EARLY CHANGES OF CORONARY ATHEROSCLEROSIS DETECTED WITH HIGH RESOLUTION TRANSTHORACIC ECHOCARDIOGRAPHY

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TABLE OF CONTENTS

Summary

Coronary artery disease (CAD) in its subclinical phase is a silent disease process accumulating over time until a catastrophic event such as myocardial infarction or death occurs often as the first presentation of the disease. Conventional risk factors do not fully explain the incidence of CAD and many people considered as low or intermediate risk for development of CAD based on these risk factors are often overlooked for primary prevention measures. However; it is often these people in which cardiovascular events occur as there is no pre existing urgency to modify important risk factors such as diet and exercise. Imaging plays an important role in this context as it may allow for targeted primary prevention measures despite a low or intermediate risk assessment using conventional methods. A novel imaging technique known as high resolution transthoracic echocardiography (HRTTE) was used to image the proximal left anterior descending coronary artery (LAD) to make measurements of the wall thickness and therefore the degree of subclinical atherosclerosis in varying cohorts of subjects.

HRTTE demonstrated that the LAD wall thickness and the external diameter of patients with CAD were significantly larger than that of normal volunteers, even when matched for age. The luminal diameter however was maintained in both groups indicating that the CAD group has undergone positive remodelling at the site measured. This objectively visualised evidence of

coronary atherosclerosis with HRTTE would likely be undetected during coronary angiography that images the lumen and not the vessel wall.

HRTTE was also able to show non-invasively the effects of recognised vasodilators on the coronary circulation. The HRTTE technique was sufficiently sensitive to detect coronary artery vasomotion and may be able to determine endothelial dysfunction, a sign of subclinical CAD. It was also found that LAD wall thickness as determined by HRTTE was able to predict future cardiovascular events in subjects free of clinical CAD and had a better predictive power than conventional cardiovascular risk factors.

HRTTE was also sufficiently sensitive to determine stabilisation of LAD wall thickness using moderate dose statin therapy in subjects with new myocardial infarction indicating that this method may assist in individual tailoring of both primary and secondary prevention therapies.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed

Rebecca Perry

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Publications

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Dedication

For the three loves of my life – Simon, Mia and Sam xxx

Chapter 1

General Introduction

1.1 Coronary artery disease

1.1.1 Prevalence of coronary artery disease

Coronary artery disease (CAD) is a major cause of mortality and morbidity in Australia and other western countries. It is a slowly progressive disease that can silently accumulate over time. Since we know that early changes of CAD can begin as early as the teenage years, [1-4] it represents a disease with a long preclinical stage. In fact it has been found that over 37% of heart transplant donors less than 30 years of age have signs of early CAD [1]. This would suggest that early primary intervention of CAD should occur at a young age. Failure to detect early, asymptomatic atherosclerosis may result in a missed opportunity for therapeutic modulation at a time when there may be the greatest chance for disease regression and primary prevention of acute coronary events.

1.1.2 Pathology of coronary artery disease

The primary pathology in CAD is atherosclerosis, a focal, pathological phenomenon characterised by the thickening and hardening of arteries due to accumulation of lipids, carbohydrates, blood products, fibrous tissue and calcium deposits (known as plaque) in the wall of the coronary artery [5]. However the mechanisms of atherosclerosis are not fully understood, especially the early changes of atherosclerosis that will be discussed further in this chapter.

1.1.3 The burden of coronary artery disease

Often (in over 70% of patients) the first presentation of CAD is acute myocardial infarction (MI) or even death. Sadly this is commonly the case with relatively young and previously healthy patients [6]. The most advantageous method for detection of this early stage disease would be rapid, inexpensive and non-invasive and would focus on early changes in the atherosclerotic process. Most current techniques for detection of early CAD are invasive, requiring cardiac catheterisation or are not able to directly image the degree of plaque burden in the coronary arteries.

The direct economic burden of CAD in Australia has been estimated as being in excess of $1.8 billion in 2004 [7]. This would indicate that primary prevention may be more cost effective than secondary prevention if patients with early atherosclerotic changes can be easily identified and treated accordingly.

1.2 Relevant coronary artery anatomy

The basic role of the coronary arteries is to supply the heart muscle and cardiac structures with blood allowing the heart to function normally.

1.2.1 Normal anatomy

In the vast majority of patients, the left main coronary artery originates from the left aortic sinus of Valsalva and after travelling about 1cm it bifurcates into the left anterior descending artery (LAD) and the left circumflex artery. The

right coronary artery originates from the right aortic sinus of Valsalva and is usually about 10 – 13cm in length before giving rise to the posterior descending artery [8] in 92% of people. Smaller branches come off these major arteries to make up the full epicardial coronary artery tree. Coronary artery anatomy has been shown to differ widely with many normal variants known [8], however the site of interest, the proximal LAD, is only a very rare site of congenital anatomic abnormality (reported as <0.1%[9]).

1.2.2 The walls of the coronary arteries

The walls of the coronary arteries consist of three circular layers. The outer vessel wall layer is known as the adventitia, the middle layer is the media and the innermost layer is the intima, which separates the arterial lumen from the rest of the vessel wall [8]. The intima consists of a layer of endothelial cells and a subendothelial layer comprising of connective tissue and smooth muscle cells. The media consists of smooth muscle cells. Separating the media from the adventitia is the external elastic membrane (EEM) which is primarily composed of elastin [8].

1.2.3 Role of endothelial cells

The endothelial cells of the intima play an important role in the normal function of the coronary artery vessel walls. The endothelial cells release endothelium-derived relaxing factor (EDRF) which is essential in coronary artery dilatation when an increase in blood flow is required [8]. Damage to the

endothelial cells may alter the function of these cells allowing plasma constituents such as lipoproteins access to the artery wall and is thought to be a precursor to the development of atherosclerosis [8].

Early in the course of coronary atherosclerosis, progressive thickening of the arterial wall by plaque is compensated for by arterial remodelling, resulting in arterial enlargement and preservation of the arterial lumen [10]. As a result remodelling renders early coronary atherosclerosis invisible to coronary angiography, which only visualises the arterial lumen.

1.3 Coronary artery remodelling

Originally it was thought that the development of coronary artery disease was a gradual growth of plaque within the lumen of the artery [6]. The EEM, intima and media were thought to remain fixed in size; therefore any plaque growth would always lead to luminal narrowing [11]. With the invention of coronary angiography by Mason Sones over 40 years ago luminal narrowing became the measure for the extent of coronary atherosclerosis in patients [11].

However, it was soon found that angiography could not always predict acute coronary syndromes [4]. Further to this it has been shown that only 14% of all clinical events occur in lesions that are greater than 70% narrowed, and the majority of coronary events occur at the site of a 'haemodynamically insignificant' coronary lesion [6]. Indeed in patients with single vessel disease, arteries with a 'normal' angiographic appearance have a mean atheroma burden of greater than 50% [12]. It is now well understood that atherosclerosis

is not a luminal disease, it is a disease of the vessel wall [4]. From numerous intravascular ultrasound (IVUS) studies it has been observed that by the time a lesion is able to be detected by angiography, there is disease involvement over much, if not all of the coronary artery network [4].

Angiography results were also not correlating with histology studies [10]. The lesions producing the most significant luminal obstruction appeared to be stable plaques, but the lesions giving rise to the acute coronary event usually did not cause a great degree of luminal narrowing due to dilatation of the EEM [13]. It was through these studies that the process of coronary artery remodelling was discovered.

1.3.1 History of remodelling

Glagov et al [10] first documented the process of coronary artery remodelling using histologic sections of the left main coronary artery from hearts obtained at autopsy. They demonstrated that the coronary arteries enlarge as the area of plaque increases. They also demonstrated that the area of the lumen does not begin to significantly decrease until the atherosclerotic lesion occupies over 40% of the vessel area [10].

Remodelling has been demonstrated in the left main and the LAD coronary arteries [10, 14] using pathological sections and was first demonstrated in vivo by McPherson et al [15] using epicardial echocardiography.

1.3.2 Types of remodelling

Since these initial studies many others have been performed using epicardial ultrasound, IVUS and histologic methods. Through these studies it has been shown that there are two types of arterial remodelling – positive and negative [16-19]. When there is an increase in the artery size to accommodate a plaque, this is known as positive remodelling [14-15, 20-22]. In fact it has been found in some studies that positive remodelling may overcompensate for early atherosclerosis [23-24]. Negative remodelling is the opposite – a decrease in the artery size.

When positive remodelling occurs this renders the plaque 'invisible' to angiography as the luminal area of the artery remains the same even when associated with a large plaque burden [25-26]. There is thinning and focal atrophy of the media induced by the atherosclerosis to accommodate the expanding plaque into the arterial wall allowing the plaque to bulge outward rather than inward [27-29].

In normal arteries remodelling occurs as a response to changes in the blood flow in order to restore normal shear stress and wall tension [30]. Nitric oxide and matrix metalloproteinases (MMPs) are central in the process of positive remodelling both in normal arteries as a response to an increase in blood flow and in atherosclerosis [30].

1.3.3 Plaque vulnerability and remodelling

It was first thought that positive remodelling was a desirable phenomenon as it kept the coronary artery lumen patent even when associated with a large plaque accumulation [20, 23]. In one study it was found that in the proximal LAD segment, the absence of positive remodelling would have caused a 92% luminal stenosis, rather than the observed 25% [14]. More recent evidence has contradicted this initial theory and it has since been shown that positive remodelling may be indicative of unstable and vulnerable plaques, which are more likely to cause an acute coronary syndrome without symptoms prior to the acute event [31]. It has been found that the presence of plaque disruption and thrombus are significantly more common in patients with positive remodelling than in patients with negative or no remodelling [32].

Further to this it has been shown that acute MI is almost exclusively associated with positive remodelling, which can therefore be extended to suggest an association between plaque rupture and positive remodelling [33].

Negative remodelling is a narrowing of the lumen due to plaque accumulation but this is usually associated with stable plaques that are less likely to rupture or produce acute coronary events [30-31, 34-35].

1.3.4 Location of remodelling

Remodelling has been shown to occur at points focal to the site of atherosclerosis i.e. the arterial enlargement occurs as a local response of the artery wall to the development of atherosclerotic plaque [26]. Adjacent segments

of the same artery not affected with atherosclerosis do not show the same enlargement as the diseased segments [25]. Atheroma is not uniformly deposited on all of the arterial walls and has variable morphological characteristics [36]. In fact there may be compensatory remodelling and lack of remodelling in different portions of the same coronary artery [37].

1.3.5 Failure of remodelling

Once the lesion occupies more than 40% of the vessel area the compensatory mechanism of remodelling fails, and there is progressive luminal narrowing [24, 38]. Knowing the slow progression of CAD, this correlates with evidence demonstrating that positive remodelling predominates in younger patients and negative remodelling predominates in older patients [32].

1.3.6 Implications of remodelling on detection of coronary artery disease

Due to the effects of remodelling, any screening test for the detection of subclinical CAD should assess the thickness of the coronary arterial walls and the total arterial diameter rather than just the luminal diameter. Therefore, the characteristics of subclinical CAD are arterial wall thickening with compensatory enlargement resulting in a preserved arterial lumen.

1.4 Atherosclerosis

Atherosclerosis is thought to be an inflammatory, systemic disease process involving the vessel wall [39-40]. It is the result of interaction between blood elements, disturbed flow and vessel wall (endothelial) abnormalities [41].

1.4.1 Components of atherosclerosis

An atherosclerotic lesion normally consists of a lipid core separated from the vessel lumen by a fibrous cap [13, 19]. The lesions contain varying proportions of connective tissue (including collagen), lipids, inflammatory cells, smooth muscle cells, thrombi and calcium [42].

The soft lipid core is rich in extracellular lipids, especially cholesterol and cholesterol esters [41]. The lipid core also contains lipid-laden macrophages (known as foam cells) which can produce large amounts of tissue factor, a substance thought to promote thrombus formation when brought into contact with blood [43].

Collagen is an important constituent of the fibrous cap and the plaque, giving the lesion strength and stability [41]. If there is a small amount of collagen content then the plaque may be weak and vulnerable but excessive collagen accumulation may lead to arterial stenosis [44]. Further to this it has been found that the density of the collagen in the plaque correlates to the type of remodelling - the higher the collagen component of the plaque the more likely for negative remodelling to occur which may make the lesion more stable [44].

1.4.2 Development of atherosclerosis

There are a number of theories regarding the development of atherosclerosis, some of which include the thrombogenic theory, the lipidic theory and the inflammation theory [5, 45-46]. In the thrombogenic theory the growth of the lesion is thought to occur by the transfer of thrombus from the lumen into the intima of the vessel wall [5]. It is now thought however that thrombus formation is more likely to occur after the atherosclerosis is present, rather than as a precursor to plaque formation [45].

The lipidic theory proposes that an imbalance between cellular lipid deposition and removal due to a damaged endothelial layer leads to a slow accumulation of lipid into the wall of the artery causing atherosclerosis [5, 45].

Ross [39] developed the inflammation theory after showing that the majority of the cellular and molecular responses to endothelial damage are part of an inflammatory disease process. These theories can be integrated into one common trigger – endothelial dysfunction [18]. Endothelial dysfunction and damage may lead to; increased permeability of the arterial wall to blood constituents (particularly low density lipoproteins [LDL]), adhesion of platelet and monocytes to the area of damage, and smooth muscle cell proliferation [39].

1.4.3 Evolution of atherosclerotic lesions

Atherosclerotic lesions have been found to have a common developmental pathway that they follow which then diverges as the lesions become more complex. The initial lesion (called a type I lesion) occurs when monocytes

accumulate in the intima from the lumen across the endothelial layer [29]. Next is the 'fatty streak' (type II) in which the intima contains lipid filled foam cells which progress to small pools of extracellular lipids (type III) [29]. Lesion types I to III progress in a predictable manner, but further lesion development do not necessarily go in a set sequence and may vary between types IV, V and VI. In a type IV lesion the extracellular lipids form a lipid core and smooth muscle cells may be detected [29]. After formation of a plaque cap the lesion is known as a type V which is further broken down into; type Va – a lesion with a large lipid core, type Vb – a lesion with heavy calcification and type Vc – a lesion with a minimal lipid core but containing collagen and smooth muscle cells. A type VI lesion is one that is complicated by thrombus [29]. Most type IV and Va lesions are not able to be detected angiographically due to positive remodelling and do not usually cause symptoms, but are the most vulnerable and have been shown to cause the majority of acute coronary events [29].

1.4.4 Thrombosis causing lesion progression

Plaque rupture, subsequent thrombosis, and fibrous thrombus organisation are important in the progression of atherosclerosis [42]. After thrombus formation the natural process of thrombolysis dissolves a variable part of the thrombus which is then replaced by smooth muscle cells and collagen as part of the healing process [29]. It has been proposed that repeated, asymptomatic plaque rupture, thrombosis and healing may cause 'wound' contraction leading to narrowing of the luminal diameter [47]. Therefore lesions that are responsible

for new stable angina may previously have had associated positive remodelling, undergone asymptomatic plaque rupture, thrombus formation, partial thrombolysis and collagen deposition with negative remodelling [33].

1.4.5 Endothelial dysfunction

Endothelial dysfunction is thought to be caused by elevated LDL, free radicals (caused by cigarette smoking), hypertension, diabetes and genetic alterations [39]. Endothelial damage and dysfunction is thought to cause inflammation that may continue indefinitely promoting further development of atherosclerosis [39]. In fact this inflammatory process is thought to be the cause of early lesion development which then in turn may lead to positive arterial remodelling [39]. The endothelium normally has anticoagulant properties however any damage to the endothelial layer may cause it to become a promoter of coagulation [39]. LDL is thought to cause a major proportion of endothelial damage, especially in the oxidised form [39]. The LDL is taken up by macrophages within the lesion which results in the formation of foam cells [5].

Chronic injury to the endothelium can be the result of a disturbance in the pattern of blood flow, which is often seen at bending points and near bifurcations in coronary arteries [42]. These alterations in the normal blood flow may cause shear stress and turbulence [39]. At sites of increased shear stress and turbulence, molecules responsible for infiltration of monocytes (e.g. vascular-cell adhesion molecule 1 [VCAM-1]) are formed on the endothelial surface [39]. The monocytes migrate into the sub-endothelium where they are

transformed into macrophages. These macrophages once lipid-enriched transform into foam cells leading to the formation of fatty streaks, the earliest form of atherosclerotic change [42]. Clinically it has been observed that bending points and bifurcations in coronary arteries are more susceptible to atherosclerotic changes supporting the hypothesis of increased chance of development of atherosclerosis at sites of shear stress and turbulence [39]. It has also been found that plaques associated with a large lipid core bear circumferential stresses less well than fibrous components of the arterial wall [48].

1.5 Plaque vulnerability

Plaque vulnerability describes the tendency of lesions to rupture or erode [49]. Plaque disruption and acute thrombosis formation which may cause luminal obstruction is thought to be the process underlying acute coronary syndromes [41]. Plaque rupture is thought to occur frequently and only occasionally leads to clinical symptoms; in fact it is only when a mature plaque is present that an occlusion and therefore clinical symptoms may develop [41, 50].

1.5.1 Factors influencing plaque vulnerability

Vulnerability to rupture depends on various factors including the size of the lipid core and the stability of the fibrous cap [41]. There is a balance between fibrous cap degradation by proteases and deposition of collagen into the plaque

cap. Inequality between these two processes may lead to either plaque vulnerability or increased stenosis respectively [4, 29].

A large lipid core, high macrophage count and thin fibrous cap are thought to be markers of vulnerability, whereas stable plaques are more fibrotic and calcified [4]. Foam cell infiltration causes weakening of the fibrous cap reducing its strength which predisposes the cap to rupture [41, 43].

Plaques with a larger lipid core and a thinner plaque cap (i.e. plaques more vulnerable to rupture) are usually found in areas of positive arterial remodelling [6]. Therefore it is the plaque composition rather than the degree of luminal stenosis that determines the risk of an acute coronary syndrome occurring [41, 51]. Histologic studies have demonstrated that plaques characterised as vulnerable were six times more thrombogenic than more stable, collagen rich lesions, suggesting that not only are these plaques more vulnerable to rupture but are also more likely to cause thrombosis and possible luminal occlusion [5].

1.5.2 Endothelial erosion and plaque disruption

Endothelial erosion is thought to be caused by endothelial cell loss and subsequent exposure of thrombogenic plaque to blood flow which usually results in the formation of small subclinical thrombi [29]. Occasionally larger areas of endothelial loss may occur giving rise to larger thrombus formation with possible acute coronary effects [29]. The larger the thrombus that is

formed, the more likely it is to occlude the coronary blood flow causing clinical symptoms.

Plaque disruption however is caused by a tear in the fibrous cap covering a plaque with a large lipid core allowing the blood from the vessel lumen to enter the lipid core. This gives rise to platelet adhesion and activation causing the formation of large thrombi which may cause an acute coronary syndrome depending on the size of the thrombus formed [29].

Whilst the major cause of acute coronary syndromes is plaque rupture, a minority may be caused by superficial erosion of the endothelium [52]. The amount of thrombogenic potential in the blood will determine whether a thrombus is formed and the size of the thrombus formed in superficial endothelial erosion [42]. Factors such as elevated LDL levels, smoking and diabetes have been found to increase the thrombogenicity of the blood, thus increasing the likelihood of a flow occluding thrombus being formed [42].

Disruption and thrombosis is usually seen in plaques with angiographically lower degrees of initial stenosis [53], whereas endothelial erosion causing thrombosis is more often seen at sites of higher grade stenoses [5]. Histologic studies on patients who died suddenly from CAD showed evidence of plaque rupture in approximately 70% of cases and superficial erosion of plaque in 30% of cases [34].

1.5.3 Fibrous cap stability

The quantity of smooth muscle cells in the fibrous cap influences its stability; the higher the number of smooth muscle cells the more stable the fibrous cap [52]. Inflammation has been proven to cause smooth muscle cell apoptosis that results in deterioration of the stability of the fibrous cap [52].

The constant motion of the coronary arteries due to cardiac contraction may also lead to fatigue and weakening of the fibrous cap leading to rupture [41]. The pressure of the blood within the coronary artery causes tension on the fibrous cap. It has been shown that the tension created in the fibrous caps of angiographically mild or moderately stenotic plaques is greater than that created in the caps of severely stenotic plaques with the same fibrous cap thickness and blood pressure [41]. This implies that angiographically smaller plaques may be more prone to rupture and therefore more vulnerable than severely stenotic plaques.

The fibrous cap is often thinnest at the junction between the plaque and the adjacent less diseased vessel wall, called the shoulder region, and it is at this point where cap disruption most frequently occurs [41, 43].

Foam cells within a lesion are then able to attract additional macrophages and smooth muscle cells into the lesion, significantly increasing plaque growth [42]. Macrophages in turn are able to release MMPs via apoptosis which digest the extracellular matrix and fibrous cap, leading to possible plaque disruption and thrombus formation [42].

1.6 Invasive detection of coronary artery disease

1.6.1 Angiography

Even though it was invented over 40 years ago, coronary angiography is still generally thought of as the 'gold standard' in the assessment of the extent of coronary atherosclerosis [54]. It is used to guide clinical decision making and is used extensively as a research tool [54]. The femoral artery is most often used for catheter access though the radial artery is being used increasingly and the catheter is introduced into the artery up through to the aorta and into the coronary arteries. Radio-opaque contrast dye is injected into the coronary artery tree so that the arteries can be visualised. It is able to provide a 'map' of the coronary arteries and usually can determine the site and severity of stenotic lesions. However, the accuracy and reproducibility of angiography has been under investigation for many years as inaccuracies have been detected through post-mortem histologic studies [54-55].

Angiography only shows a planar silhouette of the contrast filled lumen, it is not able to look at the vessel walls and as mentioned previously atherosclerosis is a disease of the vessel wall [4]. It is considered a safe investigation; however it is invasive and therefore is associated with finite morbidity and even mortality. It is also involved with bulky, expensive equipment [56]. Lesions are defined by focal narrowing of the luminal silhouette [49]. If the atheroma is uniformly deposited around the vessel wall and diffusely along the vessel wall,

angiography is unable to define the stenosis; at best it may define the vessel as 'small' with minimal stenosis [4].

To calculate percent stenosis an angiographically 'normal' reference segment is used for comparison. IVUS studies have demonstrated that this 'normal' reference site is almost always associated with some degree of atherosclerosis, therefore causing the angiographically detected stenosis to be rated less stenotic than it actually is [4, 21].

As stated earlier most MI's occur at sites that have only mild to moderate luminal obstruction and may be missed or found as non-significant lesions on angiography [34, 51]. Angiography is unable to determine the site of plaque rupture and cannot differentiate unstable from stable plaques [53].

It has been shown in numerous studies that there is significant inter-operator variability in the interpretation and analysis of angiograms and therefore computerised lesion quantification is now used [57]. Angiography is limited in arteries where positive remodelling has occurred, as the luminal diameter is often unaffected [54]. This is important because, as noted earlier positive remodelling appears to be associated with plaque vulnerability [31].

There is an inherent toxicity of the contrast agents and some patients may be allergic to the various agents used. In patients with significant kidney disease the use of radioactive contrast is contra-indicated [58]. Safety standards limit the radiation dose patients receive causing the resolution of the angiogram in some patients to be very poor [54].

Even though angiography is limited in defining arterial wall pathology when the lumen is preserved, it is able to detect advanced lesions that encroach on the lumen [40]. The ideal situation however would be to prevent these advanced lesions from occurring.

1.6.2 Intravascular ultrasound

A more precise technique to look at atherosclerotic burden in the coronary arteries is using IVUS [6]. This is an invasive diagnostic tool, which although considered to be relatively safe is associated with a number of risks similar to angiography including MI; stroke and bleeding from the puncture site [59].

IVUS allows direct visualisation of the arterial wall from within the artery [60]. A high frequency (20 – 50MHz) ultrasound catheter is placed inside the coronary artery during cardiac catheterisation and tomographic intramural images of the artery wall and lumen can be obtained [6]. Using a 20MHz transducer the resolution is approximately 0.25 x 0.1mm [26, 55].

The main clinical uses for IVUS are to plan coronary interventions[68-73], assist in optimal stent deployment to reduce stent thrombosis[74], detect early edge dissections[68, 75-76] and reduce incomplete stent apposition[77-78].

IVUS is able to give accurate vessel and luminal dimensions and has been shown to be more reproducible than angiography for determination of coronary disease serverity[61-63].

IVUS has the ability to resolve some of the morphological components of the plaque and enables measurement of the amount of remodelling that has

occurred. IVUS offers a means to identify 'non-stenotic' coronary lesions and plaque vulnerability [64]. Using known landmarks such as cardiac veins or side branches the image orientation is able to be determined [57]. Furthermore, it is able to identify ruptured plaques by detecting any disruption to the integrity of the fibrous cap [53].

Coronary plaque burden as assessed by IVUS has been found to be highly reproducible especially with the assistance of computerised edge detection [57]. Drawbacks do occur when there are large areas of plaque calcification that cause acoustic shadowing (as ultrasound cannot penetrate areas of dense calcification) making assessment of the vessel wall behind areas of calcium difficult [57]. IVUS is able to make measurement of the minimal luminal area (MLA) which above a value of 4.0mm^2 is said to predict which nonischaemic lesions do not require immediate revascularisaion[65-67].

IVUS can only display a single cross sectional image of the artery at a given point in time, it does not permit instantaneous assessment of the entire arterial tree whereas angiography to a degree can [57]. However there are computer programs which can compile all of the cross sections into a three dimensional reconstruction of the artery, though this cannot be done instantaneously [57, 60].

Although it is able to image subclinical CAD, IVUS is not suitable as a screening test for early atherosclerotic changes in the coronary arteries due to the invasive nature and the cost associated with the examination [60].

1.6.3 Optical Coherence Tomography

Optical coherence tomography (OCT) is an invasive diagnostic test that measures the interaction of light with tissue wall and can provide high resolution cross sectional images of the coronary artery wall and the components atherosclerosis. The process is similar to IVUS, however instead of measuring reflected sound waves it measures reflected light waves [79].

OCT is used to visualize the microstructure of coronary plaques including macrophages [80-81], calcium [82-83], thrombus [84-85] and lipid rich plaques [82-83, 86] and has had good correlation with histopathology.

For images to be obtained accurately, blood must be removed from the imaging field, requiring a saline flush with proximal balloon inflation [87]. Occlusion of the artery is often associated with chest pain and ischaemia during the procedure and may elevate the risk of the procedure for the patient [87].

OCT is superior to IVUS for imaging subclinical CAD; however it is not suitable as a screening test for low risk patients due to the inherent risks of the invasive procedure and the cost associated with the examination.

1.7 Non-invasive detection of coronary artery disease

1.7.1 Electron beam computer tomographic imaging

Electron beam computer tomographic (EBCT) scanning is a non-invasive technique used to detect the extent of calcium (coronary artery calcium score –

CACS) that may be present in atherosclerotic lesions [48] and more recently coronary CT angiography (CTCA) has been used to detect luminal stenosis [88] . CACS has been found to predict death, non fatal MI and the need for revascularisation in large population studies [89]. However, calcium formation usually occurs in older lesions and may also occur in the absence of significant atherosclerosis making it a limited diagnostic technique for the detection of early atherosclerosis [90]. Moreover, vulnerable plaques usually are not associated with calcium however stable obstructive plaques are often associated with higher levels of calcium [91]. Indeed, it has been found that in acute coronary syndromes there is a relative lack of calcium in the culprit lesion compared with culprit lesions in stable angina [48]. The calcium score has been proposed as an independent risk factor for all cause mortality, however in this study there were still a small number of subjects having an event despite a calcium score of zero [92].

CTCA has been shown to be valuable in selected patients for the visualisation of the coronary arteries, including the lumen and parts of the vessel wall. The major limitation in CT detection of coronary atherosclerosis has been constant cardiac motion; however advances in CT technology have overcome many issues as acquisition time has been minimised and is gated with the ECG. Using a 64-slice multi-detector CT system a complete cardiac scan can be achieved in 5-10 seconds[93]. Over many studies the negative predictive value remains high (95-100%), indicating that CTCA is a valuable tool for ruling out significant CAD in patients with low to intermediate risk factors for CAD[94-96,

[88]. However, CTCA is unable to quantify the degree of coronary stenosis severity in a reproducible manner and the presence of calcium limits the visualisation of the vessel wall and lumen[94, 88].

The presence of a significant stenosis detected by CTCA has been associated with an approximate 10 times higher risk of events. Furthermore, if any level of disease was detected there was a 4.5 time higher risk of a cardiovascular event[97].

The current appropriate use guidelines for CTCA advocate the use of CTCA in individuals with acute chest pain and negative electrocardiogram and cardiac enzyme changes to reduce hospital stay and streamline diagnostic pathways[98]. The use of CTCA in asymptomatic subjects is not supported by current data; however CACS may allow stratification of who may benefit from CTCA[99-100]. Still within the realms of research is the ability of CTCA to resolve plaque characteristics, including coronary artery remodelling and calcified or non-calcified plaques[101-103].

EBCT uses radiation, requires a steady heart rate of less than 60 beats per minute and necessitates the use of an iodine based contrast agent for CTCA. There is a lack of haemodynamic information given from an EBCT scan – it is purely used as a diagnostic tool to determine the presence or absence of CAD.

1.7.2 Magnetic resonance imaging

Magnetic resonance imaging (MRI) has also attempted to visualise early changes of coronary atherosclerosis but the poor resolution (due to poor signal

to noise ratio) of this technique makes it limited for detection of early CAD [104]. However atherosclerosis and its various components may be detected in great detail by MRI in the carotid and peripheral arteries [40]. There have been a number of attempts to visualise the coronary artery wall and plaque components by MRI [105-107]. In 2 small studies the use of delayed contrast enhancement made it possible to visualise selected coronary plaques and differentiate plaque types, however the use of contrast was required [105-106]. Using a non-contrast method of MRI known as black-blood scanning measurements of the coronary artery wall thickness and luminal diameter were able to be made and positive remodelling was able to be detected using this method [107]. Each image of the coronary artery wall takes 2-3 minutes to acquire and subjects with a high heart rate or irregular respiration are not able to be scanned with accuracy as the images are triggered to both the ECG and the motion of the diaphragm [107]. MRI has poor spatial resolution but good contrast resolution. The spatial resolution of MRI for coronary imaging is 0.46 x 0.46mm [108].

Both EBCT and MRI involve bulky, expensive equipment that does not have the portability and cost effectiveness of echocardiography.

1.7.3 Carotid intima-media thickness

The carotid intima-media thickness (IMT) has been used as a surrogate marker for subclinical coronary artery disease in many large longitudinal studies [109-113]. In some studies increased carotid IMT is associated with an increased risk

of MI and stroke, and has been found to be independent of other conventional risk factors [109-111]. However, other studies have found inconsistencies using carotid IMT and consider it not useful for risk stratification on an individual level [112-113].

It has also been found that lipid lowering may reduce the IMT of the carotid artery as measured by ultrasound [40]. The carotid IMT has an almost linear association with lipid factors after 6 months of lipid lowering treatment [114-115]. The carotid IMT is an indirect, non-invasive measurement of the degree of subclinical atherosclerosis, however using ultrasound it may be possible to directly measure the wall thickness of the coronary arteries.

1.7.4 Transthoracic echocardiography

The coronary arteries have been able to be visualised using transthoracic echocardiography (TTE) for many years [116], but accurate measurements have not been possible due to resolution difficulties. The accuracy of TTE for the detection of atherosclerotic changes did not correlate well with angiography [117]. However these studies mainly focussed on the left main coronary artery and only in the short axis view, therefore limiting the visualisation of the coronary artery walls parallel to the ultrasound beam [117-119]. Some groups [120-122] made limited success, but overall the technique was considered far too subjective with poor resolution to become a valid diagnostic screening test. Indeed, one group was only able to measure the diameter of the LAD in 44% of subjects studied [123].

The majority of these early studies gave a qualitative assessment of the coronary artery walls, without accurate measurements to quantify the wall thickness [116-117, 119, 121]. While there was much interest in imaging the distal LAD [116, 120-121] it has been shown that the bulk of atherosclerotic burden is found the proximal regions of coronary arteries [15, 124]. Many of these studies only used male subjects due to what was considered the 'limited window' access for LAD imaging which was made even smaller by mammary tissue. Even only using normal male subjects only two thirds of the study population had adequate LAD images [125]. One small study found that the mid portion of the LAD could be visualised in the parasternal long axis view to detect the location of stents, however no measurements were made and no control group was used [126].

Other studies have evaluated transthoracic echocardiographic measurement of coronary flow reserve and found this to be an accurate, non-invasive assessment of significant stenosis [127]. Coronary flow reserve is defined as the ability of the coronary arteries to increase their blood flow by demand and is determined echocardiographically by Doppler velocity assessment of coronary blood flow before and after the administration of Adenosine. Coronary flow reserve however only becomes abnormal when there is a stenosis of greater than 50% to 70% and therefore is only a valid measurement when the disease process has already gone beyond a subclinical level [127].

1.7.5 High resolution transthoracic echocardiography

Gradus-Pizlo et al [128] have demonstrated that in the parasternal long axis view, relatively long segments of the proximal LAD can be seen. They also found that HRTTE measurement of the LAD wall thickness was sensitive enough to detect differences between normal subjects and patients with CAD [128]. The maximum axial resolution provided by an 8MHz transducer is 0.19mm (assuming 2 cycles per pulse and speed of sound in soft tissue = 1540m/sec). IVUS and epicardial echocardiography studies have demonstrated that coronary atherosclerosis is a diffuse disease process and rarely spares the proximal coronary arteries especially the proximal LAD [15, 124]. In fact it has been shown that before clinical CAD is evident at least 90% of the coronary artery tree is atherosclerotic [4]. This coronary artery segment should therefore represent a good sampling site for the detection of early stage coronary artery disease.

Gradus-Pizlo and colleagues demonstrated a significant difference in the LAD wall thickness and external diameter between patients with normal coronary arteries and patients with known CAD [128]. This suggests that HRTTE may be a useful screening test for early changes of CAD. However this has not been verified by any other researchers for reliability.

In further studies by the same group the HRTTE measurement of the LAD coronary artery was compared to both IVUS and epicardial echocardiography where, during cardiac surgery, a high frequency ultrasound transducer is placed directly over the mid LAD segment after the pericardium was

opened[129-130]. They found that there was a discrepancy between the HRTTE measurements of the LAD wall thickness and the corresponding IVUS measurement. Using epicardial echocardiography it was discovered that this discrepancy could be directly related to the fact that IVUS is not able to image the adventitial layer of the coronary arteries, whereas both HRTTE and epicardial echocardiography could. Furthermore, it was found that this adventitial layer actually increased in thickness with the development of coronary atherosclerosis and is a valuable part of the artery to visualise when aiming to detect subclinical atherosclerosis as it seems to play a part in the vascular disease process[129-130]. The adventitial layer was thought to contribute approximately half of the LAD wall thickness in both normal and diseased arteries and was thought to be the main reason for the discrepancy between coronary artery wall thicknesses measured by IVUS, histology, epicardial echocardiography and HRTTE[129-130].

1.8 Risk factor analysis

Conventional cardiac risk factors do not fully explain the incidence of coronary artery disease and coronary events. Risk stratification and therapy based solely on these conventional risk factors may exclude a population who would otherwise benefit from lifestyle and risk factor modification. Additionally, risk factor stratification has been demonstrated to have significant limitations in the individual patient, which has generated a search for more specific and sensitive markers. They still remain however surrogate marker of the disease process.

If a rapid, inexpensive, non-invasive, direct, specific and sensitive measure of early coronary atherosclerosis can be developed, then early detection of coronary disease may allow precise targeting of preventative therapies at the early stages when coronary atherosclerosis may be most likely to regress, before irreversible cardiac damage has occurred. However possibly the greatest benefit of a validated non-invasive screening test of CAD may be to increase awareness of the prevalence of early stage CAD so changing individual and societal attitudes and behaviour thereby enhancing primary prevention. HRTTE measurement of the LAD wall thickness may be this modality.

1.9 Summary

Clinically silent CAD is an accumulative disease process that may go undetected for decades until a catastrophic event such as acute MI, unstable angina and in the worst case sudden death occurs. Many imaging modalities have been employed to document the degree of early atherosclerosis with limited success. This has been due to a number of factors, the main being inadequate resolution of image quality.

This thesis will explore the hypothesis that HRTTE can be used to image the LAD to detect subclinical atherosclerosis. The HRTTE method will be assessed for inter and intra operator variability to ensure that it is repeatable and reproducible and a range of normal values will be established for subjects of different age groups with no know CAD or risk factors for CAD. The

HRTTE measurement of LAD wall thickness from normal subjects will be compared with age matched patients with known CAD to determine if there are any differences and confirm the pioneering work done by Gradus-Pizlo [128]. Coronary vasoreactivity will be assessed using the HRTTE method to determine if a direct measurement of coronary endothelial function can be made. LAD wall thickness will be assessed along with conventional risk factors to determine if the LAD wall thickness is able to predict future cardiovascular events on long term follow up. It will also be compared as a screening test for CAD with exercise stress echocardiography and HRTTE will be used to determine if regression of LAD wall thickness occurs with moderate statin therapy post MI.

Chapter 2

Coronary artery wall thickness of the left anterior descending artery using high resolution transthoracic echocardiography – normal range of values

2.1 Introduction

Using high frequency transducers for echocardiography has advantages and disadvantages. The main advantage is the vast improvement in spatial resolution, particularly axial resolution (that is along the axis of the ultrasound beam). The main disadvantage is lack of penetration. The higher the transducer frequency, the greater the attenuation of the ultrasound beam thereby reducing beam penetration. Since the coronary arteries are small compared to other structures normally visualised by echocardiography (i.e. the valves and chambers of the heart), the resolution must be high for accurate measurements to occur. The proximal and mid LAD can be imaged in the near field of the field of view from a parasternal position and therefore a high level of penetration of ultrasound into the patient is not necessary.

As previously mentioned Gradus-Pizlo et al [128] found that HRTTE measurement of the LAD wall thickness differed significantly between normal subjects and patients with known coronary artery disease. However both a normal range of values for each measurement and the test-retest inter and intra operator variability of this method was not reported.

2.1.1 Aims

1) To determine a normal range of values of LAD wall thickness, luminal and external diameters using HRTTE

2) To determine the test-retest reproducibility and variability of the method of measuring the wall thickness, luminal and external diameters of the LAD

using HRTTE to test the hypothesis that HRTTE measurement of LAD wall thickness is a viable and repeatable method.

2.2 Methods

The Flinders Medical Centre Clinical Research Ethics Committee approved this study and all subjects provided written informed consent to participate (application number 89/034).

2.2.1 Subjects

Two hundred and forty two volunteer participants had a HRTTE study to measure their LAD wall thickness, luminal and external diameters. Thirty of these subjects had these measurements taken on three separate occasions by two different echosonographers. All subjects were free of clinical CAD, hypertension, hyperlipidemia and diabetes mellitus.

2.2.2 Procedures

2.2.2.1 High resolution transthoracic echocardiography:

Examinations were obtained using a commercially available ultrasound system (iE33, Philips Medical Systems, Bothell, Washington, USA) with a broadband, phased array sector high frequency transducer (S8-3). The maximum axial resolution provided by an 8MHz transducer is 0.19mm (assuming 2 cycles per pulse and speed of sound in soft tissue = 1540m/sec).

Subjects were placed in a steep left lateral decubitus position with left arm extended over the head to drop the left lung away from the mid line of the chest, allowing the heart to fall away from behind the sternum and maximising the intercostal spaces. Positioning was important because ultrasound will not

penetrate through air (i.e. the lungs) or bone. The LAD was recorded using a modified low parasternal long axis examination with a slight inferior tilt adjusted to visualise as much of the proximal and mid LAD in long axis as it runs along the interventricular septum as possible.

Machine controls were optimised by the operator at each examination. Optimisation included altering the depth, movement of focal zone to region of LAD, reduction of sector size, alteration of gain control and dynamic range. Because of the constant motion of the coronary arteries, digital clips were taken and the best four separate one-second clips that showed the LAD were captured digitally. The procedure lasted approximately 5-10 minutes. Images were stored for off-line retrieval and analysis. Each operator was required to make measurements from their own acquired data for accurate test-retest variability (see also appendix A).

Image analysis:

Readers were blinded to any clinical data. The presence of the 2 linear echoes anterior to the inter-ventricular septum in at least 3 consecutive frames was used to identify the LAD. Segments with the largest luminal diameter were selected to ensure that the measured cross-section of the artery was through the luminal centre and thus the results were not confounded by off axis images. Where possible, identification of branching vessels was used as an additional criterion. Readers were asked to identify the LAD, perform measurements of the luminal size, external elastic membrane (EEM) diameter and anterior and posterior LAD wall thickness on 3 frames where the artery was best identified

and where the luminal diameter was the largest. Segments with the largest luminal diameter were selected to ensure that the measured cross-section of the artery is through the centre and thus the coronary arterial wall thickness was not overestimated by off axis images. All vessel diameters were measured with electronic callipers according to the methods used by Gradus-Pizlo et al [128]; the LAD wall thickness was measured from the outer edge to the inner edge of the line representing the coronary artery wall. The EEM of the vessel was measured as the distance between the outer edges of the lines representing the coronary artery wall and luminal diameter was measured as the distance between the inner edges of the lines representing vascular walls (figure 2.1). Where multiple linear echoes were noted anterior to the inter-ventricular septum the thicker walled vascular structure was measured to avoid potential confusion with the great cardiac vein. Pulsed wave spectral and colour Doppler were used to further delineate the LAD from the great cardiac vein if required (figure 2.2).

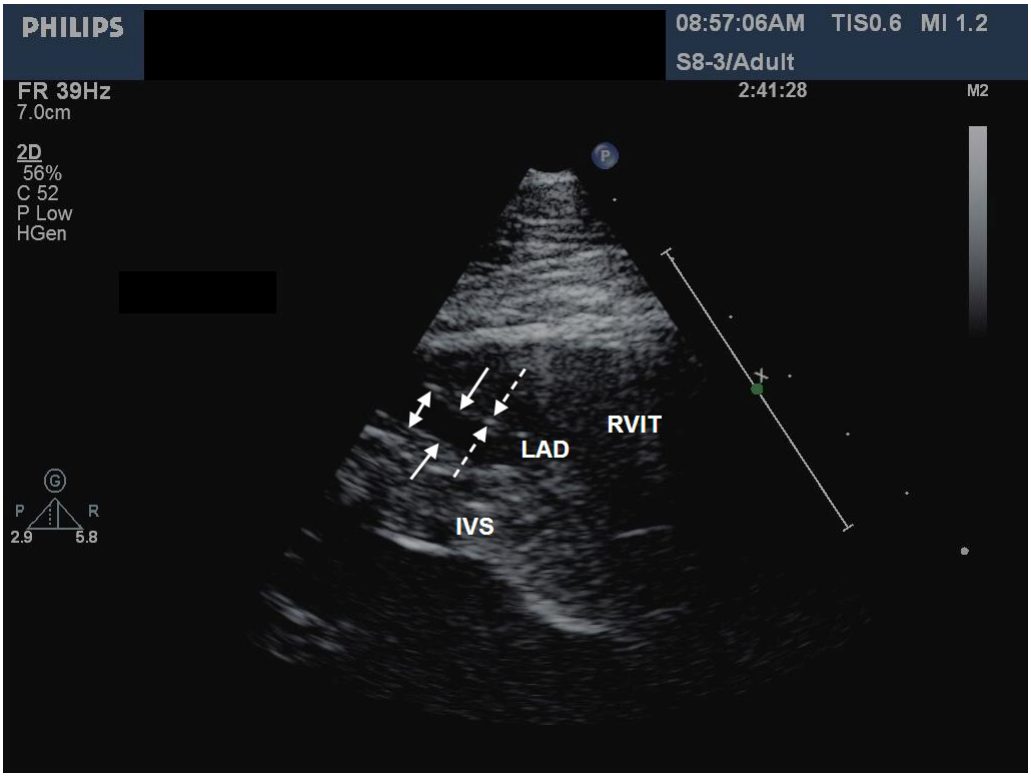

Figure 2.1: A modified parasternal long axis view showing LAD measurements. Dashed arrows demonstrate anterior wall thickness measurement, solid arrows demonstrate external elastic membrane measurement and double headed arrow demonstrates luminal measurement. RVIT – right ventricular inflow tract, LAD – left anterior descending coronary artery, IVS – interventricular septum.

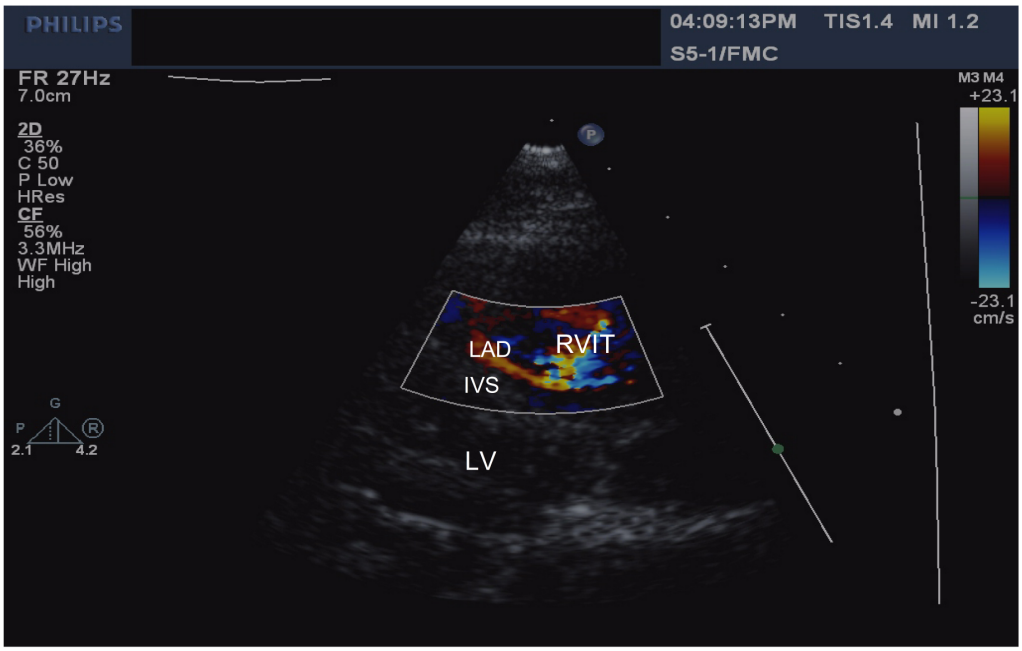

Figure 2.2: A modified parasternal long axis view to demonstrate colour flow in the left anterior descending coronary artery as it runs along the inter-ventricular septum. Some colour flow in the right ventricular inflow tract can also be detected (IVS – inter-ventricular septum, LAD – left anterior descending coronary artery, LV – left ventricular cavity, RVIT – right ventricular inflow tract)

2.2.2.2 Other procedures:

A risk assessment questionnaire was filled in by each subject (see appendix B) to determine risk factors for coronary artery disease.

Height and weight of each subject was also taken. A fasting blood test was obtained to measure total cholesterol, triglycerides, low-density lipoprotein (LDL), high density lipoprotein (HDL), C-reactive protein (CRP), homocysteine and creatinine levels.

2.2.3 Statistical analysis

As the wall thickness, luminal and external diameters were normally distributed a Pearson's correlation was used to determine the test-retest intra and inter operator variability. Continuous variables are summarised as mean ± standard deviation. Discrete variables are summarised as number and percentage. A p value of < 0.05 was considered statistically significant.

2.3 Results

The LAD was visualised and measured in 227 subjects (94%). Of the 242 subjects, there were 86 males (36%). Thirty two subjects (13%) were current smokers and 45 (19%) had a family history of premature CAD. The average age was 46.5±14.5 years and the average body mass index (BMI) was 25.9±4.8kg/m^2. Baseline characteristics are given in table 2.1.

Variable	Value
Age (years)	**47±15**
Males	**86 (36%)**
BMI (kg/m^2)	**26±5**
Total cholesterol (mmol/L)	**5.3±1.0**
Triglyceride (mmol/L)	**1.8±1.4**
LDL (mmol/L)	**2.9±0.8**
HDL (mmol/L)	**1.6±0.5**
CRP (mg/L)	**2.5±3.0**
Creatinine (µmol/L)	**71.0±14.4**
Homocysteine (µmol/L)	**10.9±4.1**
Framingham risk score	**4.1±5.2**
HTN	**0 (0%)**
Diabetes	**0 (0%)**
Smokers	**32 (13%)**
Family history CAD	**45 (19%)**

Table 2.1: Baseline subject characteristics

BMI – body mass index, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – C-reactive protein, HTN – hypertension, CAD – coronary artery disease

HRTTE data:

The average anterior wall thickness was 1.1±0.2mm, posterior wall thickness was 1.1±0.2mm, luminal diameter 2.2±0.6mm and EEM diameter 4.5±0.9mm. The anterior and posterior wall thicknesses were averaged to give an overall wall thickness measurement. There were no differences in LAD wall thickness between males and females. However, the luminal diameter was larger in males (2.4±0.8mm vs. 2.1±0.6mm, p=0.004) as was the EEM diameter (4.7±0.9mm vs. 4.5±1.0mm, p=0.03). The LAD measurements were weakly correlated with BMI. As the BMI increased there was an increase in the average wall thickness (r=0.19, p=0.004), an increase in the luminal diameter (r=0.24, p<0.001) and an increase in the EEM (r=0.25, p<0.001). Using regression analysis BMI only slightly contributed to changes in the average wall thickness (r^2=0.032, p=0.004), luminal diameter (r^2=0.06, p<0.001) and EEM (r^2=0.06, p<0.001). Some of the LAD measurements were also weakly correlated with age. As the age increased there was an increase in the average wall thickness (r=0.38, p<0.001) and an increase in the EEM diameter (r=0.35, p<0.001) but no change in the luminal diameter. Using regression analysis age only slightly contributed to changes in the average wall thickness (r^2=0.14, p<0.001) or EEM (r^2=0.12, p<0.001).

Test-retest intra and inter operator variability:

For the 30 subjects who had repeat studies performed, the correlations for test-retest variability in the same operator were r=0.86 (p<0.001), r=0.81 (p<0.001)

and r=0.85 ($p<0.001$) for LAD wall thickness and luminal and external diameters respectively (table 2.2 and figures 2.3, 2.4 and 2.5). The correlations for test-retest variability in two separate operators were r=0.82 ($p<0.001$), r=0.76 ($p<0.001$) and r=0.70 ($p<0.001$) for LAD wall thickness and luminal and external diameters respectively (table 2 and figures 2.6, 2.7 and 2.8). The coefficient of repeatability in measurements within the same operator for LAD wall thickness, luminal diameter and EEM was 0.05mm, 0.06mm and 0.16mm respectively. The coefficient of repeatability in measurements between two different operators for LAD wall thickness, luminal diameter and EEM was 0.06mm, 0.14mm and 0.22mm respectively.

	r value	p value	Bias (mm)	95% CI (mm)	-95% CI (mm)
Intra-operator wall thickness	**0.86**	**<0.001**	**0.042**	**0.32**	**-0.24**
Intra-operator luminal diameter	**0.81**	**<0.001**	**-0.060**	**0.59**	**-0.71**
Intra-operator EEM diameter	**0.85**	**<0.001**	**-0.077**	**0.77**	**-0.93**
Inter-operator wall thickness	**0.82**	**<0.001**	**0.082**	**0.39**	**-0.23**
Inter-operator luminal diameter	**0.76**	**<0.001**	**-0.077**	**0.67**	**-0.83**
Inter-operator EEM diameter	**0.70**	**<0.001**	**0.027**	**1.23**	**-1.17**

Table 2.2: Results of intra and inter operator measurements of LAD wall thickness. CI – confidence interval, EEM – external elastic membrane, LAD – left anterior descending coronary artery.

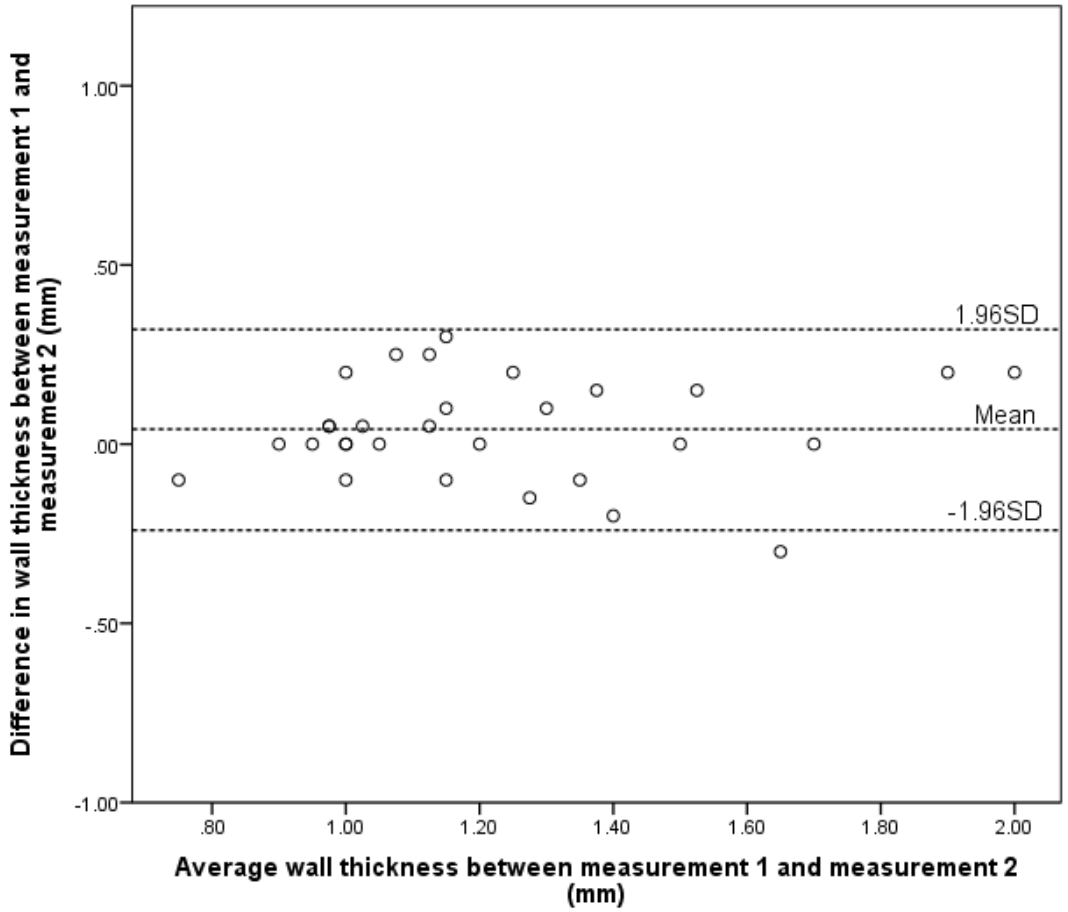

Figure 2.3: Bland-Altman graph demonstrating the test-retest agreement in LAD wall thickness measurements for the same operator.

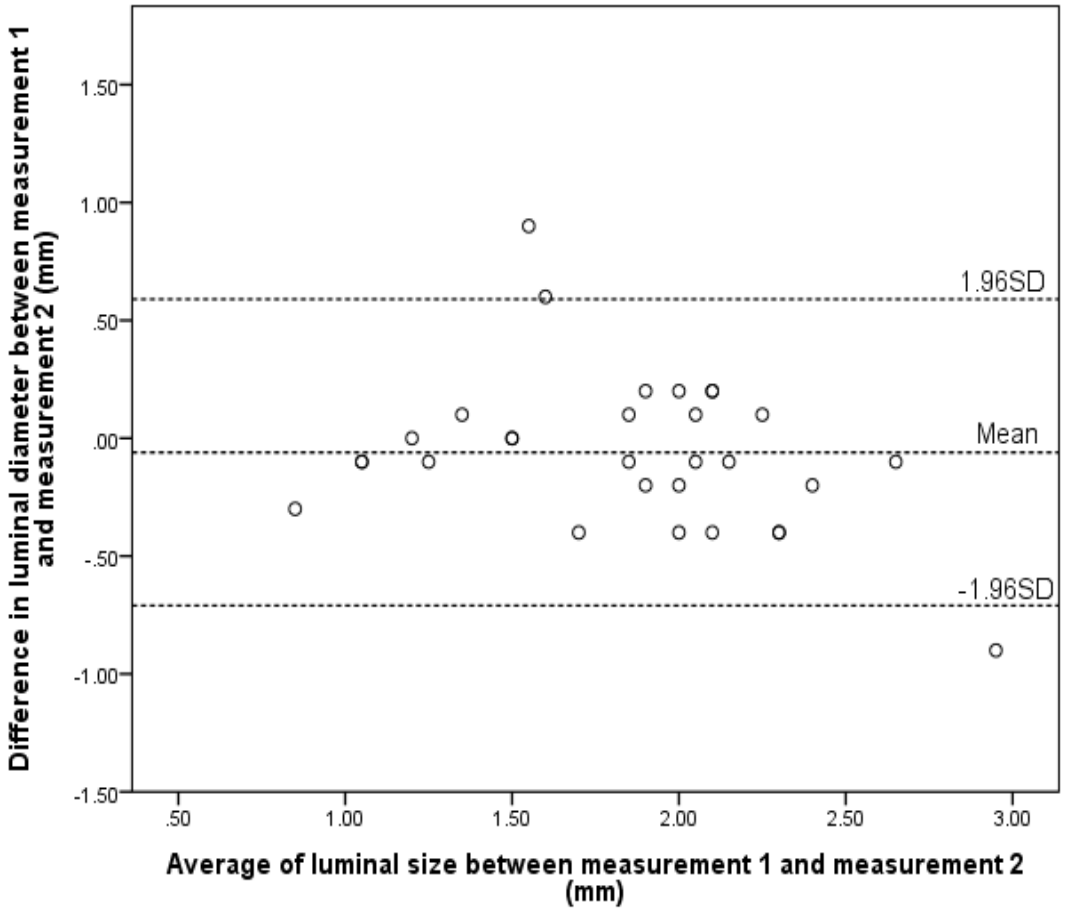

Figure 2.4: Bland-Altman graph demonstrating the test-retest agreement in LAD luminal diameter measurements for the same operator.

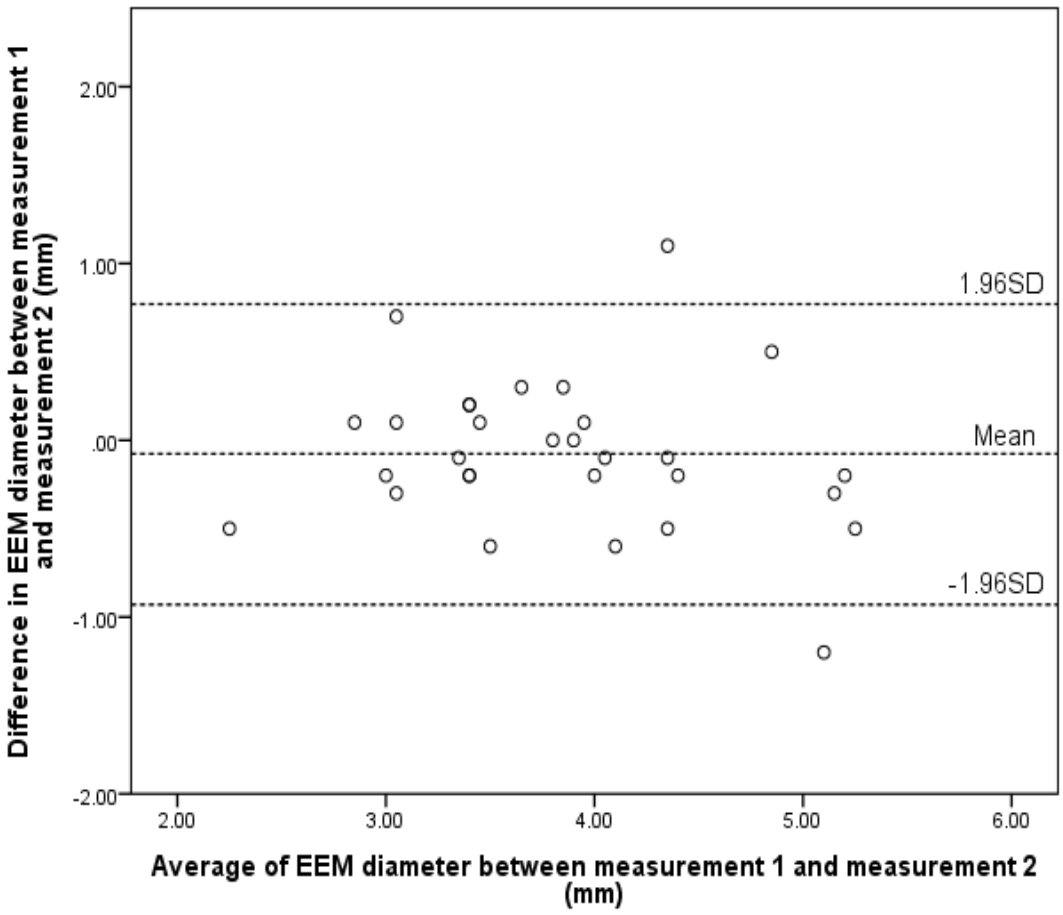

Figure 2.5: Bland-Altman graph demonstrating the test-retest agreement in LAD EEM diameter measurements for the same operator.

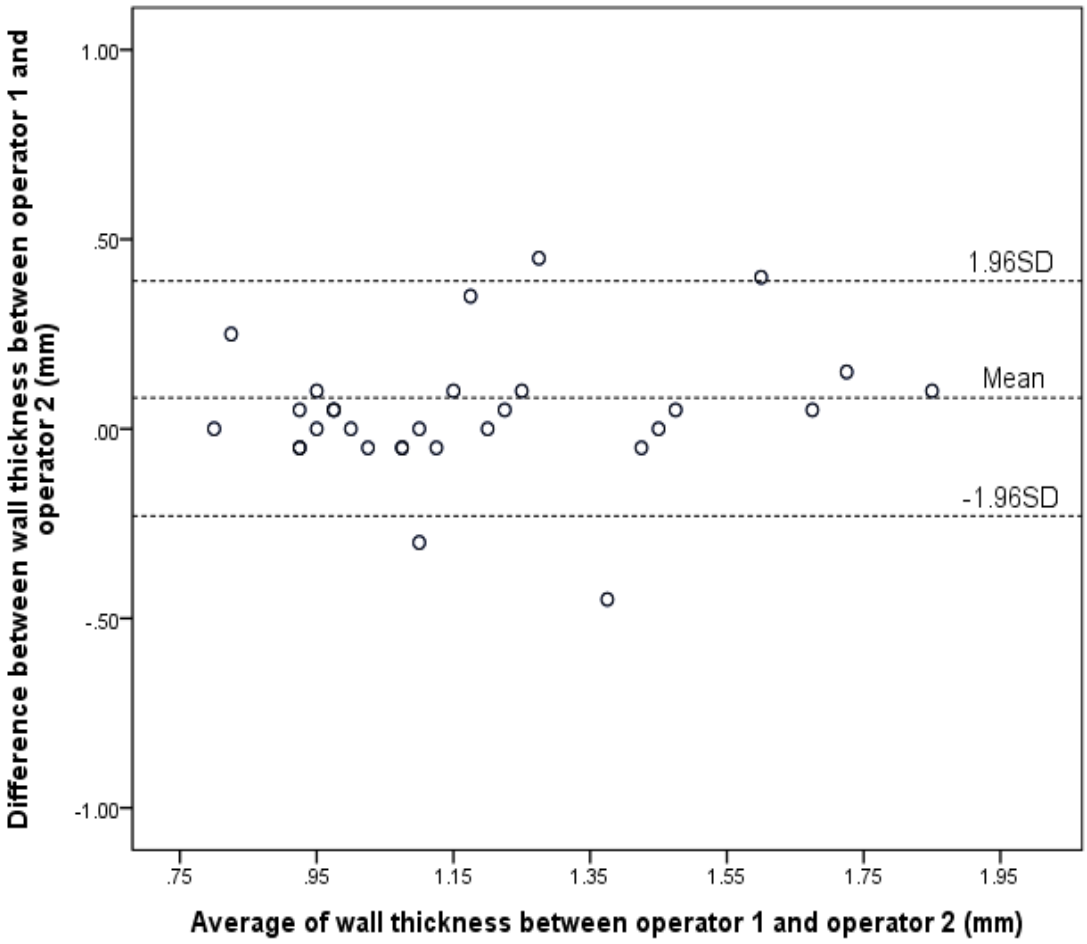

Figure 2.6: Bland-Altman graph demonstrating the test-retest agreement in LAD wall thickness measurements between 2 different operators.

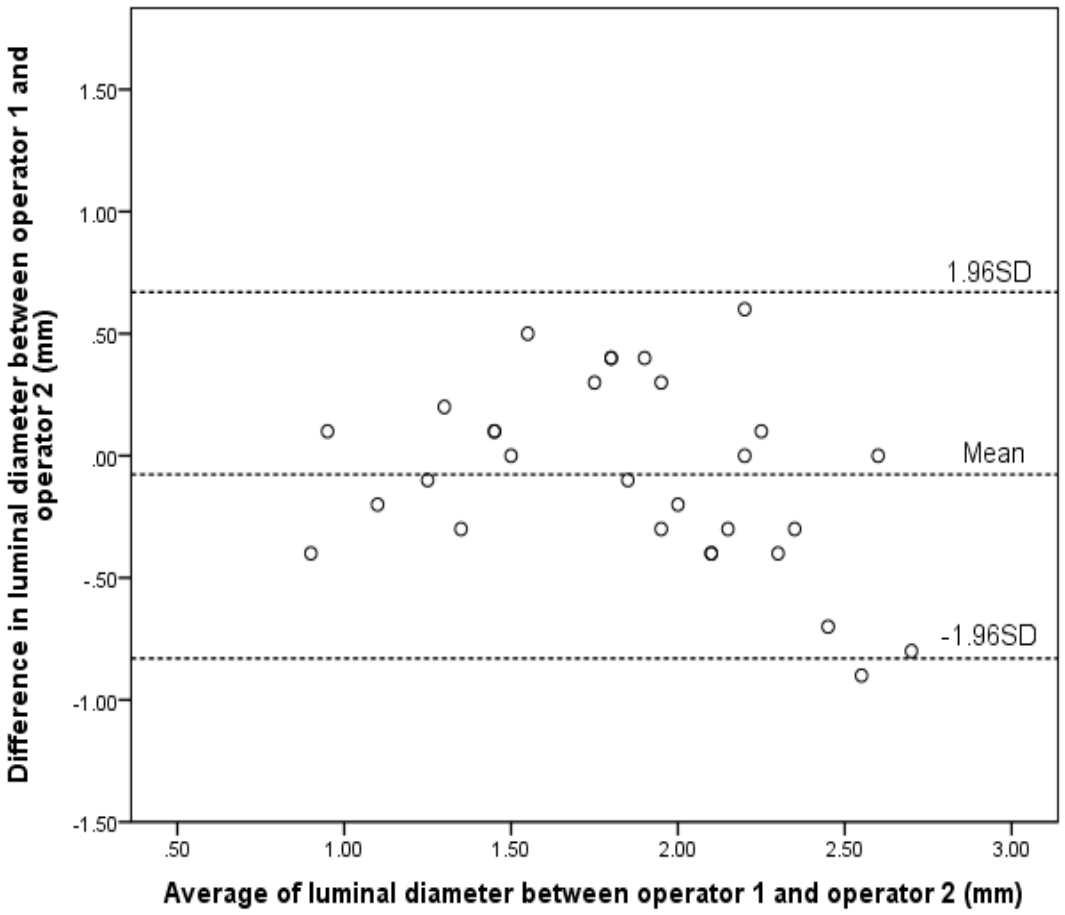

Figure 2.7: Bland-Altman graph demonstrating the test-retest agreement in LAD luminal measurements between 2 different operators.

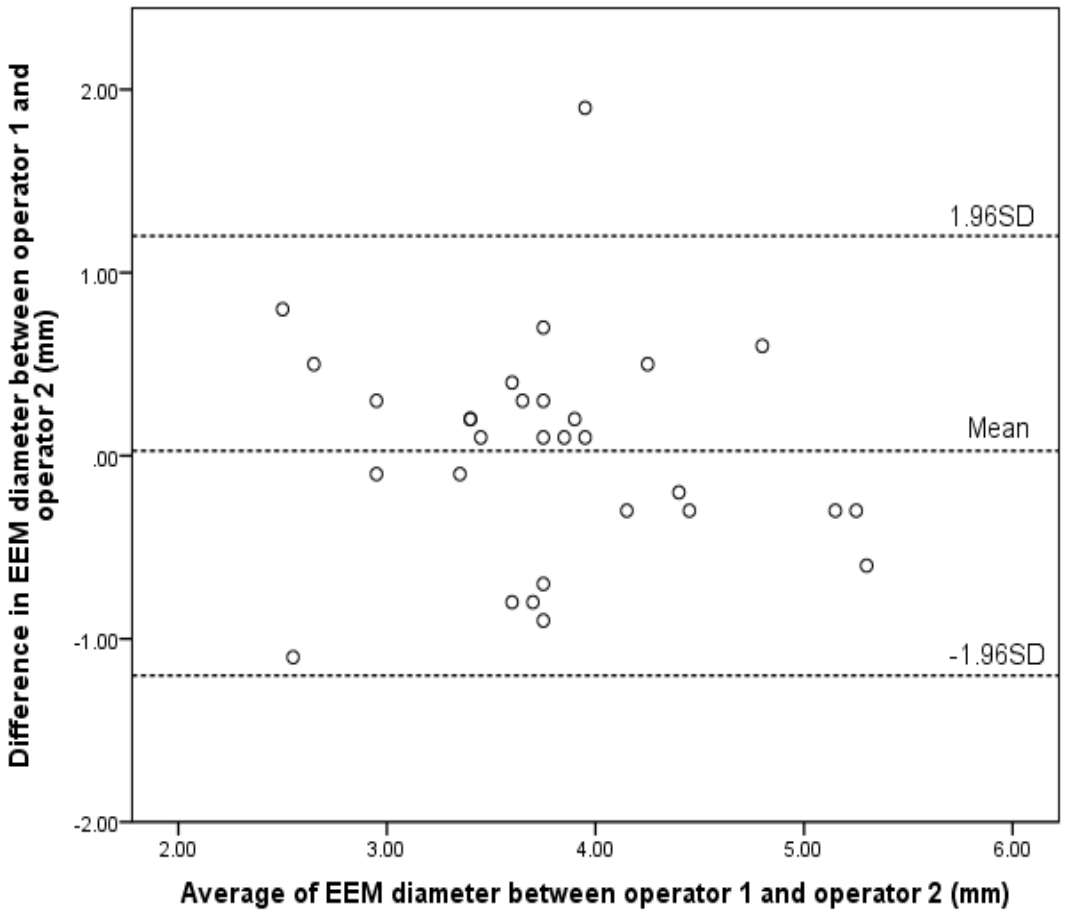

Figure 2.8: Bland-Altman graph demonstrating the test-retest agreement in LAD EEM measurements between 2 different operators.

Biochemistry results:

Baseline biochemistry results are given in table 2.1. The average Framingham risk score was calculated as 4.1±5.2%. The HDL level was lower in males than females (1.3±0.4mmol/L vs. 1.8±0.4mmol/L, $p<0.001$), the triglyceride level was higher (2.7±2.0mmol/L vs. 1.5±0.9mmol/L, $p<0.001$), the creatinine level was higher (85.1±12.6µmol/L vs. 65.8±11.3µmol/L, $p<0.001$) and the Framingham risk score was higher (8.7±7.3% vs. 2.1±1.7%, $p<0.001$). As the age increased the total cholesterol level increased ($r=0.24$, $p=0.002$), the HDL decreased ($r=-0.23$, $p=0.003$), the homocysteine level increased ($r=0.26$, $p=0.002$), the creatinine level increased ($r=0.44$, $p<0.001$) and the Framingham risk score increased ($r=0.44$, $p<0.001$). As the BMI increased the LDL increased ($r=0.23$, $p=0.004$), the HDL decreased ($r=-0.26$, $p=0.001$) and the CRP increased ($r=0.37$, $p<0.001$).

2.4 Discussion

The measurement of LAD wall thickness, luminal and EEM diameter is able to be reproduced within and between operators with a high degree of agreement. Using test-retest variation rather than intra and inter operator variability is important in this setting as it requires each operator to acquire and measure their own dataset rather than make measurements off of the same acquisition. This gives the test-retest variability greater validation in a real world setting. The variation in EEM measurement is the lowest as it is larger than both the wall thickness and luminal diameters making it easier to measure.

The majority of subjects (94%) enrolled in this study were able to have accurate measurement of their LAD wall thickness which could be due in part to improved equipment or the fact that they were all healthy volunteers thus making it easier for visualisation and measurements of the LAD to be made. Our current findings are consistent with previous studies by ourselves and others. In our study[131] we found the average wall thickness, luminal diameter and EEM in the normal cohort to be 1.2±0.3mm, 2.1±0.8mm and 4.4±0.9mm respectively. In the study by Gradus-Pizlo et al[128], the average wall thickness, luminal diameter, and EEM external diameter in the normal cohort were 0.9±0.1, 2.1±0.6, and 3.9±0.7 mm respectively. The corresponding values in this study closely agree with these values, supporting robust inter-observer variability for this novel technique.

The interesting finding of increasing wall thickness, luminal diameter and EEM diameter with increasing BMI may be significant in a number of ways.

The increase may be reflective of a higher level of atherosclerotic deposits in subjects with more body lipid composition, or may simply be a manifestation of a larger person needing a larger coronary artery for adequate blood supply. The fact that males, who are generally higher in BMI than females have a larger luminal and EEM diameter than females without an increase in their LAD wall thickness certainly reflects a larger person requiring a larger coronary artery diameter, therefore the increase in LAD wall thickness with increasing BMI in the total cohort probably does reflect an increase in subclinical atherosclerosis. . However, these findings may be incidental as regression analysis only demonstrated a slight contribution of BMI to the LAD measurements.

As the age increased there was an increase in both the LAD wall thickness and the EEM diameter, but no change in the luminal diameter that would also reflect an increase in atherosclerotic thickening certainly fitting in with what we know about the progression of silent CAD with advancing age. An increase in both the total cholesterol and LDL level resulted in an increase in the LAD wall thickness without a change to the luminal diameter, which again would suggest an increase in atherosclerotic deposit in those with higher blood cholesterol.

2.4.1 Limitations:

There is a potential risk of confusion of the LAD for the great cardiac vein as in most cases both vessels run parallel to each other along the anterior surface

of the heart. This risk was minimized however by measurement of the thicker walled vascular structure visualized and by using pulsed wave Doppler analysis to define the arterial from the venous blood flow in a subset of patients.

There were a number of subjects (6%) in which the LAD could not be imaged well enough to make accurate measurements of the wall thickness. Echocardiography, particularly at a high frequency, is limited in imaging subjects with poor intercostal spaces, significant lung disease and obesity.

Finally, although it is also possible that variations in coronary anatomy made it difficult to visualize the LAD, the proximal LAD is a very rare site of congenital anatomic abnormality (reported as <0.1%[9]) and thus congenital coronary anomalies would probably not contribute in this study.

2.5 Conclusion

HRTTE measurement of the LAD vessel is reproducible within and between operators in normal volunteers. This technique therefore warrants further study as a potential screening modality for subclinical coronary atherosclerosis to be investigated in the next chapter.

Chapter 3

Changes in Left Anterior Descending Coronary Artery Wall Thickness Detected by High Resolution Transthoracic Echocardiography

3.1 Introduction

Intra-vascular ultrasound and epicardial echocardiography studies have demonstrated that coronary atherosclerosis is a diffuse disease process and rarely spares the proximal coronary arteries, especially the proximal LAD [15, 23, 38, 54, 124, 132-133]. In fact, it has been shown that before clinical CAD is evident, at least 90% of the coronary artery tree is atherosclerotic [24, 124]. Accurate baseline measurements of the LAD luminal and external diameters and wall thickness are achievable using HRTTE [128, 134]. We sought to use this technique to demonstrate differences in coronary atherosclerosis between CAD patients and controls.

3.1.1 Aims

To determine if differences in LAD wall thickness, luminal and EEM diameters exist between normal subjects and patients with known CAD in non-LAD territories.

3.2 Methods

The Flinders Medical Centre Clinical Research Ethics Committee approved this study and all subjects provided written informed consent to participate (application number 118/034).

3.2.1 Subjects

Healthy volunteers (n=52) without a history of or risk factors for CAD (control group, no clinical hypertension, hypercholesterolemia, non-smokers, non-diabetics and no history of peripheral or cerebral vascular disease) and consecutive hospital inpatients with angiographically proven CAD (defined as a coronary artery stenosis > 50% in any coronary artery branch other than the LAD; CAD group, n=58) underwent an ultrasound scan to measure luminal and external artery calibre and anterior and posterior LAD wall thickness. The CAD group subjects were prospectively identified whilst in hospital. Subject characteristics for both the normal cohort and the CAD group are listed in table 3.1, columns A and C respectively.

3.2.2 Procedures

Echocardiography procedures and image analysis as in 2.2.2.1.

3.2.3 Statistical analysis

Continuous variables are summarised as mean ± standard deviation.

A t-test was used to determine if there was any significant difference in each measurement between the two groups. Discrete variables are summarised as

numbers and percentages and were compared with chi square analysis. A value of $p < 0.05$ was considered statistically significant.

3.3 Results

Adequate imaging of the LAD was possible in 50 of the 52 subjects (96%) in the control group (32 male) and in 50 of the 58 (86%) in the CAD group (42 male). These 10 subjects were excluded from analysis. The subjects in the CAD group were significantly older than in the control group (51±6 vs. 35±9 years, $p<0.001$) and had a higher body mass index (29±4 vs. 25±4 kg/m^2, $p=0.02$).

The anterior and posterior wall thickness significantly differed between the CAD group and controls (1.9±0.6 vs. 1.2±0.3mm, $p<0.001$ and 1.8±0.5 vs. 1.2±0.3mm, $p<0.001$, respectively). The external LAD diameter was also increased in the CAD group compared to controls (5.2±1.9 vs. 4.4±0.9mm respectively, $p=0.01$). However, there was no difference in the luminal diameter between the CAD group and the controls (1.9±0.9 vs. 2.1±0.8mm respectively, $p=0.3$). (figure 3.1).

The degree of LAD wall thickness did not correlate to the degree of coronary artery disease as determined by angiography.

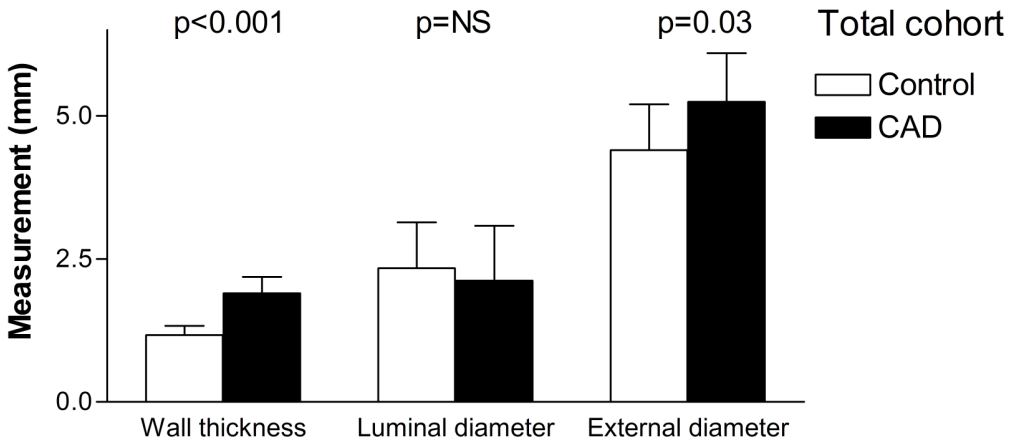

Figure 3.1: Graph demonstrating the differences in left anterior descending coronary artery wall thickness, luminal diameter and external artery diameter between normal subjects and patients with angiographically proven coronary artery disease >50% in any part of the coronary tree other than the proximal or mid left anterior descending coronary artery (CAD – coronary artery disease group).

To determine if this difference in wall thickness and external diameter was due to the effects of age a subgroup analysis was performed using control subjects above the age of 35 years and CAD subjects below the age of 55. In this subgroup analysis there were 23 control subjects (13 males) (table 3.1, column C) and 22 in the CAD group (17 males). The subgroup subject characteristics in the CAD group did not significantly differ from those of the total cohort except that they were significantly younger (table 3.1, column D). There was no difference in age or BMI between the two subgroups (age - control group, 42±5 years vs. CAD group 44±2 years, p=0.3 and BMI – control group, 27±3kg/m^2 vs. CAD group 28±4kg/m^2, p=0.2).

The anterior and posterior wall thickness significantly differed between the aged matched CAD group and controls (1.7±0.5 vs. 1.2±0.3mm, p=0.001 and 1.8±0.5 vs. 1.3±0.4mm, p=0.001, respectively). The external LAD diameter was again also increased in the age matched CAD group compared to the controls (5.2±1.9 vs. 4.5±0.8mm respectively, p=0.03). However, as seen in the total cohort there was no difference in the luminal diameter between the age matched CAD group and the controls (2.0±1.1 vs. 2.4±0.7mm respectively, p=0.5). (figure 3.2).

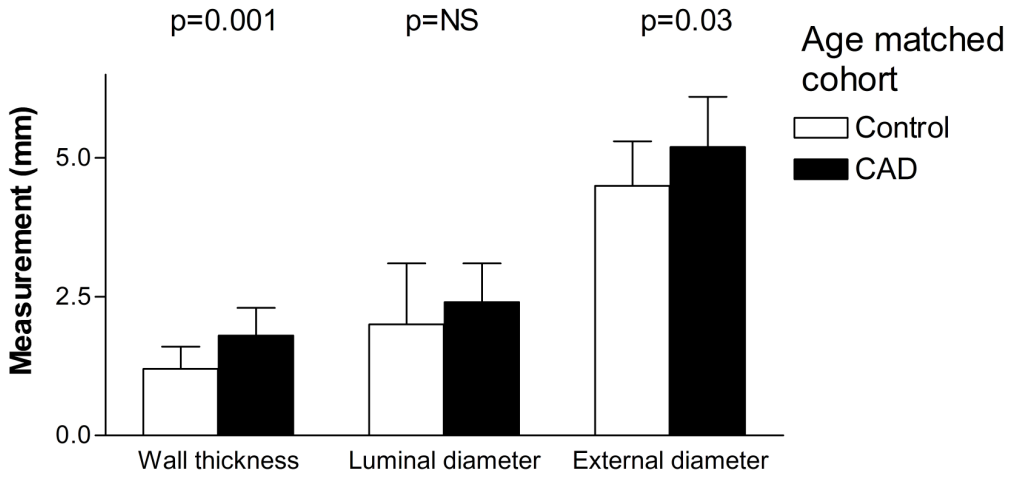

Figure 3.2: Graph demonstrating the differences in left anterior descending coronary artery wall thickness, luminal diameter and external artery diameter between age matched normal subjects and patients with angiographically proven coronary artery disease (CAD – coronary artery disease group).

Variable	A. Normal subjects (n=50)	B. Normal subgroup (normal subjects > 35 years, n=23)	C. CAD group (total CAD cohort, n=50)	D. CAD subgroup (CAD subjects <55 years, n=22)
Age (years)	35±9	42±5	51±7[#]	44±2*
Males	32 (64%)	13 (57%)	42 (84%)	17 (77%)
BMI (kg/m^2)	25±4	27±3	29±4	28±4
Total cholesterol (mmol/L)	5.2±1.0	5.3±1.0	4.9±1.7	4.9±1.6
Triglyceride (mmol/ L)	1.7±1.5	1.8±1.4	2.0±1.3	1.9±0.9
Low Density Lipoprotein (mmol/L)	2.8±0.8	2.9±0.8	3.0±1.6	3.1±1.5
High Density Lipoprotein (mmol/L)	1.6±0.6	1.6±0.5	1.4±1.2	1.2±0.3
C-Reactive Protein (mg/L)	2.3±2.6	2.5±2.9	8.0±9.4[#]	8.4±10.7[#]

Right coronary disease	**0 (0%)**	**0 (0%)**	**37 (74%)#**	**16 (73%)#**
Left circumflex disease	**0 (0%)**	**0 (0%)**	**30 (60%)#**	**14 (63%)#**
Previous statin therapy	**0 (0%)**	**0 (0%)**	**21 (42%)#**	**9 (41%)#**
Previous ACE inhibitor/ ARB	**0 (0%)**	**0 (0%)**	**9 (18%)#**	**4 (18%)#**
Previous aspirin	**0 (0%)**	**0 (0%)**	**18 (36%)#**	**7 (31%)#**
Hypertension	**0 (0%)**	**0 (0%)**	**13 (26%)#**	**6 (27%)#**
Diabetes Mellitus	**0 (0%)**	**0 (0%)**	**3 (6%)**	**1 (5%)**
Smokers	**0 (0%)**	**0 (0%)**	**17 (34%)#**	**11 (50%)#**

Table 3.1: Subject characteristics of CAD group in total cohort (column A) and subgroup analysis (column B). Continuous data expressed as mean ± standard deviation, discrete variables as number (percentage). # $p<0.05$ compared with total normal cohort and subgroup normal cohort, * $p<0.05$

compared with total CAD cohort. BMI – body mass index, CAD – coronary artery disease, ACE - angiotensin converting enzyme, ARB - angiotensin receptor blocker

3.4 Discussion

This study confirms that HRTTE can detect structural differences in proximal LAD morphology suggestive of atherosclerotic induced positive remodelling in patients with confirmed significant luminal CAD in other coronary vascular territories. We found the LAD wall thickness and the external diameter of subjects with CAD to be significantly larger than that of normal volunteers indicating atherosclerosis (figure 3.3). The luminal diameter, however, was the same for both groups indicating the well recognised phenomenon of positive remodelling at the measured site in the CAD group.

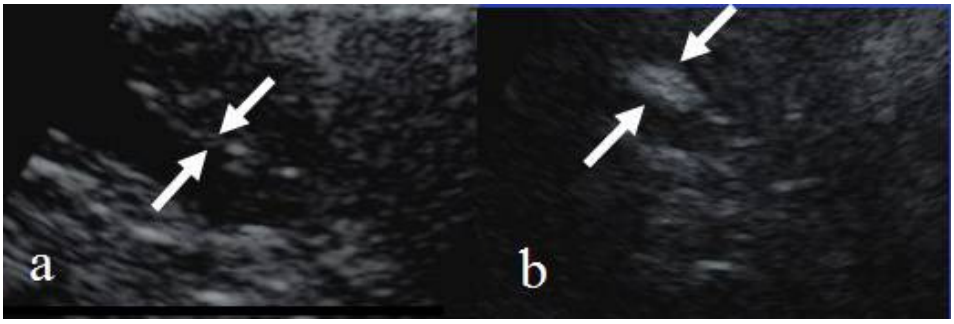

Figure 3.3: High resolution transthoracic echocardiography of LAD of subject in control group (A) and in CAD group (B). Arrows demonstrate anterior LAD wall thickness.

Subjects in the CAD group were older thereby introducing a bias that could potentially account for the differences in wall thickness and external artery diameter, a limitation also observed by the pioneering work of Gradus Pizlo et al [128]. To counter this we performed a subgroup analysis using the older control subjects (> 35 years) and the younger CAD group subjects (< 55 years). The comparative difference in visualised proximal LAD existed between the groups after this analysis thereby confirming that the differences were not solely age related.

The increased wall thickening seen in the CAD group despite the benign angiographic appearance of the proximal and mid LAD region indicates that this HRTTE method may be a more sensitive than angiography for the detection of subclinical atherosclerosis. This may have future clinical relevance in an era when high-risk subgroup targeted primary prevention therapeutic intervention exists such as statin and aspirin therapy.

In the study by Gradus-Pizlo et al [128], the average wall thickness, luminal diameter and external diameter in the CAD group was 1.9±0.4mm, 2.2±0.5mm and 6.0±1.1mm respectively and in the control group was 0.9±0.1mm, 2.1±0.6mm and 3.9±0.7mm respectively. The corresponding values in this study closely agree with these values, supporting robust inter-observer variability of this novel technique.

3.4.1 Limitations:

The CAD group had a higher BMI than the control group which may have accounted for some of the differences in the LAD wall thickness between the

two groups; however in the age matched subgroup the BMI did not differ significantly eliminating this limitation.

See also limitations section in 2.4.1.

3.5 Conclusion

HRTTE demonstrates that the LAD wall thickness and the external diameter of patients with CAD are significantly larger than that of normal volunteers, even when matched for age. The luminal diameter however is maintained in both groups indicating that the CAD group has undergone positive remodelling at the site measured. This objectively visualised evidence of coronary atherosclerosis with HRTTE would likely be undetected during coronary angiography.

Since this method is able to detect structural differences in the proximal and mid LAD between normal volunteers and subjects with known CAD it is warranted to investigate if this method is sensitive enough to detect functional changes in the LAD luminal diameter with known vasodilating drugs to determine direct coronary endothelial function. The use of HRTTE in detection of endothelial function will be discussed in the next chapter.

Chapter 4

High Resolution Transthoracic Echocardiography of the Left Anterior Descending Coronary Artery: A novel non-invasive assessment of coronary vasoreactivity

4.1 Introduction

Endothelial dysfunction is a precursor to the development of the clinical manifestations of CAD and is a significant predictor of future cardiovascular events [135-136]. The brachial or radial artery vasodilatory response may be used as a peripheral surrogate to assess coronary artery endothelial function. Differences in the endothelial function have been found between subjects with known CAD, subjects with risk factors for CAD and controls [135-140]. Endothelial function from both coronary (invasively measured) and peripheral artery vasodilator responses are able to predict long and short term CAD progression and more importantly cardiovascular event rates [141-145]. Nitroglycerin [146] (GTN) and salbutamol [147] are both coronary artery vasodilators. Salbutamol is an endothelial dependant vasodilator through the adrenergic release of nitric oxide[147] whereas GTN is an endothelial independent vasodilator acting through nitric oxide donation [148]. Together these drugs may be used in the assessment of endothelial function. Currently any changes that occur in coronary luminal diameter in response to these vasodilators have had to be measured invasively thus limiting their application to patients with some degree or suspicion of coronary artery disease rather than the subclinical target group of asymptomatic individuals [142-149].

Accurate baseline measurements of the LAD luminal and external diameters and wall thickness are achievable using HRTTE [128]. We used the HRTTE technique, combined with radial artery Pulse Wave Analysis (PWA), to study the vasomotion of the LAD in healthy male volunteers during drug challenge with salbutamol and GTN.

4.1.1 Aims

To determine if the HRTTE technique is able to detect changes in LAD luminal diameters with the administration of GTN and salbutamol.

4.2 Methods

The Flinders Medical Centre Clinical Research Ethics Committee approved this study and all subjects provided written informed consent to participate (application number 133/056).

4.2.1 Subjects

Nineteen healthy male subjects were consented for this study. Females were excluded from this pilot study as they can demonstrate significant variation in endothelial function throughout the menstrual cycle [150] and may have created confounding effects in such a small population.

4.2.2 Procedures

Healthy male subjects were administered 400µg of inhaled Salbutamol (4 puffs using a spacer) and, after 20 min and return to baseline, 300µg of sublingual GTN. This protocol has been previously validated for the non-invasive assessment of peripheral endothelial function in humans [151].

Subjects were asked to refrain from consuming alcohol and caffeine in the 8 hours before the examination and all subjects were non-smokers. Subjects were allowed to rest quietly in the supine position before baseline assessment and in between the administration of the two drugs. Blood pressure was measured non-invasively every 3 minutes (Dinamap™, DRE Inc, Louisville, KY, USA).

High Resolution Transthoracic Echocardiography:

Each subject underwent an ultrasound scan to measure luminal and external artery calibre and anterior and posterior LAD wall thickness at baseline and at 5, 10, 15 and 20 minutes post each drug challenge.

Echocardiography procedures and image analysis as in 2.2.2.1.

The same procedure was repeated on five subjects within 1- 2 weeks to determine the reproducibility of the HRTTE method.

Pulse wave analysis:

Endothelial function was assessed non-invasively using PWA method (appendix C) [141, 151-153]. Briefly, radial artery waveforms were recorded with a high-fidelity micromanometer (SPC-301, Millar Instruments) placed over the wrist of the dominant arm. A transfer function within the PWA software was then used to generate a central waveform from the radial waveform [151, 153]. The augmentation index (AIx), (the difference between the second and first systolic peaks) was then determined. The AIx indicates the extent of reflection of peripheral pressure waves to the central arteries and is a marker of arterial stiffness [153]. A decrease in the AIx is indicative of peripheral arterial vasodilatation. Endothelial function was assessed by measuring AIx at baseline and after 5, 10, 15, and 20 min post drug challenge.

4.2.3 Statistical analysis

All data are expressed as mean ± standard deviation. Each variable was analysed using one way ANOVA to detect a change over time and then each time point was compared to baseline using a post hoc *t* test with Bonferroni correction for repeated measures. The maximum change in the LAD luminal diameter was related to the maximum change in AIx, after either salbutamol or GTN, using Pearson's correlation. Intra-class correlation coefficients were calculated to assess intra-individual reproducibility of the maximum changes in LAD diameter and AIx post-drug (SPSS for Windows 11.0, SPSS Inc, Chicago, IL, USA). Statistical significance was assumed if $p < 0.05$.

4.3 Results

Nineteen healthy male subjects were enrolled into the study. The average age of the subjects was 31±5years. None of the subjects were smokers, hypertensive, diabetic or hypercholesterolemic, two of the subjects had a family history of premature atherosclerosis (age < 60 years). The baseline heart rate was 67±12bpm, systolic blood pressure 130±10mmHg and diastolic blood pressure 71±8mmHg. Heart rate and blood pressure did not significantly alter during the procedure. Baseline subject characteristics are displayed in table 4.1.

Variable	Value
Age (years)	**31±5**
Males	**19 (0%)**
Heart rate (BPM)	**67±12**
Systolic blood pressure (mmHg)	**130±10**
Diastolic blood pressure (mmHg)	**71±8**
HTN	**0 (0%)**
Diabetes	**0 (0%)**
Smokers	**0 (0%)**
Family history CAD	**2 (10%)**

Table 4.1: Baseline subject characteristics.

BPM – beats per minute, mmHg – millimetres of mercury, HTN – hypertension, CAD – coronary artery disease

High-resolution transthoracic echocardiography:

The luminal diameter of the LAD increased after salbutamol with a mean increase of 44±28% from baseline (2.8±0.8mm to 3.7±0.9mm, $p<0.001$). The maximal increase in LAD luminal diameter occurred at 10 minutes post drug inhalation, however at both 5 and 10 minutes post drug challenge the luminal diameter was significantly greater than at baseline (Figure 4.1). GTN induced greater vasodilatation of the LAD with a mean increase of 60±30% in luminal diameter from baseline (2.7±0.9mm to 4.4±1.1mm, $p<0.001$). The maximal luminal vasodilatation occurred at 5 minutes post drug challenge (Figure 4.2) but remained significantly dilated at 10 minutes compared to baseline (Figure 4.1). Corresponding changes were demonstrated in the external LAD diameter. No significant change was detected in wall thickness.

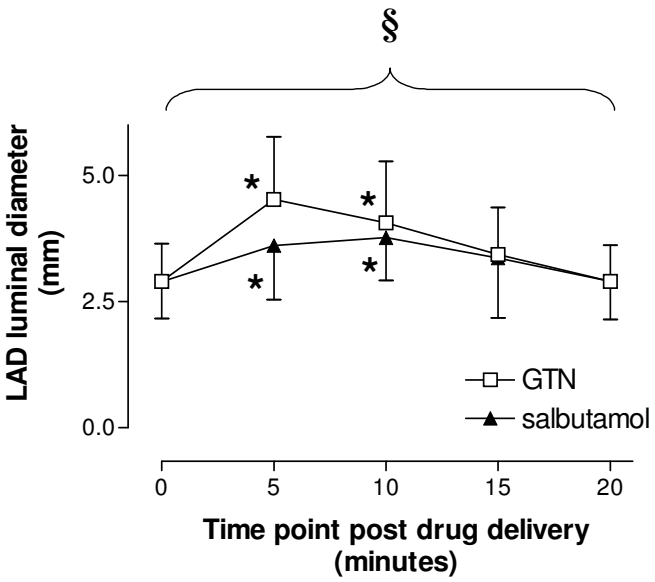

Figure 4.1: Graph demonstrating the change in left anterior descending coronary artery luminal diameter over time with administration of salbutamol (black triangles) and GTN (open squares). $^{\S}p<0.01$; *$p<0.001$ vs. baseline

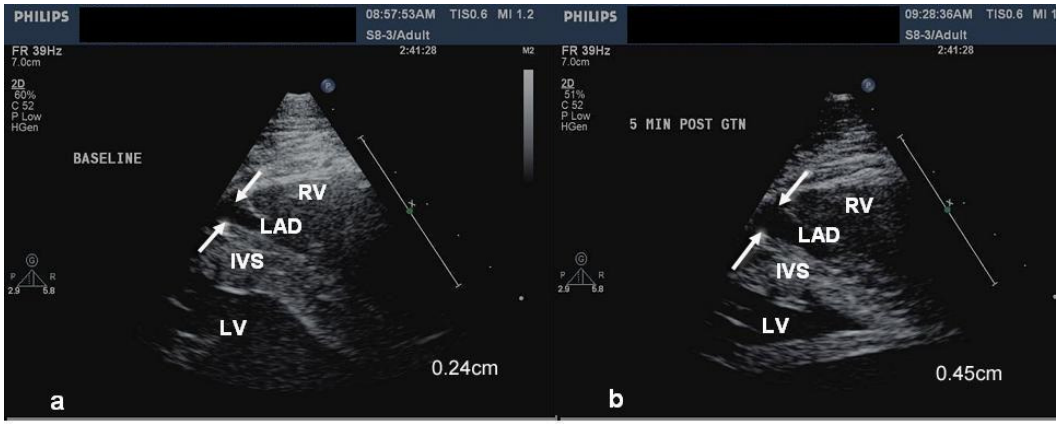

Figure 4.2: High resolution transthoracic echo image of the left anterior descending coronary artery (LAD) in a single subject at (a) baseline measuring 0.24cm and (b) 5 minutes post sublingual GTN measuring 0.45cm. Both pictures are taken at the same depth (7cm). The arrows demonstrate the LAD lumen. (IVS – inter-ventricular septum, LAD – left anterior descending coronary artery, LV – left ventricular cavity, RV – right ventricular cavity)

Pulse wave analysis:

Both salbutamol and GTN induced a significant reduction in AIx consistent with peripheral arterial vasodilatation (-13.4±6.6%, $p<0.001$ and -24.1±8.2%, $p<0.001$, respectively).

The vasodilatation of the LAD luminal diameter was related to reduction in AIx due to radial artery dilatation after both salbutamol ($r=-0.53$, $p=0.02$) (figure 4.3a) and GTN ($r=-0.57$, $p=0.01$) (figure 4.3b).

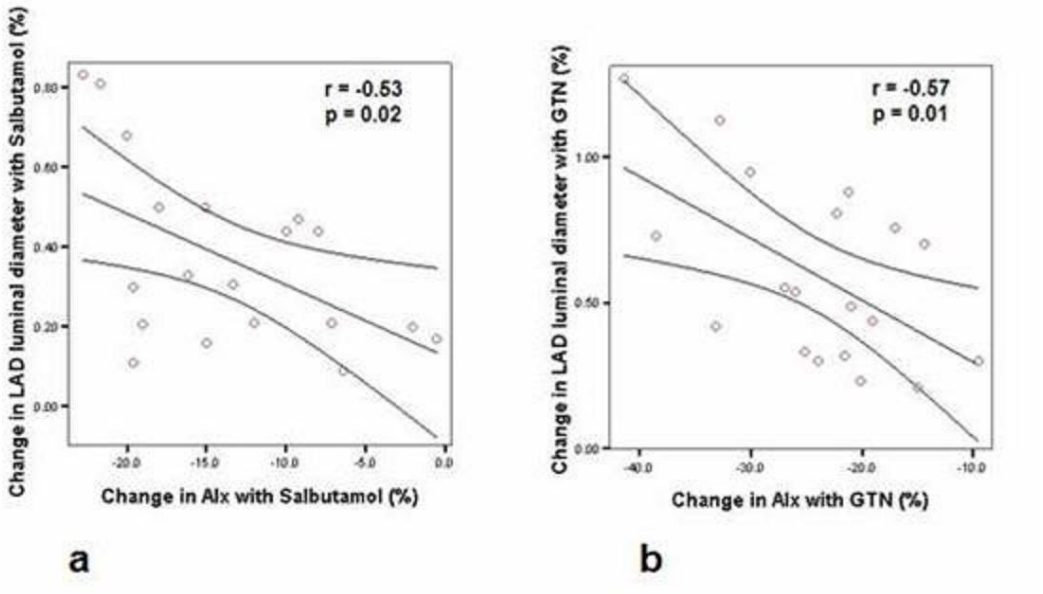

Figure 4.3: Graphs demonstrating the relationship between the change in the luminal diameter of left anterior descending coronary artery (LAD) by high resolution transthoracic echo (HRTTE) and the change in the augmentation index (AIx) by pulse wave analysis with (a) salbutamol and (b) GTN. Graphs depict line of best fit and 95% confidence interval

Reproducibility:

There was good reproducibility for the change in LAD luminal diameter with both GTN (intra-class correlation coefficient 0.84, $p<0.01$) and salbutamol (intra-class correlation coefficient 0.85, $p<0.01$).

The intra and inter operator variability for offline measurement of the luminal LAD diameter was 2.4% ($r=0.84$, $p<0.001$) and 3.3% ($r=0.78$, $p<0.001$) respectively.

4.4 Discussion

This is the first study to our knowledge directly demonstrating coronary artery vasoreactivity using HRTTE. Our results have demonstrated that HRTTE is sufficiently sensitive to detect changes in the LAD luminal diameter with known vasodilating drugs in normal volunteers. Moreover these changes are highly reproducible and correlate with peripheral endothelial function measured by PWA.

This novel technique is the first to be able to non-invasively visualise the functional status of the coronary endothelium. Whether coronary endothelial function assessed with HRTTE will have an incremental value over current cardiovascular risk factor analysis remains to be seen. However HRTTE is a rapid, non-invasive and relatively inexpensive method to directly detect coronary artery vasomotion in real time.

The ability of HRTTE to detect coronary vasomotion comes from recent advances in ultrasound technology that have allowed for higher frequency and therefore higher resolution transducers to be used enabling visualisation and accurate measurements of small structures such as the coronary arteries. The broadband transducer used in this study is designed to give excellent axial resolution with adequate penetration to reach the anterior structures of the heart.

Inter and intra operator variability:

In our hands we have found that using HRTTE to detect coronary vasomotion was highly reproducible with excellent intra and inter operator variability as also demonstrated in chapter 2.

It is well established that endothelial dysfunction is a strong risk factor for the future development of cardiovascular disease using assessment of the peripheral vasculature [145-149]. In fact the presence of peripherally measured endothelial dysfunction has been shown to have better predictive power than carotid IMT and has an incremental value above conventional risk factor analyses [154]. However the periphery may react differently to the coronary circulation and hence using HRTTE for direct assessment of coronary artery endothelial function rather than a surrogate may further improve cardiac event risk assessment.

Comparison with previous studies:

It is difficult to compare the results of our study with other studies on the effects of salbutamol and GTN on the coronary vasculature as these studies were invasive and usually involve subjects with some degree of coronary disease. Barbato et al found that in 'normal' coronary segments salbutamol induced an average 10% increase in the luminal diameter [147]. However, the subjects used were at high risk for the development of coronary disease and consequently likely had a degree of endothelial dysfunction.

Another study found that a variation in the degree of dilatation (with averages between 9 to 54%) occurred according to the location and size of the vessel [155], however it is unclear as to the degree of CAD in their cohort.

We found a mean increase in luminal diameter of 44% with salbutamol and 60% with GTN. When compared to brachial artery flow-mediated vasodilatation in normal subjects (6-10%) [156-157] this is a much larger increase. However the microvasculature of the coronary circulation differs from that of the periphery in that the coronary circulation operates as a higher resistance circuit with a large reserve capacity, this may explain the marked changes seen in the coronary luminal diameter with both drugs [158]. Also an inverse relationship between vessel diameter and degree of vessel dilatation with hyperaemia has been found in the brachial artery [135]. Therefore the smaller resting luminal diameter of the LAD may account for the magnitude of the drug-induced dilatation observed in our study.

It has been found that the change in AIx with the PWA method correlates with invasive measures of endothelial function using forearm venous occlusion plethysmography and brachial artery cannulation with administration of vasodilators [159]. This makes the PWA method the non-invasive method of choice to test the HRTTE method. We detected a modest relationship between AIx and coronary vasodilatation consistent with the premise of systemic arterial vasodilatation with these agents.

4.4.1 Limitations:

See also limitations section in 2.4.1.

This study was limited to male subjects only. Females were excluded from this pilot study as they can demonstrate significant variation in endothelial function throughout the menstrual cycle and may have created confounding effects in such a small population.

Since the study was performed on subjects without known atherosclerosis or significant risk factors, we cannot extrapolate our findings to a diseased population. However, we have shown that HRTTE is sufficiently sensitive to detect coronary vasomotion in the normal physiological state.

It is difficult to know that we have measured sequentially the same portion of the LAD due to rotation and translational effects throughout the cardiac cycle. We tried to reduce this variability by maintaining the transducer in a fixed position throughout the procedure.

Potential clinical implications:

Direct detection of coronary endothelial function using HRTTE may be able to be developed to improve risk stratification in the low to intermediate cardiovascular risk population perhaps enabling targeted primary prevention.

Due to its non-invasive nature this method could also be applied in longitudinal interventional studies to detect changes in coronary endothelial function.

4.5 Conclusion

This is the first non-invasive study directly showing the effects of recognised vasodilators on the coronary circulation. The HRTTE technique is sufficiently sensitive to detect coronary artery vasomotion and warrants further investigation as a new window to coronary artery structure and function.

As HRTTE is sensitive enough to detect both structural and functional changes in the LAD this technique may be suitable for the prediction of future cardiovascular events and this will be investigated in the next chapter.

Chapter 5

Left Anterior Descending Coronary Artery Wall Thickness Detected by High Resolution Transthoracic Echocardiography Predicts Future Ischemic Events

5.1 Introduction

Conventional cardiac risk factors do not fully explain the incidence of coronary artery disease and coronary events. Risk stratification and therapy based solely on these conventional risk factors may exclude a population who would otherwise benefit from lifestyle and risk factor modification. Additionally, risk factor stratification has been demonstrated to have significant limitations in the individual patient, this has in turn generated a search for more specific and sensitive surrogate marker of the disease process. Accurate measurements of the LAD luminal and external diameters and wall thickness have been shown to be achievable using HRTTE [128, 131, 134] which visualises the proximal LAD. This technique may demonstrate subclinical CAD and reflect the burden of coronary atherosclerosis. Intra-vascular ultrasound and epicardial echocardiography studies have demonstrated that coronary atherosclerosis is a diffuse disease process that rarely spares the proximal coronary arteries, especially the proximal LAD [15, 23, 38, 54, 124, 132-133]. In fact, it has been shown that before clinical CAD is evident, at least 90% of the coronary artery tree is atherosclerotic [24, 124]. We sought to use HRTTE to demonstrate the rates of LAD wall thickness abnormality in low, intermediate and high risk groups and to determine if the LAD wall thickness could independently predict future cardiovascular events in reportedly normal subjects.

5.1.1 Aim

To determine if LAD wall thickness by HRTTE will be predictive of future cardiovascular events in a population free of clinical CAD.

5.2 Methods

The Flinders Medical Centre Clinical Research Ethics Committee approved this study and all subjects provided written informed consent to participate (application number 119/034).

5.2.1 Subjects

Subjects were recruited via mail-out and identified through local general practice surgeries. Two thousand invitations to participate were sent and potential subjects returned a brief questionnaire on cardiovascular risk to apply to be in the study (appendix D). There were 425 responses to the invitation and 174 subjects were deemed suitable for the study. Subjects were suitable if there was no previous evidence of coronary, cerebral or peripheral atherosclerosis, no history of diabetes and they were not on any medication for hypertension, dyslipidemia or cardiac disease. In total, 131 subjects were consented to the study and underwent the following tests: HRTTE measurement of the LAD wall thickness, intima-media thickness (IMT) of the common carotid artery, a blood test for lipids, homocysteine, c-reactive protein, creatinine levels, blood pressure measurement and further cardiovascular questioning (appendix E).

5.2.2 .Procedures

Echocardiography procedures and image analysis as in 2.2.2.1.

An arbitrary cut-off value for baseline LAD wall thickness of 1.5mm was assigned with measurements under this value being considered normal and above or equal to this being considered abnormal. This cut-off value was determined from previous studies on normal subjects using the 95% confidence interval of normal wall thickness values from Chapter 2 [131, 134].

Carotid IMT:

Measurement of the common carotid artery (CCA) IMT was undertaken according to the following protocol. Examinations were obtained using a commercially available ultrasound system (iE33, Philips Medical Systems, Bothell, Washington, USA) with a high frequency linear array transducer (L11-3). Subjects lay in the supine position with a slight chin tilt away from the side being interrogated. Both the right and left carotid arteries were imaged, with the CCA being defined as the arterial segment between 2 and 6cm from the carotid bifurcation. Dedicated software (Philips Qlab, IMT quantification) was used to determine the IMT of the far wall of the CCA and both the left and right IMT was averaged to give an overall IMT.

Other testing:

A fasting blood test was obtained to measure total cholesterol, triglycerides, LDL, HDL, C-reactive protein (CRP), homocysteine and creatinine levels. Blood pressure was measured non-invasively using an automatic blood

pressure cuff (Dinamap™, DRE Inc, Louisville, KY, USA). Height and weight were measured and BMI calculated.

Subjects were followed up by phone to determine if an ischemic event had occurred in the follow-up period. Hospital records were obtained to confirm the type of event. An ischemic event on follow up was defined as either a non-ST-segment elevation acute coronary syndrome (NSTEMI) or a ST-segment elevation myocardial infarction (STEMI) with associated ischaemic discomfort and the presence of serum cardiac markers (CK-MB and troponin) in the blood.

Framingham risk score was calculated using previously published guidelines for primary risk (appendix F) [160].

5.2.3 Statistical analysis

Data are expressed as the mean ± standard deviation for continuous variables and as percentages for discrete variables.

Univariate analysis was performed to evaluate variables as predictors of an ischemic event. The independent variables examined were Framingham risk score, LAD wall thickness, carotid IMT, age, gender, BMI, total cholesterol, triglycerides, LDL, HDL, CRP, homocysteine, creatinine, systolic and diastolic blood pressure, smoking status and family history of premature cardiovascular disease. Tests included chi-square analysis for discrete variables and a student's t-test for normally distributed continuous variables and a Mann Whitney test for non-normally distributed continuous variables.

Kaplan-Meier survival using log-rank analysis was performed to assess event free survival.

All statistical analyses were performed using SPSS software for Windows (version 17.0, SPSS Inc., Chicago, Illinois, USA). A p value of < 0.05 was considered statistically significant.

5.3 Results

Adequate imaging of the LAD was made in 121 of the 131 (92%) subjects. The average age was 53±14 years and there were 39 (30%) males. The majority of subjects (113, 86%) had a low Framingham risk score (cardiovascular event rate <6% predicted over the next 10 years), with 14 (11%) at intermediate risk (6-20% event rate predicted) and 4 (3%) considered to be at high risk (≥20% event rate predicted). Baseline subject characteristics are shown in table 5.1.

Total cohort	
Age (years)	53±14
Males	39 (30%)
BMI (kg/m^2)	26±4
Total cholesterol (mmol/L)	5.2±1.0
Triglyceride (mmol/L)	1.9±1.5
LDL (mmol/L)	2.8±0.8
HDL (mmol/L)	1.6±0.5
CRP (mg/L)	2.3±2.6
Framingham risk score	4.2±5.4
HTN	0 (0%)
Diabetes	0 (0%)
Smokers	20 (15%)
Family history CAD	30 (23%)
Systolic blood pressure (mmHg)	128±19
Diastolic blood pressure (mmHg)	75±10
Mean blood pressure (mmHg)	95±14
LAD wall thickness (mm)	1.3±0.3
LAD luminal diameter (mm)	2.3±0.5
LAD external diameter (mm)	4.8±0.9
Carotid IMT (mm)	6.3±1.4

Table 5.1 Baseline subject characteristics

BMI – body mass index, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – C-reactive protein, HTN – hypertension, CAD – coronary artery disease, LAD – left anterior descending coronary artery, IMT – intima-media thickness.

Risk factor analysis:

Those in the low risk group were younger than the intermediate risk group, were less likely to be male, and had lower systolic blood pressure, creatinine level and homocysteine level. They also had a more favourable lipid profile and a smaller carotid IMT (table 5.2).

Those in the high -risk group were more likely to be male than those in the low risk group and had a less favourable lipid profile (table 5.2).

Those in the intermediate risk group had a higher HDL level than those in the high risk group (table 5.2).

	Low risk group (n=113, 86%)	Intermediate risk group (n=14, 11%)	High risk group (n=4, 3%)
Age (years)	**50.6±13.7**	**66.8±10.5[a]**	**67.5±11.9[a]**
Males	**22 (19%)**	**13 (93%)[a]**	**4 (100%)[a]**
BMI (kg/m^2)	**25.8±4.4**	**25.3±3.8**	**27.4±0.8**
Positive family history	**27 (24%)**	**2 (14%)**	**1 (25%)**
Smokers	**15 (13%)**	**3 (21%)**	**2 (50%)**
Systolic BP (mmHg)	**126.4±18.5**	**140.5±20.3[a]**	**149.3±26.6[a]**
Diastolic BP (mmHg)	**74.5±10.4**	**78.9±10.5**	**83.0±8.0**
Total cholesterol (mmol/L)	**5.2±1.0**	**5.5±0.8**	**4.9±1.4**
Triglycerides (mmol/L)	**1.6±1.1**	**3.6±2.8[a]**	**3.7±1.6[a]**
LDL (mmol/L)	**2.8±0.8**	**3.0±0.8**	**2.6±0.5**
HDL (mmol/L)	**1.7±0.5**	**1.3±0.3[a]**	**0.8±0.3[ab]**
Creatinine (µmol/L)	**68.4±12.2**	**92.1±15.6[a]**	**83.0±17.8**
Homocysteine	**10.4±3.5**	**15.1±6.5[a]**	**15.9±5.7**

(µmol/L)			
CRP (mg/L)	**2.3±2.7**	**2.7±2.5**	**2.3±1.2**
Carotid IMT (mm)	**6.1±1.3**	**7.4±0.9[a]**	**7.0±1.8**
LAD wall thickness (mm)	**1.3±0.3**	**1.4±0.2**	**1.3±0.2**
Abnormal LAD wall thickness	**27 (24%)**	**6 (43%)**	**2 (50%)**
Events	**6 (5%)**	**2 (14%)**	**1 (25%)**

Table 5.2: Baseline subject characteristics according to Framingham risk group – low (<6% over the next 10 years), intermediate (6-20% risk) or high (≥20% risk).

BMI – body mass index, BP – blood pressure, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – c-reactive protein, IMT – intima media thickness, LAD – left anterior descending coronary artery.

a- $p<0.05$ c/w low risk group, b-$p<0.05$ c/w intermediate risk group

LAD wall thickness:

Using the cut-off value of <1.5mm as a normal LAD wall thickness there were 86 (65%) subjects under this value and 35 (27%) above this value. In 10 (8%) subjects the LAD was unable to be visualised adequately, however none of these patients had an ischemic event. Those with an abnormal LAD wall thickness were older, were more likely to be male, had a higher Framingham risk score, had higher systolic and diastolic blood pressure, had a higher carotid IMT and were more likely to have had an event. Baseline characteristics of the 2 groups are shown in table 5.3.

The LAD wall thickness and the carotid IMT was weakly correlated ($r=0.33$, $p<0.001$, figure 5.1).

	Normal LAD group (n=86, 66%)	Abnormal LAD group (n=35, 27%)
Age (years)	**51.2±13.6**	**58.8±15.0[a]**
Males	**21 (24%)**	**15 (43%)**
Framingham risk score (%)	**3.8±5.7**	**5.7±5.4[a]**
BMI (kg/m^2)	**25.6±4.3**	**25.8±4.2**
Positive family history	**20 (23%)**	**9 (26%)**
Smokers	**13 (15%)**	**5 (14%)**
Systolic BP (mmHg)	**124.3±19.6**	**138.2±17.4[a]**
Diastolic BP (mmHg)	**72.9±10.3**	**80.0±9.1[a]**
Total cholesterol (mmol/L)	**5.1±0.9**	**5.3±0.9**
Triglycerides (mmol/L)	**1.8±1.3**	**2.0±1.9**
LDL (mmol/L)	**2.7±0.8**	**2.9±0.8**
HDL (mmol/L)	**1.6±0.5**	**1.6±0.4**
Creatinine (µmol/L)	**70.2±13.9**	**75.6±16.5**
Homocysteine (µmol/L)	**10.9±4.3**	**12.1±4.3**
CRP (mg/L)	**2.1±2.3**	**2.7±3.4**
Carotid IMT (mm)	**6.1±1.2**	**6.8±1.6[a]**

LAD wall thickness (mm)	**1.2±0.1**	**1.6±0.2[a]**
Events	**1 (1%)**	**8 (23%)[a]**

Table 5.3 Baseline characteristics between subjects with normal LAD wall thickness (<1.5mm) and abnormal LAD wall thickness (≥1.5mm).

BMI – body mass index, BP – blood pressure, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – c-reactive protein, IMT – intima media thickness, LAD – left anterior descending coronary artery.

a- $p<0.05$ c/w normal LAD group.

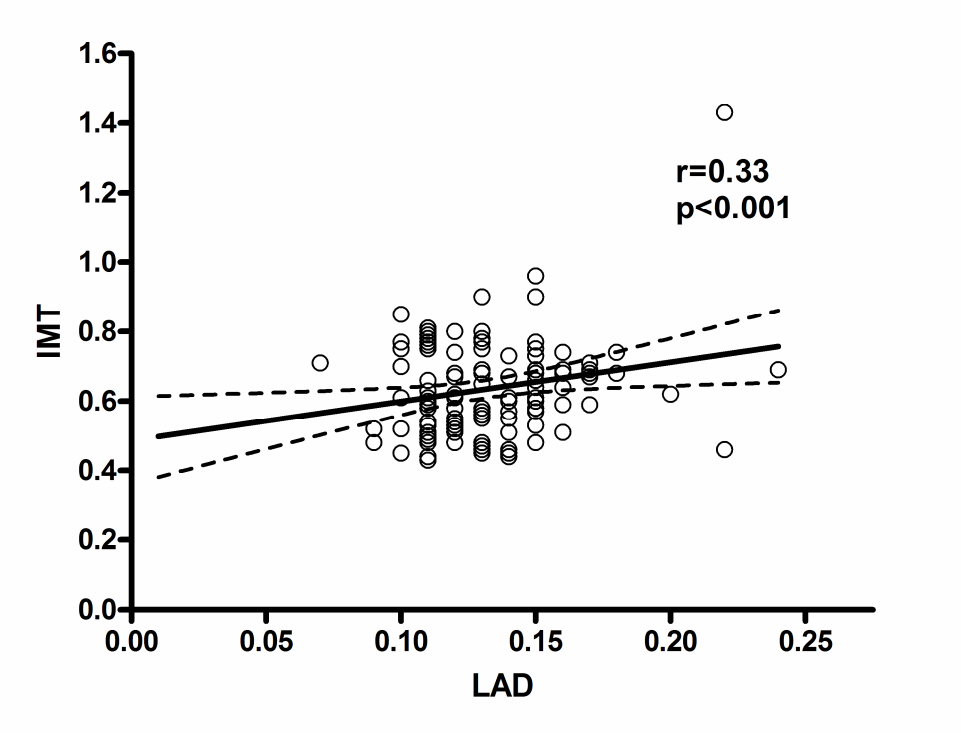

Figure 5.1: A scatter plot demonstrating the correlation between carotid intima-media thickness (IMT) and left anterior descending coronary artery wall thickness (LAD).

Event rate:

The average length of follow up was 39.0±5.7 months. One subject (1%) was lost to follow-up. Overall there were 9 (7%) documented ischemic events on follow up. There were 4 STEMIs and 5 NSTEMIs on follow up. Those subjects who had an event were older than those who did not have an event and had a larger LAD wall thickness. Baseline characteristics of the 2 groups are shown in table 5.4.

Kaplan-Meier event free survival analysis demonstrated that the baseline LAD wall thickness was able to predict event rate in this cohort (p=0.006, figure 5.2). Using receiver operator curves a cut-off value of 55 years for age was then used in a Kaplan-Meier analysis. An age above this cut-off was also able to predict event rate in this cohort (p=0.028, figure 5.3). Traditional risk factors and carotid IMT was unable to predict an event in this cohort.

To account for age only those subjects aged over 55 years were used to determine if the LAD wall thickness could independently predict event rate, which it was able to do (p=0.02, figure 5.4).

	No event group (n=122, 93%)	Event group (n=9, 7%)
Age (years)	52.1±14.4	66.2±9.8[a]
Males	36 (29%)	3 (33%)
Framingham risk score (%)	4.2±5.5	6.1±5.0
BMI (kg/m^2)	25.8±4.3	25.1±2.9
Positive family history	30 (24%)	0 (0%)
Smokers	20 (16%)	0 (0%)
Systolic BP (mmHg)	127.7±19.0	140.9±22.1
Diastolic BP (mmHg)	74.8±10.1	81.7±10.8
Total cholesterol (mmol/L)	5.2±1.0	5.6±1.0
Triglycerides (mmol/L)	1.9±1.5	2.5±1.6
LDL (mmol/L)	2.8±0.8	3.3±0.6
HDL (mmol/L)	1.6±0.5	1.5±0.5
Creatinine (µmol/L)	70.4±13.9	78.2±14.1
Homocysteine (µmol/L)	10.8±3.8	15.4±7.8
CRP (mg/L)	2.3±2.6	2.6±1.6
Carotid IMT (mm)	6.2±1.4	6.9±1.1

LAD wall thickness (mm)	**1.3±0.3**	**1.6±0.2[a]**

Table 5.4 Baseline characteristics between subjects without and event and with an event on follow up.

BMI – body mass index, BP – blood pressure, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – c-reactive protein, IMT – intima media thickness, LAD – left anterior descending coronary artery.

a- $p<0.05$ c/w non event group.

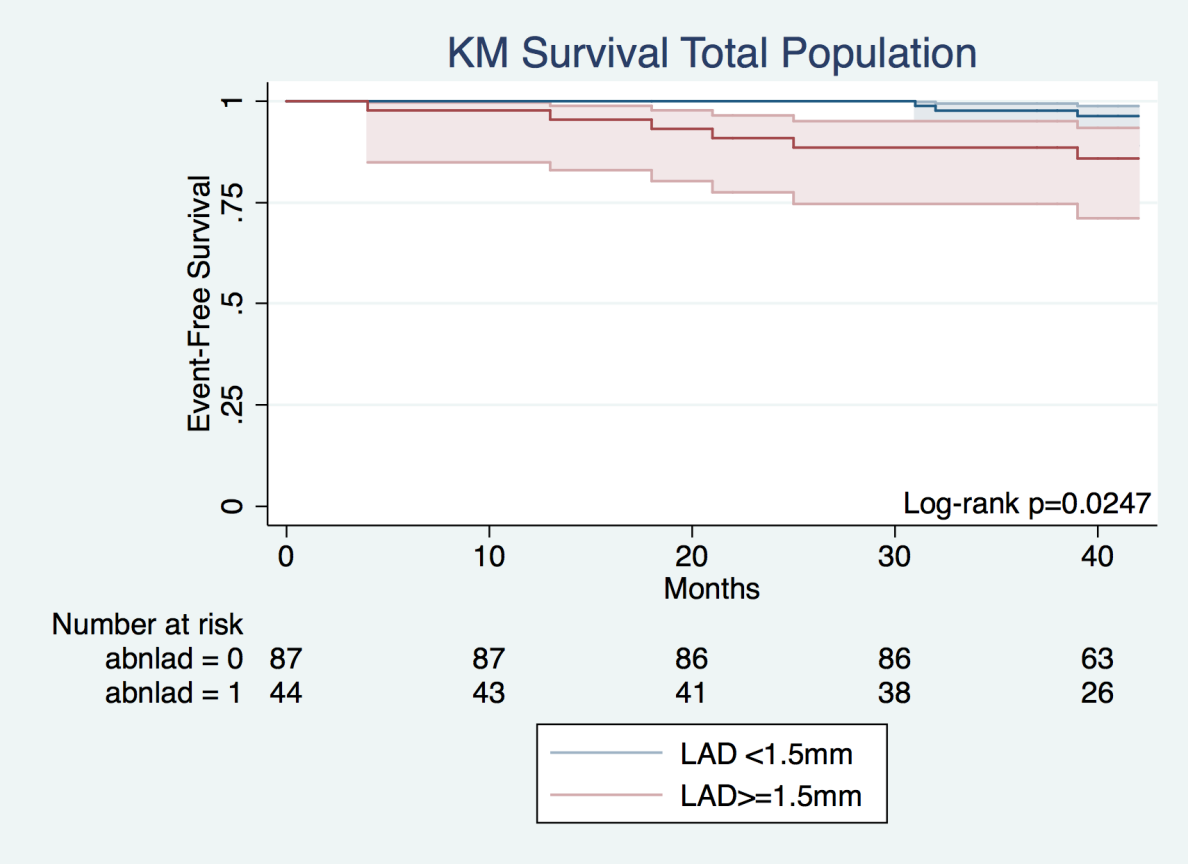

Figure 5.2: A Kaplan-Meier survival graph using the cut-off value of the baseline LAD wall thickness as the factor for analysis.

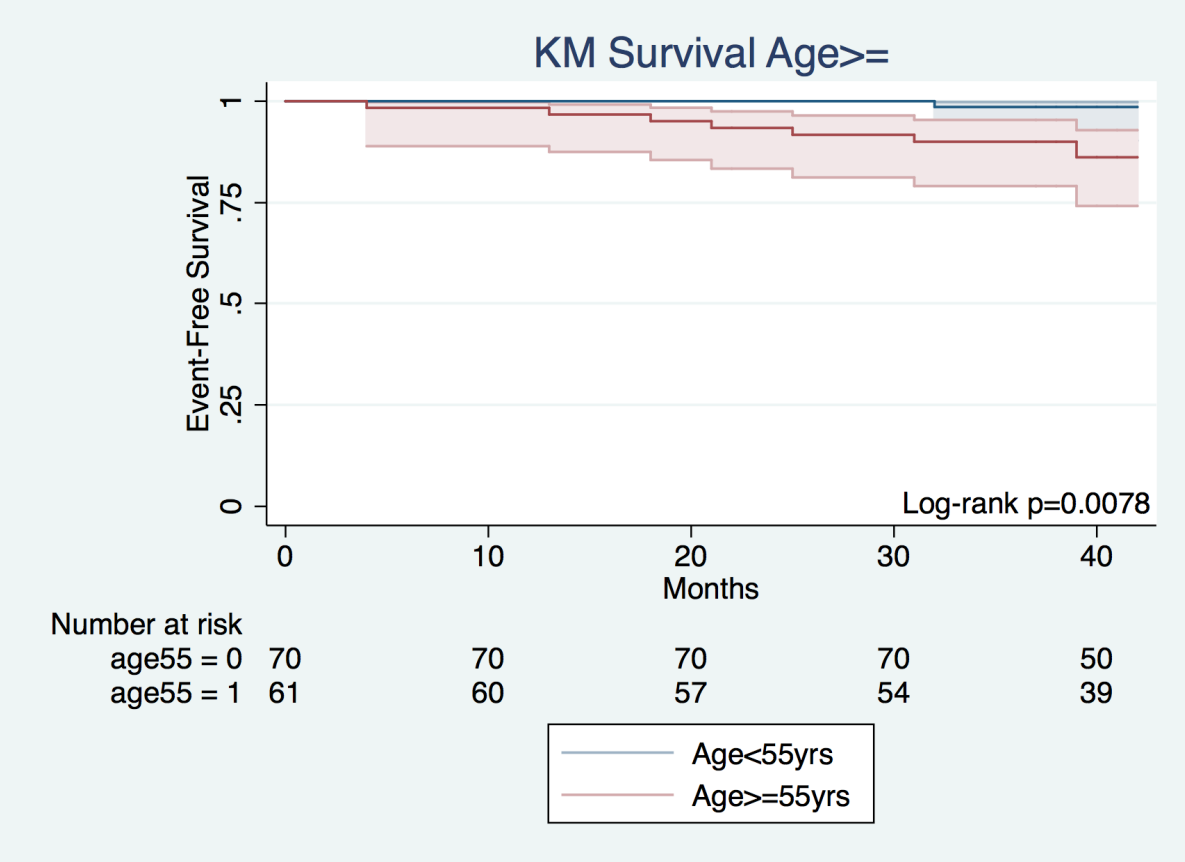

Figure 5.3: A Kaplan-Meier survival graph with age as the factor for analysis.

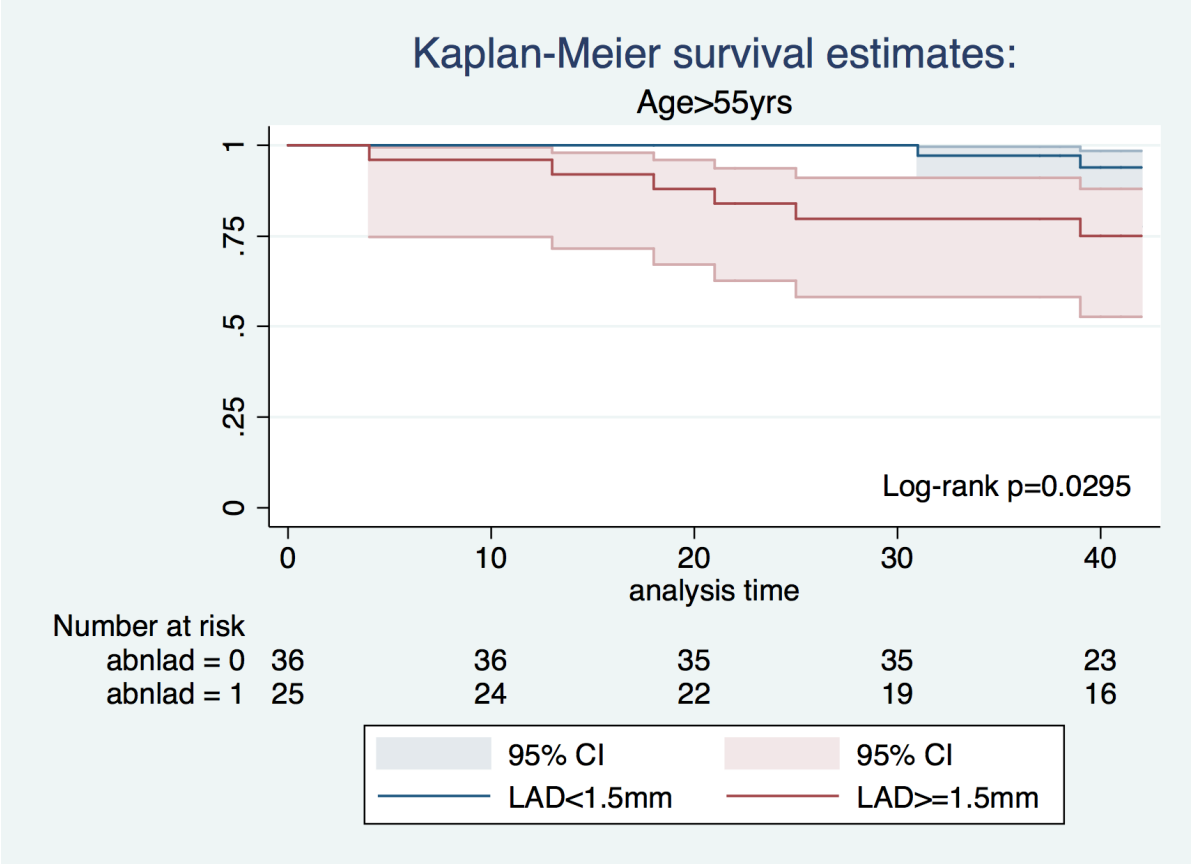

Figure 5.4: A Kaplan-Meier survival graph using the cut-off value of the baseline LAD wall thickness as the factor for analysis in subjects over 55 years of age.

5.4 Discussion

This is the first study using the novel method of coronary artery wall thickness measurement with HRTTE to determine future events in a self-deemed normal population. All subjects were selected on the basis of minimal reported cardiac risk and all were not on any cardiac medications. Other than age, which is a known and non-reversible risk factor, the only predictor of an event was the baseline LAD wall thickness in this small sample. However to minimise the influence of age on this analysis only those subjects aged > 55 years were analysed and the ability of the LAD wall thickness to predict an event remained rather than any other conventional risk factor. This association supports the premise that LAD wall thickness as determined by HRTTE may reflect coronary atherosclerotic burden.

Risk factor analysis:

As expected the majority of subjects fitted into the low risk category using Framingham risk assessment. There were however a higher percentage of intermediate and high risk subjects (14%) than expected demonstrating the need for more education and awareness of cardiovascular risk at a population level.

The Framingham risk results were sent to both the subject and their general practitioner and many in the intermediate and high-risk groups underwent lifestyle modification (quitting smoking, dieting to reduce cholesterol etc) in

an attempt to lower this risk. Whilst this may have added a bias to the result by potentially reducing the event rate in these subjects, non-disclosure of the results would have been unethical. Furthermore, a significant difference in event rate at 39 months despite lifestyle modification in motivated subjects (self selected) strengthens the significance of our findings. No subject commenced cardiac medication due to the risk results.

There was no difference in the baseline LAD wall thickness between the 3 risk factor groups.

Event rate:

Standard conventional risk profiling was unable to accurately predict event rates in this subject cohort. In fact, other than age, only a LAD wall thickness of ≥1.5mm was predictive of future events. Early coronary atherosclerosis is a disease of the vessel wall and this non-invasive measurement of the LAD wall thickness appears to be able to detect threatening coronary atherosclerosis.

There was no difference in the LAD luminal measurement between the event and non-event groups indicating that this wall thickening determined by HRTTE would appear to reflect the overall burden and threat of coronary atherosclerosis and may have remained undetected by standard coronary angiography. This is consistent with the well-recognised phenomenon of positive remodelling in atherosclerotic coronary disease; in this setting the vessel wall is abnormal with atherosclerotic plaque while the lumen is preserved.

Invasive procedures such as intravascular ultrasound are better able to determine the stability and composition of atherosclerotic plaques whereas HRTTE can only measure the thickness of the proximal LAD wall. The wall thickness measurement does not give any information about the structure or vulnerability of the atherosclerosis; however as a screening tool for early detection of CAD it is ideal. It is a relatively inexpensive technique that in experienced hands is rapid and easy to perform. Compared to other imaging techniques, it is non-invasive without radiation exposure. Unlike coronary computed tomography (CT) scanning the HRTTE technique does not need the presence of calcium to determine the wall thickness and therefore earlier and less stable plaques may be able to be detected.

5.4.1 Limitations:

Due to the low number of events performing multivariate analysis on this cohort was not possible so the only way we were able to account for age was to analyse a subgroup of subjects aged > 55 years to determine if the LAD wall thickness was still able to predict an event. However, age is a risk factor that cannot be altered whereas the LAD wall thickness may regress or at least be attenuated with early primary prevention strategies.

5.5 Conclusion

The HRTTE method of LAD wall thickness was able to predict future cardiovascular events despite standard risk profiling being unable to do so. It is possible that this technique may be a simple, non-invasive measure of subclinical atherosclerotic burden and allow targeted primary prevention to reduce event rates in a low to intermediate risk population.

This technique should be compared with other risk stratifying tests including exercise stress echocardiography which will be discussed in the next chapter.

Chapter 6

Echocardiographic Left Anterior Descending Coronary Artery Wall Thickness and Future Events: a Comparison with Exercise Stress Echocardiography

6.1 Introduction

Exercise stress echocardiography (ESE) is an imaging based assessment of ischaemia which visualises left ventricular function and augmentation during physical exercise [161]. Although ESE cannot determine anatomic narrowing of the coronary arteries, it can determine the functional significance of coronary luminal obstruction. Consequently it is an indirect test of CAD. ESE only addresses the importance of fixed luminal obstruction at the time of the stress test and will not detect the presence of atherosclerosis if the obstruction is not haemodynamically significant [162] or indeed the overall burden of coronary atherosclerosis. This is an important shortcoming since pathophysiological studies have shown that in >70% of cases it is rupture of non-flow limiting lesions that precipitate an acute coronary event [6, 163].

ESE can also provide information on risk stratification in population studies. A negative ESE portends low risk of cardiovascular event in the short to medium term [164-171]. However, because minor plaques, which may be at risk of rupture are not detected with ESE longer term risk assessment of the individual patient is not addressed with this investigative modality.

Given the premise that HRTTE of the LAD reflects coronary atherosclerotic burden we used this technique to determine if the LAD wall thickness could add incremental risk information in the individual patient undergoing ESE.

6.1.1 Aims

To determine if LAD wall thickness as measured by HRTTE adds incremental risk information to the screening test of ESE.

6.2 Methods

The Flinders Medical Centre Clinical Research Ethics Committee approved this study and all subjects provided written informed consent to participate (application number 126/034).

6.2.1 Subjects

Forty five consecutive hospital inpatients without previous CAD referred for ESE for assessment of possible cardiac ischemia underwent HRTTE prior to their ESE.

6.2.2 Procedures

Echocardiography procedures and image analysis as in 2.2.2.

An arbitrary cut-off value for baseline LAD wall thickness of 1.5mm was assigned with measurements under or equal to this value being considered normal and above this being considered abnormal. This cut-off value was determined from previous studies on normal subjects using the 95% confidence interval of normal wall thickness values from Chapter 2 [131, 134].

6.2.2.1 Exercise stress echocardiography

ESE was undertaken according to the American Society of Echocardiography guidelines [172] using the Bruce treadmill protocol [173] (appendix G).

A fasting blood test was obtained to measure total cholesterol, triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL) levels. Height and weight were also measured and BMI calculated.

Subjects were followed up by phone or mail and using the hospital database to determine if a new ischemic event had occurred since ESE. Hospital records were obtained to confirm the type of event. An ischemic event on follow up was defined as either a non-ST-segment elevation acute coronary syndrome (NSTEMI) or a ST-segment elevation myocardial infarction (STEMI) with associated ischaemic discomfort and the presence of serum cardiac markers (CK-MB and troponin) in the blood.

Framingham risk score was calculated using previously published guidelines for primary and secondary risk (appendix F) [160].

6.2.3 Statistical analysis

Data are expressed as the mean ± standard deviation for continuous variables and as percentages for discrete variables.

Univariate analysis was performed to evaluate variables as predictors of an ischemic event. The independent variables examined were Framingham risk score, LAD wall thickness, carotid IMT, age, gender, BMI, total cholesterol, triglycerides, LDL, HDL, CRP, homocysteine, creatinine, systolic and diastolic blood pressure, smoking status and family history of premature cardiovascular disease. Tests included chi-square analysis for discrete variables and a student's t-test for normally distributed continuous variables and a Mann Whitney test for non-normally distributed continuous variables. Kaplan-Meier survival using log-rank analysis was performed to assess event free survival.

All statistical analyses were performed using SPSS software for Windows (version 17.0, SPSS Inc., Chicago, Illinois, USA). A p value of < 0.05 was considered statistically significant.

6.3 Results

Forty five consecutive hospital inpatients without previous CAD were consented for the study. Adequate imaging of the LAD was achieved in 39 of the 45 (87%) subjects. The data from the 6 subjects without LAD imaging data was excluded from analysis. Three patients had a positive ESE result requiring revascularisation and were excluded from the remainder of the analysis as only the negative ESE result patients were of interest for this study. Of the 36 remaining subjects (mean age 54±7 years), 17 (47%) were male. Baseline subject characteristics are listed in table 6.1, column A. None of the subjects had a high Framingham risk score, the majority were low risk (<6% over the next 10 years, n=30, 83%) with the remainder having an intermediate risk score (6-20%, n=6, 17%). The average length of follow up was 51.7±13.4 months. Of the 36 subjects, there were 4 (11%) clinically relevant ischemic events.

Exercise stress echo results:

Results from the ESE are shown in table 6.2. There were 17 (47%) patients who developed non-specific ST changes and 3 (8%) who had atypical chest pain inconsistent with angina.

HRTTE LAD results:

The average LAD wall thickness was 1.6±0.5mm. Using the cut-off value of LAD wall thickness >1.5mm as an abnormal LAD wall thickness 13 (36%) subjects were found to have an LAD wall thickness above this value.

Those with an abnormal LAD wall thickness were older (57±7 vs. 51±6 years, p=0.013), more likely to be male (79% in abnormal group vs. 27% in normal group, p=0.003), had a higher Framingham risk score (7.4±4.4 vs. 2.4±2.1%, p<0.001), had a higher triglyceride level (2.3±1.1 vs. 1.7±0.9mmol/L, p=0.036) and were more like to have an ischemic event (31% in abnormal group vs. 0% in normal group, p=0.014) (table 6.1, columns B and C).

	A. Total cohort (n=36)	B. LAD wall thickness ≤ 1.5mm (n=23)	C. LAD wall thickness >1.5mm (n=13)
Age (years)	**54±7**	**51±6**	**57±7***
Males	**17 (47%)**	**6 (27%)**	**10 (79%)***
BMI (kg/m^2)	**28±5**	**28±7**	**28±3**
Framingham risk score (%)	**4.2±3.9**	**2.4±2.1**	**7.4±4.4***
Total cholesterol (mmol/L)	**5.2±1.1**	**5.2±0.9**	**5.3±1.3**
Triglyceride (mmol/L)	**1.8±1.0**	**1.7±0.9**	**2.3±1.1***
LDL (mmol/L)	**2.7±0.9**	**2.7±0.8**	**2.8±1.1**
HDL (mmol/L)	**1.7±0.4**	**1.7±0.4**	**1.5±0.5**
CRP (mg/L)	**4.7±7.2**	**3.8±3.5**	**6.0±10.6**
HTN	**13 (36%)**	**6 (26%)**	**7 (54%)**
Diabetes	**3 (8%)**	**1 (4%)**	**2 (15%)**
Smokers	**5 (14%)**	**4 (17%)**	**1 (8%)**
Family Hx CAD	**19 (53%)**	**12 (52%)**	**7 (54%)**
Ischemic event	**4 (11%)**	**0 (0%)**	**4 (31%)***

Table 6.1: Baseline subject characteristics. Continuous data expressed as mean ± standard deviation, discrete variables as number (percentage). BMI -

body mass index, CAD – coronary artery disease, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – C reactive protein, HTN – hypertension, Family Hx CAD – family history of premature coronary artery disease, primary male relative < 50 years and/or primary female relative < 60 years. *$p<0.05$ compared with LAD wall thickness ≤ 1.5mm group.

Variable	Mean	Standard deviation
Exercise time (mins)	9.3	2.5
Metabolic equivalents (MET)	11.1	2.6
Resting heart rate (BPM)	81.1	11.2
Peak exercise heart rate (BPM)	161.2	16.1
Percentage of max. age predicted heart rate (%)	96.7	9.2

Table 6.2: Exercise stress echocardiogram results.

Ischemic events:

There were 4 subjects with a negative ESE result that had an ischaemic event at 51 months follow up. These ischemic events consisted of 3 non-ST-segment elevation MI and 1 ST-segment elevation MI all with a troponin rise greater than the upper limit of normal. These events occurred beyond a 2 year period.

The subjects that had an event were older (52±6 vs. 59±4 years, p=0.03), more likely to be male (34% vs. 100%, p=0.01), had a higher Framingham risk score (3.3±3.6 vs. 9.0±3.2%, p=0.004) and had a larger LAD wall thickness (1.5±0.5 vs. 2.2±0.4mm, p=0.012) (table 6.3).

On univariate analysis, event rate was positively correlated with age (r=0.53, p=0.001), sex (r=0.51, p=0.001), LAD wall thickness (r=0.33, p=0.04) and creatinine level (r=0.45, p=0.006) (table 6.4).

Kaplan-Meier event free survival analysis demonstrated that an abnormal LAD wall thickness >1.5mm was able to predict event rate in this subgroup (p=0.003, figure 6.1).

	Non event group (n=32)	Event group (n=4)
Age (years)	**52±6**	**59±4***
Males	**11 (34%)**	**4 (100%)***
BMI (kg/m^2)	**27±6**	**29±2**
Framingham risk score (%)	**3.3±3.6**	**9.0±3.2***
Total cholesterol (mmol/L)	**5.4±0.9**	**5.1±1.5**
Triglyceride (mmol/L)	**1.8±0.9**	**2.3±1.1**
LDL (mmol/L)	**2.8±0.7**	**2.7±1.9**
HDL (mmol/L)	**1.7±0.4**	**1.3±0.2**
CRP (mg/L)	**3.0±3.2**	**3.8±3.8**
HTN	**10 (31%)**	**3 (75%)**
Diabetes	**3 (9%)**	**0 (0%)**
Smokers	**5 (16%)**	**0 (0%)**
Family Hx CAD	**18 (56%)**	**2 (50%)**
LAD wall thickness (mm)	**1.5±0.5**	**2.2±0.4***

Table 6.3: Baseline subject characteristics of subjects with and without an event. Continuous data expressed as mean ± standard deviation, discrete variables as number (percentage). BMI - body mass index, CAD – coronary

artery disease, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – C reactive protein, HTN – hypertension, Family Hx CAD – family history of premature coronary artery disease, primary male relative < 50 years and/or primary female relative < 60 years, LAD – left anterior descending coronary artery. *$p<0.05$ compared with non-event group.

Variable	Univariate analysis (r)	p valve
Age	**0.53**	**0.001**
Sex	**0.51**	**0.001**
LAD wall thickness	**0.33**	**0.04**
BMI	**0.07**	**0.67**
Total cholesterol	**-0.20**	**0.24**
Triglyceride	**0.19**	**0.26**
LDL	**-0.18**	**0.31**
HDL	**-0.21**	**0.22**
CRP	**0.10**	**0.59**
Homocysteine	**0.20**	**0.29**
Creatinine	**0.45**	**0.006**
HTN	**0.27**	**0.10**
Diabetes	**-0.13**	**0.45**
Smokers	**-0.17**	**0.31**
Family history CAD	**-0.07**	**0.68**

Table 6.4: Univariate analyses of predictors of ischemic event. LAD - left anterior descending coronary artery, BMI - body mass index, LDL – low

density lipoprotein, HDL – high density lipoprotein, CRP – C reactive protein, HTN – hypertension, CAD – coronary artery disease.

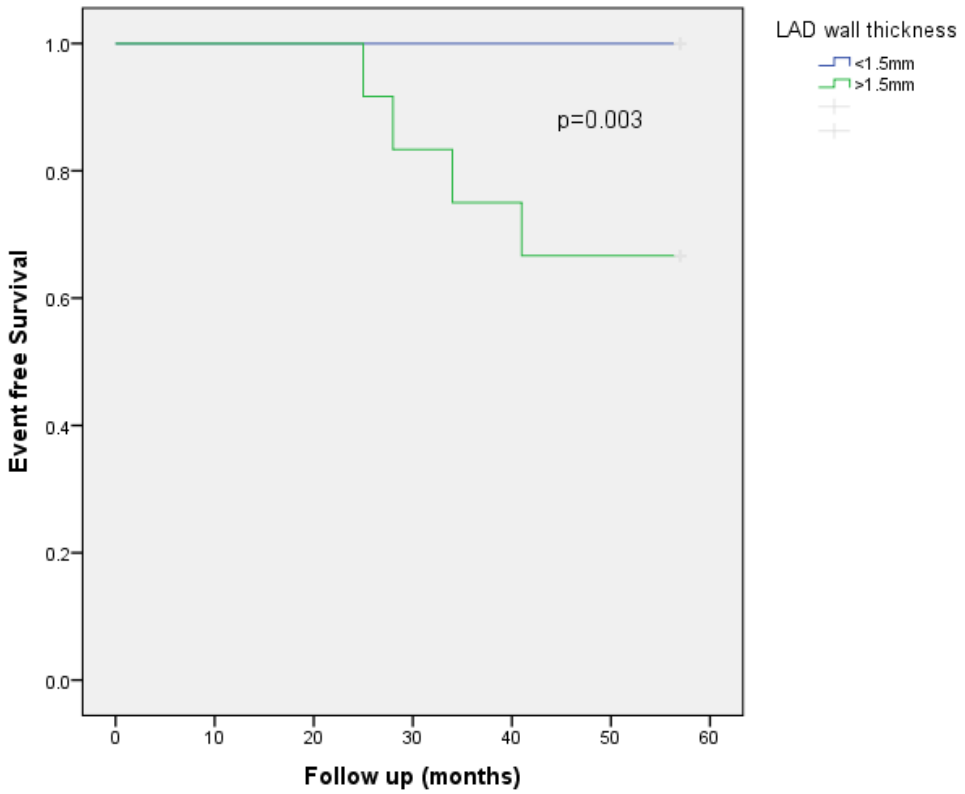

Figure 6.1: A Kaplan-Meier survival graph in those with a negative exercise stress echocardiography result demonstrating event free survival based on left anterior descending coronary artery wall thickness

6.4 Discussion

The results of this study hold promise for individual risk factor analysis in those who have had a negative ESE result. The HRTTE method of imaging LAD wall thickness is fast, reliable and non-invasive and may identify a subgroup who warrant primary prevention. Our cohort consisted of only low or intermediate risk patients, however despite this there was still an 11% event rate over the prolonged follow-up period.

There were 4 subjects who had an ischemic event after a negative ESE and all of these subjects had an abnormal LAD wall thickness. Survival analysis demonstrated that an LAD wall thickness > 1.5mm could predict long term (> 2 years) future events. This is particularly important as atherosclerosis is an insidious, chronic, clinically silent disease process with catastrophic final consequences and the ability to visualize the burden of coronary wall disease may assist in targeted primary prevention. The negative ESE group may be an ideal group to target with the HRTTE method of LAD wall thickness as we know a positive ESE results in a poorer prognosis (since the patient usually has a fixed CAD obstruction); however the ESE has little ability to predict long term risk due to atherosclerotic burden in the absence of flow limiting stenosis. This represents an excellent screening opportunity to address both short and long term prognoses at the same visit.

In keeping with previous studies our short term (up to 12 months) event rate post negative ESE is very low [174]. Indeed our 12 and 24 month event rate was

0% and in the longer term, the 3 year event rate was 5% and 4 year event rate was 11%. Pathophysiologically one could propose that the HRTTE LAD wall thickness is measuring the relatively early and subclinical stages of CAD and that it may take many years before an event occurs, whereas the ESE will give information on significant, fixed luminal obstructions where the event risk is more immediate (<1year).

The HRTTE technique is attractive in that it is quick in experienced hands (the LAD was identified within 5±2 minutes of scanning) and can be performed on the same equipment as the ESE, limiting cost and patient inconvenience.

6.4.1 Limitations:

See also limitations section in 2.4.1.

This is a small cohort and confounding results may have occurred due to the small number of subjects and the small number of events; however this is a proof of concept study and paves the way for larger trials to explore this technique and its long term predictive power.

6.5 Conclusion

Our study suggests that the incorporation of direct coronary visualization using HRTTE with standard stress imaging may provide incremental value in predicting long term events.

The combination of ESE and LAD wall thickness gives anatomical, functional and disease burden information to the clinician in the one test and may potentially allow for more targeted risk management on an individual level. We have demonstrated that HRTTE is sensitive enough to show structural and functional changes in the LAD; however we need to determine if it is sensitive enough to detect changes in the LAD due to treatment. The impact of statin therapy will be discussed in the next chapter.

Chapter 7

Predictors of Statin Induced Regression of Left Anterior Descending Coronary Artery Wall Thickness as Measured by High Resolution Transthoracic Echocardiography

7.1 Introduction

Statin therapy reduces future cardiac events in patients with coronary heart disease and in those following MI [175-179]. A 30% reduction in mortality post MI has been observed with statin based lipid lowering over an 8 year follow up period [180]. However, this reduction in events does not necessarily correlate to a reduction in coronary artery disease as demonstrated by angiography [179]. In fact the typical angiographic reduction in stenosis was approximately 1 to 3% compared with a 25 to 75% reduction in acute coronary events [175, 179]. As plaques stabilize it is thought that remodelling occurs causing limited changes in their angiographic appearance despite a marked reduction in cardiovascular events [13].

Statin based lipid lowering has been shown to reduce the rate of progression of atherosclerosis and the development of new lesions compared with a control group in IVUS trials using high dose lipid lowering therapy [181] and regression using very high dose lipid lowering therapy [182].

Accurate baseline measurements of the LAD luminal and external diameters and wall thickness have been shown to be achievable using HRTTE [128, 131, 134]. We sought to use this technique to assess the statin induced changes in LAD wall thickness in previously statin naive patients post non-LAD MI over a 12 month period.

7.1.1 Aims

To determine whether statin therapy causes regression of the LAD wall thickness as measured by HRTTE in subjects with their first documented CAD event occurring in a non-LAD coronary branch.

7.2 Methods

The Flinders Medical Centre Clinical Research Ethics Committee approved this study and all subjects provided written informed consent to participate (29/045).

7.2.1 Subjects

Thirty volunteer participants who presented to Flinders Medical Centre with their first episode of an acute coronary syndrome involving any artery other than the LAD, who were previously statin naive and were subsequently commenced on statin therapy underwent HRTTE measurement of the LAD wall thickness prior to discharge and at 3, 6, and 12 month intervals. At each visit a fasting blood sample was taken to determine lipid profile (total cholesterol, HDL, LDL, triglycerides), high sensitivity CRP, creatinine and homocysteine levels.

7.2.2 Procedures

Echocardiography procedures and image analysis as in 2.2.2.1

A fasting blood test was obtained to measure total cholesterol, triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL) levels. Height and weight were also measured and body mass index (BMI) calculated.

7.2.3 Statistical analysis

Data are expressed as the mean ± standard deviation for continuous variables and as percentages for discrete variables. Each variable was analysed using one way ANOVA to detect a change over time and then each time point was compared to baseline using a post hoc *t* test with Bonferroni correction for repeated measures.

Univariate analysis was performed to evaluate variables as predictors of change in LAD wall thickness. The independent variables examined were age, gender, baseline LAD wall thickness, BMI, total cholesterol, triglycerides, LDL, HDL, CRP, homocysteine, creatinine, location of MI, blood pressure, presence of diabetes, smoking status and family history of premature cardiovascular disease. Tests included Spearman's correlation for non-normally distributed and Pearson's correlation for normally distributed data. All statistical analyses were performed using SPSS software for Windows (version 17.0, SPSS Inc., Chicago, Illinois, USA). A p value of < 0.05 was considered statistically significant.

7.3 Results

Adequate imaging of the LAD was achieved in 27 of the 30 (90%) subjects. The data from the 3 subjects without LAD imaging data was excluded from analysis. Five subjects were lost to follow up and 2 subjects died within the study period, one from a subsequent MI and the other from a non-cardiac cause. This data was also excluded. Of the remaining 20 subjects (mean age 53±7 years), 15 were male and 5 were female. All subjects were commenced on 40mg Atorvastatin during their hospital stay, except for 2 who were alternatively prescribed 40mg Simvastatin instead. Baseline subject characteristics are listed in table 7.1.

There was a decrease in total cholesterol (23% reduction), triglycerides (19% reduction) and LDL (41% reduction) at the 3 month visit from baseline ($p=0.01$) which was sustained over the study period (figure 7.1). There was no change in HDL level.

Variable	Value
Age (years)	**53±7**
Males	**15 (75%)**
BMI (kg/m^2)	**29±4**
Total cholesterol (mmol/L)	**5.2±1.3**
Triglyceride (mmol/L)	**2.1±1.0**
LDL (mmol/L)	**3.2±1.3**
HDL (mmol/L)	**1.2±0.3**
CRP (mg/L)	**7.1±10**
RCA infarct	**5 (25%)**
LCx infarct	**11 (55%)**
Both RCA and LCx infarct	**5 (25%)**
HTN	**4 (20%)**
Diabetes	**1 (5%)**
Smokers	**7 (35%)**
Family Hx CAD	**10 (50%)**

Table 7.1: Baseline subject characteristics. Continuous data expressed as mean ± standard deviation, discrete variables as number (percentage). BMI - body mass index, CAD – coronary artery disease, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – C reactive protein, RCA – right coronary artery, LCx – left circumflex artery, HTN – hypertension.

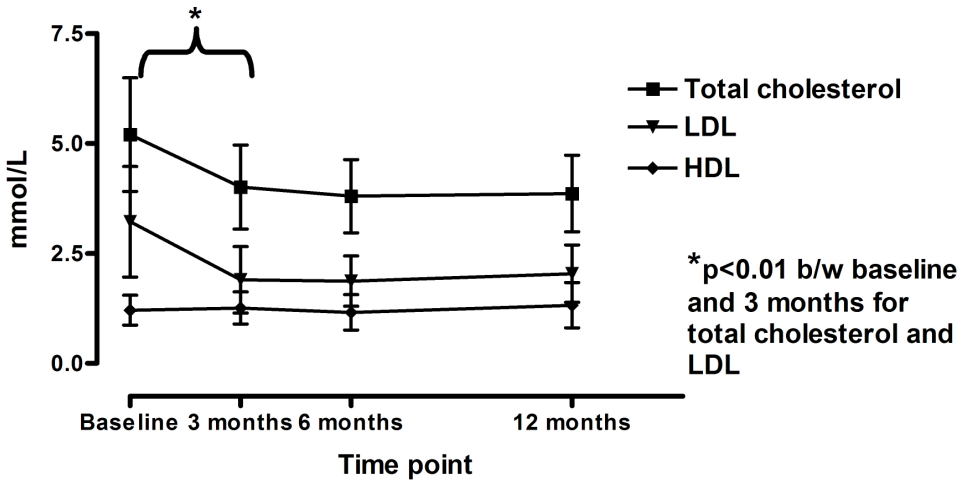

Figure 7.1: Graph demonstrating the change in blood cholesterol levels over the study period (HDL – high density lipoproteins, LDL – low density lipoproteins)

Overall, there was no change in the LAD wall thickness, external or vessel luminal diameter over the 12 month period (figure 7.2). There were 11 (55%) subjects who demonstrated some degree of regression despite there being no overall regression in the total cohort. The percent change in LAD wall thickness was negatively correlated with age ($r= -0.53$, $p=0.04$) and positively correlated with baseline LAD wall thickness ($r=0.76$, $p=0.001$) (table 7.2).

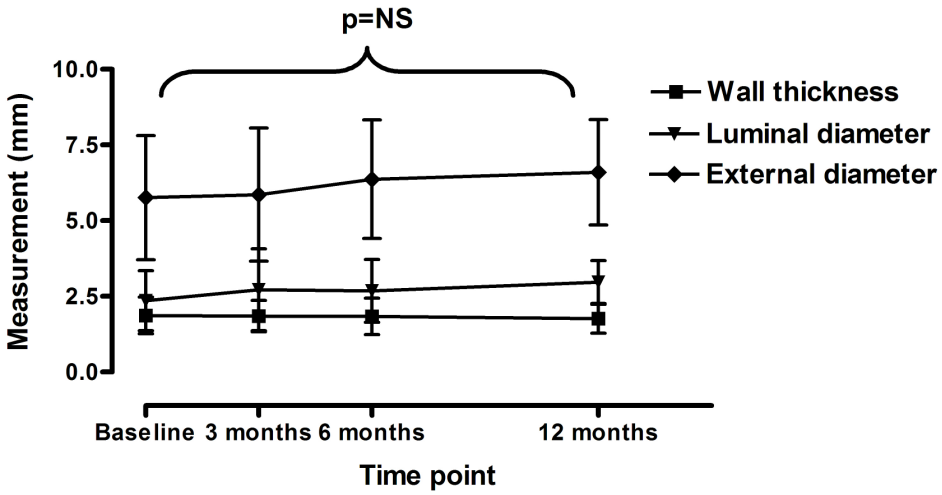

Figure 7.2: Graph demonstrating the change in left anterior descending coronary artery wall thickness, luminal diameter and external diameter over the study period

Variable	Univariate analysis (r)	p valve
Age	**-0.53**	**0.04**
Sex	**-0.34**	**0.16**
Baseline LAD wall thickness	**0.76**	**0.001**
Baseline BMI	**0.12**	**0.67**
Baseline total cholesterol	**-0.38**	**0.89**
Baseline triglyceride	**-0.16**	**0.56**
Baseline LDL	**-0.11**	**0.74**
Baseline HDL	**-0.29**	**0.36**
Baseline CRP	**0.18**	**0.62**
Homocysteine	**0.18**	**0.70**
Creatinine	**-0.36**	**0.18**
Location of MI	**-0.23**	**0.43**
HTN	**0.36**	**0.18**
Diabetes	**0.35**	**0.15**
Smokers	**-0.19**	**0.44**
Family history CAD	**0.13**	**0.66**

Table 7.2: Univariate analyses of predictors of percentage change in left anterior descending coronary artery (LAD) wall thickness. BMI - body mass index, CAD – coronary artery disease, LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – C reactive protein, RCA – right coronary artery, LCx – left circumflex artery, MI – myocardial infarction, HTN – hypertension.

Changes in the lipid, CRP, homocysteine and creatinine levels, which can affect the development of coronary atherosclerosis were not related to the baseline LAD wall thickness in univariate analyses, suggesting that baseline LAD wall thickness can predict percentage change in LAD wall thickness independently of the aforementioned variables (table 7.3).

Variable	Univariate analysis (r)	p valve
Δ Total cholesterol	**0.24**	**0.41**
Δ Triglycerides	**0.34**	**0.22**
Δ LDL	**0.16**	**0.65**
Δ HDL	**-0.22**	**0.54**
Δ CRP	**-0.04**	**0.93**
Δ Homocysteine	**0.69**	**0.09**
Δ Creatinine	**0.48**	**0.08**

Table 7.3: Univariate analyses between baseline left anterior descending coronary artery (LAD) wall thickness and the changes in serum lipids, CRP, homocysteine and creatinine. LDL – low density lipoprotein, HDL – high density lipoprotein, CRP – C reactive protein.

7.4 Discussion

This study supports the ability of HRTTE to detect subclinical atherosclerosis in the LAD. Moreover these measurements are reproducible over time and may be used to detect stabilization of plaque. IVUS studies have demonstrated a reduction of plaque burden with intensive high dose statin therapy [182] and stabilization of plaque burden with high dose statin therapy [181-182], thus in our cohort, stabilization or minor progression of LAD wall thickness was a likely outcome due to the moderate statin dose given. However unlike IVUS, HRTTE is non-invasive, fast, and technically simple and essentially risk free.

As expected there was a decrease in total cholesterol, triglycerides and LDL at the 3 month visit which was sustained at subsequent visits. However, the change in serum lipid levels was not able to predict percent change in the LAD wall thickness. In fact the only predictor of percent change of LAD wall thickness was the baseline LAD wall thickness, in that the larger the LAD wall thickness was at baseline the more likely it was to demonstrate regression. This is a similar finding to what was observed in both the REVERSAL [181] and ASTEROID [182] trials where the area with the highest plaque burden underwent the greatest degree of regression, even when the total atheroma volume in the target vessel did not regress. This is intuitive, as a higher plaque burden at baseline results in a larger potential for absolute reduction.

Those with baseline LAD wall thickness ≥ 1.8mm tended towards regression of wall thickness. There was no difference in the change in total cholesterol, triglycerides, LDL and HDL levels between the subjects with LAD wall thickness above and below the cut-off value. It is possible that with more aggressive lipid lowering regression in the LAD wall thickness may have been seen, particularly in subjects with a higher plaque burden initially.

At baseline all subjects demonstrated abnormal levels of LAD wall thickness, and despite a level of regression in some subjects none reduced to a normal level as determined in our previous studies [131]. On univariate analysis the only other variable to be associated with wall thickness was age; intriguingly this was an inverse relationship. The impression that younger patients seemed to have more potential for LAD wall thickness reduction on HRTTE might be of major future clinical significance, particularly in the primary preventative setting. Whilst older patients clearly have more coronary atherosclerosis the regression in younger patient's coronaries might reflect a more active stage of coronary atherosclerosis and a window where statin therapy might be of greatest clinical value.

Therapeutic intervention and risk analysis of the individual patient post MI may be assisted by the HRTTE method. The simple non-invasive nature of the test makes it easily applicable and could potentially be used in follow up and dose titration of preventative medications, however this requires further validation.

7.4.1 Limitations:

This is a small cohort and because of this may not have reached statistical significance, however given the final sample size of n=20, the power of the trial to detect a 30% reduction in LAD wall thickness associated with statin therapy exceeded 90%. This is a proof of concept study and paves the way for larger trials to explore this technique.

See also limitations section 2.4.1.

7.5 Conclusion

The increased wall thickening seen in our cohort despite the benign angiographic appearance of the proximal and mid LAD region indicates that this HRTTE method may be a useful tool for the detection of subclinical atherosclerosis. Furthermore this method may have potential in the evaluation of response to preventative therapies and allow individual tailoring of both primary and secondary prevention strategies.

Chapter 8

Summary and conclusions

8.1 Summary of findings

The main aim of this thesis was to determine if HRTTE is able to be used to detect subclinical atherosclerosis and enhance current risk factor analysis for CAD.

Firstly a normal range of values for LAD wall thickness, luminal and external diameters was determined (chapter 2). It was also determined that this method was reproducible within and between operators, with 3% intra-operator variability and 6% inter-operator variability for the wall thickness measurement. Using this normal range of values a cut-off value was determined from the 95% confidence interval of the wall thickness to be used in future studies.

It was then determined that subjects with known CAD in a non-LAD territory had a larger LAD wall thickness and external diameter than normal volunteers, however the luminal diameter was the same indicating that positive remodelling had occurred in the CAD group at the measured site (chapter 3). This is a similar study to the pioneering study performed by Gradus-Pizlo et al [128], however we were able to age match both the normal and the CAD groups to show that the differences in LAD wall thickness and external diameter were not age related.

One of the precursors of clinical atherosclerosis is endothelial dysfunction which has been measured peripherally for many years. Using the HRTTE

technique we were able to directly measure the response of the proximal LAD to both Salbutamol (an endothelial dependent vasodilator) and GTN (an endothelial independent vasodilator – chapter 4). The HRTTE technique is sufficiently sensitive to detect changes in the LAD luminal diameter due to the vascular activity of these drugs and whilst this was only performed on normal volunteers it has the potential to assist in risk factor stratification in people with suspected subclinical CAD.

Using the assumption that the LAD wall thickness is a measure of subclinical atherosclerotic burden we used the HRTTE technique in a cohort of normal volunteers (chapter 5) and in subjects referred for ESE (chapter 6) to determine if LAD wall thickness could predict future cardiovascular events. The LAD wall thickness was a predictor of events in both the self-deemed normal cohort (as was age) and in the negative ESE group, whereas conventional cardiovascular risk factors (other than age) were not.

Finally we analysed the changes in the LAD wall thickness over time in a previously statin naïve group of post MI patients (chapter 7) to assess regression, progression or stabilisation of their atherosclerotic burden. Overall there was no change to the LAD wall thickness in this group, which indicated stabilisation of plaque burden – an expected finding with moderate statin therapy. However, it demonstrated that the HRTTE technique may be sufficiently sensitive for monitoring of secondary preventative therapies.

8.2 Conclusions

Using the HRTTE measurement of LAD wall thickness, luminal and external diameters we found that an LAD wall thickness greater than 1.5mm was able to predict future events and may assist in targeted primary prevention strategies.

8.3 Future Studies

Our findings will need to be confirmed in larger, multi-centre studies. Validation of the LAD coronary measurements should be performed with comparison between the HRTTE technique and IVUS or coronary CT angiography. Studies looking at the other coronary arteries to determine the best site for wall thickness and coronary artery diameter measurements may also be instructive. Blinded primary prevention studies using subjects with an abnormal LAD wall thickness should also be performed to determine if future cardiovascular events may be reduced by targeting this group. This may involve drug intervention (statin therapy for example) or lifestyle modification. Perhaps the most important outcome measure would be changes to individual and societal attitudes towards CAD and risk of future cardiovascular events. If the HRTTE method of measuring LAD wall thickness was able to increase awareness of the silent disease process that is CAD this may prove to be the most rewarding outcome by focusing individual behaviour towards a healthy lifestyle.

Appendix A:

Imaging the left anterior descending coronary artery (LAD) using the Philips iE33 ultrasound machine.

- Use the S8-3 probe
- Change optimisation settings to 'HRes' to give maximum resolution
- Harmonic imaging must be used to reduce near field artifact and improve image quality
- Obtain a standard parasternal long axis image
- Reduce depth and field of view to focus on anterior side of inter ventricular septum
- Move focus to the level of the inter ventricular septum
- Reduce compression/ dynamic range to make a more black and white image
- Use X-res function to improve the signal to noise ratio
- Reduce gains to remove background noise
- Whilst live look for double lined structure running anterior to inter ventricular septum
- Freeze image and scroll to frame with clearest view of LAD
- Using the region of maximum luminal diameter measure the anterior wall thickness from outer edge to inner edge of the line representing the anterior wall

- Repeat for luminal diameter, posterior wall thickness and external elastic membrane
- Where 2 vascular structures are visualised use colour (and pulsed wave) Doppler to determine the coronary artery from the coronary vein
- For colour imaging reduce colour scale to 30-40cm/s and reduce wall filter
- Acquire live images where the LAD is seen to come in and out of view

Appendix B:

SUBJECT DATA SHEET

Name: _____

Age:_____

Sex:_____

Height:_____

Weight:_____

Risk factors:

Do you have (or are you on medication for) hypertension?

☐ Yes ☐ No

Is your total cholesterol level greater than 5.5mmol/L or are you on medication for high cholesterol?

☐ Yes ☐ No

Have you smoked in the last 3 years?

☐ Yes ☐ No

Do you have diabetes (type I or II)?

☐ Yes ☐ No

Do you have a positive family history of coronary artery disease (CAD) - primary male relative (father, brother, uncle) with CAD at age 60 or less or primary female relative (mother, sister, aunt) with CAD at age 50 or less?

☐ ☐

Yes No

Do you have a history of atherosclerosis (including CAD, peripheral vascular disease and cerebral vascular disease [transitory ischaemic attack, cerebrovascular accident])?

☐ ☐

Yes No

Thank you for your participation in this study

Appendix C:

Pulse wave analysis [151-153]:

Pulse wave analysis is a non-invasive technique which involves the use of a high-fidelity micromanometer (a small pencil-like probe) placed over the wrist of the dominant arm. The radial artery needs to be slightly flattened under the sensor by pressure from the operator. The arterial pulse pressure causes the piezo-electric crystal to vibrate and transform this vibration into an electrical signal. This signal is then converted into a pressure waveform signal (figure C.1). A consistent radial waveform must then be attained to ensure accuracy before any measurements can be made. A number of waveforms (usually 10) are averaged and a transfer function within the PWA software is then used to generate a central waveform from the radial waveform.

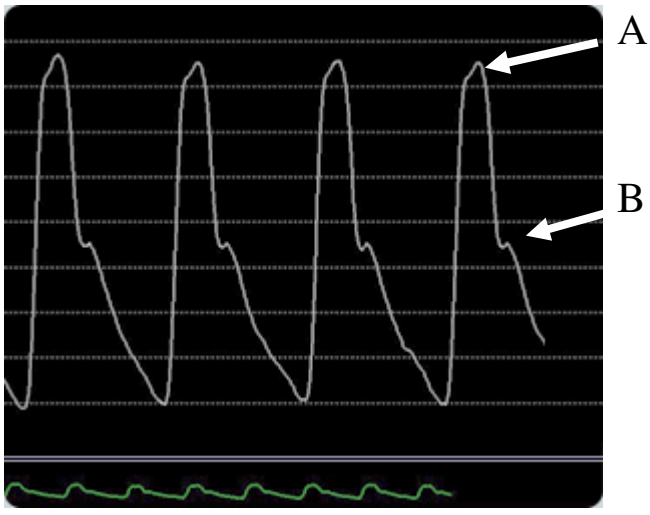

Figure C.1: An example of radial waveforms given from a pulse wave analysis assessment. Point A is showing the peak of the transmitted wave and point B is showing the peak of the reflected wave.

The augmentation index (AIx) is a measure of systemic arterial stiffness and is calculated as the difference between the transmitted (A) and reflected (B) systolic peaks expressed as a percentage of the pulse pressure. The AIx indicates the extent of reflection of peripheral pressure waves to the central arteries and is a marker of arterial stiffness. A decrease in the AIx is indicative of peripheral arterial vasodilatation.

Appendix D:

Flinders Medical Centre

Flinders University

Participant Information Sheet

Normal value ranges of coronary artery wall thickness using high resolution transthoracic echocardiography

This is a research project and you do not have to be involved. If you do not wish to be involved, your medical care will not be affected in any way.

You are invited to take part in a research study. This study involves research on heart disease. It aims to examine the early changes in the blood vessels supplying blood to the heart using ultrasound pictures of the heart.

Heart ultrasound or echocardiography is considered a very safe method of obtaining pictures of the heart. It can provide doctors with a large amount of information about the heart's structure and function. It will not cause you any discomfort and is not dangerous to you in any way.

While there may not be any direct benefit to you by participating in this study, the study is being performed to determine how useful this new way of using

ultrasound is in detecting changes inside the blood vessels of the heart. These findings may help to improve early detection of heart disease in the arteries of the heart.

Should you agree to participate in this research study, you would undergo the following:

- An ultrasound picture (known as echocardiography) of one of the blood vessels around your heart will be taken.
- A short questionnaire (taking approximately 10 minutes) will be filled out in which we will be asking you about any heart problems.
- A blood test will be taken.

These assessments will take approximately 30 minutes to complete.

The ultrasound will not cause you any discomfort, and is not known to have any side effects. It does not give out any radiation.

You will be asked to give a blood sample that will be taken by a qualified nurse. The sample will be 10mL (about 2 teaspoonsful) to be collected with a needle from a vein in your arm. Rarely people can experience pain, irritation, bruising and a feeling of faintness after a blood sample has been taken. If you experience any of these symptoms please tell the nurse immediately.

Your participation in the study is entirely voluntary and you have the right to withdraw from the study at any time. If you decide not to participate in the study or if you withdraw from the study, you may do this freely without prejudice to any treatment at Flinders Medical Centre. Your doctor receives no financial incentive for enrolling you in this study.

You may be reimbursed for your study related visit. The cost of parking can be met by re- imbursement of parking fees.

All records containing personal information will remain confidential and no information which could lead to your identification would be released.

If you as a participant of this research suffer injury, compensation may, at the discretion of Flinders Medical Centre, Division of Medicine, be paid without litigation. However compensation is not automatic and you may have to take legal action in order to receive payment.

Should you require further information about the study, either before or during or after the study, you may contact Ms Rebecca Perry, Ms Amy Penhall or Ms Lynn Brown on (08) 8404 2004.

This study has been reviewed by the Flinders Clinical Research Ethics Committee. Should you wish to discuss the study with someone not directly

involved, in particular in relation to matters concerning policies, your rights as a participant, or should you wish to make a confidential complaint, you may contact the Administrative Officer-
Research, Ms Carol Hakof, at the Flinders Medical Centre (8204 4507).

If you are interested in taking part, please fill in this form, place it in the enclosed stamped envelope and post.

Name: _____

Date of Birth: _____

Contact phone number: _____

Preferred contact time (please tick):

☐ ☐ ☐

Morning (9am-11am) Afternoon (12pm – 3pm) Other: please specify time___

Please tick the box if you have/ have had any of the following medical conditions:

☐ Hypertension (high blood pressure)

☐ High cholesterol

☐ Diabetes

☐ Heart attack

Please list your current medications (if any): _____

_____

_____

_____

_____

Thank you for you time in filling out this form. One of our friendly researchers will contact you shortly.

Appendix E:

Name:_____ BP:

Date of birth:_____ Mean BP:

Height:_____ cm/ft Weight:_____ kg/lb

Sex: M / F

Current medications: _____

_____

_____

_____

1. Do you have diabetes?

☐ ☐ ☐

Yes No Unknown

2. Do you have high blood pressure or are you on medication for your blood pressure?

☐ ☐ ☐

Yes No Unknown

3. Do you have high cholesterol or are you on medication for high cholesterol?

☐ ☐ ☐

Yes No Unknown

4. Have you had any of the following (please tick appropriate box if yes):

☐ Heart attack

☐ Coronary bypass surgery

☐ Angiogram

☐ Angioplasty

☐ Deep vein thrombosis (blood clot in your leg)

☐ Pulmonary embolus (blood clot in your lungs)

☐ Stroke

5. Smoking history:

☐ Never ☐ Current ☐ Recently quit ☐ Quit for more than 12 months

6. Has anyone closely related to you (by blood) had a heart problem at a young age (males less than 60 and females less than 50 years) particularly parents, sisters and brothers and uncles and aunties?

☐ ☐ ☐

Yes No Unknown

If you would like your results sent out to your local doctor please write their name and address (if not Seacombe Medical Centre) below:

_____

_____

_____

If you would like your results sent to you please fill in your address below:

_____

_____

_____

If your ultrasound results of your heart or neck arteries are abnormal would you be interested in being contacted about another study we are running involving medication to possibly reverse these changes?

☐ ☐

Yes No

Are you happy to be contacted either by mail or with a phone call in 12 months time just to check on your cardiac health?

☐ ☐

Yes No

Thankyou for your time in filling out this form.

Appendix F:

Framingham risk score calculation [160]:

For men without coronary heart disease at baseline examination:

Age	
Years	Points
30-34	-1
35-39	0
40-44	1
45-49	2
50-54	3
55-59	4
60-64	5
65-69	6
70-74	7

LDL (mmol/L)	
LDL	Points
<2.59	-3
2.60-3.36	0
3.37-4.14	0
4.15-4.92	1
>4.92	2

HDL (mmol/L)	
HDL	Points
<0.90	2
0.91-1.16	1
1.17-1.29	0
1.30-1.55	0
>1.55	-1

Blood Pressure					
Systolic (mmHg)	Diastolic (mmHg)				
	<80	80-84	85-89	90-99	≥100
<120	0 pts				
120-129	0 pts				
130-139	1 pt				
140-159	2 pts				
≥160	3 pts				

Diabetes	
No	0
Yes	2

Smoker	
No	0
Yes	2

Coronary heart disease risk	
Points total	10 year risk
<-3	1%
-2	2%
-1	2%
0	3%
1	4%
2	4%
3	6%
4	7%
5	9%
6	11%
7	14%
8	18%
9	22%
10	27%

11	33%
12	40%
13	47%
≥14	≥56%

For women without coronary heart disease at baseline examination:

Age	
Years	Points
30-34	-9
35-39	-4
40-44	0
45-49	3
50-54	6
55-59	7
60-64	8
65-69	8
70-74	8

LDL (mmol/L)	
LDL	Points
<2.59	-2
2.60-3.36	0
3.37-4.14	0
4.15-4.92	2
>4.92	2
HDL (mmol/L)	
HDL	Points

<0.90	5
0.91-1.16	2
1.17-1.29	1
1.30-1.55	0
>1.55	-2

Blood Pressure					
Systolic (mmHg)	Diastolic (mmHg)				
	<80	80-84	85-89	90-99	≥100
<120	-3 pts				
120-129	0 pts				
130-139	0 pts				
140-159	2 pts				
≥160	3 pts				

Diabetes	
No	0
Yes	4

Smoker	
No	0

Yes	2

Coronary heart disease risk	
Points total	10 year risk
≤-2	1%
-1	2%
0	2%
1	2%
2	3%
3	3%
4	4%
5	5%
6	6%
7	7%
8	8%
9	9%
10	11%
11	13%
12	15%
13	17%
14	20%
15	24%

16	27%
≥17	≥32%

Appendix G:

Exercise stress echo (ESE) protocol [172-173]:

- Select appropriate stress echo protocol
- Optimise image according to standard procedure
- Use tissue harmonic imaging to improve image quality
- Use contrast imaging where 2 or more wall segments are not clearly visualised
- Acquire a parasternal long axis image, parasternal short axis image, apical 4 chamber image and apical 2 chamber image with the patient at rest
- Patient to exercise to maximum level according to the Bruce treadmill protocol, unless significant chest pain or electrocardiogram changes are noted
- Treadmill to be stopped when patient reaches maximum workload and heart rate according to age
- Post exercise images are then obtained as soon as possible post exercise, within 90 seconds of exercise termination
- The endocardial excursion and wall thickening for 17 segments of the left ventricle are then analysed with comparison between the rest and post exercise images
- For a normal (negative) result to be given the wall motion for all segments at rest must be normal and post exercise must by hyperkinetic

(increase in endocardial excursion/ wall thickening) with a reduction in the left ventricular end systolic volume

- For an abnormal (posititve) result to be given the wall motion for all segments at rest must be normal (for this study) and post exercise one or more segments stay normal or become hypokinetic (decrease in endocardial excursion/ wall thickening), akinetic (no endocardial excursion/ wall thickening) or dyskinetic (one of more segments increase in volume in systole) with no change or an increase in the left ventricular end systolic volume

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