 M mode ultrasound

M Mode, or motion mode is a stationary narrow beam that produces a one-dimensional view of the anatomical structures over time (Bunce et al, 2004). Time is displayed on the horizontal axis and depth on the vertical axis (Figure 2.10). Movement of structures provides information on structural movement towards or away from the transducer. It records the amplitude of the US beam and rate of motion in real-time by repeatedly measuring the distance of the object being examined from the single transducer at a given moment. It has been mostly used in the past for cardiac and fetal imaging with its high temporal resolution allowing visualisation of tiny structures such as heart valves and accurate measurements of size and distances, and has also been used to determine flow mediated dilatation and small changes in arterial diameters during the cardiac cycle.

![M-Mode image measuring the diameter of the aorta.](image)

The diameter of the aorta (Ao) is measured in both diastole (D) and systole (S) with time shown on the horizontal axis and depth on the vertical axis.

(Figure taken from Cavalcante JL et al, 2011).
2.4.2 B mode ultrasound

Also known as brightness modulation this is a 2D display of B Mode data and is the most widely used form of US. B Mode is based on brightness which depends on the intensity of the echo. Sweeping a narrow ultrasound beam through the area being examined while transmitting pulses and detecting echoes along closely spaced scan lines produces B-Scan images. Vertical positions of the dots are determined by the time delay from pulse transmission to receiving of the echo and horizontal dots are determined by the location of the receiving transducer element. Repeated sweeping of the US beam generates a rapid series of 2D images that show motion. Varying intensities create varying shades of grey on the display that represent variations in the texture of organs or tissues being imaged. The appearance of fluid as being black and solid areas being white is what is referred to as grey scale. Typically between 15 and 100 images per second are shown with improved resolution as frame rates rise. To achieve the effect of real-time 16 or greater images per second are required.

2.4.3 Doppler ultrasound

The Doppler principle, first described in the early 19th century, is the phenomenon in which sound transmitted from a moving object is perceived by a stationary observer to be of a different frequency depending upon the velocity and direction of travel. The classical illustration is of a train whistle, which increases in pitch (frequency) as the train approaches and decreases as the train moves away (Taylor P, 2003). Doppler US uses the scattering of ultrasound waves seen above in Figure 2.4 that is generated by the movement of blood cells through vessels. Doppler can provide the user with information on velocity and direction of blood flow within individual vessels and the heart. Valuable information can be obtained in conjunction with the various other US modes on the function of structures in the body. Doppler US analyses the difference between the transmitted frequency and the received frequency, also
known as the frequency shift. The signal processing of the Doppler frequencies or frequency shift is a process called spectral analysis. The signal is maximal when blood is flowing directly towards or away from the transducer and the value of cosine tends towards unity (Figure 2.11).

![Diagram of the Doppler principle]

**Figure 2.11** The Doppler principle.

This relies on the change in frequency of the sound wave, which is reflected from a moving object. The magnitude of the frequency change is related to the velocity of the moving object and the cosine of the angle of incidence \( \theta \). To maximize the value of \( \cos \theta \) the angle of incidence should be as close as possible to the direction of flow i.e. \( \theta \) should be as close as possible to 0° or 180°.

(Figure and legend taken from Taylor P, 2003).

The different Doppler formats are briefly presented below:

(i) Continuous wave doppler.

Here, the changes in pitch of the sound waves are used to produce information about flow through vessels or heart valves. Sounds produced enable the examiner to quickly ascertain whether or not there is flow in a vessel or valve. The advantage of this is that information is continuously analysed and therefore provides information on velocity and direction of flow in areas of high velocity blood flow, for example in calculating gradients across stenotic valves in the heart or a significant carotid artery narrowing.
(ii) Pulse wave doppler.

In contrast to continuous wave doppler, pulse wave doppler uses a transducer element that is activated with a short burst or pulse and the returning signal is received by the same element. The interval between pulses is termed the pulse repetition frequency (PRF). High PRF’s are seen when vessels are near the surface or when the blood flow is fast, and conversely low PRF’s are used to sample blood flow in deeper vessels or when blood flow is slow. A specific area of interest, for example the centre of an artery, can be selected for analysis. This region is separated from the rest of the image in terms of analysis by means of an electronic gate that is placed in the centre of the artery and only signals generated from within this region are accepted for analysis of flow. Information is presented audibly and also by graphical representation.

(iii) Colour flow doppler.

Colour Flow imaging (CFI) is predominately used to study blood flow, but is also increasingly being used to assess organ expansion and function. CFI provides a real-time blood velocity component and direction of blood flow is displayed in colour which is superimposed on the screen. Typically red denotes flow toward the probe and blue away from it with varying intensities of red and blue corresponding to the velocities of blood flow (Carson et al, 2009).

(iv) Power doppler.

Power Doppler, also known as Ultrasound Angiography or Colour Power Angiography demonstrates the amplitude of the Doppler shift in the auto-correlation sequence without estimating velocity or direction (Hamper UM et al, 1997). Here only the Doppler signal power or intensity is displayed superimposed on the B-Mode image (Figure 2.12). It is 3 times more sensitive at detecting flow than CFI as it is dependant on the reflected power that is generated by moving blood cells and can produce useful images even close to 90° to the transmitted beam. This allows imaging of small vessels and those with low-velocity flow.
Figure 2.12 Power Doppler of the carotid artery bifurcation. (Figure taken from http://www.medical.siemens.com/siemens/en_US/rg_marcom_FBAs/images/presskits/ACC_2008/USD/X300_PWR_Doppler.jpg).

(v) Duplex ultrasound

This form of US is mainly used in vascular US and incorporates both 2D B-Mode US and Colour Doppler imaging to assess anatomy, direction and velocity of blood flow through vessels.

This project will be utilizing 2D and 3D B-mode ultrasound, however a knowledge of doppler imaging can be helpful to confirm vascular anatomy for example, distinguishing venous from arterial structures and also aiding in the correct identification of the internal carotid from the external carotid artery.
2.5. Imaging artefacts

Modern US machines make certain basic assumptions when generating images that lead to imaging artefacts, which need to be understood when interpreting images. These artefacts are image errors and are usually caused by physical properties that affect the US beam in some way so that it does not follow the machines basic assumptions listed below:

- The US beam only travels in a straight line with constant attenuation rates.
- The velocity of sound in all body tissues is 1540m/s.
- The US beam is finitely thin with all echoes coming from its central axis.
- The depth of a reflector is accurately determined by the time it takes for sound to travel from the source to the reflector and return to the source.

In fact, the ultrasonic energy propagates through tissue as a beam of a finite size determined by the properties of the transducer (Kossoff G, 2000). Velocity of sound is not the same in all tissues throughout the body and this may lead to deviation of the beam from the assumed direction of propagation resulting in misplotting of echoes on the image from their true position in space. The main artefacts encountered in US are described below:

- Reverberation: These artefacts appear as multiple equally spaced lines along a beams path. This occurs when the US beam is repeatedly reflected between two strong specular reflectors before the US waves return to the transducer. This can result in an apparent extra structure further from the transducer (Figure 2.13). Changing the angle of the transducer, applying more gel or rotating the patient can resolve these artefacts.
Figure 2.13 Reverberation artefact caused by a metallic foreign body.

In this case a biopsy needle, which has been introduced to the subcutaneous tissues during an ultrasound guided biopsy.

(Figure taken from http://www.vaultrasound.com/educational-resources/ultrasound-physics/artifacts/).

- Mirror images: These are similar to reverberation artefacts in that a structure is anomalously placed on the display due to redirection of the beam as it interacts with strong reflectors (Baun J, 2009). Reproduction of tissue interfaces is called reverberation artefact, whereas the reproduction of objects is termed mirror image. Mirror-image artefacts are produced when an object is located in front of a highly reflective surface at which near total reflection takes place. The surface will act as a mirror and reflect the beam to another tissue interface. The US machine assumes that the second interface is beyond the first surface and this is where it appears on the scan (Figure 2.14).
Figure 2.14 Mirror image artefact.

This a longitudinal image of the trachea with the air-mucosa interface visualized just below the tracheal wall. A=reverberation artefact from the air-mucosal interface. B=mirror image artefact of the cricoid cartilage at the cartilage-soft tissue interface. CRI=Cricoid cartilage. RINGS=Cartilaginous rings within the anterior wall of the trachea.

(Figure taken from http://new.sinaiem.us/artifact-3-mirror-in-the-wall/).

- Ring down artefacts: These are produced when objects such as air bubbles (in the abdomen for example) or cholesterol crystals resonate at the same frequency as the US beam and emit sound. The sound is emitted after the transducer receives the initial reflection and so the machine thinks the echo is coming from a deeper structure. These artefacts appear a solid streak or a series of parallel bands radiating away from the air (Figure 2.15). They occur from a large mismatch or large difference in acoustic impedance between two kinds of tissues, such as air and water (Bollinger C et al, 2009).
Figure 2.15 Ring down artefact.

Transverse image of an internal jugular vein during an ultrasound guided percutaneous central venous access procedure. Note the ring down artefact resulting from a needle tip that is not clearly seen on this image but which casts a narrow shadow inferiorly. The artefact in this case can be used as a guide to the accurate placement of the needle tip. IJ=Internal jugular vein.

(Figure taken from http://www.sonoguide.com/line_placement.html).

• Acoustic shadowing: This is the result of decreased energy within the beam as a result of reflection and absorption. It can be seen with arterial (Figure 2.16) and valvular calcification, or gallstones and calculi where most of the ultrasound waves are reflected back to the transducer preventing visualization of anything beyond the calcification which appears as a dark posterior shadow. Spatial compound imaging can decrease the effect of
acoustic shadowing and will be discussed later in this chapter. Figure 2.16 illustrates the appearances of different types of plaque with different acoustic properties.

**Figure 2.16** Acoustic shadowing artefact.

Longitudinal ultrasound images of the common carotid artery illustrating arterial plaques with different acoustic properties. Image at the top of the figure demonstrates a diffusely thickened homogenous far wall intima-media thickness (IMT). A=Posterior acoustic shadowing caused by dense calcific plaque in the CCA. B=Homogenous ulcerated plaque in a segment of CCA. C=Homogenous plaque in a CCA segment with an echo-poor central area of haemorrhage.

(Figure taken from Bathala L et al, 2013).

- **Acoustic enhancement:** This presents as abnormally high brightness and occurs when sound travels through a medium with an attenuation rate lower than the surrounding tissues (Aldrich J, 2007). Posterior enhancement occurs when fluid structures attenuate the sound less than solid structures with the strength of the pulse increasing after passing through fluid compared to passing through a solid structure (Bollinger C et al, 2009). This is illustrated in Figure 2.17.
Figure 2.17 Posterior acoustic enhancement in a simple breast cyst.

Ultrasonography reveals a well-circumscribed anechoic cyst with dense echoes in the posterior wall and acoustic enhancement deep to the lesion.

(Figure taken from https://iame.com/online/sonographic_evaluation_of_benign_and_malignant_breast_masses_/content.php).

2.6 Limitations of 2D ultrasound

- In 2D US the sonographer needs to mentally create a 3D picture of the anatomy that he/she is examining and this can often be difficult especially for the inexperienced sonographer.
- 2D US is known to be very subjective and have variable reproducibility, which can lead to diagnostic errors.
- Measurement of length and volume use a simple measurement of width in only 2 planes to calculate volumes, which can lead to inaccurate
• Many organs or anatomical locations in the body are difficult to visualise due to angulation difficulties and variable anatomy. It is also difficult to image the exact same location on each successive occasions thus making it difficult to perform quantitative follow up studies and accurately monitor response to treatment.

2.7 Introduction to 3D ultrasound

Real-time quantitative 3D ultrasound has recently become available and may overcome many of the afore mentioned limitations of 2D ultrasound (Fenster et al, 2001). With 2D imaging, the sonographer must mentally transform a series of 2D images into volumetric information in order to obtain a 3D impression of the anatomy. The ability to do to this varies greatly with experience.

The goal of 3D US imaging is to overcome these limitations thus providing clinicians with a complete view of the anatomy of the target organ. It also has many advantages over conventional 2D US. This section will outline how 3D US can overcome the various limitations of 2D US and will also illustrate the basic principles of 3D US, how images are created and processed as well as its many clinical applications.

2.7.1 Basic principles

3 Dimensional Ultrasound is a data set that contains a large number of 2D images or planes (x, y and z). Each individual 2D plane may be thought of as a page in a book and the book itself the entire data set. The data set in this instance is a “snapshot” of the imaged object in 3D taken over a specific time period, i.e. a series sequential 2D slices in 3 planes taken over approximately 1 second and put together to form a 3D image. The units of stored 2D information are called pixels and the stored 3D units are termed voxels. One is
able to leaf through the individual 2D images following acquisition of a 3D data set. This process is known as translation. The data set or volume can be flipped in any direction to view the 3D object in different planes. The volume can also be dissected in any plane to achieve multiplanar imaging. 4D ultrasonography is a 3D image displayed in real-time and is produced by the rapid display of sequential 3D data sets. The faster the data sets are acquired the less interrupted the motion of the 4D image becomes. 3D US machines must meet or exceed the specifications of modern 2D machines in order to produce quality images and a 3D image is only as good as its 2D capabilities. A typical 3D US transducer is illustrated in Figure 2.18.

![Probe Movement during Acquisition of volume](http://www.gehealthcare.com/usen/ultrasound/education/products/cme_3d4d.html)

**Figure 2.18** B Mode transducer in a housing that swivels on a motor in fan like motion.

(Figure taken from http://www.gehealthcare.com/usen/ultrasound/education/products/cme_3d4d.html)
2.7.2 Data acquisition techniques

There are 2 methods that have been developed for data acquisition. The first uses a series of 2D images created by 1D arrays and the second uses 2D arrays to produce 3D images directly (Downey et al, 2000). In each case the relative position and angulation of the 2D image must be known and the images must be acquired rapidly or with gating thereby avoiding artifacts secondary to respiratory, cardiac or patient movement.

There are 4 types of 3D US systems: Mechanical scanners, Tracked freehand systems, Untracked freehand systems and 2D matrix arrays. These are explained below.

1. Mechanical scanners: This method utilises a motorised assembly to rotate or translate the transducer while 2D images are taken at predefined spatial or angular intervals. This method can be cumbersome but may provide very accurate 3D reconstructions. The three types of mechanical transducer are linear, tilt and rotational (Figure 2.19).
   a) Linear scanning: Here the transducer moves along the patients skin in a linear fashion. When tilted away from the vertical plane at an angle 3D colour and power doppler can be performed. Intervals between digitized images can be adjusted for proper sampling and reconstruction of data sets is fast and efficient because images are parallel and separated by a predefined interval.
   b) Tilt scanning: Here the transducer is tilted about it’s face and images are digitized at a predetermined angle. As seen in Figure 2.19 the mechanical device is quite small and allows for easy manipulation when held. However, due to the fan-like digitized images the space between them increases as depth increases leading to poorer resolution.
   c) Rotational scanning: The transducer is rotated about a central axis producing propellorlike digitized images. Here the sampling distance increases and resolution decreases as the distance from the rotational axis increases. Due to images intersecting close to the transducer patient movement creates artifacts at the centre of the image.
2. Tracked freehand systems: Here the sonographer holds a mechanism composed of a transducer with an attachment and manoeuvres it over the area being imaged. 2D images are digitized as the transducer moves. The exact relative position and angulation must be known for each image and the operator ensures no significant gaps are left when the transducer is moved.

(Figure taken from Downey D et al, 2000).
along the anatomy. The three types of tracked free-hand systems are accoustic, articulated arm and magnetic field tracking (Figure 2.20).

a) Accoustic tracking: Microphones are placed above the patient with three sound emitters mounted on the transducer. The sound emitting devices are activated when the transducer is moved. The relative distance and timing of the emitted sound waves that are received by the microphones are used to calculate the position of the transducer.

b) Articulated arm tracking: Here the transducer is mounted on a mechanical arm with multiple moveable joints. Potentiometers at the joints are used to measure their relative rotation thereby allowing the continuous measurement of the position and angulation of the transducer.

c) Magnetic field tracking: Tracking with magnetism involves detecting changes in a spatially varying magnetic field that is produced by a transmitter attached to the transducer. The detector contains three orthogonal coils that measure the strength of the magnetic field. By measuring the local magnetic field the relative position of the transducer can be determined. To minimise electromagnetic interference the detector is positioned close to the transducer. Also, there should be no ferrous or conductive materials such as pacemakers in the vicinity (Hoppenrath M, 2006).
Figure 2.20 Tracked freehand 3D scanning.

(a) Acoustic tracking. Note the triangular device mounted on the transducer containing 3 sound emitters. As the transducer is moved the sound emitters emit pulses that are detected by three microphones positioned in different locations above the patient. The time delay between sound emission and detection is measured allowing for continuous monitoring of the angulation and position of the transducer. (b) Articulated arm tracking. Transducer mounted on a mechanical arm with numerous moveable joints containing potentiometers allowing for continuous monitoring of angulation and position of the transducer. (c, d) Magnetic field tracking. (c) Note the small electromagnetic device attached to a transducer. The adjacent box detector registers nearby changes in the electromagnetic field. Transducer position is calculated secondary to changes in the signal detected by the box detector. (d) Abdominal scan using a magnetic field tracking device attached to a transducer (T) with a black plastic cover (arrow) and adjacent box detector (D). (Figure and legend taken from Downey D et al, 2000).
3. Untracked freehand systems: In this method the sonographer moves the transducer at a preselected linear or angular velocity whilst 2D images are digitised. From these images a reconstructed 3D data set is made, however, as there is no direct information regarding positions of these digitised 2D images measurements such as volume, distance etc. will be inaccurate and should not be used.

4. 2D matrix arrays: In this method the transducer remains stationary and a built in beam former makes an electronic sweep in a pyramidal fashion over the area of interest. In these sophisticated transducers reside many thousands of equally sized piezoelectric crystal elements that steer the beam in a phased array manner (as previously described) in all three planes. The transducer used to perform 3D scans in this thesis will be a 2D matrix array transducer.

2.7.3 Image reconstruction

Image reconstruction refers to the process of generating a 3D representation of the anatomy by first placing the acquired 2D images in their correct relative positions and orientations in the 3D image volume, and then using their pixel values to determine the voxel values in the 3D image (Fenster A et al., 2001). Two methods are used for volume reconstruction: a 3D surface model and a voxel-based volume model.

- 3D surface model: Here the boundaries of the desired features are extracted from the 2D images and a 3D surface model of the anatomy is displayed. This is done either manually which can be tedious and time-consuming, or computer assisted using various algorithms. With this method information is reduced which helps with rendering and the data files are not as large leading to shorter reconstruction times, greater efficiency and assisting with storage considerations. Contrast is artificially increased between adjacent structures making them distinct from one another, however this may distort or misrepresent subtle image features and cause loss of valuable information especially in areas with very subtle tissue differences.
• Voxel-based volume model: Here each digitised 2D image is placed into its correct position in the 3D voxel-based volume i.e. each 2D image pixel is placed at its correct 3D voxel co-ordinate (x, y and z). This is the most popular approach as no information is lost during reconstruction and it makes possible a variety of rendering techniques. The downside is that volume data-sets are very large which can slow processing and have storage implications.

2.7.4 3D Ultrasound image display

After 3D reconstruction the image can be viewed interactively using 3D visualisation and rendering software. The three main rendering techniques are surface rendering, multiplanar reformatting and volume-based rendering. These are discussed below.

• Surface rendering: Here a wire frame representation is created with the operator delineating structures to be rendered either manually or computer assisted. These boundaries are shaded and illuminated giving optimal visualisation of structures or organs.

• Multiplanar reformatting: In this method one of two approaches can be used. The first presents the user with three orthogonal or perpendicular planes that are taken from the volume and displayed simultaneously. They can be rotated to view the structure from different angles. The second technique views the 3D volume as a polyhedron and the appropriate imaging plane is “painted” on each face of the polyhedron which is then manipulated. The advantage with this method is that the operator can always relate the manipulated plane to the anatomy (Figure 2.21).
Figure 2.21 3D multiplanar ultrasound images of the brachial artery.

Note that the X, Y and Z planes transect the centre of the artery. 1. Sagittal view. 2. Transverse view. 3. Top-down view. The image in the bottom right of the figure is a volume rendered image of the artery which can be manipulated as a polyhedron.

- Volume-based rendering: In this method the entire volume of data and not just selected planes are viewed in the desired orientation. This is made possible by ray casting whereby a 3D image is projected onto a 2D plane by casting rays through the 3D image. The algorithm produces a projection that is directed through a row of 3D voxels in the set of image data. When the ray encounters each voxel a value is assigned to that particular voxel that weights the volume elements and then sums them to produce varying degrees of translucency. For example, when voxel values are multiplied by a factor of 1 and then added they form a radiographic type image. When values are
multiplied by factors they appear translucent. This method allows for the display of many different effects such as maximum intensity projection images wherein only the voxel with the greatest intensity along each ray is displayed. In this way, internal features can be visually explored throughout the entire data volume (Hoppenrath M, 2006). Figure 2.22 illustrates a combination of multiplanar and volume rendering techniques.

![Image of fetal face](http://3dviz.ucsd.edu/~3DUS/Home.html)

**Figure 2.22** Multiplanar and volume rendered display of fetal face.

The top two images and the bottom left image represent the multiplanar images with the bottom right image representing the volume rendered display.

(Figure taken from http://3dviz.ucsd.edu/~3DUS/Home.html).

### 2.7.5 3D Imaging artifacts

In general terms the same artifacts that occur in 2D US imaging occur in 3D US imaging and at approximately the same rate (Nelson et al, 2000). Artifacts need to be minimised in the 2D image in order to achieve good quality 3D data sets. All suspected 3D US artifacts in rendered images should be confirmed.
with multiplanar images or 2D scanning to avoid misdiagnosis and error. Multiplanar images may identify the source of the artifact which can be confirmed or refuted with 3D US. Also, the acquisition of data from multiple angles can reduce the impact from artifacts.

2.7.6 Clinical applications of 3D ultrasound

Until recently the fields of cardiology and obstetrics and gynaecology have had the most to gain from utilising 3D US. Today however, many more specialities are benefiting from its use in clinical practice. The clinical applications of 3D ultrasound are extensive but some of the more well known clinical applications are listed below:

• Obstetrics and gynaecology: Use of 3D US either transabdominally or transvaginally is used to aid in assisted conception when assessing tubal patency, predicting IVF outcome (Kupesic S et al, 2002) and diagnosing uterine anomalies (Salim R et al, 2003). It is also used in the preoperative assessment of submucosal fibroids, where 2D US is limited as it provides views in the sagittal and coronal planes only (Smith A et al, 2006). In addition it aids in the assessment of adnexal masses, endometrial cancer, follicular cysts and intrauterine device position.

• Fetal imaging: Improved visualisation of fetal features including cardiac imaging and imaging of the face and limbs improves anomaly identification and helps parents-to-be in their understanding of diagnoses. It is a useful supplement to 2D imaging.

• Cardiology: Valve geometry and motion assessment can be performed using transoesophageal and transthoracic 3D US. It also has a role in intracoronary ultrasound, determination of ventricular motion and volumes (Corsi C et al, 2005), mass and ejection fraction and prior to cardiac surgery (Mor-Avi V et al, 2008). It has also been used successfully in transoesophageal valve repair (Chikwe J et al, 2012).
• Interventional: 3D US provides accurate identification of needle or catheter placement (Zhao Y et al, 2013). It is also useful in transjugular intrahepatic portosystemic shunt (TIPS) procedures during main portal vein access (Rose SC et al, 2002).

• Ophthalmology: 3D reconstruction of orbit with retinoblastoma provides unique views that are unavailable with 2D US and is used in evaluation and follow up after treatment (Finger PT et al, 2002), and when used in conjunction with fundoscopy provides equal diagnostic accuracy to that of CT (Ghosh S et al, 2010).

• 3D Transrectal and transabdominal ultrasound imaging: This involves use of a rotational side-firing linear array transducer and is used in prostate biopsy (Figure 2.23), permanent implant prostate brachytherapy and prostate segmentation. Transabdominal 3D US is used in the delivery of prostate external beam radiotherapy (Carson PL et al, 2009).

![Figure 2.23](image)

**Figure 2.23** Registered 3D transrectal ultrasound image on the left and a corresponding MR image of the same patient on the right.

Two suspicious lesions are circled in red and green on the MR image and then superimposed on the corresponding US image. The lesions are subsequently targeted for biopsy using a 3D guided biopsy system.

(Figure taken from Fenster A et al, 2013).
• **Breast imaging:** 3D US can complement 2D US in providing information about both site and differential diagnoses of breast lesions (Kotsianos-Hermle et al, 2009) and also in determining breast density and volume (Moo WK et al, 2011).

• **Vascular imaging:** 3D US may become an alternative to X-ray angiography in the assessment of arterial stenosis. Studies have shown 3D colour doppler sonography to be similar to digital subtraction angiography in the assessment of internal carotid artery stenosis (Wessels T et al, 2004) and may be useful when more invasive imaging is contraindicated. It has also been found to be useful in detecting carotid artery plaque composition changes following treatment with statins (Awad J et al, 2010) and also was found to be more reliable than 2D US in detecting carotid artery plaque ulcers (Heliopoulos J et al, 2011). 3D US has recently proved to be accurate in determining local arterial wall strain and vessel displacement in abdominal aortic aneurysms with use of 3D speckle tracking programs (Karatolios K et al, 2013).

### 2.7.7 Advantages and limitations of 3D US

Recent advances in 3D data acquisition techniques, image reconstruction and acquisition and rendering have provided 3D US with an exciting future. The added dimension facilitates surgical planning in ways previously only available with CT or MRI. It can also be used intra-operatively to monitor progress and results of the procedure. It is relatively cheap in comparison to other imaging modalities (yet still more expensive and not as widely accepted as 2D), and doesn’t expose the patient to the radiation risks seen in other procedures. When using MRI, even though acquisition of images is less time consuming than before, infants may need general anaesthesia, whereas with 3D techniques adequate images may be acquired in 3-4 cardiac cycles. MRI has certain contraindications where metallic instrumentation or prostheses are concerned whilst 3D US does not. As stated earlier, 2D US is inaccurate when calculating volume and length of a structure due to the limited capabilities of having only 2 dimensions whereas in 3D US the added dimension allows for
much more accurate and reproducible measurements. 3D US also allows for the imaged data set to viewed from any angle and also allows for significant rendering and post processing of data described above, which is not possible in 2D, US.

The main disadvantage with 3D US imaging is the time taken to perform image reconstruction and rendering although with modern US machines this has improved greatly. A single 3D data set also has considerably more data than a 2D image of the same area. This may have storage implications especially if 3D US is used in a clinical setting. The learning curve for performing 3D US varies between those who have limited experience and those considered to be expert sonographers. In many radiology departments there are a set of standardised protocols in place for different imaging studies. Such protocols allow for consistency when performing and reporting the scan. With 3D US becoming more popular protocols must be put in place to allow for similar standardization.

2.8 Recent advances in image resolution

Spatial resolution is the ability to differentiate closely spaced objects in the beams trajectory as separate entities and is dependent on lateral, elevatory and axial resolution (Fischetti AJ et al, 2007). Lateral resolution depends on the scanners ability to resolve structures perpendicular to the beam axis and elevatory resolution to structures along its slice thickness. Axial resolution is dependent on resolving structures along the axis of the US beam. Some of the major technologies that have lead to improved spatial resolution are briefly discussed below:

• Digital beam formers: Beam formers provide pulse delay sequences that are responsible for transmission, focusing and reception of US waves. A greater number of beam formers in a transducer means more piezoelectric elements and therefore better lateral resolution. A pulser provides the initial voltage to the piezoelectric elements. A delay generator allows for beam
transmission focusing. The beam former automatically adjusts for frequency, pulse repetition and amplitude and then digitizes and sums returning echoes. The receiving focus is dynamic and changes automatically thereby accurately tracking reflector depth and improving signal to noise ratio and spatial resolution. A dynamic aperture limits the variations in beam width and depth and maintains a narrow width for optimal resolution. Digital beam formers allow for a wide range of signal frequencies and decrease unwanted artefacts.

- Coded pulse/coded excitation: Amplitude and frequency of pulses determine the depth at which we can image effectively. Amplitude is limited in clinical imaging due to safety considerations and high frequencies can provide very clear images but have limited penetration. Coded pulse technology uses long, high energy pulses that dramatically improve echo signal and decrease noise in deeper tissues whilst not sacrificing spatial resolution, thereby overcoming previous limitations.

- Tissue harmonic imaging (THI): When sound propagates through tissue its shape is distorted leading to the production of harmonic frequencies. The echo signal from tissue not only contains the original frequency (fundamental frequency) but also its multiples of (harmonic frequencies) (Weinstein SP et al, 2006). Therefore if a transducer uses a frequency of 4 MHz for example, the tissues will produce harmonic frequencies of 8 MHz. In THI the fundamental frequency is separated from the harmonic frequency via signal processing. This results in an improvement of clinically useful artefacts such as acoustic enhancement and shadowing and a reduction in artefacts that hamper diagnosis such as side lobe and ring down artefacts. There is also improved resolution and signal to noise ratio and decreased speckle, noise and reverberation.

- Spatial compounding: Speckles are variations in intensity imposed on the image due to the interference of echoes on the tissue resulting in decreased image quality. Spatial compounding uses a phased array transducer to generate a single real-time image from multiple fused frames. In this way speckles can be reduced by combining partially correlated or non-correlated images of the same region of interest produced by transducers with different spatial locations (Behar V et al 2003). When used with spatial compounding images are further improved but at the expense of frame rate.
An important point to note is that artefacts that sometimes help with diagnosis such as posterior acoustic enhancement and shadowing are reduced. The effects of spatial compounding are illustrated in Figure 2.24 below.

**Figure 2.24** Example of an irregular echogenic plaque located at the carotid bifurcation and the effects of spatial compounding.

Due to acoustic shadowing, the plaque texture is poor in the conventional B-mode scans (C and D). With spatial compound imaging there is a reduction in shadowing and improved resolution (A and B).

(Figure taken from Kern R et al, 2004).

### 2.9 Summary

An understanding of the physics of ultrasound, its limitations and advantages along with a knowledge of imaging artefacts, has enabled me to optimise the images taken for analysis throughout this project. For all images a high
resolution ultrasound scanner with a broadband linear array (2D) and a broadband volume linear array transducer (3D) were used. The methods will be discussed in the chapters that follow.
Chapter 3. A comparison of novel 3 dimensional and established 2 dimensional flow mediated dilatation
3.1 Introduction

With the evolution of flow mediated dilatation numerous limitations of this 2D US assessment have been made evident. The most significant of these is variable reproducibility, both inter-individual (Sejda T et al, 2005) and between study reproducibility (Hardie KL et al, 1997). Furthermore, as the 2D model uses the longitudinal plane only, probe malalignment may occur thereby resulting in the US beam inaccurately bisecting the artery resulting in inaccurate measurements of arterial diameter.

Further limitations of the 2D US approach include a lack of standardised protocols and the technically challenging nature of the assessment which requires significant training.

To our knowledge this is the first study to evaluate flow mediated dilatation using 3D US.

Hence we conducted this study wherein two hypotheses were tested, namely that:

- Use of 3D ultrasound images to quantify flow mediated dilatation eliminates the systematic underestimation of diameter that occurs when 2D ultrasound is used, and hence estimates gained utilising 3D ultrasound images will be greater than estimates gained utilising 2D ultrasound images.

- Quantification of flow-mediated dilatation gained using 3D ultrasound images will be more reproducible than quantification using 2D ultrasound images.
3.2 Methods

3.2.1 Study design

This was a cross sectional reproducibility study with 30 males recruited to participate. The study was performed over 2 visits separated over approximately 2 weeks. The study compared the accuracy and reproducibility of 2D and 3D ultrasound in the assessment of flow mediated dilatation. Both 2D and 3D FMD were performed on visit 1 and on visit 2 thereby facilitating assessment of reproducibility.

The study was approved by the Beaumont Hospital Ethics (Medical Research) Committee of Beaumont Hospital, Dublin and the research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

All participants received a coded study number with the patients initials and a number assigned to them chronologically according to their participation in the study. This facilitated the blinded measurements of images performed off-line. All electronic and paper data was stored using the coded study number.

For example FMD – 1 – 001 – AB.


001 = study number, first patient to participate in the study.

AB = patient initials.

And

NID – 1 – 001 – AB.

Where NID – 1 = Nitrate induced dilatation – Visit 1.

001 = study number, first patient to participate in the study.

AB = patient initials.
3.2.2 Study population

The volunteers were aged between the 18 and 80 years and were eligible if they were willing to provide informed and written consent. Volunteers were excluded from the study if they had an acute illness or were suffering from any significant chronic illness or if their medication was likely to be changed 2 weeks prior to starting or 2 weeks following completion of the study. Intolerance to sub-lingual glyceryl trinitrate was also deemed an exclusion factor. All subjects were Caucasian and of Western European descent.

3.2.2.1 Population recruitment

Subjects were identified from amongst attendees of Beaumont hospital outpatient clinics and also the general public in the Dublin area. The participants were approached and given both verbal and written information in the form of an invitation letter and participant information leaflet about the study. An example of this letter, the consent form and a sample letter that was sent to the participants General practitioner following the study, is given in Appendix 1. Approximately 50 letters of invitation were sent to achieve the required participation giving a study uptake of 60%. Interested participants either contacted the study doctor directly for more information, or were contacted by a follow up telephone call one week after the letter of invitation was sent. Following screening for inclusion and exclusion criteria a study visit was arranged at the RCSI Clinical Research Centre, Beaumont Hospital.

3.2.2.2 Study conduct

There was a total of 2 visits per participant separated by a period of approximately 2 weeks. Volunteers abstained from caffeine containing beverages and alcohol for 24 hours prior to both study days and abstained from smoking for at least 4 hours prior to the visit. All assessments were made
in the morning and subjects were fasted from midnight the night before. All studies were performed in the same room with ambient temperature maintained within the thermo neutral zone (19°C - 22°C). All study visits and ultrasound scans were performed in the Vascular imaging suite in the Smurfit Building (Clinical Research Centre), Beaumont hospital in Dublin.

The following assessments were performed prior to Ultrasound on the first study day only unless stated:

- Medical history and examination; Lifestyle assessment including diet and smoking history; Alcohol consumption; Family history; Current drug treatments; Exercise patterns were defined as none, occasional (up to 3 times per week) or regular (> 3 times per week, ≥ 20 minutes each session). Height & weight measurement.

- A family history of heart disease included a history of MI, angina, CABG, coronary artery disease, coronary stenting, cardiomyopathy or valvular disease. A family history of DM included both IDDM and NIDDM in parents, brothers or sisters. A family history of dyslipidaemia included those on either dietary or medical treatment.

- Clinic BP measurement (both study days).

- Phlebotomy: Fasting glucose, Lipid profile, Serum creatinine.

### 3.2.3 Clinical and laboratory measurements

#### 3.2.3.1 Clinical measurements

All participants had their height and weight measured in kilograms. Body mass index was calculated as weight in kilograms divided by height in meters squared.
3.2.3.2 Blood pressure measurements

Blood pressure was taken from the right arm in accordance with the recommendations of the British Hypertension Society (Ramsey et al 1999) with the patient resting in the supine position for 5 minutes. Measurements were obtained using a regularly calibrated semi-automated blood pressure machine (OMRON® HEM 705CP, Omron Healthcare, Sussex, UK (O’Brien et al 1996)). Blood pressure was measured 3 times and the mean of the second and third measurements was taken to be representative of brachial artery blood pressure.

3.2.3.3 Laboratory measurements

Fasting glucose, lipid profile and serum creatinine and electrolytes were measured on all patients with analysis being performed in the central hospital laboratory in Beaumont hospital. Patients were deemed to have hypercholesterolaemia if they had a total cholesterol ≥ 5 mmol/l or if they were on current treatment for hypercholesterolaemia.
3.2.4 Determination of flow mediated dilatation using ultrasound

The equipment used for ultrasound imaging was a Philips iU22 Premium High Resolution Vascular Ultrasound Scanner. The Phillips L17-5 broadband linear array transducer (2D) and the VL13-5 broadband volume linear array transducer (3D) were used.

Figure 3.1 Philips iU22 Ultrasound Scanner (left), 2D (middle) and 3D (right) transducers used in determining FMD.

Following 15 minutes of supine rest, a baseline cineloop of a straight segment of the right brachial artery was performed. The arm was extended in a comfortable supinated position. Baseline longitudinal images were obtained 5cm above the antecubital fossa using 2D US with the L17-5 high frequency broadband linear array transducer for approximately 15 seconds (or until a satisfactory image was obtained). Images were deemed satisfactory when both the near and far walls of the artery were clearly visualized, and the artery was visualized throughout the entire field of view in a horizontal fashion in an attempt to minimize skew (Figure 3.2). Gain was optimized with focusing set at the far wall. Depth was typically set at approximately 3cm. The artery was marked for subsequent images using a skin marker. A record of anatomical location was documented to ensure imaging of the same segment of artery.
during subsequent scans performed that day and during the follow up visit. All images were ECG gated and heart rate was recorded prior to starting the procedure.

**Figure 3.2** 2D ECG gated longitudinal image of the brachial artery with the entire segment under evaluation included in the field of view and orientated in line with the probe.

Flow mediated dilatation (endothelium dependant dilatation) is based upon the arteries ability to dilate in response to increased blood flow (hyperaemia) and increased shear stress. This environment is created by inflating a blood pressure cuff to 50 mmHg above the systolic BP. After 5 minutes the cuff is rapidly deflated creating a reactive hyperaemia with arterial dilatation occurring primarily in response to NO and prostacyclin released from the healthy or intact endothelium on which it is dependant. A cineloop was recorded between 50 and 70 seconds post deflation. Maximal dilatation is known to occur at 60 seconds (Uehata A et al, 1997).

Endothelium independent dilatation (nitrate induced dilatation (NID)) was performed 20 minutes later following a period of rest and a return to baseline brachial arterial diameter. The same segment of the brachial artery was identified with the aid of the skin marker, and a 15 second cineloop was
performed to record baseline brachial artery diameter. Two puffs (800mcg) of sublingual glyceryl trinitrate (S/L GTN) were administered to achieve NID. A further cineloop was performed after 3 minutes and 50 seconds for a period of 20 seconds (maximal NID is known to occur 4 minutes after GTN administration). This was performed as a control. The FMD procedure was performed according to the guidelines issued in 2002 by the International brachial artery reactivity task force (Corretti MC et al, 2002). Figure 3.3 summarises the FMD procedure.

![Figure 3.3](image)

**Figure 3.3** Flow mediated dilatation.

Schematic on the left demonstrates brachial artery FMD following cuff deflation. Schematic on the right demonstrates the blood pressure cuff on the upper arm with the transducer positioned above the antecubital fossa. The differences in brachial arterial diameter between baseline imaging, imaging following deflation of cuff and following administration of GTN are also illustrated.

(Figure taken from Corretti MC et al, 2002).

After a further 20 minutes of rest to allow a return to baseline artery diameter, the process was repeated using 3D US and the VL 13-5 volume linear array
probe. The basic principles of the 3D FMD process were identical to the 2D assessment however, important differences to be noted when using 3D US are the following:

1. Baseline diameter imaging was performed by capturing a 3D volume data set with the 3D probe held in position over the region of interest. All images were ECG gated with the beginning of the 3D sweep triggered by the R wave of the QRS complex.
2. 3D sweeps took approximately 1 second to complete.
3. 3 X 3D sweeps / volume data sets were captured at each stage of the process. For example, when recording images post deflation; 3 X 3D sweeps were captured between 50 and 70 seconds post deflation. For recording images during NID, 3 X 3D sweeps were taken at the time of maximal dilatation i.e. after 3 minutes and 50 seconds.

3.2.4.1 Summary of calculations in the assessment of 2D and 3D flow mediated dilatation

• Determination of brachial FMD utilising 2D ultrasound.
• Determination of brachial nitrate induced dilatation utilising 2D ultrasound.
• Determination of brachial FMD utilising 3D ultrasound.
• Determination of brachial nitrate induced dilatation utilising 3D ultrasound.
3.2.5 Measurements

3.2.5.1 Image labelling / image selection

All 2D cineloops were ECG gated so as to capture images in end-diastole (peak of R wave). 3D volume data sets were also ECG gated and triggered to acquire a ‘sweep’ at end-diastole (peak of R wave). The time to acquire a full ‘sweep’ took approximately 1 second.

Following each individual scan, the cineloops and 3D data sets were transferred electronically from the iU22 ultrasound scanner in DICOM format to a PC for analysis offline. All images were carefully reviewed and the best 2D images and 3D data sets were identified and saved to a USB disk. The software used to select images for analysis was QLAB (3DQ Advanced, QLAB 7, Philips, The Netherlands).

2D measurements were performed at the following points in the cineloops:

• 2D brachial artery baseline diameter prior to FMD and NID was measured at three different points in the cineloop during diastole at the peak of the R wave.

• 2D brachial diameter post cuff deflation (FMD) and post administration of GTN (NID) was also measured at three different points in the cineloop taken following the appropriate interval as outlined above.

3D measurements were performed at the following points in the 3D sweeps:

• 3D brachial artery baseline diameter prior to FMD and NID was measured using three 3D sweeps triggered by the R wave.

• 3D brachial diameter post cuff deflation (FMD) and post administration of GTN (NID) was measured from three 3D sweeps triggered by the R wave.
In all cases the diameter measurement was taken to be the median of the three measurements.

3.2.5.2 Offline diameter measurement using Arterial Measurement System

Diameter was defined as the mean distance from the leading edges of the intima-lumen interfaces of the near wall and lumen-intima of the far wall. This is illustrated below in figure 3.4.

![Image of ultrasound image and schematic](image)

**Figure 3.4** A longitudinal B-mode 2D Ultrasound image of a segment of the brachial artery illustrating the calculation of lumen diameter from the echogenic interfaces.

The schematic on the right demonstrates lumen diameter $\text{I}_3$ (leading edge of second echogenic zone in near wall) – $\text{I}_5$ (leading edge of first echogenic zone in far wall). The distance between $\text{I}_5$ and $\text{I}_7$ is the intima-media thickness (IMT).

$I_3$=near wall intima-lumen interface, $I_5$=far wall intima-lumen interface, $I_7$=far wall media-adventitia interface.

Individual diameter measurements were performed on a separate PC using a software called AMS (Arterial Measurement System) version 1.102 (Chalmers University, Gotenburg, Sweden). This is a semi-automated and histologically validated software for the calculation of arterial diameter at each stage in the process as summarised in section 3.2.10. The software evaluates lumen
diameter by measuring approximately 150 points over a 1 cm segment of the artery thus reducing the standard error of the measurement (Wendelberg et al, 1997). It uses a multi-scale dynamic programme algorithm based on echo intensity, intensity gradient between pixels and boundary continuity.

All images are initially calibrated to scale into mm/pixel. The calibration distance is 10 mm and is carefully chosen by the operator prior to analysing each set of images. A box is then placed around the region of interest to be measured representing a 1cm segment of the brachial artery. The echogenic interfaces are chosen automatically by the software and the accuracy of the border detection is visually inspected by the operator. Figure 3.5 below illustrates an example of the border detection.

![Figure 3.5 Longitudinal 2D ultrasound image of the brachial artery with a box placed around the region of interest.](image)

I3=near wall intima-lumen interface, I5=far wall intima-lumen interface, I7=far wall media-adventitia interface.

The figure above demonstrates how after placing the box around the ROI, the software automatically detects the echogenic borders of the near and far wall in order to calculate the diameter. This is an image of the artery in end-diastole as indicated by the ECG tracing at the bottom of the figure. Lumen diameter is
the distance between near wall intima-lumen interface and the far wall intima-lumen interface.

In the case where the software automatically detects an interface that is not the true border to be measured the user can adjust the border detection in a semi-automated fashion. By clicking the mouse on the correct interface the software will readjust and try to seek the true border. It is recommended that user intervention in this regard be limited to a maximum of 3 attempted adjustments in order maintain objective accurate measurements. An example of incorrect border detection and its subsequent correction using this method is illustrated in figure 3.6 below.

![Figure 3.6](image)

**Figure 3.6** Semi-automated border correction performed by AMS software.

(a) The software has automatically and incorrectly detected a border for I5 and I7. The correct interfaces of I5 and I7 are illustrated by the **white arrows**. (b) Following user intervention by clicking on the correct interface (the I5 interface) the software correctly identifies the I5 and I7 borders.

The user also has the option to manually trace the borders however this is not recommended as it is likely to lead to systematic bias and give different measurements to those achieved via the semi-automated method. If the proximal or distal ends of the ROI are felt by the user to have detected borders incorrectly, then that particular segment of the artery can be ‘cut’ or excluded.
from measurement. This results in a decrease in the number of diameter measurements per segment (i.e., it will be less than 150 individual measurements) and therefore it is recommended not to exclude more than 20% of the segment so as to maximise accuracy. Full instructions on how to use AMS software are found in Appendix 2.

Arterial diameter measurements from the 3D data sets are performed on a longitudinal image from the 3D data set. Choosing a 2D longitudinal image from the 3D data set for the purposes of diameter measurement is performed by accurately bisecting the artery in the transverse, sagittal and craniocaudal (view from above) planes. This ensures that the artery is not skewed and that a true diameter measurement is performed. This is illustrated in figure 3.7 below.

*Figure 3.7* 3D data set of the brachial artery optimised for diameter measurement by accurately bisecting the artery in 3 planes.

(1) Longitudinal or sagittal view. (2) Transverse view. (3) Craniocaudal view or ‘view from above’. Note how the vertical and horizontal straight lines placed in the center of the artery by the user, accurately bisect the vessel ensuring a true measurement of diameter. The image in (4) is a 3D volume that can be rotated in any direction, however this is not useful in terms of calculating arterial diameter. It can be useful when imaging larger body parts such as in obstetrics, where in can aid in detection of fetal anomalies.
When the 3D image has been optimised it is transferred via USB to another PC in order to calculate diameter using AMS. The same process is followed as for 2D assessments including careful calibration, and a ROI in the longitudinal image is selected for measurement. This is illustrated in figure 3.8 below.

**Figure 3.8** Close up view of a longitudinal image of the brachial artery taken from a 3D data set with calculation of arterial diameter using AMS.

### 3.2.6 End-points

The primary end-point is brachial FMD (endothelium dependent dilatation), and the secondary end-point is brachial nitrate induced dilatation (endothelium independent dilatation).
3.2.6.1 Brachial diameter endpoints in both 2D and 3D

Brachial diameter at baseline.

Brachial post-ischaemic diameter.

Brachial diameter at baseline.

Brachial nitrate induced diameter.

3.2.6.2 Calculation of end-points

FMD and NID were expressed as a percentage of the average diameter of the artery during the resting / baseline scan.

The following equations were applied:

\[
FMD = \left( \frac{\text{post ischaemic diameter} - \text{baseline diameter}}{\text{baseline diameter}} \right) \times 100\%
\]

\[
NID = \left( \frac{\text{NID diameter} - \text{baseline diameter}}{\text{baseline diameter}} \right) \times 100\%
\]

3.2.7 Data handling / storage

Data was stored using Excel (Microsoft Excel 97, Microsoft Corporation, Redmond, WA, USA).
3.2.8 Statistical analyses

All analyses were performed using DataDesk 6.1 software (Data Description Inc., NY, USA). All descriptive data were expressed as mean +/- SD or as median (interquartile range). Mean values for visit 1 and visit 2 were calculated as were the between visit mean differences. Bland Altman plots were then constructed using these values to assess between visit 2D and 3D FMD and NID reproducibility (Bland JM et al, 1986).

Mean values at visit 1 and visit 2 were also used to construct correlation plots and $r^2$ values to assess between visit baseline diameter, FMD and NID correlation using 2D and 3D US.

3.2.9 Power of study

It was anticipated that mean +/- SD of FMD, in these participants would be approximately 15% +/- 10%, and that the SD of the differences between repeated measurements would be approximately 5%. Hence with 30 participants this study had 90% power to detect a 3% difference in brachial FMD when quantified using 3D ultrasound as opposed to 2D ultrasound, with alpha of 0.05.
3.3 Results

3.3.1 Population recruitment

In total 30 men were recruited to the study. No patients met the exclusion criteria as outlined previously, however 2 patients were unable to attend for the second visit and were therefore excluded from analysis. A further patient was excluded from analysis as the images obtained were unsuitable due to the brachial artery of this thin patient lying superficially almost beneath the skin surface. This prohibited accurate analysis of the near wall which is essential for measurement of lumen diameter. Therefore, a total of 27 patients were analysed.
### 3.3.2 Participant characteristics of study population

**Table 3.1 Patient characteristics**

Data are given as n (%) or mean ± standard deviation (SD).

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>44 ± 12</td>
</tr>
<tr>
<td>Smoking habit</td>
<td></td>
</tr>
<tr>
<td>- current</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>- ex-smoker</td>
<td>10 (37%)</td>
</tr>
<tr>
<td>- never</td>
<td>10 (37%)</td>
</tr>
<tr>
<td>Alcohol (units/week)</td>
<td>22 ± 13</td>
</tr>
<tr>
<td>Exercise habit</td>
<td></td>
</tr>
<tr>
<td>- none</td>
<td>3 (11%)</td>
</tr>
<tr>
<td>- occasional</td>
<td>14 (52%)</td>
</tr>
<tr>
<td>- regular</td>
<td>10 (37%)</td>
</tr>
<tr>
<td>Salt added</td>
<td>21 (85%)</td>
</tr>
<tr>
<td>Fruit and veg (portions/day)</td>
<td>3.44 ± 1.53</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84 ± 9</td>
</tr>
<tr>
<td>Height (cms)</td>
<td>177 ± 6</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Systolic BP (visit 1)</td>
<td>128 ± 12</td>
</tr>
<tr>
<td>Diastolic BP (visit 1)</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Systolic BP (visit 2)</td>
<td>126 ± 8</td>
</tr>
<tr>
<td>Diastolic BP (visit 2)</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>Heart rate (visit 1)</td>
<td>60 ± 9</td>
</tr>
<tr>
<td>Heart rate (visit 2)</td>
<td>60 ± 7</td>
</tr>
</tbody>
</table>
3.3.3 Cardiovascular risk factors of participants

Cardiovascular risk factors of the 27 participants are shown in Table 3.2

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker (current)</td>
<td>7   (26%)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>12  (44%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4   (15%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1   (4%)</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>2   (7%)</td>
</tr>
<tr>
<td>Family history of ischaemic heart disease</td>
<td>7   (26%)</td>
</tr>
</tbody>
</table>

3.3.4 Biochemical characteristics of participants

Biochemical characteristics of the 27 participants are shown in Table 3.3

<table>
<thead>
<tr>
<th>Variables</th>
<th>Data are given as mean ± SD.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.83 ± 1.21</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.96 ± 0.73</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>85 ± 10</td>
</tr>
</tbody>
</table>
The participants were between 32 and 56 years of age. Only 37% had never smoked with 26% classed as current smokers. Mean alcohol consumption was 22 units/week, slightly above the recommended limit. Most subjects exercised occasionally or on a regular basis. Mean body mass index was slightly above the recommended at 27. Salt was added to food by 85% of subjects. Overall the lifestyle habits of participants were deemed to be somewhat poor.

44% of participants were classed as having hypercholesterolaemia. The cardiovascular risk factor profile was otherwise deemed to be generally good with only 4% having diabetes, 15% with hypertension (average systolic BP over the 2 visits was $127 \pm 10$ mmHg) and only 7% with a history of ischaemic heart disease.
### 3.3.5 Baseline diameter correlation between visit 1 and 2 using 2D and 3D ultrasound

Mean 2D baseline diameter (pre-cuff inflation diameter) was 4.167 mm at visit 1 and 4.223 mm at visit 2 (mean difference -0.055mm) with a SD of 0.22 mm. The $r^2$ value was 0.68 using 2D (Figure 3.10 (a)).

Mean 3D baseline diameter was 4.894 mm at visit 1 and 4.920 mm at visit 2 (mean difference -0.025 mm) with a SD of 0.280 mm. The $r^2$ value using 3D was 0.66. (Figure 3.10 (b)).

![Figure 3.9 Baseline diameters (mean ± SD) using 2D and 3D Ultrasound at visit 1 and visit 2.](image)

3D and 2D baseline diameter reproducibility and between visit correlation was similar between the two methods with a SD of 0.22 mm for 2D and 0.28 mm for 3D. The $r^2$ values were also similar with 0.68 and 0.66 for 2D and 3D respectively.
Figure 3.10 Correlation plots demonstrating between visit correlation of (a) 2D and (b) 3D US determined baseline brachial diameters prior to cuff inflation.
(a) Bland Altman plot illustrating between visit 2D baseline diameter reproducibility.

(b) Bland Altman plot illustrating between visit 3D baseline diameter reproducibility.

**Figure 3.11** Bland Altman plots demonstrating between visit reproducibility of (a) 2D and (b) 3D US determined baseline brachial diameters prior to cuff inflation.
3.3.6 Flow mediated dilatation correlation between visit 1 and 2 using 2D and 3D ultrasound

The mean FMD using 2D was 2.43% (SD 3.63%) at visit 1 and 2.55% (SD 5.28%) at visit 2.

The between visit difference in FMD using 2D was -0.12% +/- 4.5% with an $r^2$ value of 0.28.

The mean FMD using 3D was 2.58% (SD 3.83%) at visit 1 and 2.16% (SD 3.63%) at visit 2.

The between visit difference in FMD using 3D was 0.42% +/- 4.03% with an $r^2$ value of 0.17.

Correlation and reproducibility of between visit FMD was slightly better using 2D with a smaller between visit difference of -0.12% +/- 4.5% with an $r^2$ value of 0.28 versus 0.42% +/- 4.03% with an $r^2$ value of 0.17 when using 3D.
Figure 3.12 Correlation plots demonstrating between visit correlation of (a) 2D and (b) 3D US determined Flow mediated dilatation.
Figure 3.13 Bland Altman plots demonstrating between visit reproducibility of (a) 2D and (b) 3D US determined Flow mediated dilatation.
3.3.7 Nitrate induced dilatation correlation between visit 1 and 2 using 2D and 3D Ultrasound

The mean NID using 2D was 18.04% (SD 7.25%) at visit 1 and 18.19% (SD 6.94%) at visit 2.

The between visit difference in NID using 2D was -0.15% +/- 8.49% with an $r^2$ value of 0.08.

The mean NID using 3D was 6.87% (SD 6.09%) at visit 1 and 6.42% (SD 6.70%) at visit 2.

The between visit difference in NID using 3D was 0.44% +/- 6.02% with an $r^2$ value of 0.32.

Between visit difference and reproducibility in NID was better in 3D than 2D with 0.44% +/- 6.02% with an $r^2$ value of 0.32 in 3D versus -0.15% +/- 8.49% with an $r^2$ value of 0.08 using 2D. Mean 2D NID was greater at around 18% with 3D NID measuring approximately 6%.
Figure 3.14 Correlation plots demonstrating between visit correlation of (a) 2D and (b) 3D nitrate induced dilatation.
Bland Altman plot illustrating between visit 2D NID reproducibility.

Bland Altman plot illustrating between visit 3D NID reproducibility.

Figure 3.15 Bland Altman plots demonstrating between visit reproducibility of (a) 2D and (b) 3D nitrate induced dilatation.
3.4 Discussion

Due to hormonal influences on FMD during the menstrual cycle (Masayoshi et al, 1995), an all male population was chosen. This made the logistics of the study easier in terms of booking patients and as we were comparing two different modalities and not study populations, this was deemed appropriate.

Only one patient was excluded from the study due to technical difficulties. This patient had extremely thin arms and the brachial artery was positioned too superficially to get an accurate and measureable image. This is a well described limitation but did not severely affect the results or power of the study. Two further subjects were unable to complete the study, meaning 27 subjects (90%) completed the study.

A broad range of cardiovascular risk factors were spread evenly over the study population which was felt to be an accurate representation of the general male population. This also provided a broad range of brachial artery diameters, flow mediated dilatation and nitrate induced dilatation.

Our hypothesis that 3D US would provide a greater diameter measurement than 2D was found to be correct. Our results show that the overall average baseline diameter using 3D was 4.907 mm and the equivalent 2D measurement was 4.195 mm. This represents an underestimation in diameter of 14.5 %. This occurs because 3D US more accurately disects the artery in the mid axial, sagittal and coronal planes thereby giving a true estimate of arterial diameter (Figure 3.16). We feel 2D diameter measurements can never be 100% accurate as the probe may be malaligned or aligned obliquely over the artery when in the longitudinal plane.
Figure 3.16 3D diameter is greater than 2D diameter and gives a more accurate measurement due to the ability to dissect the artery in all three planes as shown above.

Numerous studies have compared diameter measurements of abdominal aortic aneurysms (AAA) obtained using 2D US with CT (the current gold standard in assessing AAA > 5 cm). In 2003 Sprouse RL et al, compared axial diameters obtained using US and CT in 334 subjects following AAA endograft repair. They found CT yielded a significantly greater diameter in 95% of subjects with significant discrepancies between the 2 modalities. The mean maximum diameter obtained by CT was 5.69 +/- 0.89 cm versus 4.74 +/- 0.91 cm using US (P < 0.001) (Sprouse RL et al, 2003). In 2009 Manning BJ et al, compared AAA diameter in 109 patients obtained by CT with 3D reconstruction software, against those using standard 2D. Maximum aortic diameter for CT was measured in 4 slightly different planes; the anteroposterior plane (CT-AP), along the maximum ellipse (CT-ME), perpendicular to the maximum ellipse (CT-PME) and perpendicular to the centerline of flow (CT-PCLF). Maximum anteroposterior diameter measurements were also performed using US. They found all of the CT measurements to be significantly larger than the US measurement and in the case of CT-ME this difference was 9.6 mm +/- 8.0 mm (mean +/- SD) (Manning BJ et al, 2009).
Our results have also found smaller diameter measurements using 2D ultrasound, albeit when compared to 3D US and not CT. In order to be completely certain that 3D is more accurate than 2D US in the assessment of arterial diameter a bench study would have to be performed on an arterial phantom of a known diameter comparing the 2 US modalities. Future studies may prove this to be the case thereby further supporting our findings.

Both methods had an average FMD of < 3%. A normal FMD is quoted as being between 7 and 10% (Moens AL et al, 2005). Our population was therefore deemed to lie in the at risk category which would fit with our population characteristics as seen in Tables 3.1, 3.2 and 3.3 above. Another possible explanation of the decreased FMD in our study population is that it has been shown that FMD exhibits diurnal variation and can be decreased in the morning time as opposed to later in the evening (Otto ME et al, 2004). Etsuda H et al, examined FMD in the morning, midday, in the evening and at night in 13 healthy young males. They found FMD to be significantly reduced with a mean FMD of 4% in the morning as opposed to the evening where the mean FMD was 9.7%, and 6.9% at night (Etsuda H et al, 1999).

Inflation of the blood pressure cuff at the upper arm was used as opposed to at the forearm, which is a viable alternative. It has been shown that using cuff inflation at the forearm demonstrates a lesser FMD than when using cuff inflation over the upper arm (Vogel RA et al, 2000, Mannion TC et al, 1998). There is no consensus as to which technique is better, however it is suggested that inflation of the cuff on the upper arm can be technically challenging as the image can be distorted by the collapse of the artery and the shift in soft tissues making accurate data acquisition more difficult (Corretti MC et al, 2002). This was not felt to be the case in this study where the vast majority of images were deemed suitable for analysis.

The major limitation of our study was that real-time 3D US, or 4D US currently is not capable of the temporal resolution necessary to capture such minute changes required during FMD. Therefore, whilst we found 3D to be a more accurate measure of brachial artery diameter, it is not a real-time measurement. In order to account for this, the 3D sweep was triggered by the
R wave of the ECG. This meant that the volume data set captured was acquiring the arterial diameter from end diastole and through systole and then into diastole again. Therefore at least 1 systolic cycle is included in the data set as the sweep takes on average 1 second to complete. If the heart rate is above 60 the heart will contract further during the sweep and a further systolic measurement will be included in the data set. The 3D diameter measurements in our study were greater than the 2D measurements and it is likely that this is due in part to the fact that the 3D sweep is including some systolic data. It has to be said that systole occurs over a relatively short period of time throughout the cardiac cycle so that by far the majority of measurements or data captured by the 3D sweep will be during diastole. Taking the average diameter measurement in 2D FMD over the entire cardiac cycle has been shown not to reduce accuracy (Kizhakekuttu TJ et al, 2010) when compared with FMD measurements taken at the same time throughout the cardiac cycle. In a way it can also be said that the 3D sweep is taking a volume data set from the entire cardiac cycle thus giving an average diameter measurement, thereby not reducing accuracy.

Nitrate induced dilatation was greater using 2D US than 3D US. One possible explanation for this may be due to a hangover effect of the GTN following the 2D NID examination. Although a 20 minute washout period was given prior to the start of the 3D examination, it is conceivable that some GTN was still metabolically active, resulting in a lesser NID during the 3D examination. If future studies are to be performed perhaps carrying out 2D and 3D examinations on different days may help to clarify this discrepancy. Nitrate tolerance between the 2D and 3D examinations is a further, but less likely explanation or contributing factor.
3.4.1 Conclusion

Our findings suggest that 3D US gives a greater and more accurate measurement of brachial artery diameter and we believe that 2D US underestimated diameter by approximately 14%. A bench study using an arterial phantom of known dimensions, directly comparing 2D and 3D US diameters would be needed in future studies to further support our findings. Baseline diameter measurements were similarly reproducible and between visit FMD correlation and reproducibility was only slightly better with 2D, with NID being slightly better using 3D. To our knowledge this is the first study to compare 2D and 3D US assessment of flow mediated dilatation.

With real-time high resolution 4D US likely to provide better temporal resolution, the advent of 4D FMD is only around the corner. This is likely to be more accurate, reproducible and user friendly than 2D and may soon find its way into clinical practice as a means of identifying those at future risk, enabling preventive measures to be put in place. We believe that the process of identifying 3D US as a useful and comparable tool to 2D US will provide a stepping stone for this to happen, thereby facilitating better quantification of endothelial function.
Chapter 4. Ultrasound assessment of subtle alterations in vascular structure and function in young women with and without a history of gestational hypertension or preeclampsia
4.1 Introduction

Pre-eclampsia is a placentally mediated multisystem disorder characterized by hypertension and proteinuria in pregnancy. It usually occurs following 20 weeks gestation and resolves approximately 3 months post-partum. It affects between 2-8% of all pregnancies (Duley L, 2009). Pre-eclampsia occurs as a result of failure in the vascular remodelling of the maternal spiral arteries with resultant hypoperfusion of the placenta. This is turn leads to the release of inflammatory cytokines and vascular endothelial growth factor-1 amongst other antiangiogenic proteins, causing systemic endothelial dysfunction and vasoconstriction, with subsequent systemic hypertension.

Pre-eclampsia is associated with an increased prevalence of cardiovascular risk factors. The Cardiovascular Health After Maternal Placental Syndromes study (CHAMPS study) evaluated over 1 million women, none of whom had cardiovascular disease prior to their first pregnancy. This retrospective cohort study included those with both pre-eclampsia and gestational hypertension. It demonstrated a 12-fold increased risk of CVD in those with a history of pre-eclampsia and metabolic syndrome versus those without such a history (Hazard ratio [HR] 11.7; 95% confidence interval [CI]: 4.9 to 28.3) (Ray JG et al, 2005).

Numerous studies have shown pre-eclampsia to also be associated with future cardiovascular risk. The Rochester Family Heart study evaluated 626,272 Norwegian women over a 25-year period, many of whom had a history of pre-eclampsia during their first delivery. They found that women with a history of pre-eclampsia and a pre-term delivery, had an 8.12 fold higher risk of death from cardiovascular causes than those with a normal pregnancy (HR 8.12; 95% CI: 4.31 to 15.33) (Irgens HU et al, 2001). Furthermore, a recent meta-analysis of 43 studies found that women with a history of eclampsia or pre-eclampsia had an increased risk of developing hypertension (HR 3.13; 95% CI: 2.51 to 3.89), cerebrovascular disease (HR: 1.76; 95% CI: 1.43 to 2.21) and an increased risk of developing CVD (resulting in death or adverse clinical outcome) (HR: 2.28; 95% CI: 1.87 to 2.78) (Brown MC et al, 2013).
Pre-eclampsia is also associated with increased IMT. In 2006, Blaauw J et al, examined common femoral artery and CCA IMT in 3 groups of women. Group 1 consisted of 22 nulliparous women, group 2 had 22 primiparous women with a normal pregnancy and group 3 had 22 primiparous women with pre-eclampsia. Groups 2 and 3 were followed up a year post-partum. They found increased femoral IMT in the pre-eclampsia group (mean 0.63 mm) compared with the normal pregnancy (mean 0.55 mm) and nulliparous groups (mean 0.52 mm). Common carotid artery IMT was also higher in the pre-eclampsia group (mean 0.65 mm) versus the nulliparous group (mean 0.59 mm) with no significant IMT difference between the pre-eclampsia and normal pregnancy group (mean 0.62 mm) (Blaauw J et al, 2006). The same study group followed up these patients approximately 5 years later in a case control study and found no difference in IMT or progression of IMT between 17 pre-eclampsia subjects and 16 controls. They concluded that severe pre-eclampsia was not associated with increased IMT after 5 years and suggested a transient adaptive response of the arteries of those with a history of pre-eclampsia (Blaauw J et al, 2014).

Conversely, Ciftci FC et al, examined the carotid IMT of 33 mild pre-eclampsia patients 5 years post-partum and 29 healthy volunteers. They also performed echocardiography looking at coronary flow reserve (CFR) and high-sensitivity CRP (hs-CRP) in all patients. They found a lower CFR and increased IMT in the PET group when compared with the controls (0.59 ± 0.15 versus 0.46 ± 0.1). These negative effects of pre-eclampsia were also significantly correlated with hs-CRP (Ciftci FC et al, 2014).

Pre-eclampsia is also associated with arterial stiffness. A systematic review and meta-analysis including 23 relevant studies demonstrated a significant increase in all combined arterial stiffness indices in those with pre-eclampsia versus those with normotensive pregnancies (Hausvater A et al, 2012). In a small study conducted by Evans CS et al in 2011, the group examined 18 pre-eclampsia patients and 50 uncomplicated pregnancies with a mean follow up of 16 months post-partum. They found that arterial stiffness persists in the peripheral arteries of pre-eclampsia sufferers post-partum by way of increased heart to brachial PWV. Central stiffness measured by heart to femoral and
heart to carotid PWV was not affected (Evans CS et al, 2011). Recently, vector velocity imaging (VVI) was used to demonstrate increased peripheral vascular stiffness in the CCA’s of 24 pre-eclampsia patients versus 34 normotensive pregnant women. These patients were not followed up however to determine if this increased arterial stiffness persisted post-partum (Ma XJ et al, 2012).

Elevated PWV in the second trimester is also associated with an increased risk of developing pre-eclampsia (Savvidou MD et al, 2011).

A previous case control study was performed by Dr Catherine Brown in the Clinical Research Centre (Smurfit Building), Beaumont Hospital, Dublin (unpublished data). 124 participants were recruited. 43 controls had achieved normal pregnancy, 34 experienced gestational hypertension (GH) and 47 pre-eclampsia (PET). Large vessel vascular structure and function were assessed by carotid artery ultrasound, brachial reactivity, carotid to femoral PWV (C-F PWV), and various non-invasive assessments of vascular stiffness. The study found the PET and GH groups to have greater IMT and common carotid wall cross sectional area than the control group. These results were not explained by differences in established risk factors such as hypertension, dyslipidaemia, smoking, diabetes or a family history of heart disease. There was no significant difference in cross sectional compliance, distensibility or elasticity between the control, GH or PET groups. However, greater arterial stiffness in the form of increased C-F PWV was found in the PET and GH groups when compared to the controls. This was explained by a greater mean arterial pressure in those with pregnancy-induced hypertension.

The PET group also demonstrated greater endothelial dysfunction versus those in the control group, and this could not be explained by established risk factors.

The study concluded that those with pregnancy-induced hypertension have structural abnormalities of large vessels and endothelial dysfunction, thereby putting them at risk of developing future cardiovascular events.
Hence, in order to test for more subtle alterations in vascular structure and function, this study was initiated, testing the five following hypotheses:

- Women with a history of pre-eclampsia will demonstrate different IMT values according to angle in the distal CCA than women with a history of gestational hypertension or normotensive pregnancies.
- Women with a history of pre-eclampsia will demonstrate different IMT values between the proximal CCA, the distal CCA, the carotid bulb and the ICA than women with a history of gestational hypertension or normotensive pregnancies.
- Women with a history of pre-eclampsia will illustrate different IMT compression patterns, according to angle in the distal CCA than women with gestational hypertension and normotensive pregnancies.
- Women with a history of pre-eclampsia will illustrate different IMT compression patterns, according to vascular tree than women with gestational hypertension and normotensive pregnancies.
- Women with a history of pre-eclampsia will illustrate different common carotid compliance patterns from women with normotensive pregnancies.

4.2 Methods

The objectives of the study were as follows:

1. To assess if alterations in the distribution of IMT thickness throughout the bifurcation provides additional distinction between the 3 groups of women.

Therefore the following assessments are performed:

- IMT to be compared by angle at the level of the distal common carotid artery (anterolateral versus lateral versus posterolateral projection).
- IMT to be compared along the vascular tree (proximal CCA versus distal CCA versus Carotid bulb versus ICA).
2. To assess if the distribution of IMT compression (systolic - diastolic difference) throughout the bifurcation provides additional distinction between the 3 groups of women.

Therefore the following assessments are performed:

- IMT compression patterns are compared by angle at the level of the distal common carotid artery (anterolateral versus lateral versus posterolateral projection).
- IMT compression patterns are compared along the vascular tree (proximal CCA versus distal CCA versus carotid bulb versus ICA).

3. The distribution of compliance between the proximal and distal CCA provides additional distinction between the 3 groups of women.

4.2.3 Study population

This was phase 2 of a cohort study involving a total of 40 women who had at least one pregnancy within the last 5 years. Women were eligible to participate if they were aged between 18 and 40 years. Women were excluded from the study if they had chronic hypertension, defined as; current BP ≥160/100, currently on BP lowering medications, or history of BP > 140/90 in first 20 weeks of pregnancy. They were also excluded if they had diabetes, dyslipidaemia (total cholesterol ≥ 7.0, TG ≥ 3.0), and chronic obstructive airways disease or if they had significant endocrine, hepatic, gastric (peptic ulcer disease), renal or inflammatory disease or any other major illness.

Women who were pregnant or lactating at the time of the study and those using vasoactive drugs were also excluded. All participants were Caucasian and of Western European descent.

In this study there were a total of 13 women who had a normotensive pregnancy, 13 who had gestational hypertension and 14 who had suffered pre-eclampsia.
Gestational hypertension is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg in a previously normotensive pregnant woman who is ≥20 weeks of gestation and has no proteinuria or new signs of end-organ dysfunction (American Congress of Obstetricians and Gynaecologists, ACOG task force 2013).

Pre-eclampsia is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg in a previously normotensive pregnant woman who is ≥20 weeks of gestation and has proteinuria (please note that this criterion was revised by ACOG in late 2013 stating that proteinuria is no longer essential for the diagnosis of pre-eclampsia) or signs of end-organ dysfunction.

4.2.4 Study design

4.2.4.1 Population recruitment

124 women were invited to participate. All had already taken part in phase 1 of this cohort study entitled “A comparison of the vascular structure and function of large and small arterial vessels in young women with and without a history of gestational hypertension or preeclampsia”. They were invited initially by letter (see Appendix 3) followed by a telephone call, further explaining to them the reasons for the study and answering any additional questions they may have had. Following an expression of interest a participant information leaflet was sent providing further details of the study (See Appendix 3). Out of the 124 women invited to participate approximately 32% were recruited for the current study (Table 5.1). All participants were willing and able to provide both written and informed consent (See Appendix 3).

A breakdown of the recruitment process is illustrated in Table 4.1 below.
Table 4.1 Breakdown of the recruitment process for study 2.

GH=Gestational hypertension, PET=Pre-eclampsia.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GH</th>
<th>PET</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number invited</td>
<td>43</td>
<td>34</td>
<td>47</td>
<td>124</td>
</tr>
<tr>
<td>Number recruited</td>
<td>13</td>
<td>13</td>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>Percentage uptake</td>
<td>30</td>
<td>38</td>
<td>30</td>
<td>32</td>
</tr>
</tbody>
</table>

4.2.4.2 Study conduct

The study was approved by the Beaumont Hospital Ethics (Medical Research) Committee of Beaumont Hospital, Dublin and the research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

All participants of phase 2 of the study were assigned the same coded participant ID number they were assigned in phase 1 of the study. For example:

PIH-2-023

Where PIH-2-023 = Pregnancy induced hypertension-study phase 2-participant 23.

4.2.4.3 Summary of study visit

The pre-study preparation for participants, medical history and examination, BP and laboratory measurements (fasting glucose, lipid profile, serum creatinine) were conducted in the same manner as the FMD study which has been previously described in Chapter 3. Following the study visit a letter was sent to the participants GP with a summary of the visit and the pertinent clinical and laboratory measurements (Appendix 3).
4.2.5 Carotid artery, carotid bifurcation and internal carotid artery ultrasound protocol

4.2.5.1 Instrumentation

A Philips iU22 Premium High Resolution Vascular Ultrasound Scanner and a Phillips L9-3 broadband linear array transducer were used to acquire all cineloops.

4.2.6 Ultrasound examination

All ultrasound scans were performed in the Vascular Imaging Suite in the Clinical Research Centre (Smurfit Building), Beaumont Hospital, Dublin. The entire procedure was explained to the participant before commencing the scan and the following information was entered into the scanner:

- Patient initials
- Date of birth
- Subject ID number
- Sonographer initials

Following a period of 5 minutes rest in a comfortable supine position the participant’s neck was extended and slightly rotated to the contralateral side. With the probe positioned over the artery and the operator positioned behind the patient the right CCA was scanned with the right hand and left CCA using the left hand. Prior to capturing cineloops a transverse and longitudinal scout scan of the carotid artery was performed to assess the anatomy. All cineloops were ECG gated to end diastole (peak of the R wave) and end systole (end of the T wave).
Images were acquired with the assistance of the Meijer carotid Arc (see Figure 4.1 below).

**Figure 4.1** The Meijer carotid Arc.

This tool is designed to assist and guide the sonographer through a multi-angle IMT B-mode Ultrasound scan protocol. Note the angles marked on the arc. On the left side 210, 240 and 270 degrees (where the probe is positioned in the above illustration) correspond to anterolateral, lateral and posterolateral projections. The corresponding angles are marked on the right side as 150, 120 and 90 degrees.

(Figure taken from Bots ML et al, 2003).
Figure 4.2 Head position and orientation of the probe when examining the right common carotid artery.

The above illustration demonstrates the angle of interrogation of the right common carotid artery. This is performed in an anterolateral, lateral and posterolateral projection with the head tilted slightly in the contralateral direction. The exact opposite is performed when examining the left CCA.

(Figure taken from Stein JH et al, 2008).
Figure 4.3 Right common carotid artery angles of interrogation.

The illustration above demonstrates the different segments of the right carotid artery. From left to right are the internal carotid artery (ICA), the external carotid artery (ECA), the bifurcation (BIFUR) and the common carotid artery (CCA). The distal right CCA is examined from different angles of interrogation in the longitudinal plane and the figure depicts these as 90°, 120°, 150° and 180° (note our study did not examine from 180°).

(Figure taken from http://www.meijermedicalultrasound.com/media/doc/MMU%20%20ARC%20instructions%202010.pdf).

4.2.6.1 Ultrasound scanning protocol

Clear cineloops were obtained for approximately 5 seconds or 3-4 cardiac cycles of the right and left proximal and distal CCA, carotid bifurcation (BIF) and the internal carotid artery (ICA). The depth was set at 4cm and the focus was placed at the far wall for all cineloops. Dynamic range and gain were optimized for clear images of both the near and far wall of the artery being examined. The frame rate per cineloop was approximately 50 frames per second at 45 Hz. The acquired cineloops were exported in DICOM format and stored on a local network for later retrieval and offline analysis.
The following protocol was used for both the right and the left side:

1. Longitudinal scan of the most proximal segment of the CCA above the clavicle obtained at an angle that gave the clearest views of both the near and far wall (hereinafter referred to as the proximal CCA or CCA PROX).

2. Longitudinal scan of the CCA immediately prior to the bifurcation at 90, 120 and 150 degrees on the right side and 270, 240 and 210 degrees on the left side corresponding to posterolateral, lateral and anterolateral views respectively (hereinafter referred to as R CCA 90, 120, 150 and L CCA 270, 240 and 210). These projections are illustrated clearly in section 5.4 above.

3. Longitudinal scan of the bifurcation at an angle that gave the clearest views of the far wall proximal to the flow divider (hereinafter referred to as BIF). See figure 5.3 above.

4. Longitudinal scan of the internal carotid artery at an angle giving the clearest views of the near and far wall distal to the flow divider (hereinafter referred to as ICA).

5. Transverse scan of the proximal CCA just above the clavicle at an angle providing the clearest views of the near and far walls (hereinafter referred to as CCA TRANS PROX).

6. Transverse scan of the distal CCA immediately prior to the bifurcation at an angle providing the clearest views of the near and far walls (hereinafter referred to as CCA TRANS DIST).

It is important to note at this stage that clear images of the near wall were obtained for the purpose of measuring diameter only. Intima-media thickness of the near wall is deemed unreliable (as explained in section 1.5.9 of Chapter 1) and IMT measurements in this study were taken from the far wall.

On screen annotations provide the reader with information identifying each segment under analysis and the angle of insonation in the case of the CCA. The bifurcation is always positioned at the left side of the monitor with an on screen vertical marker line at the left side of the image positioned over the flow divider as illustrated in figure 4.4 below. This signifies the end of the bifurcation and the beginning of the ICA.
The segments of the carotid artery are illustrated below in Figure 4.4.

Figure 4.4 Segments of the carotid artery and positioning of the vertical marker.

Note the vertical marker positioned at the tip of the flow divider with the image selected for analysis of the right bifurcation.

**R BIF**=Right bifurcation, **ICA**=Internal carotid artery, **CCA**=Common carotid artery.

**R/B/NF**=Right side, bifurcation, near and far walls.
4.2.7 Measurements of common carotid artery, bifurcation and internal carotid artery intima-media thickness and lumen diameter from longitudinal images

A single observer (LK), blinded to all the individual participants data carried out all measurements of common carotid, bifurcation and internal carotid artery intima-media thickness and lumen diameter. Measurements were performed using Vascular Research Tools Carotid Analyser for Research Version 6.0.1 from Medical Imaging Applications, LLC 2012 ©. This is a validated software program that performs fully automated and semi-automated analysis of carotid ultrasound images and cineloops (Mancini GB et al, 2004). This software has been used in many large studies of carotid artery structure and function including the Muscatine Offspring Study (Dawson JD et al, 2009). In addition, a similar version of this software was recently approved by the FDA for use in the clinical setting. The Carotid Analyzer uses an automated method for near and/or far wall border and near and/or far wall intima border detection and vessel diameter as well as intima-media thickness measurement. When analyzing a sequence of images of the same vessel location, the method automatically learns properties of the analyzed vessel in one frame of the sequence that is analyzed under the operator’s supervision. The vessel properties are reflected in the cost function used in a graph-search-based border detection (Sonka M et al, 2007). The software tracks the near and far wall intima (I line) and media (M line) lines throughout numerous cardiac cycles performing a frame by frame analysis of the intima-media thickness and lumen diameters.

The images were sent from the iU22 ultrasound scanner to a PC in DICOM format for offline analysis. When a series of images is opened by the software calibration is performed prior to analysis. This was done in mm/pixel by manually selecting 2 calibration markers on the ultrasound image. The calibration markers used were the markers denoting centimetres on the right of
Following this, a region of interest (ROI) is placed over a 1 cm segment of artery to be analysed. This produces a rectangular box as illustrated in Figure 4.5 below.

Figure 4.5 Region of interest box placed over a longitudinal section of the proximal right CCA imaged above the clavicle.

The figure above illustrates a 1 cm region of interest box placed over the proximal CCA. Note the pink lines in the near and far walls represent the media lines or M lines, and the yellow lines represent the near and far wall intima lines or I lines. The ROI is selected at the start of the cineloop and the software subsequently performs automated near and far wall IMT and diameter measurements throughout the entire cineloop.

R CCA PROX = Right proximal common carotid artery. R/C/NF = Right side, common carotid artery, near and far walls.

If the near and far wall boundaries are not accurately detected by the software the operator can stop the cineloop and manually click on the correct boundary. The software subsequently makes a semi-automated adjustment and tracks this correct boundary for the remaining images in the cineloop.
The steps required to select a region of interest (ROI), identify the near and far wall boundaries to be measured and make the necessary adjustments to the intima line (I line) or the media line (M line) if desired prior to starting the tracking process are illustrated in Figure 4.6 below. Diameter is taken to be the distance between near and far wall I lines. IMT is taken from the far wall and is the distance between the I and M lines.
Figure 4.6 Detection of the media and intima interfaces.

(a) ROI box placed over a longitudinal segment the carotid artery for analysis.
(b) The software will display the media lines in red. In this example the software has incorrectly identified the near wall intima line as the media line. The arrow points to the correct media line interface. The far wall media line has been correctly identified.
(c) The user right clicks on the near wall media line and the red line is shifted to detect the correct interface. Again the near and far wall media lines are depicted in red.
(d) The near and far wall intima lines are correctly detected by the software and depicted as yellow lines. Media lines of near and far wall are depicted in red.

N=near wall. F=Far wall.
The M and I lines are tracked by the software throughout the cineloop at the end of which the relevant frames in end diastole and end systole are reviewed by the operator and corrected in a semi-automated fashion if necessary. Three sequential frames were chosen for end-diastole, with the first frame at the end of the R wave. In addition, three sequential frames were chosen for end-systole, with the first frame at the end of the T wave. An illustration of an edited frame of the R CCA at 150 degrees in end-diastole immediately after the peak of the R wave is shown in Figure 4.7 below.

![Edited frame of a cineloop taken of the distal right common carotid artery at 150 degrees during end-diastole.](image)

**Figure 4.7** Edited frame of a cineloop taken of the distal right common carotid artery at 150 degrees during end-diastole.

Note the R wave in the bottom left corner. The vertical marker is positioned at the start of the bifurcation and a 1cm ROI is placed at the distal CCA prior to the bulb. The I line is highlighted in yellow and the M line in pink. Far wall IMT is 0.55mm.

**R/C/NF**=Right, common carotid artery, near and far walls.
Figure 4.8 ROI box in the same patient zoomed up for illustrative purposes showing I and M lines of both the near and far wall of the distal CCA.

Figure 4.9 Image of the same segment of common carotid artery in the same subject taken during end systole.

Note the ECG in the bottom left corner with the cineloop stopped at the end of the T wave. **R/C/NF**=Right, common carotid artery, near and far walls.
Figure 4.10 Analysis of IMT in the bifurcation and in the internal carotid artery.

(a) Image of the bifurcation taken during end diastole. Note that the segment of far wall bifurcation is downward sloping in the image. The software automatically recognises this and has an angle correction built into the algorithm to account for it. (b) The same patient with analysis of the proximal segment of the ICA. Note the difficulty in gaining accurate IMT measurements in the near wall of the ICA. The M line is tracked with accuracy by the software, however the I line on the near wall is poorly visualised and therefore is not tracked. N=near wall, F=far wall.

As previously stated, 3 individual frames were measured following the end of the R wave for each segment analysed in end diastole. 3 measurements were also taken during the same cardiac cycle at the end of the T wave in end systole. Where possible, a total of 3 cardiac cycles were analysed in this way for each individual segment of artery imaged. The mean of the 3 measurements in both end diastole and end systole was taken as representative for the particular segment being analysed at the relevant stage of the cardiac cycle. The mean end diastole and end systole measurements of the 3 cardiac cycles were then taken as the final representative value.

Both far wall IMT and lumen diameter were measured for the CCA. With regards to the BIF and ICA only the far wall IMT was measured. This was due to the fact that many images of the near wall in these segments were
significantly degraded by noise and artefact. The IMT is defined as the thickness between the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface. The diameter is defined as the distance from the near wall blood-intima interface to the far wall blood-intima interface, or the distance between near and far wall I lines.

Transverse images were also performed of the right and left proximal and distal CCA as described above in section 4.4.1 (see Figure 4.11 below). This was performed in order to calculate vessel compliance at different segments of the CCA. Measurements of diameter were calculated as per all other cineloops.

Figure 4.11 Transverse images of the left distal common carotid artery in end diastole and end systole.

(a) Transverse image of the left distal CCA in end diastole illustrating the tracking of the near and far wall M and I lines. (b) Image of the same segment during end systole. This was performed to assess diameter change during the cardiac cycle and therefore assist in calculating vessel compliance. This was also performed for the proximal CCA facilitating comparisons in compliance at different segments of the CCA.
4.2.7.1 Carotid ultrasound end-points

4.2.7.2 Longitudinal measurements

The following measurements were made on longitudinal ultrasound images of the common carotid artery, the carotid bifurcation and the internal carotid artery:

Diameter measurements:

1. Proximal common carotid artery immediately above the clavicle.
2. Distal common carotid artery immediately prior to the bifurcation at 90, 120 and 150 degrees on the right side and 270, 240 and 210 degrees on the left side.

Intima media thickness measurements:

1. Proximal common carotid artery immediately above the clavicle.
2. Distal common carotid artery immediately prior to the bifurcation at 90, 120 and 150 degrees on the right side and 270, 240 and 210 degrees on the left side.
3. Carotid bifurcation.
4. Internal carotid artery.

4.2.7.3 Transverse measurements

The following measurements were made on transverse ultrasound images of the common carotid artery.

Diameter measurements:

1. Proximal common carotid artery immediately above the clavicle.
2. Distal common carotid artery immediately prior to the bifurcation.
Diameter was again measured in end diastole and end systole over 3 cardiac cycles as previously described with the mean value taken as representative.

The above longitudinal and transverse measurements were used to calculate the following.

Longitudinal:

1. Intima media thickness compression during the cardiac cycle.

Where \( \text{Diastolic IMT} - \text{Systolic IMT} \times 100 = \% \text{ IMT compression}. \)

Diastolic IMT

Transverse:

2. Cross-sectional compliance, CC (mm\(^2\)/kPa) = \(\Delta A/\text{PP}\)

3. Distensibility coefficient, DC (10\(^{-3}\)/kPa) = \((\Delta A/A_d)/\text{PP}\)

Where

\( A_d \) (mm\(^2\)) = \(\pi \times (LD_d/2)^2\)

\( A_s \) (mm\(^2\)) = \(\pi \times (LD_s/2)^2\)

\( \Delta A \) (mm\(^2\)) = \(A_s - A_d\)

All of the above measurements and calculations aided in achieving and answering the objectives and hypotheses listed in the following section.

4.2.8 Data handling / storage

Data was stored using Excel (Microsoft Excel 97, Microsoft Corporation, Redmond, WA, USA).
4.2.9 Statistical analysis

The above listed measures of arterial function are compared across the 3 groups using ANOVA.

Modelling was performed using a repeated measures, linear mixed effects model with the dependent variable of interest measured multiple times according to angle or vascular tree depending on which hypothesis was being tested. Individual participants formed the random intercept whilst pregnancy group, angle, and vascular tree were the fixed effects. The hypotheses we attempted to answer are outlined above.

For each of these hypotheses we were primarily interested in the interaction terms. The model with group and angle as fixed effects has pregnancy group x angle as its default interaction term. The model with group and vascular tree level as fixed effects has pregnancy group x vascular tree as its default interaction term. Statistical significance of the interaction term proves that the mean of the dependent variable differed across the interacting variables. For example in the first hypothesis, a statistically significant pregnancy group times angle interaction will demonstrate that the mean IMT values in the distal CCA differs significantly across pregnancy groups for different levels of the angle; or the means differ significantly across angles for each level of the pregnancy group.

Calculations were carried out using lmer function in R Core Team software version 3.1.2. 2015. We used a 5% level of significance to test the above hypotheses, all of which were considered to be two-sided.
4.3 Results

4.3.1 Participant characteristics of study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>GH</th>
<th>PET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35 ± 4</td>
<td>35 ± 5</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>Smoking habit</td>
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<tr>
<td>- current</td>
<td>2 (15%)</td>
<td>1 (8%)</td>
<td>0 (0%)</td>
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<tr>
<td>- ex-smoker</td>
<td>5 (38%)</td>
<td>3 (23%)</td>
<td>9 (64%)</td>
</tr>
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<td>- never</td>
<td>6 (46%)</td>
<td>9 (69%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Alcohol (units/week)</td>
<td>6 ± 6</td>
<td>5 ± 6</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>Exercise habit</td>
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</tr>
<tr>
<td>- none</td>
<td>4 (31%)</td>
<td>5 (38%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>- occasional</td>
<td>6 (46%)</td>
<td>5 (38%)</td>
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</tr>
<tr>
<td>- regular</td>
<td>3 (23%)</td>
<td>3 (23%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Salt added</td>
<td>8 (62%)</td>
<td>4 (31%)</td>
<td>10 (71%)</td>
</tr>
<tr>
<td>Fruit &amp; veg (portions/day)</td>
<td>3.69 ± 1.38</td>
<td>3.46 ± 1.27</td>
<td>4 ± 1.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67 ± 8</td>
<td>75 ± 18</td>
<td>75 ± 20</td>
</tr>
<tr>
<td>Height (cms)</td>
<td>168 ± 3</td>
<td>161 ± 5</td>
<td>166 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 3</td>
<td>29 ± 7</td>
<td>27 ± 8</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>121 ± 12</td>
<td>144 ± 19</td>
<td>133 ± 13</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>73 ± 9</td>
<td>84 ± 11</td>
<td>82 ± 9</td>
</tr>
<tr>
<td>Heart rate</td>
<td>62 ± 8</td>
<td>67 ± 16</td>
<td>66 ± 8</td>
</tr>
<tr>
<td>Parity</td>
<td>2.07 ± 0.64</td>
<td>1.77 ± 0.73</td>
<td>2.07 ± 0.73</td>
</tr>
</tbody>
</table>

Data are given as n (%) or mean ± standard deviation (SD). N=Normal, GH=Gestational hypertension, PET=Pre-eclampsia.
4.3.2 Cardiovascular risk factors of participants

Cardiovascular risk factors for the 40 participants are shown in Table 4.3

Table 4.3 Cardiovascular risk factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>GH</th>
<th>PET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker (current)</td>
<td>2 (15%)</td>
<td>1 (8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>5 (38%)</td>
<td>7 (54%)</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1 (8%)</td>
<td>5 (38%)</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Family history of ischaemic heart disease</td>
<td>5 (38%)</td>
<td>5 (38%)</td>
<td>7 (50%)</td>
</tr>
</tbody>
</table>

Data are given as n (%). N = Normal, GH = Gestational hypertension, PET = Pre-eclampsia.
4.3.3 Biochemical characteristics of participants

Biochemical characteristics of the 40 participants are shown in Table 4.4

**Table 4.4 Biochemical characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>GH</th>
<th>PET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.59 ± 0.93</td>
<td>5.11 ± 0.77</td>
<td>5.03 ± 0.93</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.65 ± 0.42</td>
<td>4.49 ± 0.37</td>
<td>4.8 ± 0.30</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>65 ± 10</td>
<td>57 ± 10</td>
<td>59 ± 6</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD. N=Normal, GH=Gestational hypertension, PET=Pre-eclampsia.

From the preceeding tables in can be seen that 15% of the normotensive group were current smokers, with only 8% in the GH group and no current smokers in the PET group. However, 64% of the PET group admitted to being ex-smokers. When looking at the general health of the 3 groups we see that the GH and PET group have a raised BMI of 29 ± 7 and 27 ± 8 (mean ± SD) respectively. Mean systolic BP was also slightly raised in the 2 groups being 144 ± 19 mmHg in the GH group and 133 ± 13 mmHg in the PET group. Additionally, both groups had mean cholesterol levels greater than 5 mmol/l, albeit only slightly above the recommended limit. Those with normotensive pregnancies, demonstrated mean values of the aforementioned parameters within the recommended reference ranges, and therefore it can be assumed that they were in better overall health.
4.3.4 Hypothesis 1

Intima-media thickness distribution in the distal common carotid

The first hypothesis that was tested was that women with a history of pre-eclampsia demonstrate different IMT values according to angle in the distal CCA than women with a history of gestational hypertension or normotensive pregnancies.

Figure 4.12 below illustrates the mean IMT values across the 3 groups as analysed in the distal CCA according to angle. This is shown for both right and left sides and also for mean IMT values when the corresponding values on the right and left side are combined i.e. the combined mean values of 90° on the right and its corresponding angle of 270° on the left (corresponding to the anteromedial wall) etc.

The least squares estimates of means, corresponding standard errors and 95% confidence intervals for the highest level interactions between the fixed effects (pregnancy group and angle) are listed in table 4.5 below.

ANOVA found the main effect, angle, and the interactive effect, pregnancy group x angle, to be statistically significant. Hence it can be concluded that IMT is thicker in the anteromedial and medial walls compared to the posteromedial walls in all women, and that these differences according to angle are greatest in women with a history of preeclampsia.
Figure 4.12 Plots illustrating the mean CIMT values with error bars, representing the 95% confidence interval for the mean, for the 3 groups according to (a) angle and side, and (b) according to angle alone when both right and left mean CIMT values are combined.
Table 4.5 Intima-media thickness according to angle in the distal common carotid artery across the 3 groups

Data are given as mean ± SE with 95% confidence intervals (CI)  
N=Normal, GH=Gestational hypertension, PET=Pre-eclampsia.

<table>
<thead>
<tr>
<th>Group</th>
<th>Angle</th>
<th>Mean IMT (mm)</th>
<th>SE (mm)</th>
<th>Lower CI (mm)</th>
<th>Upper CI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>Anteromedial wall</td>
<td>0.6286</td>
<td>0.0150</td>
<td>0.5983</td>
<td>0.6589</td>
</tr>
<tr>
<td>GH</td>
<td>Anteromedial wall</td>
<td>0.6118</td>
<td>0.0154</td>
<td>0.5806</td>
<td>0.6431</td>
</tr>
<tr>
<td>NORMAL</td>
<td>Anteromedial wall</td>
<td>0.6049</td>
<td>0.0154</td>
<td>0.5737</td>
<td>0.6360</td>
</tr>
<tr>
<td>PET</td>
<td>Medial wall</td>
<td>0.6334</td>
<td>0.0149</td>
<td>0.6032</td>
<td>0.6636</td>
</tr>
<tr>
<td>GH</td>
<td>Medial wall</td>
<td>0.5977</td>
<td>0.0155</td>
<td>0.5664</td>
<td>0.6290</td>
</tr>
<tr>
<td>NORMAL</td>
<td>Medial wall</td>
<td>0.6118</td>
<td>0.0154</td>
<td>0.5806</td>
<td>0.6430</td>
</tr>
<tr>
<td>PET</td>
<td>Posteromedial wall</td>
<td>0.6121</td>
<td>0.0149</td>
<td>0.5820</td>
<td>0.6423</td>
</tr>
<tr>
<td>GH</td>
<td>Posteromedial wall</td>
<td>0.5859</td>
<td>0.0156</td>
<td>0.5545</td>
<td>0.6173</td>
</tr>
<tr>
<td>NORMAL</td>
<td>Posteromedial wall</td>
<td>0.6023</td>
<td>0.0155</td>
<td>0.5710</td>
<td>0.6336</td>
</tr>
</tbody>
</table>
4.3.5 Hypothesis 2

Intima-media thickness distribution along the carotid vascular tree

This hypothesis tested whether women with a history of pre-eclampsia demonstrate different IMT values along the vascular tree when compared to women with a history of gestational hypertension or normotensive pregnancies. We examined the IMT in the proximal CCA, distal CCA, carotid bulb and the ICA.

The mean IMT values for the 3 groups along the vascular tree on both the right and left sides, and also with the combined mean IMT values of the right and left sides are shown in figure 4.13 below.

The least squares estimates of means, corresponding standard errors and 95% confidence intervals for the highest level interactions between the fixed effects (pregnancy group and vascular tree) are listed in table 4.6 below.

ANOVA found the main effect vascular tree, and interactive effects pregnancy group x vascular tree, to be statistically significant. These differences are greatest within the PET group who demonstrated greater IMT in the proximal CCA, distal CCA and the ICA. No difference in IMT was found at the bifurcation. There is also a stepwise increase in IMT along the vascular tree, with it being thinnest in the proximal CCA and gradually increasing in thickness within the distal CCA and the bifurcation. For the GH and the normotensive groups the IMT then decreases in the ICA relative to the bifurcation. However in the PET group there is a further increase of the IMT within the ICA. Hence, it can be concluded that those with PET have different and greater values of IMT along certain levels of the vascular tree than those with GH or normotensive pregnancies.
Figure 4.13 Plots illustrating the mean CIMT values with error bars representing the 95% confidence interval for the mean, across the 3 groups according to (a) vascular tree and side, and (b) according to vascular tree alone when both right and left mean CIMT values are combined. CCA PROX=Proximal CCA, CCA DIST=Distal CCA, BIF=Bifurcation, ICA=Internal carotid artery.
Table 4.6 Intima-media thickness according to vascular tree across the 3 groups

Data are given as mean ± SE with 95% confidence intervals (CI)  

N=Normal, GH=Gestational hypertension, PET=Pre-eclampsia.

ICA=Internal Carotid artery, BIF=Bifurcation, CCA=Distal common carotid artery, CCA PROX=Proximal common carotid artery

<table>
<thead>
<tr>
<th>Group</th>
<th>Vascular tree</th>
<th>Mean IMT (mm)</th>
<th>SE (mm)</th>
<th>Lower CI (mm)</th>
<th>Upper CI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>ICA</td>
<td>0.6895</td>
<td>0.0162</td>
<td>0.6572</td>
<td>0.7217</td>
</tr>
<tr>
<td>GH</td>
<td>ICA</td>
<td>0.5780</td>
<td>0.0162</td>
<td>0.5455</td>
<td>0.6104</td>
</tr>
<tr>
<td>NORMAL</td>
<td>ICA</td>
<td>0.5802</td>
<td>0.0159</td>
<td>0.5483</td>
<td>0.6121</td>
</tr>
<tr>
<td>PET</td>
<td>BIF</td>
<td>0.6721</td>
<td>0.0154</td>
<td>0.6413</td>
<td>0.7029</td>
</tr>
<tr>
<td>GH</td>
<td>BIF</td>
<td>0.6626</td>
<td>0.0156</td>
<td>0.6313</td>
<td>0.6938</td>
</tr>
<tr>
<td>NORMAL</td>
<td>BIF</td>
<td>0.6801</td>
<td>0.0157</td>
<td>0.6485</td>
<td>0.7117</td>
</tr>
<tr>
<td>PET</td>
<td>CCA</td>
<td>0.6254</td>
<td>0.0144</td>
<td>0.5963</td>
<td>0.6544</td>
</tr>
<tr>
<td>GH</td>
<td>CCA</td>
<td>0.6010</td>
<td>0.0145</td>
<td>0.5717</td>
<td>0.6303</td>
</tr>
<tr>
<td>NORMAL</td>
<td>CCA</td>
<td>0.6055</td>
<td>0.0148</td>
<td>0.5755</td>
<td>0.6355</td>
</tr>
<tr>
<td>PET</td>
<td>CCA PROX</td>
<td>0.5833</td>
<td>0.0149</td>
<td>0.5533</td>
<td>0.6134</td>
</tr>
<tr>
<td>GH</td>
<td>CCA PROX</td>
<td>0.5411</td>
<td>0.0150</td>
<td>0.5109</td>
<td>0.5714</td>
</tr>
<tr>
<td>NORMAL</td>
<td>CCA PROX</td>
<td>0.5387</td>
<td>0.0155</td>
<td>0.5075</td>
<td>0.5699</td>
</tr>
</tbody>
</table>
4.3.6 Hypothesis 3

Intima-media thickness compression patterns in the distal common carotid artery

Intima-media thickness compression throughout the cardiac cycle was measured in terms of the difference in IMT values, measured in mm, between the diastolic and systolic measurements and expressed in terms of percentage of IMT compression during the cardiac cycle.

Figure 4.14 below illustrates IMT % compression differences across the 3 groups according to angle.

Table 4.7 below demonstrates IMT compression patterns measured in percentage IMT compression throughout the cardiac cycle according to angle in the distal CCA.

ANOVA found the main effect angle and the interaction effects group x angle to be significant. The PET group had the greatest IMT compression amongst the 3 groups within the posteromedial and medial walls, with the GH group demonstrating a comparatively slightly increased IMT compression within the anteromedial wall. The 3 groups demonstrated greatest % compression in the posteromedial wall, then the medial wall, with the anteromedial wall having the least amount of IMT compression (Figure 4.15 (b)). This was true for all groups and angles apart from the GH group within the anteromedial wall where % compression was greatest in this group. Hence it can be concluded that IMT compression in PET differs from GH or Normal groups according to angle and that compression is greatest in the PET group within the posteromedial and medial walls.
Figure 4.14 Plots illustrating the mean % compression differences in IMT values between diastole and systole, with error bars representing the 95% confidence interval for the mean, across the 3 groups according to (a) angle and side, and (b) according to angle alone when the mean IMT differences are combined for left and right.
Table 4.7 Intima-media thickness compression patterns according to angle measured as percentage compression in the distal CCA

Data are given as mean ± SE with 95% confidence intervals (CI)

N=Normal, GH=Gestational hypertension, PET=Pre-eclampsia.

<table>
<thead>
<tr>
<th>Group</th>
<th>Angle</th>
<th>Mean IMT % compression</th>
<th>SE (%)</th>
<th>Lower CI (%)</th>
<th>Upper CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>Anteromedial wall</td>
<td>-2.4723</td>
<td>0.4340</td>
<td>-3.3341</td>
<td>-1.6105</td>
</tr>
<tr>
<td>GH</td>
<td>Anteromedial wall</td>
<td>-3.2604</td>
<td>0.4188</td>
<td>-4.0956</td>
<td>-2.4252</td>
</tr>
<tr>
<td>NORMAL</td>
<td>Anteromedial wall</td>
<td>-1.9421</td>
<td>0.4075</td>
<td>-2.7561</td>
<td>-1.1280</td>
</tr>
<tr>
<td>PET</td>
<td>Medial wall</td>
<td>-4.1802</td>
<td>0.4161</td>
<td>-5.0085</td>
<td>-3.3519</td>
</tr>
<tr>
<td>GH</td>
<td>Medial wall</td>
<td>-2.6116</td>
<td>0.4268</td>
<td>-3.4618</td>
<td>-1.7613</td>
</tr>
<tr>
<td>NORMAL</td>
<td>Medial wall</td>
<td>-2.8357</td>
<td>0.4189</td>
<td>-3.6709</td>
<td>-2.0004</td>
</tr>
<tr>
<td>PET</td>
<td>Posteromedial wall</td>
<td>-4.4354</td>
<td>0.4144</td>
<td>-5.2605</td>
<td>-3.6102</td>
</tr>
<tr>
<td>GH</td>
<td>Posteromedial wall</td>
<td>-3.0769</td>
<td>0.4476</td>
<td>-3.9661</td>
<td>-2.1878</td>
</tr>
<tr>
<td>NORMAL</td>
<td>Posteromedial wall</td>
<td>-3.6238</td>
<td>0.4273</td>
<td>-4.4749</td>
<td>-2.7728</td>
</tr>
</tbody>
</table>
4.3.7 Hypothesis 4

Intima-media thickness compression patterns along the vascular tree

This hypothesis tested if women with a history of pre-eclampsia demonstrate different IMT compression along the vascular tree than those with a history of gestational hypertension or normotensive pregnancies.

Figure 4.15 below illustrates the IMT compression differences expressed in terms of % compression across the 3 groups according to vascular tree.

Table 4.8 illustrates IMT % compression along the vascular tree.

The PET group illustrated different IMT % compression patterns according to vascular tree when compared with the GH and normotensive group. The pattern of compression in all 3 groups increases as we move along the vascular tree from proximal to distal CCA with further increased compression in the bifurcation. The IMT compression then slightly decreases within the ICA relative to the bifurcation. The PET group demonstrates the greatest compression in the distal CCA but not within the bifurcation or ICA.

Using ANOVA we found that main effect vascular tree and interactions group x vascular tree to be significantly different. Hence, women with PET do illustrate different IMT compression percentages, according to vascular tree when compared to women with GH or normotensive pregnancies.
Figure 4.15 Plots illustrating the mean IMT % compression difference, with error bars representing the 95% confidence interval for the mean, across the 3 groups according to (a) vascular tree and side, and (b) according to vascular tree alone when the mean IMT differences are combined for left and right. CCA PROX=Proximal CCA, CCA DIST=Distal CCA, BIF=Bifurcation, ICA=Internal carotid artery.
Table 4.8 Intima-media thickness % compression along the vascular tree according to the 3 groups

Data are given as mean ± SE with confidence intervals (CI)

N=Normal, GH=Gestational 95% hypertension, PET=Pre-eclampsia.

ICA=Internal Carotid artery, BIF=Bifurcation, CCA=Distal common carotid artery, CCA PROX=Proximal common carotid artery

<table>
<thead>
<tr>
<th>Group</th>
<th>Vascular tree</th>
<th>Mean IMT % compression</th>
<th>SE (%)</th>
<th>Lower CI (%)</th>
<th>Upper CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>ICA</td>
<td>-3.5099</td>
<td>0.5704</td>
<td>-4.6316</td>
<td>-2.3882</td>
</tr>
<tr>
<td>GH</td>
<td>ICA</td>
<td>-4.3400</td>
<td>0.5700</td>
<td>-5.4613</td>
<td>-3.2188</td>
</tr>
<tr>
<td>NORMAL</td>
<td>ICA</td>
<td>-3.0327</td>
<td>0.4922</td>
<td>-4.0037</td>
<td>-2.0618</td>
</tr>
<tr>
<td>PET</td>
<td>BIF</td>
<td>-4.0807</td>
<td>0.4784</td>
<td>-5.0243</td>
<td>-3.1372</td>
</tr>
<tr>
<td>GH</td>
<td>BIF</td>
<td>-4.6371</td>
<td>0.4999</td>
<td>-5.6223</td>
<td>-3.6518</td>
</tr>
<tr>
<td>NORMAL</td>
<td>BIF</td>
<td>-5.0976</td>
<td>0.4684</td>
<td>-6.0225</td>
<td>-4.1727</td>
</tr>
<tr>
<td>PET</td>
<td>CCA</td>
<td>-3.7813</td>
<td>0.3418</td>
<td>-4.4667</td>
<td>-3.0959</td>
</tr>
<tr>
<td>GH</td>
<td>CCA</td>
<td>-3.0758</td>
<td>0.3487</td>
<td>-3.7751</td>
<td>-2.3765</td>
</tr>
<tr>
<td>NORMAL</td>
<td>CCA</td>
<td>-2.7575</td>
<td>0.3421</td>
<td>-3.4459</td>
<td>-2.0692</td>
</tr>
<tr>
<td>PET</td>
<td>CCA PROX</td>
<td>-2.1766</td>
<td>0.4233</td>
<td>-3.0143</td>
<td>-1.3389</td>
</tr>
<tr>
<td>GH</td>
<td>CCA PROX</td>
<td>-2.6692</td>
<td>0.4374</td>
<td>-3.5340</td>
<td>-1.8045</td>
</tr>
<tr>
<td>NORMAL</td>
<td>CCA PROX</td>
<td>-2.1609</td>
<td>0.4393</td>
<td>-3.0301</td>
<td>-1.2917</td>
</tr>
</tbody>
</table>
4.3.8 Hypothesis 5

Compliance and distensibility patterns in the common carotid artery

This hypothesis examined whether women with a history of pre-eclampsia demonstrate different compliance and distensibility patterns within the proximal and distal CCA than those with a history of GH or normotensive pregnancy.

Figures 4.16 and 4.17 below illustrate the mean compliance and distensibility coefficients respectively within the CCA across the 3 groups as measured in the proximal CCA and the distal CCA.

Tables 4.9 and 4.10 below illustrate the mean compliance coefficients (CC) and distensibility coefficients (DC) respectively within the proximal and distal CCA.

Using ANOVA we found compliance and distensibility coefficients demonstrated significantly different values in the PET group when compared with the GH and normotensive groups. This was most noticeable within the distal CCA where the GH group had the stiffest vessels, followed by the PET group with the normotensive group demonstrating greatest compliance and distensibility. The CCA was less compliant in the proximal CCA than in the distal CCA for all groups. Distensibility increased from the proximal CCA to the distal CCA in the normotensive group. However, in the PET group there was only a minimal increase in distension when compared with the proximal CCA, and in the GH group there was actually a slight decrease in distal CCA distensibility when compared with the proximal CCA. It is clear from our results that both the GH and PET groups demonstrated increased stiffness in the form of less compliant and distensible arteries in the distal CCA when compared to normotensives.
Figure 4.16 Plots illustrating the mean compliance coefficient with error bars, representing the 95% confidence interval for the mean, across the 3 groups within the proximal CCA and the distal CCA for (a) both right and left sides, and (b) according to vascular tree alone. CCA TRANS PROX=Proximal CCA transverse, CCA TRANS DIST=Distal CCA transverse.
Table 4.9 Mean compliance coefficients within the proximal and distal CCA

Data are given as mean ± SE with N=Normal, GH=Gestational 95% confidence intervals (CI) hypertension, PET=Pre-eclampsia.

CCA TRANS DIST=Distal CCA transverse, CCA TRANS PROX=Proximal CCA transverse

<table>
<thead>
<tr>
<th>Group</th>
<th>Vascular tree</th>
<th>Mean CC mm²/kPa⁻¹</th>
<th>SE mm²/kPa⁻¹</th>
<th>Lower CI mm²/kPa⁻¹</th>
<th>Upper CI mm²/kPa⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>CCA TRANS DIST</td>
<td>0.9238</td>
<td>0.0674</td>
<td>0.7877</td>
<td>1.0598</td>
</tr>
<tr>
<td>GH</td>
<td>CCA TRANS DIST</td>
<td>0.7054</td>
<td>0.0698</td>
<td>0.5645</td>
<td>0.8462</td>
</tr>
<tr>
<td>NORMAL</td>
<td>CCA TRANS DIST</td>
<td>1.0954</td>
<td>0.0693</td>
<td>0.9555</td>
<td>1.2353</td>
</tr>
<tr>
<td>PET</td>
<td>CCA TRANS PROX</td>
<td>0.8035</td>
<td>0.0668</td>
<td>0.6685</td>
<td>0.9385</td>
</tr>
<tr>
<td>GH</td>
<td>CCA TRANS PROX</td>
<td>0.6627</td>
<td>0.0694</td>
<td>0.5226</td>
<td>0.8029</td>
</tr>
<tr>
<td>NORMAL</td>
<td>CCA TRANS PROX</td>
<td>0.7593</td>
<td>0.0694</td>
<td>0.6190</td>
<td>0.8995</td>
</tr>
</tbody>
</table>
Figure 4.17 Plots illustrating the mean distensibility coefficient with error bars, representing the 95% confidence interval for the mean, across the 3 groups within the proximal CCA and the distal CCA for (a) both right and left sides, and (b) according to vascular tree alone. CCA TRANS PROX=Proximal CCA transverse, CCA TRANS DIST=Distal CCA transverse.
Table 4.10 Mean distensibility coefficients within the proximal and distal CCA

Data are given as mean ± SE with confidence intervals (CI). DC=distensibility coefficient

N=Normal, GH=Gestational 95% hypertension, PET=Pre-eclampsia.

CCA TRANS DIST=Distal CCA transverse, CCA TRANS PROX=Proximal CCA transverse

<table>
<thead>
<tr>
<th>Group</th>
<th>Vascular tree</th>
<th>DC $10^{-3}$/kPa$^{-1}$</th>
<th>SE $10^{-3}$/kPa$^{-1}$</th>
<th>Lower CI mm$^2$/kPa$^{-1}$</th>
<th>Upper CI mm$^2$/kPa$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>CCA TRANS DIST</td>
<td>27.1434</td>
<td>1.8192</td>
<td>23.4771</td>
<td>30.8097</td>
</tr>
<tr>
<td>GH</td>
<td>CCA TRANS DIST</td>
<td>22.3027</td>
<td>1.8811</td>
<td>18.5103</td>
<td>26.0952</td>
</tr>
<tr>
<td>NORMAL</td>
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4.4 Discussion

The IMT in the distal CCA was significantly different across all angles of interrogation in the PET group when compared with the other 2 groups, and the PET group also demonstrated greater IMT values across all angles, with IMT being thicker in the anteromedial and medial walls in all groups.

We also found the PET group to have significantly different and greater values of IMT along certain levels of the vascular tree than those with GH or normotensive pregnancies, notably within the proximal CCA and the ICA.

IMT compression throughout the cardiac cycle in the PET group differs significantly from GH or Normal groups according to angle, with compression greatest in the PET group within the posteromedial and medial walls. Women with PET also illustrate significantly different IMT compression according to vascular tree when compared to women with GH or normotensive pregnancies.

Both the GH and PET groups demonstrated significantly increased vascular stiffness in the form of less compliant and distensible arteries in the distal CCA when compared to normotensives.

If we consider our analysis of the IMT in the distal CCA according to angle, our results are in line with a study carried out by Tajik et al in 2011. This group demonstrated an asymmetrical and helical like distribution of atherosclerosis in the carotid artery by examining the carotid arteries of 3364 subjects across 4 multicenter international trials (Tajik et al, 2011). Ultrasound was performed at 10 different angles of interrogation using the Meijer arc. In the distal CCA we found similar patterns of asymmetrical IMT distribution in the anteromedial, medial and posteromedial walls as in this large study. The IMT across all angles was statistically different in the PET group than the other 2 groups, and demonstrated greater IMT values across the majority of angles for both right and left sides albeit not at a significant level. All angles demonstrated greatest IMT in the PET group. When angles are combined for an overall average IMT value in the distal CCA the PET group had a greater IMT value than the other
groups, albeit not at a statistically significant level. This is in line with previous studies demonstrating increased CIMT in those with pre-eclampsia (Blaauw J et al, 2006). To our knowledge however this is the first study to examine the distribution of IMT according to angle in such a group.

In our study there is a stepwise increase in IMT as we travel distally along the vascular tree. This was previously described by Espeland MA, et al in 1994. This group examined the carotid arteries of 899 asymptomatic low-risk participants aged between 40 and 79. They demonstrated that IMT increases gradually in the common carotid artery, peaks within the bifurcation and then decreases gradually within the ICA (Espeland MA, et al 1994). Our results mirror this pattern in the GH and normotensive group and also in the PET group as far as the bifurcation. The further stepwise increase of IMT within the ICA in the PET group may be suggestive of an early atherosclerotic process in this asymptomatic group of women. To our knowledge this is the first study to demonstrate the differences in IMT along the vascular tree in such a group of women.

In phase 1 of this cohort study performed by Dr Catherine Brown (unpublished data) the IMT value for the PET group in the distal CCA was 0.46 ± 0.05 mm (mean ± SE). Our results demonstrate IMT in this group to be 0.6254 ± 0.0144 mm (mean ± SE). The between study difference in IMT values may be explained by smaller numbers in the follow-up study, difference in technique and equipment with more measurements taken at 3 different angles in phase 2, or indeed progression in IMT over time.

The patterns of IMT within the distal CCA according to angle and within the vascular tree are due to the local haemodynamic forces acting within the artery resulting in atherosclerosis formation at branch points, the outer walls of the bifurcation and the inner walls of curvatures. These are regions where endothelial shear stress (ESS) is lowest and tensile stress greatest. ESS also has an impact on NO, which is also involved in the generation of atherosclerosis and has been explored in detail in chapter 1.

This pattern of increasing IMT compression from the anteromedial wall to the posteromedial wall does not follow the overall pattern of IMT thickness being
greatest in the anteromedial and medial walls. One explanation for this would be the fact that the exact anatomical orientation of the carotid bifurcation and its location varies somewhat between individuals and would therefore produce some variation in haemodynamics at the bifurcation between individuals (Masawa et al, 1994). A greater arterial expansion is likely to result in greater thinning of the artery and resultant increased IMT compression. This of course would be extremely difficult to prove in practice, however the pattern of increasing compression from the anteromedial wall to the posteromedial wall is interesting, and as seen with different IMT values in the distal CCA according to angle, is likely related to differences in shear stress and local haemodynamics.

The fact that the PET group had the greatest IMT compression amongst the 3 groups may also be secondary to possible alterations in the structural and biomechanical properties of the arterial wall that may exist within this group. This would provide a similar explanation to that which Polak et al explored in 2012. They found a positive relationship between IMT compression and pulse pressure. This group hypothesized altered wall mechanics resulted in increased IMT compression due to greater arterial expansion in the radial direction and therefore greater compression of the IMT. However they also noted a positive relationship between age and LDL levels and IMT compression. They found this relationship difficult to explain as age and LDL are associated with increased arterial stiffness (Polak et al, 2012).

The pattern of increasing IMT compression as we travel distally along the vascular tree, with peak compression occurring within the bifurcation, is likely secondary to haemodynamic factors. These factors include relatively high shear stress and low tensile stress in the proximal CCA and decreased shear stress and high tensile stress within the distal CCA and bifurcation.

To our knowledge this is the first study to examine the IMT compression patterns according to interrogation angle in the distal CCA and along the vascular tree in such a group of women.

Compliance and distensibility coefficients demonstrated significantly different values in the PET group when compared with the GH and normotensive
groups, most noticeably within the distal CCA where the GH group had the stiffest vessels, followed by the PET group with the normotensive group demonstrating greatest compliance and distensibility. The CCA was less compliant in the proximal CCA than in the distal CCA for all groups, likely secondary to haemodynamic forces in the distal CCA being more turbulent in this region when compared with the proximal CCA where there is more laminar blood flow. The distending pressure in the distal CCA will exert a greater radial tensile stress, thereby resulting in an increase in coefficient values closer to the bifurcation. As explained in section 1.6.2, the pressure wave increases as we travel distally along the vascular tree, which may also explain the overall increase in compliance coefficients in the distal CCA. Our results show that both the GH and PET groups demonstrated increased stiffness in the form of less compliant and distensible arteries in the distal CCA when compared to normotensives. This is suggestive of an early atherosclerotic process occurring in these women. Interestingly, the pulse pressure, an indirect marker of arterial stiffness, in the GH group was 60 ± 14 mmHg and in the PET group 51 ± 7 mmHg (mean ± SD). Pulse pressure in the normotensive group was the lowest among the groups at 48 ± 6 mmHg. Systolic blood pressure, another indirect marker for arterial stiffness, was also greater in the GH and PET groups as seen in table 6.1 above, further suggesting an overall increase in arterial stiffness in these groups when compared to the normotensive group.

The study had a number of limitations including relatively low numbers. Although 124 women were invited to participate only 40 agreed to take part. The relatively low numbers were offset by the extensive analysis of the carotid arteries however, with approximately 16,000 individual images analysed between the 40 study participants.

Another limitation is that brachial blood pressure was used to calculate pulse pressure, which was in turn used to determine carotid artery compliance and distensibility. This method has been used by other groups to calculate arterial stiffness and gives a reasonable estimation of pulse pressure (Van Dijk RA et al, 2000), however, it may in fact overestimate stiffness in the peripheral arteries (Laurent S et al, 2006). Applanation tonometry used to estimate local arterial pulse pressure provides a more accurate estimate of stiffness.
4.4.1 Future work

Those with a history of pre-eclampsia are at increased risk of future cardiovascular events and our results demonstrate they have increased IMT when compared to those without such a history. These women should be advised of this increased risk and prescribed optimal cardiovascular protective lifestyles and therapies such as BP lowering and statin therapy.

Future studies should take into account the asymmetrical distribution of IMT in the distal CCA, as a single IMT measurement from a single or inconsistent angle will no doubt make IMT measurements less reliable, accurate or indeed representative. As such multiple measurements taken from at least 3 angles of interrogation are recommended.

Further studies should be performed on those with pre-eclampsia, or indeed other asymptomatic groups who have an increased risk of future cardiovascular events, to examine whether the IMT in the ICA increases relative to the bifurcation and CCA and what impact this may have on their future cardiovascular health.

If future studies are to examine IMT compression patterns and stiffness, the measurement of local arterial blood pressure using tonometry may be helpful to assess if this has a significant influence. In particular the use of applanation tonometry in our study may have been able to show local pressure differences within the proximal and distal common carotid artery, thus providing a possible explanation for the increased compliance patterns observed in the distal CCA when compared to the proximal CCA. Novel methods of measuring local arterial stiffness such as PWI, UltraFast imaging, shear wave elastography, speckle tracking and other emerging techniques as discussed in section 1.7, may further our understanding of local arterial stiffness in asymptomatic groups. They may also aid in our understanding of IMT compression differences according to angle in the distal CCA and along the vascular tree. Indeed, if there is to be a follow up study of these patients, perhaps using a novel ultrasound approach such as speckle tracking imaging could be employed to assess arterial stiffness and IMT distribution in these groups of
women. This is sure to further our understanding of early subclinical atherosclerosis in such asymptomatic groups.

### 4.4.2 Conclusion

We demonstrated different IMT values according to angle in the distal CCA between the PET, GH and normotensive groups and a pattern of IMT distribution in the distal CCA with the medial wall having the greatest IMT values amongst all 3 groups. This was statistically significant, and provides further evidence that those with a history of pre-eclampsia demonstrate greater IMT than those without such a history, and as such, may put them at increased risk of developing future cardiovascular events. There is also a stepwise increase in IMT values along the carotid vascular tree that peaks in the bifurcation and then decreases in the ICA. This was true for all groups except the PET group who demonstrated a further increase in IMT in the ICA. The PET group had significantly different IMT values and greater IMT differences along the vascular tree when compared with the GH and normotensive groups. These patterns of IMT distribution have been demonstrated in previous studies however, to our knowledge never in such a group of asymptomatic women. Also the stepwise increase in IMT in the ICA may signify an accelerated atherosclerotic process in the PET group and warrants further evaluation in other pre-eclampsia sufferers and perhaps similar asymptomatic groups. The percentage compression of IMT throughout the cardiac cycle was significantly different amongst the 3 groups in both the distal CCA according to angle and along the vascular tree. The IMT demonstrated increased compression from the anteromedial to the posteromedial wall and also from the proximal CCA to the distal CCA and bifurcation with subsequent decreasing compression in the ICA. The PET group had the greater compression in the distal CCA. IMT compression patterns are not easily explained from our study but we hypothesise they may be secondary to anatomical variations, haemodynamic factors or perhaps structural and biochemical properties within the vessel wall. Both carotid compliance and distensibility were significantly different amongst
the 3 groups with the GH and PET groups demonstrating increased vascular stiffness in comparison to the normotensive groups. This is suggestive of an early atherosclerotic process which again, may put these women at risk for future cardiovascular events. Both IMT compression and arterial stiffness should be further examined in future studies using more novel non-invasive ultrasonic techniques measuring arterial stiffness and vascular wall mechanics.
Chapter 5. Conclusion
Conclusions

This thesis attempted to improve our understanding and detection of early vascular disease using ultrasound and consisted of 2 ultrasound based studies. The first study compared an established 2D US based method of assessing flow-mediated dilatation with a novel 3D US approach. The second study used US to assess for subtle differences in vascular structure and function in women with and without a history of pre-eclampsia or gestational hypertension.

Throughout this process I have gained valuable knowledge on how to design a research project. This included gaining ethics approval and the recruitment of participants. It also involved meeting with hardware providers and sourcing the best available analysis software. There was extensive background reading required to understand 2D and 3D ultrasound, endothelial dysfunction, atherosclerosis and its non-invasive methods of assessment. Furthermore, knowledge of pre-eclampsia, intima-media thickness and arterial stiffness and their non-invasive means of assessment were also required.

In order to complete the studies, it was necessary to be proficient in ultrasound techniques. Having had no prior US experience, this meant a steep learning curve to progress to a level capable of performing consistent vascular US prior to commencing the examinations. This process has no doubt helped me in my career as a radiologist.

Following the US studies came extensive image analysis and compilation of the results. This gave me a valuable insight into statistical analysis and in particular ANOVA.

Flow-mediated dilatation is an endothelial dependant process reflecting the dilatation of a conduit artery when exposed to increased blood flow and subsequently increased shear stress. It requires an intact and healthy endothelium and therefore is suppressed in those with cardiovascular risk factors. 2D FMD is limited as a research tool due to difficulties in assessment of true arterial diameter, reproducibility and technical difficulties. We
hypothesised that 3D US would eliminate probe malalignment errors that occur in 2D resulting in underestimation of arterial diameter. We also tested to see if 3D FMD was more reproducible. Our findings suggest that 3D gives a greater and more accurate measurement of arterial diameter. This should be confirmed in future bench studies comparing diameter measurements of arterial phantoms using 2D and 3D US. We found similar 2D and 3D FMD reproducibility.

We also looked to assess for subtle alterations in the vascular structure and function in young women with and without a history of pre-eclampsia or gestational hypertension. Pre-eclampsia is associated with increased future cardiovascular risk including increased IMT and arterial stiffness. Using 2D US we assessed for alterations in the distribution of IMT, and for IMT compression patterns, at multiple angles of insonation in the distal CCA and along the carotid arterial tree. We found the PET group had greater IMT in areas of the arterial tree with a predilection for atherosclerosis i.e. the medial wall and the ICA. In addition to the gestational hypertension group, they also demonstrated increased arterial stiffness in the distal CCA when compared to the normotensive group. These findings suggest an accelerated atherosclerotic process and provide further evidence that having a history of pre-eclampsia may put these asymptomatic women at future risk of cardiovascular events. The IMT compression patterns in the PET group differed according to vascular tree and angle of insonation. The reasons for this are not easily explained however they may be secondary to anatomical variations, haemodynamic factors or perhaps structural and biochemical properties within the vessel wall. Our extensive US examination of the carotid arterial tree supports other studies assessing carotid vascular structure and function and provides a unique detailed assessment of how the artery is affected following pre-eclampsia and gestational hypertension. This may aid in our understanding of how this particular disease process affects the vascular health of these women and other asymptomatic groups at risk of future cardiovascular events.

Real-time high resolution 4D US will likely provide improved temporal and spatial resolution. We believe that by identifying 3D US as a useful and comparable tool to 2D US in assessing FMD, that we have provided a stepping
stonewall for this to happen and therefore facilitate a more accurate assessment of endothelial function. Furthermore, our US evaluation of women with a history of pre-eclampsia and gestational hypertension further improves our phenotypical knowledge of how these conditions affect the carotid arterial tree and how they may result in increased future cardiovascular risk.