IMAGING BIOMARKERS OF SUBCLINICAL CARDIOVASCULAR DISEASE IN ASYMPTOMATIC EUROPEAN WHITES AND INDIAN ASIANS:

A POPULATION STUDY

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**ABSTRACT**

**Background:** Subclinical biomarkers of cardiovascular disease (CVD) are being increasingly applied in clinical settings to refine cardiovascular risk stratification and also as an epidemiological tool to enhance the understanding of disease mechanisms. Indian Asians living in the United Kingdom have at least a 50% higher risk of CVD mortality compared to European whites. The excess mortality risk cannot be explained by the pattern of classical risk factors which are generally lower in Indian Asians, and there are no tools that identify their elevated CVD risk.

**Objectives:** To explore potential CVD mechanisms using established and novel imaging biomarkers of subclinical atherosclerosis and left ventricular (LV) function, in a large bi-ethnic cohort of asymptomatic European white and Indian Asian subjects. Mechanisms potentially responsible for the excess CVD mortality observed in UK Indian Asians will also be examined.

**Methods:** A total of 2,439 subjects were recruited from the LOLIPOP study, of which 2,288 were free from clinical CVD. All subjects underwent carotid ultrasonography and echocardiography with tissue Doppler imaging for estimation of LV filling pressure (E/Ea).

**Results:** In hypertensives, increased LV mass is independently associated with impaired LV function and increased LV filling pressure, whereas increasing degrees of concentric remodeling are associated with attenuated diastolic function but augmented systolic function, possibly representing an adaptive response to pressure overload physiology. Subclinical carotid plaque disease, rather than IMT, is more closely related to LV systolic dysfunction and increased LV filling pressure. Compared to European whites, Indian Asians intrinsically have attenuated longitudinal LV function, higher E/Ea and demonstrate a greater degree of
concentric remodeling independent of other demographic and clinical parameters. Despite their lower LV mass, an increased prevalence of LVH amongst Indian Asian men compared to European white men was observed.

**Conclusions:** Novel relationships between biomarkers of subclinical LV geometry, LV function and carotid atherosclerosis have been presented. Given that the burden of carotid atherosclerosis was similar amongst both ethnic groups, this thesis suggests that further research is required into blood pressure aetiology, pro-hypertrophic mediators, end-organ damage and factors involved in acute arterial plaque destabilisation, to further understand the excess risk of CVD in Indian Asians.
DECLARATION OF ORIGINALITY

I, Navtej S. Chahal, confirm that the work presented in this thesis is original and my own. Where information has been derived from other sources, this has been appropriately referenced.
This thesis is dedicated to my wife, Pooja,

our son, Yuvaraj and our baby daughter, Suhana.
ACKNOWLEDGEMENTS

I am indebted to my principal supervisor, Professor Roxy Senior, for giving me the opportunity to undertake this wonderful project and also for providing an excellent environment in which to conduct research at Northwick Park Hospital. I would like to acknowledge his commitment to ensuring the necessary resources were available to maintain the quality of the study, his indulgence of my ideas (however fanciful), his supporting my attendance at various conferences which helped significantly with my educational development. Most importantly I am grateful for all the theoretical knowledge and practical skills that he has imparted to me.

I would like to thank Professor Kooner and John C. Chambers who devised the main LOLIPOP study and who both gave me early, invaluable insights into which direction to take my thesis. I would also like to thank John in his capacity as a co-supervisor of my research - I have appreciated your support throughout the project, your critique of the final thesis and help in preparing for the viva.

I am grateful to Darrel Francis for also agreeing to be a co-supervisor and for his advice, particularly with regards to conducting reproducibility experiments and defining normative values for tissue Doppler parameters.

This thesis would not have been possible without the work of two of my colleagues, TK Lim and Piyush Jain. TK, was truly the backbone of the project before my arrival, setting the template and standards required for the study. Piyush’s generosity, industry and knowledge helped me settle in quicker than I had hoped, and ensured recruitment was kept on track.

I would like to thank all the research and administration staff at the Northwick Park Hospital Cardiovascular Research Department. In particular I am indebted to Chris Kinsey for his unparalleled database design skills, Minoo and Mary for running a tight ship and digging out journals from the library, Leah for getting my echo skills up-to-speed and Brijesh for providing stimulating intellectual insights into research governance, the mechanics of myocardial function and reverse swing.

Lastly, I would like to thank the almost two-thousand five hundred residents of Ealing and Southall who participated in this study. They have made a unique contribution, that will undoubtedly further our understanding of cardiovascular disease prevention at the individual and population level in years to come.
**Publications arising from this thesis**


**Manuscripts currently submitted for publication**

1. The Increased Prevalence of Left Ventricular Hypertrophy and Concentric Remodeling in UK Indian Asians Compared to European Whites. Chahal NS, Tiong K. Lim, Piyush Jain, John C. Chambers, Jaspal S. Kooner, Roxy Senior
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<tr>
<td>2-D</td>
<td>Two-dimensional</td>
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<td>3-D</td>
<td>Three-dimensional</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BP</td>
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<td>BSA</td>
<td>Body surface area</td>
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<td>CHD</td>
<td>Coronary heart disease</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>CECU</td>
<td>Contrast-enhanced carotid ultrasound</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>EBCT</td>
<td>Electron beam computer tomography</td>
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<td>EF</td>
<td>Ejection fraction</td>
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<td>IMT</td>
<td>Intima-media thickness</td>
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<td>LA</td>
<td>Left atrium</td>
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<td>LAVI</td>
<td>Left atrial volume index</td>
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<td>LBM</td>
<td>Lean body mass</td>
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<td>LOLIPOP</td>
<td>London Life Sciences Prospective Population study</td>
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<tr>
<td>LV</td>
<td>Left ventricular</td>
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<td>LVH</td>
<td>Left ventricular hypertrophy</td>
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<td>LVMI</td>
<td>Left ventricular mass index</td>
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<tr>
<td>RWT</td>
<td>Relative wall thickness</td>
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<td>TDI</td>
<td>Tissue Doppler imaging</td>
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<td>UK</td>
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PART I – BACKGROUND, OBJECTIVES & METHODS
CHAPTER 1. STUDYING SUBCLINICAL CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is widespread in the general population, affecting > 4 million people living in the United Kingdom (UK) (1,2). CVD refers to four major disease areas, namely coronary heart disease (CHD), cerebrovascular disease, peripheral vascular disease and atherosclerotic/aneurysmal disease of the aorta. Death rates from CVD have been falling in the UK since the early 1970s, and by as much as 40% in the last ten years, with the majority (58%) of this decline in mortality attributed to reductions in major risk factors, particularly smoking (1). Developments in pharmacological therapies and revascularisation procedures for acute myocardial infarction together with widespread application of secondary preventative measures, are responsible for the remaining mortality decline (1).

However, despite the improvements in mortality and morbidity achieved over the past 40 years, CVD remains the main cause of death in the developed world, accounting for almost 191,000 deaths each year in the UK (1). Moreover, the reductions achieved in CVD mortality rates are under threat due to an aging population, rising prevalence of obesity and the adoption of increasingly sedentary lifestyles (2). Consequently, in 2008, the Department for Health in the UK announced a national “vascular check” programme, shifting the emphasis away from disease treatment, to disease prevention (2). The current practice in preventing the occurrence of clinical cardiovascular events has evolved around targeted assessment of cardiovascular risk factors with interventions directed at lifestyle and use of medications to modify these factors and possibly prevent the disease, in individuals deemed to be at high-risk of future CVD events. Algorithms, such as the Framingham Risk Score,
reflect the overall burden of risk factors, with risk factor modification therapy reserved for those deemed to have a high (>20%) 10-year risk of suffering a clinical event. However, risk scoring algorithms are imperfect, and imperfectly applied by physicians. Although levels of conventional cardiovascular risk factors (gender, age, blood pressure (BP), diabetes, cholesterol, smoking and family history) are associated with the extent and severity of atherosclerosis, there is substantial variation in the amount of atherosclerosis for every level of risk factor exposure (3). This variation in disease is probably due to genetic susceptibility, combinations and interactions with other risk factors and duration of exposure to the various risk factors. It is also important to acknowledge that high-risk groups contain only a fraction of the individuals that will ultimately suffer a clinical cardiovascular event. In the Prospective Cardiovascular Munster (PROCAM) study 6.5% of the population were classified as high risk, 14% as intermediate and 79.5% as low risk. At 10 years, 33% of myocardial infarctions occurred in the high-risk group, 35% in the intermediate- and 32% in the low-risk group (4). This is explained by the low-risk group being very large, with at least 30% of those who went on to suffer an event not having any of the conventional cardiovascular risk factors. Therefore, a “detection gap” exists, which may be defined as the difference between CVD cases or events currently detected and the total burden of disease or events among the population (5).

Given the poor predictive capacity of risk prediction models based on conventional cardiovascular risk factors, a better understanding of mechanisms involved in the development of clinical CVD across all risk strata has become of paramount importance, and would enable the development of more robust risk prediction tools.
1.1 Enhancing Cardiovascular Disease Risk Prediction

Although it is agreed that a detection gap in CVD prognosis exists, the precise size of this gap is unknown but may be substantial. Even the available diagnostic tests have limitations. Functional, stress imaging of the heart is only capable of detecting haemodynamically significant lesions. Conventional, coronary angiography is an invasive procedure and unacceptable as a screening tool. Moreover, it provides only a “luminogram” that does not impart information about plaque instability. As mentioned earlier, the variation in disease susceptibility is largely due to gene-environment interactions, risk factor combinations and duration of exposure. Unsurprisingly the “preventative” approach adopted thus far is less successful in identifying which individuals will or will not develop CVD based on conventional risk factors (6). The problem is exacerbated amongst non-Caucasians, to whom reference ranges for independent risk factors may not apply(7).

The poor predictive value of conventional risk factors and limitations of current diagnostic tools has encouraged research into novel and established biomarkers of subclinical CVD to help improve risk prediction in both Caucasian and non-Caucasian populations. This approach essentially represents a form of disease “screening”, as in other areas of medicine allowing the detection and modification of a potential clinical entity at its earliest stages. Technological developments have made the quantification of subclinical disease processes more feasible and accurate, providing detailed information that relates directly to pathology. Biomarkers that can identify an individual’s vulnerability to clinical CVD events broadly fall into two categories: the imaging modalities, which can detect arterial disease; and the blood biomarkers, which characterise the pathophysiology of the atherothrombotic process (figure 1.1).

The concept of a “vulnerable plaque” is well established, referencing the complications that arise from arterial plaque rupture, with resultant thrombus formation and
vessel occlusion (8). Plaque vulnerability can be quantified by the assessment of its morphological features such as thinning of the plaque cap, presence of a large lipid core, evidence of fissured plaque and a luminal stenosis >90%. Traditionally this has been evaluated in the coronary arteries using invasive techniques such as intravascular ultrasound. However, using non-invasive techniques, the importance of a more comprehensive assessment of atherosclerosis burden rather than individual plaque characteristics is also being recognised (8). Modalities such as electron beam computed tomography (EBCT), detect the calcification of, hence atherosclerosis burden in, the three major coronary arteries. As arterial disease is rarely confined to one vascular bed, ultrasonography of either the carotid or an accessible peripheral artery, offers a “pan arterial” assessment that represents subclinical peripheral, cerebro- and coronary vascular disease. Moreover, assessments of plaque morphology undertaken in a peripheral artery are reflective of plaque characteristics coexisting in the coronary or cerebral vascular territories. The concept of plaque instability existing simultaneously in multiple vascular beds has been described and echolucent carotid plaques have been shown to be strongly and independently associated with future coronary events in patients with stable CVD (9,10).

In addition to detecting the anatomical presence of atherosclerosis or quantifying plaque vulnerability, other factors are involved in the development of acute, thrombotic, occlusive events. Blood thrombogenicity (vulnerable blood) or myocardial susceptibility to fatal arrhythmia (vulnerable myocardium), also determine the clinical course of an acute plaque event. To recognise the collective importance of these systems it has been proposed that at risk individuals be referred to as “vulnerable patients” (8). Vulnerable blood may be identified through serum markers of atherosclerosis (abnormal lipoprotein profile), inflammation (high-sensitivity CRP), metabolic disorders (blood glucose, triglycerides and homocysteine) and through coagulation disorders (fibrinogen, factor V Leiden, increased
coagulation factors, decreased anticoagulant factors). Vulnerable myocardium may exist in individuals without prior history of ischaemic heart disease. Conditions such as subclinical dilated cardiomyopathy or left ventricular hypertrophy (LVH) are also associated with an increased risk of arrhythmia and sudden death. Other underlying pathological processes that increase myocardial vulnerability include valvular heart disease, sympathetic hyperactivity, impaired vagal tone, primary electrical disturbances, myocardial bridging and anomalous coronary artery anatomy.
Screening for subjects at risk for cardiovascular complications: blood biomarkers/risk factors and/or markers of subclinical disease. Apo indicates apolipoprotein; BP, blood pressure; CT, computed tomography; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein; Lp(a), lipoprotein a; Lp-PLA2, lipoprotein-associated phospholipase A2; MRI, magnetic resonance imaging; and Syn, syndrome. Reprinted from Naghavi M et al. Am J Cardiol. 2006;98(suppl):I–15I.
1.2 **Understanding Disease Mechanisms**

Prospective epidemiological studies have traditionally relied on the occurrence of clinically overt events, such as myocardial infarction, stroke or death, to identify and validate factors predictive of disease manifestation. However, more recently epidemiological studies are utilising biomarkers of subclinical CVD (11-13), in addition to prospectively following-up subjects for development of clinical end-points, to enhance the understanding of disease mechanisms.

The risk of clinical events associated with subclinical disease measures has been shown to be graded and continuous (1, 2, 4, 11), similar to risk associated with conventional CVD risk factors such as BP and serum cholesterol, suggesting that interventions yielding even modest reductions in levels of subclinical disease may have a significant impact on reducing CVD risk throughout the general population. There are several advantages to using measures of subclinical disease in the study of disease aetiology, particularly when examining potential mechanisms amongst higher-risk groups (11). Firstly, measuring clinical events only may lead to distortion of risk relations due to under detection and biased ascertainment of disease. Secondly, because subclinical disease is asymptomatic, its detection is unlikely to have a direct impact on health behaviours subsequently, such as lifestyle modification or medication use that may alter the relations of risk with disease. Thirdly, measurement of subclinical disease can enhance studies of CVD risk and prevention by allowing examination of its early stages, with other subclinical markers as well as associative factors. Finally, the continuous nature of most subclinical measures greatly increases power to detect risk associations compared with discrete measures, that is, the presence or absence of clinical disease. However, it is important that the identification of subclinical disease
biomarkers are not undertaken for their own sake, but so that they can be modified, and through this modification, can reduce the burden of disease.

1.3 **Imaging Subclinical Cardiovascular Disease**

Imaging techniques can detect subclinical CVD by either directly visualising the anatomical presence of arterial disease or by measuring the effects of disease processes, such as hypertension or diabetes, upon cardiac and vascular function.

1.3.1 **Subclinical atherosclerosis**

The burden of subclinical arterial disease has been extensively investigated as a biomarker of CVD risk, and has been correlated with prognosis, risk factors and treatment efficacy. Non-invasive arterial testing offers a method for cardiovascular risk assessment based on several important considerations. Alterations in arterial function and structure predate clinical manifestations of occlusive atherosclerotic disease with changes tending to be widespread rather than being limited to a single arterial bed. Identification of such abnormalities in an accessible peripheral artery, such as the carotid artery, provides a mean for early detection of presymptomatic vascular disease. Arterial ultrasonography is an attractive modality for detecting early disease that is non-invasive and that does not expose the patient to ionising radiation.

Within general cardiology practice, carotid ultrasonography is most frequently performed (figure 1.2) to further risk stratify patients about to undergo coronary artery bypass grafting. With the risk factors for cerebrovascular and coronary heart disease being the same, it is intuitive to explore the carotid arteries for stenotic disease in individuals with known stenotic coronary disease. The incidence of coexisting flow limiting coronary and carotid artery disease in recent literature varies from 2-14% (14) with the incidence of carotid artery stenosis >50% documented as being as high as 22% in patient requiring CABG (15).
The converse also holds true and for many years physicians have been aware that patients with high-grade carotid stenoses are also likely to have significant coronary artery disease who should be considered for myocardial ischemia testing prior to carotid endarterectomy. The degree of atherosclerosis within the cerebral and coronary vascular beds is also similar, with the atherosclerotic burden in a carotid artery and coronary artery being the same as between any two coronary arteries (16).

1.3.1.1 Carotid intima-media thickness

A conventional duplex ultrasonography protocol, which principally identifies presence or absence of obstructive stenoses based on Doppler signal velocities, can miss the early changes associated with atherosclerotic disease. These subtle alterations in arterial wall structure predate clinical manifestations of occlusive atherosclerotic disease encroaching upon the lumen and are also widespread rather than limited to a single arterial bed. The combined thickness of the carotid artery intima and media can be measured with high-resolution ultrasonography and provides an independent measure of cardiovascular risk (figure 1.3).
Figure 1.2 - An ultrasound image of a normal carotid artery demonstrating its bifurcation into the internal (ICA) and external (ECA) carotid arteries.

![Ultrasound image of carotid artery bifurcation](image1)

Figure 1.3 – Examples of IMT measurement in a subject with normal IMT (left) and increased IMT (right) using semi-automated computer software. (IMT = intima-media thickness)

![IMT measurement examples](image2)
Carotid intima-media thickness (IMT) has been used predominantly as a research tool, as either an outcome variable in epidemiological studies or a surrogate end-point in trials of disease intervention. Clinically, identification of such abnormalities in an accessible artery provides a means for early detection of presymptomatic vascular disease and improved cardiovascular risk stratification.

Carotid IMT has been shown to correlate well with the degree of carotid atherosclerosis found at autopsy (17) which in turn has been found to correlate with atherosclerosis in other vascular beds (18). Increased carotid IMT is associated with cardiovascular risk factors (19,20), prevalent cardiovascular disease (21,22) and the presence and extent of angiographically determined coronary atherosclerosis (23,24). The presence of a mean carotid IMT of > 1mm is associated with an 18-fold increase of having an abnormally thickened coronary artery IMT and a seven-fold increased risk of having a significant coronary stenosis as determined by intra-vascular ultrasound (25). Several studies show that increased carotid IMT is predictive of cardiovascular events in asymptomatic individuals including myocardial infarction, stroke and death (12,26-28). Subclinical carotid artery disease has been shown to have a graded and continuous association with future CVD events (12,27).

Reported normative median IMT values vary amongst the large prospective studies although a value greater than 1.2mm is considered clearly abnormal (29,30). In the ARIC (Atherosclerosis Risk in Communities) study, a mean carotid IMT > 1mm was associated with a hazard ration for CHD events of 2.07 in women and 1.85 in men (12). IMT is dependent on age and gender, and normative values with respect to these demographic factors are available in different populations (31).
1.3.1.2 Carotid plaque disease

Distinct from diffuse intima-media hypertrophy is the formation of atherosclerotic plaque and represents an advanced stage of atherosclerosis, whether obstructive or not (figure 1.4). Some studies suggest that plaque may be a better predictor of cardiovascular events than IMT, probably as it better reflects established disease (32-35). Whereas IMT needs to be interpreted in the context of the individual’s age and gender, the presence of plaque is always abnormal.

Figure 1.4 – Non-obstructive carotid artery plaque disease at both the near and far walls

1.3.1.3 Plaque morphology

There is also growing evidence that the morphological characteristics of carotid plaques as determined by ultrasonography reflect their propensity to rupture and cause clinical events (36-40). Carotid plaque composition or echogenicity can be assessed in a standardised and reproducible manner with ultrasonography by visually assessing the degree of plaque echogenicity. Mature plaques which are calcified, fibrotic and more echogenic are less
vulnerable than “soft”, echogenic plaques rich in lipids, haemorrhage and elastin. Ethnic heterogeneity in carotid plaque morphology has been demonstrated in one small study using MRI which showed Chinese patients to have morphologically more vulnerable plaques compared to Europeans (41).

1.3.1.4 The adventitial vasa vasorum
The observation that excessive growth of neovessels in the arterial wall precedes the development of luminal plaques has led to much interest in developing technologies capable of imaging blood flow through the vasa vasorum. Adventitial vasa vasorum are functional endarteries that are present at birth but their extent and distribution increases disproportionately with arterial wall disease. The primary functions of the vasa vasorum are to deliver nutrients to and remove waste products from the vessel wall, however, pathological plaque neovascularisation is thought to play a central role in the process of atherosclerosis and plaque destabilisation (42). Acute plaque instability seems to be triggered by recurrent episodes of intraplaque haemorrhage caused by disruption and leakage of the immature intraplaque neovessels that originate from the adventitial vasa vasorum. Experimental hypercholesterolaemia has been demonstrated in animal models to induce prominent adventitial neovascularisation that precedes endothelial dysfunction, intima-medial thickening and plaque formation. Detection of these angiogenic vessels using contrast-enhanced, ultrasound-based technologies is technically feasible now in humans (43,44). Although the pathological observation of plaque angiogenesis within plaques is well documented and associated with increased risk of future events, the clinical implications of detecting adventitial vasa vasorum in the absence of plaque disease remains unknown and requires clinical validation.
1.3.1.5 Clinical role of carotid intima-media thickness and plaque assessment

Given the clear association of carotid IMT with coronary atherosclerosis and future cardiovascular events, IMT measurement may be useful in refining risk stratification of individual patients, particularly in the intermediate (10-year risk 10-20%) risk category. The predictive value of algorithms based on traditional cardiovascular risk factors are unsatisfactory with more than half of subjects with CHD having no major cardiovascular risk factor or only one (45). Data from the Framingham Heart Study showed that carotid IMT is independently associated with 10-year CHD risk, supporting its usefulness as a prognostic marker (46). A limitation of the Framingham risk equations is that they are heavily influenced by age despite there being great variation in atherosclerotic burden at any given chronological age. It has been proposed that chronological age should be replaced by vascular age determined from age specific IMT values determined from healthy cohorts (47). Using this approach almost 50% of individuals assigned to intermediate–risk category by the original Framingham CHD risk algorithms can be reclassified into higher or lower risk categories. Automated computerised edge-detection software has decreased variability and improved precision of IMT measurements. On-line algorithms can also be used to integrate IMT values with demographic data to determine an individual’s vascular age. Carotid IMT testing is quick, non-invasive, safe, relatively inexpensive and allows identification of minor and major (stenotic) disease.

The incremental value of carotid IMT and plaque assessment beyond traditional risk factor assessment was recently confirmed in a study assessing different predictive models including these two variables (48). The authors evaluated the additional predictive value of IMT, plaque presence, or both carotid ultrasound findings to CHD risk prediction. They found that the area under the receiver operating characteristic curve for traditional risk factor
prediction of CHD events (0.742) was significantly increased by the addition of increased IMT (0.750) or carotid plaque presence (0.751), and that the combination of risk factors, IMT, and plaque yielded the highest area under the curve (0.755). The paper also clearly demonstrated the power of IMT and plaque assessment to appropriately reclassify CVD risk, with 37.5% of patients in the 5% to 10% CVD risk stratum (based on risk factors) and 38.3% of patients in the 10% to 20% risk stratum being reclassified when carotid ultrasound data were considered, with plaque presence being more important than increased IMT among women. Adding either IMT or carotid plaque presence to traditional risk factors led to net clinical reclassification indices of 16.7% and 17.7%, respectively; 21.7% when used together. On the basis of this work, the 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults gave a Class IIa recommendation, with a B level of evidence for measurement of carotid IMT on ultrasound to provide cardiovascular risk assessment in asymptomatic individuals at intermediate risk (49).

Although there is now strong evidence demonstrating the incremental value of carotid ultrasonography in risk prediction, this data is based on observational studies. To convincingly demonstrate the value of atherosclerosis imaging requires prospective, randomised studies comparing a strategy of imaging-guided risk factor modification to risk factor modification alone. In this regard, randomised data showing the efficacy of any atherosclerosis imaging strategy are scant.

1.3.2 Subclinical cardiac dysfunction and remodeling

Increasingly echocardiographic parameters of left ventricular (LV) structure and function are being utilised as subclinical measures of CVD and prognosis. The Framingham study pronounced LVH as a risk factor 21 years ago (50), and also noting poorer outcome in those with asymptomatic systolic and/or diastolic function (51). Since the advent of novel,
quantitative techniques for assessing LV function, the field of “echoepidemiology” continues to evolve (52), with echocardiographic protocols almost ubiquitously incorporated into prospective epidemiological studies of cardiovascular risk. Conventional echocardiographic predictors of poor outcome, such as LV ejection fraction (EF) and a restrictive filling pattern have recently been supplemented by tissue Doppler imaging (TDI). This modality, in particular provides an opportunity to enhance the understanding of myocardial mechanics, the potential aetiologies of subclinical dysfunction and its progression to heart failure.

1.3.2.1 Left ventricular systolic function

Normal LV systolic function requires coordinated, 3-dimensional pattern of ventricular pattern of activation and contraction. During a cardiac cycle, the LV wall thickens (radial motion), shortens (longitudinal motion) and twists (torsion) along the long axis. Radial function can be quantified by the LV EF, a well established index of global systolic function, representing the fractional change in LV end-diastolic and end-systolic volumes. Longitudinal function and torsion can be quantified by using echocardiographic techniques such as tissue Doppler and strain rate imaging respectively.

Detection of subclinical LV dysfunction in patients without overt coronary artery disease and impaired EF is predictive of subsequent congestive cardiac failure, cardiovascular and all-cause death (51,53). Presence of global LV dysfunction has been demonstrated in epidemiological studies to be associated with cardiovascular outcomes but there is recent evidence that even the presence of segmental wall motion abnormalities in subjects with undiagnosed coronary artery disease is also a strong prognosticator in subjects with no prior history of cardiovascular disease (54). The discovery of previously incipient LV dysfunction also provides an opportunity to alter the natural history of the syndrome by prompting a search for its aetiology, which usually begins by either diagnosing or excluding obstructive
coronary artery disease. Early initiation of angiotensin-converting enzyme inhibitors can potentially delay onset of symptomatic heart failure (55) and revascularisation may be considered, particularly if viable myocardium is at stake.

Assessment of longitudinal systolic function can provide important diagnostic and prognostic information over and above global measures of LV EF and volumes. (56-60). Long-axis motion of the left ventricle is an important component of LV systolic and diastolic function and the subendocardial fibres that contribute to long-axis function are particularly sensitive to various diseases and pathologies. Recently, TDI (figure 1.5) has emerged as a robust modality for quantifying regional myocardial longitudinal shortening (systolic S’ velocity) and lengthening (early diastolic E’ velocity) function, and also as a powerful prognosticator in patients with established CVD (61). TDI performed in asymptomatic cohorts has also demonstrated relationships between subclinical myocardial function and increased risk factor burden in subjects without clinical CVD (62). However, the prognostic role of subclinical LV longitudinal systolic dysfunction in healthy populations is yet to be proven.
1.3.2.2 Left ventricular diastolic function and filling pressure

Patients who present with dyspnoea and evidence of LV dysfunction as evidenced by slow LV relaxation and increased LV stiffness but with a normal LV EF are frequently referred to as having the syndrome of diastolic heart failure or heart failure with normal EF, which has emerged over the last two decades as a separate clinical entity to systolic heart failure. Approximately half of the patients presenting with symptoms of congestive heart failure exhibit a near normal LV systolic function at rest (63), which is thought to be caused by a predominant abnormality in diastolic function. Diastolic heart failure is generally considered to have a better prognosis than systolic heart failure, but the frequency of hospitalisations is comparable. Prevalence of diastolic heart failure increases with age and is also associated
Determinants of diastolic function include myocardial relaxation and passive properties of the ventricular wall, such as myocardial stiffness, wall thickness and chamber geometry. Diastolic function is traditionally assessed using echocardiographic indices. The most familiar of these are the mitral inflow velocities; the E and A waves that correspond, respectively, to early flow during LV relaxation and subsequent contribution of atrial contraction. With impaired relaxation, atrial contraction contributes relatively more to ventricular filling (A velocity>E velocity, with prolonged deceleration of the E wave). This state is common with increasing age and may identify patients at risk for diastolic heart failure. When LV diastolic pressure increases to the point that atrial contraction contributes little to filling, the E wave again becomes predominant but with rapid deceleration, first in a pseudonormal pattern and ultimately in a restrictive pattern (high E wave velocity, usually more than twice the A wave velocity). However, transmitral Doppler assessments offer a less reproducible and less capable method than TDI of identifying an elevated LV filling pressure - the hallmark of symptomatic diastolic dysfunction (13,66), and are no longer recommended as a first-line test for detecting diastolic dysfunction. The ratio of mitral E velocity divided by TDI derived E’ correlates closely with LV filling pressure. Mitral E depends on left atrial (LA) driving pressure, LV relaxation kinetics, and age but E’ depends mostly on LV relaxation kinetics and age. Hence, the E/Ea ratio theoretically eliminates the effects of LV relaxation kinetics and age and represents a measure of LA driving pressure or LV filling pressure. Elevated LV filling pressure carries strong prognostic value in patients with heart failure, and its estimation using TDI is a similarly powerful prognosticator amongst patients with heart failure, hypertension or clinical CVD (59,60,67,68). However, increases in E/Ea
within the normal range are not necessarily indicative of relatively higher filling pressure, and in asymptomatic individuals its usefulness in predicting future cardiovascular events remains to be elucidated.

1.3.2.3 Left ventricular mass

LVH refers to an absolute increase in LV mass above a predefined cut-off value and is a form of cardiac remodeling that is strongly associated with major cardiovascular events, independent of BP, known risk factors and coronary artery disease (50,69-71). The presence of LVH is important clinically because it is associated with increases in the incidence of heart failure, ventricular arrhythmias, death following myocardial infarction, decreased LV EF, sudden cardiac death, aortic root dilation, and cerebrovascular events. The increased cardiac risk associated with LVH is probably due in part to myocardial ischaemia that can be induced by a variety of factors. In hypertrophied myocardium, there is a reduced density of capillaries, with the enlarged muscle mass directly compressing the endocardial capillaries and limiting the ability of the coronary arteries to dilate in response to decreased perfusion or during vasodilatory stress (72). Both of these factors can decrease coronary reserve and can have a number of important clinical implications. Firstly, coronary occlusion is associated with a greater degree of infarction and a higher mortality rate than observed in non-hypertrophied ventricles (73). Secondly, hypertrophied myocardium may be more susceptible than normal myocardium to the effects of ischaemia. In a study of patients with sudden cardiac death, those with hypertension and LVH who died suddenly had less extensive coronary disease and were less likely to have thrombi in the coronary vessels than normotensives (74).

The development of heart failure with LVH results from depressed LV systolic function and/or diastolic dysfunction. The deleterious effect of LV remodeling may be an
important determinant of progression to overt heart failure. LVH also causes several
electrophysiologic changes or electrical remodeling, including nonuniform action potential
prolongation, altered repolarization and increased dispersion of recovery, and the easily
provocable early afterpotentials, which are associated with increased vulnerability to atrial
fibrillation, ventricular arrhythmias, especially torsades de pointes, and sudden death.

1.3.2.4 LV geometric pattern

The geometric pattern of LV remodeling may also carry independent prognostic information
to LVH (75). The relative wall thickness (RWT) is a ratio of the LV cavity dimension to the
wall thickness and provides a measure of the degree of concentric remodeling present. Pure
concentric remodeling is defined as an increased RWT in the absence of increased LV mass,
whereas concentric hypertrophy refers to a concentrically remodelled LV in the presence of
LVH. Both these patterns of disease represent physiological adaptation to pressure overload
physiology such as hypertension or aortic stenosis. A third pattern of LV remodeling,
eccentric hypertrophy, occurs in states of volume overload such as aortic or mitral
regurgitation resulting in an increased LV mass but a normal RWT. Of the different patterns
of LV remodeling, concentric hypertrophy is associated with the greatest risk of future
cardiovascular events (76) and is believed to ultimately progresses to LV dilatation and
failure in hypertensives (77-79). There is also evidence that non-hypertrophic concentric
remodeling is associated with worse prognosis compared to subjects with normal LV
geometry (80-82).

1.3.2.5 Left atrial volume

In the absence of mitral valve disease, anaemia or athletes heart, an enlarged left atrium
provides physiological and morphological evidence for chronic elevation in LA pressure or
LV filling pressure. The relationship between LA volume and prognosis has been confirmed
in patients with pre-existing cardiovascular disease such as atrial fibrillation (83), LV dysfunction (84) and myocardial infarction (85,86).

There is substantial biologic plausibility to the prognostic significance of an increased LA volume. The LA is most commonly thought of as a transporting chamber, receiving blood from the pulmonary veins and conveying it to the left ventricle through both passive and active diastolic filling. However, the LA also functions as a volume sensor of the heart, releasing natriuretic peptides in response to stretch and other neurohormones whilst also generating a reflex tachycardia in response to increased venous return (Bainbridge reflex). The LA reflects LV filling pressure and is capable of remodeling in response to its elevation. As an ongoing transducer of sustained elevations in LV filling pressures, LA size offers itself as a plausible biomarker of incipient CVD. Spectral and tissue Doppler indices at best present only a snapshot view of diastolic function, susceptible to variations in loading conditions. LA volume provides an assessment of chronic LV diastolic pressures and whether or not the patient has the “disease” of diastolic dysfunction. An analogy can be drawn with the two biomarkers used to assess glycaemia control in diabetics. Whereas serum glucose reflects transient control, measurement of serum Hb A1C reflects the longer-term glycaemic state. Similarly conventional and tissue Doppler techniques of assessing LV filling pressure provide a measurement of transient LV loading conditions. However, LA size is a long-term biomarker of average LV diastolic pressure, and hence, when increased, of diastolic dysfunction.
CHAPTER 2. CARDIOVASCULAR DISEASE IN MIGRANT INDIAN ASIANS

People originating from the Indian subcontinental countries of India, Pakistan and Bangladesh (referred to in this thesis as being of “Indian Asian” ethnicity), have been noted in several parts of the world to suffer from high rates of stroke and myocardial infarction. Although there is considerable genetic heterogeneity amongst people from the Indian subcontinent, high CVD rates appear to be ubiquitous to Indian Asian groups of different geographical origin, religion and language.

Indian people have migrated to other countries for many centuries, initially through the colonial system of indentured labour in 1834(87,88) to Mauritius, the West Indies, Natal, Fiji and East Africa. After the indentured labour system was abolished in 1917, the next wave of migration occurred from 1966-67 to Britain, predominantly from Gujarat and Punjab. The earliest reports of higher CVD rates in Indians compared with other groups was from Singapore in 1957. In a series of <9000 autopsies undertaken from 1950-54, coronary artery disease with myocardial involvement was seven times higher in Indian Asians than Chinese (89), with a threefold excess in CHD remaining in Indians compared to Chinese some 25 years later (90). In Uganda 43% of deaths among Indian Asian men in Kampala were certified as due to CHD, whilst amongst the indigenous Ugandans the disease was said to be non-existent (91). Excess mortality rates for CVD amongst Indian Asians of at least >40%, compared to the native population, have also been documented in South Africa, Fiji and Trinidad (92-94).
The predisposition of CVD amongst migrants of Indian Asian descent has been particularly well studied in the UK. Elevated CHD rates were first observed at the time of the 1971 Census with mortality rates from CHD and stroke ~20% higher in Indian Asian men and women born in the Indian subcontinent, compared to the general population (95). This disparity had widened further by the time the 1991 Census data had been examined, with at least a 50% higher mortality reported (96). Not only had the relative risk of CVD mortality increased amongst UK Indian Asians but health inequalities between ethnic groups in the UK have widened (97). Death rates from CHD amongst Indian Asians had declined at a slower rate than the indigenous population (1), and CHD mortality rates were observed to be twice as high amongst Indian Asian men aged < 40 years (98). The most recent Census-based analysis of trends in CHD and stroke mortality among migrants in England and Wales showed again at least a 50% excess risk in Indian migrants. There was an alarming two- to three-fold excess risk observed amongst Pakistani and Bangladeshi individuals; representing a doubling in their CVD mortality rates over the past 20 years (99).

Epidemiological studies have demonstrated that migrants from the Indian Asian subcontinent to the UK have a less favourable cardiovascular risk profile compared with their siblings who do not migrate, with the migrants demonstrating higher serum cholesterol, apo B, blood glucose and lower HDL-cholesterol concentrations (100). There is also evidence that second and third generation Indian Asians seem to be displaying many of the same risk characteristics that make them prone to coronary heart disease as their parents and grandparents (101).

2.1 **Cardiovascular Disease in the Indian Subcontinent**

According to World Health Organisation data, the reported prevalence of CHD has risen 4-fold over the last 40 years (to a present level of around 10%), and even in rural areas the
prevalence has doubled over the past 30 years (to a present level of around 4%) (102). In India CVD has overtaken infectious diseases as the leading cause of death, accounting for 29% of all deaths in 2005 (102). About 50% of reported myocardial infarctions occur in Indian men under the age of 50 years, with 25% under the age of 40 years(103). In addition, 30% to 40% of cardiovascular deaths occur between 35 and 64 years of age.

Marked differences in rural-urban CHD rates within India have been previously observed. Two surveys of CHD prevalence have been conducted in northern India (104,105), and the results have been compared with those of a survey in Tecumseh, USA (106). In Chandigarh, capital city of Punjab and Haryana, > 2000 adults aged > 30 years were examined, while in rural Haryana a total village community of this age was included for study. The prevalence (rate per 1000) of electrocardiographic Q waves in men aged 40-59 years was 38 in Chandigarh, 26 in Tecumseh, and 5 in rural Haryana, suggesting that CHD rates in urban Indian populations were significantly higher, and were similar to Indian Asians residing in the USA. Another study conducted in two rural settings in north India showed a graded increase in risk of coronary artery disease prevalence and higher social class (107), implying a link to the pronounced differences in education, diet and lifestyle. These studies suggest that the harmful effects of migration upon cardiovascular health occur amongst individuals relocating to cities within India, as well as amongst those who emigrate to foreign countries.

2.2 Cardiovascular Disease Risk Stratification in Indian Asians

The inherent problems of accurate CVD risk prediction based upon conventional risk factors, as discussed earlier (chapter 1.1), is exacerbated amongst non-Caucasians to whom reference ranges for independent risk factors may not apply and are felt to be set too high for Indian Asians. The prevalence of traditional risk factors such as smoking, hypercholesterolaemia
and hypertension are generally lower in Indian Asians than in European whites (7). In Asians the risk of diabetes starts to increase rapidly when the body-mass index or waist circumference are well within the accepted range for Europeans (108). Nonetheless, the management of conventional risk factors remains as important amongst Indian Asians as in Caucasian individuals. The INTERHEART study demonstrated that acute myocardial infarction mortality amongst native Indian Asians could be explained by 9 potentially modifiable risk factors, with the presence of 4 main risk factors (smoking history, high ApoB100/Apo-I ratio, history of hypertension, history of diabetes) ubiquitous across to all the Indian Asian ethnic subgroups (109).

The poor predictive value of conventional risk factors and limitations of current diagnostic tools has encouraged research into novel and established markers of cardiovascular risk to help improve risk prediction in both Caucasian and non-Caucasian populations. This is of paramount importance amongst Indian Asians, who comprise one-quarter of the world’s population and who are projected to account for ~40% of the global CVD burden by 2020 (110).

2.3 Potential Mechanisms Underlying the Excess Cardiovascular Disease Mortality in Indian Asians

2.3.1 Insulin resistance and inflammation

Novel risk factors have been sought to account for the excess CVD risk amongst Indian Asians. With levels of glucose intolerance, central obesity, triglyceride and insulin found to be uniformly elevated across the Indian Asian subgroups, it is widely believed that a greater prevalence of the insulin resistance or metabolic syndrome is likely to explain the greater susceptibility of Indian Asians to CVD (111). However, data assessing the predictive power of insulin resistance in other cohorts remains conflicting and outcome data with respect to
Indian Asians is lacking. Moreover, there remains no clinically feasible marker of insulin resistance, and reversal of the insulin resistant and proinflammatory state is not easily achieved.

Potential mechanisms responsible for the association between insulin resistance and an increased risk of CVD events have also been investigated. Inflammation is widely recognised as a central feature of atherogenesis and in particular plays a critical role in destabilisation of the fibrous cap tissue, predisposing to plaque rupture and acute thrombosis. C reactive protein (CRP) is an acute phase reactant that has been shown to reflect underlying atherosclerotic burden or activity. CRP levels are elevated in Indian Asians, explained by a greater degree of central adiposity (112) which is consistent with experimental studies suggesting that abdominal adipose tissue is a major source of cytokines, including interleukin-6, an important determinant of CRP synthesis. Although this raises the possibility that inflammatory mechanisms may underlie part of the increased CVD risk amongst Indian Asians, a recent genome-wide association study suggests that the relationship between CRP and CHD is likely to represent an epiphenomenon, rather than be causal in nature (113).

The reason for the enhanced susceptibility to insulin resistance amongst Indian Asians is not fully understood. Given the increased prevalence of atherosclerotic disease following exposure to western lifestyles, either as a consequence of urban migration within India or emigration, a genetic predisposition has been suggested. The “thrifty” gene concept was first postulated in 1962 (114) by James Neel who suggested the existence of metabolically thrifty genes that permitted more efficient food utilisation, fat deposition and rapid weight gain at times of food abundance, thereby better equipping the gene-bearer to survive a subsequent famine. Examples of a thrifty gene would include those resulting in high levels of insulin, leptin (a hormone released by fat cells that regulates appetite) or triglycerides (115). Such
genes would be advantageous under conditions of unpredictably alternating feast and famine, which has characterised the history of the Indian subcontinent, but would lead to obesity and diabetes in the modern world when the same individuals would stop exercising and foraging for food whilst consuming high-calories meals on daily basis.

2.3.2 Environmental factors

As discussed earlier, there appears to be a graded relationship between more favourable socioeconomic status and increased risk of CVD amongst individuals living within India, supporting the role of significant environmental component to the elevated risk of CVD amongst migrant Indian populations. Any, putative genetic predisposition would be exaggerated by products low in linolenic acid and high in trans fatty acids such as ghee cooking oils, typically consumed in the Indian subcontinent. Studied of rural populations have been found 300-500g per day of whole grain such as wheat, rice, millet and pulses to be consumed using mustard oil, rich in n-3 fatty acids, for cooking purposes (116-118). However, urban dwellers and immigrants substitute potato and refined carbohydrates for grains, and use proatherogenic ghee and clarified butter in place of oils, inadequate consumption of fruit and vegetables (117,118) with consequent deficiency in antioxidants vitamin A and C.

2.3.3 Emerging risk factors

Other, novel risk factors distinct from the syndrome of insulin resistance have been investigated as potential mediators of the elevated CVD risk in Indian Asians. An increased level of Lp(a) lipoprotein has been associated with an increased risk of coronary artery disease, carotid atherosclerosis and stroke in cohort studies (119-122). Plasma levels of Lp(a) lipoprotein vary substantially amongst persons, being determined at birth by variations in the LPA gene, and have been found to be significantly higher amongst Indian Asians.
compared to European whites (100). Although homocysteine levels are higher in Indian Asians compared to European whites (123), its role in causing CVD is unclear. Recent randomized trials of homocysteine lowering have not demonstrated a reduction in CHD (124,125). Thus, the role of novel risk factors as an important cause for CVD in Indian Asians is likely to be small.

2.3.4  **Anatomical coronary disease and burden of atherosclerosis**

Coronary vessels are generally smaller in Indian Asians than European whites, although these differences are removed after indexation for body size (126). Although these findings imply that the smaller arteries are proportionate to their body size, such vessels may give rise to technical difficulties during surgical or percutaneous revascularisation, which may dissuade cardiologists or cardiac surgeons from pursuing these treatments amongst Indian Asian patients. In a study assessing severity of coronary artery disease amongst UK Indian Asians, Indian Asians living in India and UK European whites, there were no significant differences in the prevalence of triple vessel disease, total number of lesions and proximal disease between the three groups (127).

2.3.5  **Imaging markers of subclinical cardiovascular disease amongst Indian Asians**

There have been very few prospective, population-based studies of subclinical atherosclerosis amongst Indian Asians. In one relatively small community-based study, Indian Asians demonstrated lower age- and gender-adjusted IMT than native European whites living in Canada, in contrast to the higher prevalence of clinical CVD observed in the cohort under investigation (128). Subclinical atherosclerosis can also be assessed in the coronary arteries by measuring the degree of calcification using EBCT. However, there are no studies to-date comparing calcium scores between Indian Asians and other ethnic groups.
Studies assessing the association of ethnicity with LVH have predominantly been conducted in American black, white and Hispanic populations (129,130), but the prevalence of LVH amongst other ethnic groups is poorly defined with very little LV mass data published in Indian Asian groups. In a study by Kumaran et al., echocardiography was performed in 435 subjects living in Mysore, South India (131). The mean LV mass in Indian Asian men and women and noted to be lower than published values in Western populations, although LVH prevalence could not be ascertained due to the lack of ethnicity-specific partition values for increased LV mass. The study did confirm that the positive correlation between increased LV mass and risk of CVD existed in individuals of Indian Asian ethnicity, with significantly higher LV mass observed in the 45 subjects with known CVD compared to controls. The relationship of LA volume with ethnicity has also not been examined sufficiently to-date, although similar LA size has been reported between African-Caribbean and Caucasian individuals (30,35), there is no published data in Indian Asians.

Ethnicity-related differences in systolic and diastolic properties of the left ventricle have not been previously reported amongst healthy individuals. Although an ASCOT substudy did demonstrate significantly worse TDI parameters of diastolic function amongst UK African-Caribbeans compared to European whites, recruited subjects were hypertensive precluding meaningful conclusion to be drawn concerning intrinsic myocardial function and ethnicity(132).

2.4 Understanding Cardiovascular Disease Risk in Indian Asians - The London Life Sciences Prospective Population (LOLIPOP) Study

The LOLIPOP study is a large, prospective population study of cardiovascular risk factors in Indian Asians and European whites residing in West London. The objectives of the study are to further understand the contribution of conventional and novel risk factors to CVD in
European whites and Indian Asians, and whether they identify the excess CVD mortality in the latter group. This will be achieved by the cross-sectional analysis of subclinical markers of CVD using imaging, genomic, metabolomic and proteomic techniques and by determining risk relationships through long-term follow-up of the cohort.

The LOLIPOP study builds on a collaboration which has already been established between 58 West London general practitioners and the Department of Cardiology at Ealing Hospital to deliver National Service Framework Standards 3 and 4 for cardiovascular risk assessment in Primary Care (figure 2.1). As part of this collaboration, all men and women aged 35-74 were invited for detailed cardiovascular assessment at their GP practice which included questions on medical history, cardiovascular risk factors, alcohol intake, physical activity and drug history. Indian Asian and European White subjects attending for cardiovascular assessment were invited to participate in the LOLIPOP cohort study. Recruitment to the study commenced in 2002, and was completed in December 2007 with ~30,000 subjects enrolled.
Figure 2.1 - The LOLIPOP Programme

12000 Indian Asians
London Life Sciences Prospective Population (LOLIPOP) Cohort Study
West London Cardiovascular Health Programme

12000 Northern Europeans
CHAPTER 3.  THESIS OBJECTIVES AND METHODS

3.1 Objectives

This thesis examines the initial, cross-sectional data collected from the LOLIPOP-ATHEROSCLEROSIS substudy (figure 2.1). Biomarkers of subclinical atherosclerosis, subclinical LV dysfunction and abnormal cardiac remodelling will be the principle dependent variables under examination. Subclinical disease has been used as an outcome measure in this study, allowing mechanistic insights into the early pathogenesis of clinical disease to be explored in a bi-ethnic population. The two principle objectives of this thesis are to study:

i) Factors that influence LV function - the relationships between atherosclerosis burden and LV geometry with cardiac function will be defined in both European white and Indian Asian individuals.

ii) Factors that may identify the increased risk of CVD amongst Indian Asians - the atherosclerosis burden and the prevalence of abnormal LV remodelling between the two ethnic group will be documented.

This data will also afford the opportunity to derive cohort-specific normative reference values for quantitative parameters of LV function, mass and relative wall thickness.

This study will be performed in the context of a well powered, prospective population study which will accommodate the long-term follow up of this cohort. Correlation of the aforementioned imaging biomarkers of subclinical CVD with future, clinical events will permit:
1) determination of characteristics related to progression of subclinical CVD to clinical CVD

2) assessment of age, sex and ethnic differences in risk of subclinical CVD progression and rates of clinical CVD

3) validation of potential risk relationships identified from the cross-sectional analysis and to determine their incremental predictive value over established risk factors

3.2 Methodology

3.2.1 The LOLIPOP-ATHEROSCELROSIS substudy

Subjects successfully enrolled into the main LOLIPOP cohort, as described above, were then invited to participate into the LOLIPOP-ATHEROSCELROSIS substudy (figure 2.1). The principle inclusion criteria were that subjects should be of: i) Indian Asian or European white ethnicity ii) aged of 35-75 years and iii) be free of clinical CVD (prior history of myocardial infarction, stroke, peripheral vascular disease, revascularisation procedures) and/or any life-limiting illness. Subjects were defined as Indian Asian if all four grandparents were born in the Indian subcontinent and European White if all four grandparents were born in Northern Europe. Subjects were selected for invitation at random using computer software.

7056 potential participants were then contacted by invitation letter, and the response rate for this substudy was 36.6%. The characteristics of responders and non responders are given in Table 3.1. Responders were more likely to be male, and had more favourable risk factor profiles with respect to physical activity, smoking status, waist-hip ratio (WHR), body mass index (BMI), BP and cholesterol. Responders were also more likely to have a family
history of heart disease. There was no difference in response rates between Indian Asians and European whites.

Responders were then invited for interview at Ealing Hospital and inclusion criteria were confirmed. Response rates from the first letter of invitation averaged 58.9%. Non-responders received a second letter of invitation. Limited data was available on the non-responders, such as name, age, sex and postcode. Non-responders were more likely to be male and of younger age.
Table 3.1 - Demographic and risk factor data for responders vs non-responders for the LOLIPOP-ATHEROSCLEROSIS substudy

<table>
<thead>
<tr>
<th></th>
<th>Responders (n = 2540)</th>
<th>Non responders (n = 4516)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>European White (%)</td>
<td>44</td>
<td>44</td>
<td>0.35</td>
</tr>
<tr>
<td>Male (%)</td>
<td>65</td>
<td>57</td>
<td>&lt;0.001</td>
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<tr>
<td>Diabetes (%)</td>
<td>15</td>
<td>15</td>
<td>0.9</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>5.7(1.8)</td>
<td>5.9(1.9)</td>
<td>0.4</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>6.9(1.5)</td>
<td>7.0(1.4)</td>
<td>0.3</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>28</td>
<td>27</td>
<td>0.3</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80(10)</td>
<td>81(11)</td>
<td>0.03</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>132(19)</td>
<td>133(20)</td>
<td>0.003</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.4</td>
<td>5.45</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.3(0.3)</td>
<td>1.4(0.3)</td>
<td>0.5</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>3.5(0.8)</td>
<td>3.4(0.8)</td>
<td>0.1</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>27.3(4.4)</td>
<td>27.6(4.9)</td>
<td>0.008</td>
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<td>Body Fat (kg)</td>
<td>30.9(8.5)</td>
<td>32.0(8.9)</td>
<td>&lt;0.001</td>
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<tr>
<td>WHR</td>
<td>0.93(0.08)</td>
<td>0.94(0.08)</td>
<td>0.02</td>
</tr>
<tr>
<td>10 yr Framingham score</td>
<td>9%</td>
<td>12%</td>
<td>&lt;0.001</td>
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<td>Smoker (%)</td>
<td>44</td>
<td>56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history IHD (%)</td>
<td>25</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BP = blood pressure, HDL = high-density lipoprotein, LDL = low-density lipoprotein, BMI = body mass index, WHR = waist hip ratio, IHD = ischaemic heart disease. SD in brackets.
Accurate ethnic group classification was not possible for non-responders, although their name provided an indication of likely ethnicity. Ethnicity was allocated to a sample of non-responders according to a typical Indian Asian (1350) or European white (1350) name. Indian Asian men and women were less likely to respond to the LOLIPOP cardiovascular risk prevention clinic than European whites. However, Indian Asian non-responders were more likely to be of younger age than European whites and after adjustment for age, there was no difference in non-response rates between the two groups.

During the cardiovascular risk assessments, clinical information was collected by study nurses at the GP surgeries and corroborated by clinical notes. Standardised study protocols were adhered to, with regular data checking and quality control. Nurses collected information about medical and family history, current prescribed medication, and smoking history. Country of birth of participants, parents, and grandparents were also recorded, together with language and religion for assignment of ethnic subgroups. Smoking was defined as never, former smoker or current smoker along with quantity of cigarettes smoked per day to calculate pack years. Venesection was performed to provide whole blood, serum and plasma samples which were analysed for routine biochemistry, serum glucose, glycosylated haemoglobin (HbA1c) and lipid profiles. Samples were frozen permitting future biochemical/genetic analysis. Physical assessments included anthropometric measurements, BP, and 12 lead electrocardiogram (ECG).

Height was measured using a stadiometer, mounted on a hard, flat surface. Patients were measured without shoes, standing with their back to the height rule, with both feet together, and with head, buttocks and heels touching the wall. Patients were asked to look directly ahead, and the stadiometer bar moved down to touch the top of the head, pressing the
hair flat. For patients with turbans, the turban was removed. Height was recorded to the nearest 0.1 cm. Weight and body fat percentage were recorded using digital Tanita Body Composition Analyser scales. The scales were mounted on a hard, flat surface. The patient was weighed after an overnight fast, wearing light clothing only without shoes or socks. Weight was recorded to the nearest 0.1 kg. The fat percentage was calculated using bioelectrical impedance analysis, allowing lean body mass to be derived. A small amount of electric current is passed through the body and the resistance encountered from different tissues is calculated. The resistance encountered from lean tissue or muscle is lower than that from adipose tissue, allowing fat percentage to be estimated and lean body mass (LBM) to be derived.

Waist-hip ratio was measured after an overnight fast, with the patient undressed except for underwear, and standing erect, with feet together. Waist was defined as the point midway between iliac crest and lowest rib, identified by palpation, and waist circumference measured as the minimum circumference at this level. Hip circumference was defined as the maximum circumference over the greater trochanters and buttocks. Measurements were made using a non-stretchable measuring tape, applied around the body without twists, and gently touching skin, without compressing soft tissue. All measurements were recorded in centimetres, to the nearest 0.1 cm. BMI was calculated by dividing the participant’s weight in kilograms by the square of his/her height in meters (kg / m²).

A total of 2,439 subjects were recruited from LOLIPOP, of which 2,288 were free from clinical CVD. All subjects enrolled into the substudy were consented to undergo echocardiography, carotid ultrasonography, venesection and 24-hour ambulatory BP monitoring, which were all performed during the same visit. Consenting subjects also agreed to undergo EBCT for coronary artery calcium scoring, performed on a separate visit.
(Wellington Hospital, London). Consenting subjects also agreed to participate in the follow-up programme involving interview every 5 years at Ealing Hospital for documentation of incident cardiovascular events. Follow-up will also be achieved by interrogation of multiple sources namely GP and local hospital records, the Office for National Statistics, Virtual Organisation for Trials and Epidemiological Studies (VOTES) and Hospital Episode Statistics (HES).

Relevant approval had been obtained to conduct the study from the Research Ethics committees of Northwick Park and Ealing Hospitals.

3.2.1.1 Power calculations

Power to detect risk associations between putative risk factors and the primary endpoint of carotid plaque was calculated for the whole cohort, using logistic regression for a cohort study design. Estimates of the baseline prevalence of carotid plaque were obtained through the pilot study and the published literature at 39%. Power to detect ethnicity-specific differences in carotid plaque prevalence was considered by treating ethnicity as a risk factor with 50% prevalence.

Table 3.2 - Power calculations

<table>
<thead>
<tr>
<th>Prevalence of Risk Factors in Control Group e.g. hypertension, ethnicity</th>
<th>Odds Ratio of Plaque disease</th>
<th>Power Calculation Whole Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>1.5</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>30%</td>
<td>1.5</td>
<td>&gt; 95</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>50%</td>
<td>1.5</td>
<td>&gt; 95</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>&gt; 95</td>
</tr>
</tbody>
</table>

For all estimates, alpha was set at 0.05; tests were two-tailed. All estimates were obtained using the PASS module of NCSS 2000 (NCSS, Utah, USA).
Assuming 2,000 subjects are recruited to the study, this affords >95% power to detect ethnicity-related differences in plaque prevalence with an odds-ratio of 1.5 (p<0.05).

### 3.2.2 Carotid ultrasonography

Carotid duplex scans were conducted using a high-resolution, non-harmonic B-mode ultrasound system (Philips IE33) with an 11- to 3-Mhz transducer. Images of the right and left common carotid arteries, their bifurcations, the internal and external carotid arteries were captured. Studies were repeated in 15 subjects to provide measures of reproducibility and inter-observer variability, by calculating the coefficient of variance (standard deviation of difference/mean of one observer).

Values for mean and mean maximal IMT were derived from at least 3 measurements at the common carotid artery and its bifurcation, for both the left and right arteries. If there was evidence of carotid plaque formation, care was taken not to include the plaque in the IMT measurement. The left and right carotid arteries were then systematically interrogated in short- and long-axis views for detection of atherosclerotic plaque formation by using colour-Doppler and pulse wave Doppler imaging. A plaque was defined as a focal structure encroaching into the arterial lumen by at least by 0.5mm, or a distinct area of intima-medial thickening that is at least 50% greater than the adjacent wall or is more than 1.5mm in thickness (133). The maximal plaque thickness, the peak systolic and diastolic Doppler velocities at the point of the maximal lesion were measured. Each plaque was classified as according to Gray-Weale (134) classification.
3.2.3 Two- and three-dimensional echocardiography

3.2.3.1 Left ventricular dimensions and global systolic function

Transthoracic two-dimensional (2-D) echocardiograph was performed by experienced sonographers using a digital commercial harmonic imaging ultrasound system with an S3 3-MHz phased-array transducer (Philips IE33, Philips Medical Systems, Holland). Full studies were repeated in 15 subjects by two sonographers to provide measures of reproducibility and inter-observer variability.

LV dimensions were obtained in the parasternal long axis view, with measurement of interventricular septal thickness, LV cavity dimensions and posterior wall thickness in systole and diastole allowing estimation of LV mass, calculation of RWT and determination of LV geometrical pattern. LV volumes and systolic function were determined from Simpson’s apical bi-plane technique by tracing a contour around the LV cavity during end-diastole and end-systole(135). LV volumes and radial function were further quantified with real-time three-dimensional (3-D) echocardiography using a matrix array ultrasonic transducer (X3-1 transducer, Philips IE33, Philips Medical Systems). Measurement of 3-D volumes was performed off-line (Q-Lab 5, Philips Medical System). Frames for end-diastolic and end-systolic volumes were identified by the same method as for 2-D echocardiography. Contour tracing was performed with semi-automatic border detection after first identifying the apex and mitral annulus, a pre-configured ellipse is fitted to the endocardial borders of each frame and adjusted as required.

Longitudinal myocardial function was assessed using TDI with an on-line, pulse-wave Doppler technique. Sample volumes were placed at the junction of the LV wall with the mitral annulus of the septal and lateral myocardial segments from the four-chamber view and inferior and anterior myocardial segments from the two-chamber view. Right ventricular
myocardial tissue velocities were also derived from the junction of the RV free wall and tricuspid valve annulus and radial velocities derived in the mid-segment of the posterior wall in the parasternal short axis view. Peak velocities during systole (Sa) were measured on-line at all 6 myocardial segments and from the RV free wall annulus.

3.2.3.2 Diastolic function

Left atrial volume

LA volume was calculated from three measurements of LA dimension taken at ventricular end-systole using the formula for an ellipse. Indexation to body surface area was then performed.

Transmitral flow

The mitral flow velocities were recorded using pulse waved Doppler with the sample volume placed at the tip of the mitral valve leaflets in the apical view. From the mitral valve inflow velocity curve the following measurements were made: peak E-wave velocity and its deceleration time; peak A-wave velocity; ratio of E-wave to A-wave (E:A) velocities and the isovolumic relaxation time was measured from the aortic valve closure to mitral valve opening.

Longitudinal diastolic function and estimate of end-diastolic pressure

The early (Ea) and late (Aa) diastolic velocities were measured from 6 myocardial segments using the same tissue Doppler pulsed wave signal obtained to measure the systolic myocardial velocity. An estimate of LV filling pressure could then be obtained from the ratio of the transmitral E-wave velocity to the tissue Doppler Ea velocity (E/Ea ratio).
3.2.4 Venesection

Subjects underwent venesection to provide blood samples which were divided into routine biochemistry gel serum separation tubes and into tubes anticoagulated with K-EDTA. Gel tubes were allowed to clot and both tubes then centrifuged. Following serum and plasma separation, they were divided into aliquots, frozen then stored at -20°C for 24 hours and then -70°C until analysis (St George’s Hospital, London).

3.2.5 Electron beam computed tomography

Subjects were also consented to undergo EBCT which was undertaken at The Wellington Hospital, London for determination of coronary artery calcium scores. This data has not been presented in this thesis, although the data was used to help define a reference, “healthy” subgroup who were free of CVD, cardiovascular risk factors and significant subclinical atherosclerosis. This healthy subgroup was studied for intrinsic ethnicity-related differences in cardiac structure and function, as well as to determine normative values for TDI parameters and partition values for LV mass. An abstract of the data published comparing coronary artery calcium scores between Indian Asians and European whites has been published (136), and is presented in Appendix 1.

3.2.6 Personal contribution to the study

The echo and carotid ultrasonographic protocols for subjects enrolled to the LOLIPOP-ATHEROSCLEROSIS substudy were devised by Prof. Senior. Initial recruitment commenced in August 2004, which was performed by his previous research fellow (TK Lim), until January 2007. From February 2007 I assumed responsibility for overseeing the recruitment of subjects into the LOLIPOP-ATHEROSCLEROSIS substudy, which was performed at Ealing Hospital. Approximately 700 subjects were enrolled from February 2007 to November 2007 when study recruitment was terminated. I personally performed ~ 450
studies upon recruited subjects at Ealing Hospital, which involved full 2-D and 3-D transthoracic echocardiography, and bilateral carotid ultrasonography for IMT and plaque assessment. At Northwick Park Hospital I analysed ~900 studies offline, performing quantitative analysis of the 3-D volumetric datasets and measuring carotid IMT. I also assisted in the offline, qualitative analysis of plaque morphology in >600 carotid artery ultrasound scans with Prof. Senior. After all data had been collected, analysed and entered into the database, I undertook a quality control and data validation exercise, cross checking outlier variables with the original, recorded echocardiographic and ultrasonic images. I was involved in the conception of the hypotheses for all the manuscripts presented and was responsible for undertaking all statistical analyses, with the assistance of a statistician.
PART II – STUDIES AND RESULTS
CHAPTER 4. STUDY I - NORMATIVE REFERENCE VALUES FOR

THE TISSUE DOPPLER IMAGING PARAMETERS OF LEFT

VENTRICULAR FUNCTION

4.1 Abstract

Objectives: TDI is used routinely to quantify LV function and filling pressure. However, there remains a lack of percentile-based normative reference values for these clinically important parameters.

Methods: 453 healthy subjects aged 35-75 years were included for analysis from the LOLIPOP study. Subjects were free of manifest CVD, cardiovascular risk factors and significant coronary artery disease as determined by EBCT. They underwent 2-D and Doppler echocardiography for assessment of left heart structure and function. TDI was performed at the septal and lateral mitral annular sites enabling on-line derivation of myocardial Sa velocity, Ea velocity and the ratio of Ea to transmitral E-wave (E/Ea).

Results: Reference ranges (5th and 95th percentile values) for septal, lateral and average mitral annular Sa velocity, Ea velocity and E/Ea ratio were derived for the whole cohort and for each of the four age groups (35-44, 45-54, 55-64, 65-75). Increasing age was associated with a significant attenuation in myocardial velocity when averaged from both the septal and lateral mitral annulus, exerting a greater influence upon average Ea velocity (p<0.001) compared to average Sa velocity (p=0.04). Average E/Ea ratio increased significantly with advancing age (p<0.001).
**Conclusion:** The reference ranges presented for the TDI parameters of Sa velocity, Ea velocity and E/Ea ratio will help to standardise the assessment of LV function by tissue Doppler echocardiography.
4.2 **Introduction**

Assessment of longitudinal myocardial function using TDI has emerged as a new prognostic tool for CVD within the field of non-invasive cardiac imaging (61). The ability to quantify long-axis function of the myocardium in a reproducible manner has also significantly refined the assessment of LV function (137). The TDI derived parameters of myocardial Sa velocity, Ea velocity and LV filling pressure (E/Ea) are used routinely in clinical echocardiography and their measurement forms the cornerstone of the recent European guidelines concerning the diagnosis of diastolic heart failure (138). The importance attached to these parameters has prompted attempts to derive their normative values. However, studies that have addressed this issue have tended to report average values for TDI parameters (139-145) rather than standard deviation or percentile derived reference ranges, which provide a more standardised and validated framework for partitioning echocardiographic variables (146,147).

Methodological heterogeneity in determining the myocardial time-velocity curve also exists. Myocardial velocities are usually obtained from the on-line, spectral pulsed tissue Doppler time-velocity curve in clinical practice but for research purposes they can also be reconstructed off-line from colour-coded images superimposed on the 2-D echocardiographic images. However, off-line reconstruction results in lower myocardial velocities than are achieved by on-line spectral analysis resulting in wide variation in published normative values. On-line assessment must be the technique of choice in the busy echo laboratory and using this study defines normative reference ranges for Sa velocity, Ea velocity and LV E/Ea. Subjects representing a biethnic, highly-phenotyped cohort free of clinical CVD, traditional cardiovascular risk factors and significant coronary artery disease were studied.
4.3 Methods

From the 2,293 subjects recruited into the LOLIPOP-ATHEROSCLEROSIS substudy (figure 2.1, chapter 3.2.1), a subset of 453 “healthy” individuals were identified for the purposes of this analysis. In addition to being free of clinical CVD, this subset was free of conventional cardiovascular risk factors, treatment for cardiovascular risk factors and significant coronary plaque disease. Individuals were excluded from the analysis if they had either: a coronary calcium score > 10 Agatston units; or a prescription for cardioactive medications (antihypertensives, antianginals, hypoglycaemic agents, lipid lowering therapy, thienodipyridine anti-platelets) or any traditional cardiovascular risk factors (systolic BP > 140mmHg, diastolic BP > 90 mmHg, total cholesterol > 6.0 mmol/L, fasting glucose > 7.1 mmol/L, BMI > 30 kg/m², current smoking).

Assessment of all LOLIPOP-ATHEROSCLEROSIS substudy participants was performed by a trained nurse using a standard protocol including questions on medical history, family history, cardiovascular risk factors, alcohol intake, physical activity and drug history (verified from the practice computerised records). Consenting subjects had a physical assessment including BP determination, anthropometric measurements and an electrocardiogram. Subjects were then invited to undergo echocardiography, electron EBCT for coronary calcium score determination (Agatston score) and provide fasting plasma and serum samples for biochemical analysis stored at -80°C. The study was approved by the Northwick Park Hospital and Ealing Hospital Research Ethics Committees.
Echocardiography

Left heart dimensions and ejection fraction

Transthoracic 2-D echocardiography was performed by experienced sonographers using a digital commercial harmonic imaging ultrasound system with an S3 3-MHz phased-array transducer (Philips IE33, Philips Medical Systems, Holland) at a single centre. LV dimensions were obtained in the parasternal short axis view and LV mass was calculated using the Devereux formula (146) and indexed to height to give LV mass index (LVMI). Quantitative 2D methods (biplane Simpson’s) were performed to obtain LV EF. LA volume was calculated from three measurements of LA dimension using the formula for an ellipse (148-151): \( \frac{\pi}{6} \times PLAX \times A4C1 \times A4C2 \) where PLAX = LA dimension measured in the parasternal long axis view and A4C1 and A4C2 are measurements of the long- and short-axis in the apical four chamber view. LA volume was then indexed to BSA.

Transmitral flow and E/Ea ratio

The transmitral flow velocities were recorded using pulsed wave Doppler with the sample volume placed at the tip of the mitral valve leaflets in the apical-four chamber view. From the mitral valve inflow velocity curve the following measurements were made: peak E-wave velocity (cm/sec); peak A-wave velocity (cm/sec) and the ratio of E-wave to A-wave (E:A) velocities.

Tissue Doppler imaging

Myocardial velocities were measured on-line using a standard pulse-wave Doppler technique. Colour-coded images were acquired during a breath hold over two consecutive cardiac cycles using low-velocity, high-intensity myocardial signals at high frame rate (>150MHz). The imaging angle was adjusted to ensure as near perpendicular alignment of the beam as possible.
with the myocardial segment of interest. The sample volume was placed at the junction of the LV wall with the mitral annulus of the septal and lateral myocardial segments in the apical four-chamber view. Peak Sa and Ea velocities (cm/s) were measured on-line from both mitral annular sites segments and the corresponding E/ Ea ratios calculated. Mean values taken from the two mitral annular sites for annular velocity and E/Ea ratio sites were then derived.

**Electron beam computed tomography**

Coronary calcium imaging was performed using EBCT at a single centre with a modified GE Imatron C-150 (San Francisco, CA, USA) scanner specially equipped with high-resolution detectors. Scan time was 100 msec per slice, synchronized to 40% of the R-R interval. All areas of calcification within the borders of a coronary artery with an optical density above 130 Hounsfield units and an area greater than 1 mm² were computed. All calcium scores were calculated on an Aquarius workstation (TeraRecon, Inc., San Mateo, USA). The output from EBCT scans was quantified into Agatston scores.

**Statistical analysis**

Continuous variables are summarised as the mean ± 1 standard deviation. Continuous variables and their association with age group were assessed using one-way analysis of variance (ANOVA) and categorical data assessed by chi-squared test. Reference ranges for TDI parameters are presented as values denoting the 5th and 95th percentiles. The effect of age upon longitudinal function and LV filling pressure was assessed by Pearson’s correlation. Statistical analysis was performed using SPSS version 15 with values of p < 0.05 considered statistically significant.
Interobserver variability

Echocardiographic measurements were repeated by two sonographers in 15 subjects to assess reproducibility and interobserver variability. For mean Sa velocity, mean Ea velocity and mean E/Ea ratio the coefficient of variance was 11.4%, 8.7% and 8.0% respectively.

4.4 Results

Of the 2,293 subjects recruited, 453 fulfilled the inclusion criteria for the purposes of this study. Their clinical characteristics stratified by age group are illustrated in table 4.1. The mean age of the subjects was 51 years with 43% being of European white ethnicity and 56% of male gender. Systolic BP, pulse pressure and total cholesterol both increased significantly with age whereas there was no significant relationship between coronary calcium score and aging. The cut-off for abnormal EF was 52% based on the 5th percentile value.

Table 4.2 displays the echocardiographic and Doppler measurements for the cohort. The E/A ratio decreased (p<0.001) whereas LAVI (p < 0.001), LVMI (p < 0.001) and EF (p = 0.004) all increased with advancing age. Age-specific and whole cohort averages with reference ranges (5th and 95th percentile) for Sa velocity, Ea velocity and the E/Ea ratio are provided in table 4.3. Myocardial Sa velocity measured at the septal mitral annulus was not significantly associated with age. However, Sa velocity measured at the lateral mitral annulus was significantly attenuated in the older subjects (p = 0.006). The average Sa velocity of the septal and lateral annuli also decreased with age (p=0.04). The Ea velocity measured at both septal and lateral mitral annular segments, and their averages, decreased significantly with aging resulting in a reciprocal and significant increase in the E/Ea ratio when measured at either annular site and when averaged between the two.
Table 4.1- Demographics and clinical characteristics of the cohort stratified by age

<table>
<thead>
<tr>
<th>Age</th>
<th>35-44</th>
<th>45-54</th>
<th>55-64</th>
<th>65-75</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>121</td>
<td>189</td>
<td>116</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>41 ± 2</td>
<td>50 ± 3</td>
<td>59 ± 3</td>
<td>69 ± 2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male (%)</td>
<td>54</td>
<td>60</td>
<td>47</td>
<td>67</td>
<td>0.07</td>
</tr>
<tr>
<td>European white (%)</td>
<td>36</td>
<td>44</td>
<td>47</td>
<td>53</td>
<td>0.24</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>114 ± 12</td>
<td>118 ± 11</td>
<td>120 ± 10</td>
<td>126 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74 ± 7</td>
<td>76 ± 8</td>
<td>74 ± 7</td>
<td>74 ± 8</td>
<td>0.14</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>40 ± 8</td>
<td>43 ± 7</td>
<td>46 ± 8</td>
<td>52 ± 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 ± 2.8</td>
<td>24.8 ± 2.5</td>
<td>24.6 ± 2.9</td>
<td>25.5 ± 2.4</td>
<td>0.30</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.0 ± 0.8</td>
<td>5.2 ± 0.7</td>
<td>5.3 ± 0.7</td>
<td>5.2 ± 0.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Agatston score (Au)</td>
<td>0.2 ± 1.1</td>
<td>0.4 ± 1.4</td>
<td>0.4 ± 1.4</td>
<td>0.3 ± 0.8</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Continuous variables presented as mean ± standard deviation. BP = blood pressure, BMI = body mass index, Au = Agatston units
Table 4.2 - 2D echocardiographic and Doppler data stratified by age

<table>
<thead>
<tr>
<th>Age</th>
<th>35-44</th>
<th>45-54</th>
<th>55-64</th>
<th>65-75</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2-D echo parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (cm/s)</td>
<td>75.0 ± 15.3</td>
<td>72.5 ± 14.7</td>
<td>74.1 ± 16.7</td>
<td>64.7 ± 14.8</td>
<td>0.01</td>
</tr>
<tr>
<td>A (cm/s)</td>
<td>57.5 ± 12.4</td>
<td>63.1 ± 13.9</td>
<td>72.0 ± 17.9</td>
<td>71.8 ± 16.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LAVI (ml/m²)</td>
<td>13.9 ± 4.5</td>
<td>14.9 ± 4.2</td>
<td>16.1 ± 4.6</td>
<td>16.7 ± 4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI (g/m)</td>
<td>80.9 ± 20.5</td>
<td>84.3 ± 21.5</td>
<td>88.2 ± 23.6</td>
<td>97.4 ± 21.1</td>
<td>0.001</td>
</tr>
<tr>
<td>ESVI (ml/m³)</td>
<td>15.7 ± 4.7</td>
<td>15.0 ± 4.9</td>
<td>13.4 ± 4.3</td>
<td>15.0 ± 4.6</td>
<td>0.001</td>
</tr>
<tr>
<td>EDVI (ml/m³)</td>
<td>40.0 ± 10.4</td>
<td>40.0 ± 10.3</td>
<td>36.5 ± 9.9</td>
<td>38.7 ± 9.7</td>
<td>0.02</td>
</tr>
<tr>
<td>EF (%)</td>
<td>61 ± 5</td>
<td>63 ± 6</td>
<td>63 ± 5</td>
<td>62 ± 6</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>TDI parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sa_septal (cm/s)</td>
<td>8.2 ± 1.4</td>
<td>8.2 ± 1.6</td>
<td>8.2 ± 1.6</td>
<td>7.6 ± 1.2</td>
<td>0.20</td>
</tr>
<tr>
<td>Sa_lateral (cm/s)</td>
<td>10.6 ± 2.4</td>
<td>10.3 ± 2.4</td>
<td>9.6 ± 2.3</td>
<td>9.9 ± 2.3</td>
<td>0.006</td>
</tr>
<tr>
<td>Sa_mean (cm/s)</td>
<td>9.4 ± 1.7</td>
<td>9.3 ± 1.7</td>
<td>8.9 ± 1.7</td>
<td>8.7 ± 1.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Ea_septal (cm/s)</td>
<td>9.5 ± 2.1</td>
<td>8.7 ± 1.7</td>
<td>8.1 ± 1.8</td>
<td>7.5 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ea_lateral (cm/s)</td>
<td>14.0 ± 3.0</td>
<td>12.3 ± 2.8</td>
<td>10.7 ± 2.5</td>
<td>10.5 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ea_mean (cm/s)</td>
<td>11.7 ± 2.2</td>
<td>10.5 ± 2.0</td>
<td>9.4 ± 1.8</td>
<td>9.0 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/Ea_septal ratio</td>
<td>8.2 ± 2.1</td>
<td>8.5 ± 1.9</td>
<td>9.5 ± 2.6</td>
<td>8.9 ± 2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/Ea_lateral ratio</td>
<td>5.5 ± 1.4</td>
<td>6.1 ± 1.6</td>
<td>7.3 ± 2.4</td>
<td>6.3 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/Ea_mean ratio</td>
<td>6.9 ± 1.5</td>
<td>7.3 ± 1.6</td>
<td>8.4 ± 2.4</td>
<td>7.6 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation. E = peak velocity of early filling, A = peak velocity of atrial filling, LAVI = left atrial volume index, LVMI = left ventricular mass index, ESVI = end-systolic volume index, EDVI = end-diastolic volume index, EF = ejection fraction.
Table 4.3 - Age-specific and whole-cohort reference ranges for Sa velocity, Ea velocity and E/Ea ratio

<table>
<thead>
<tr>
<th>Age</th>
<th>35-44</th>
<th>45-54</th>
<th>55-64</th>
<th>65-75</th>
<th>35-75</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Septal annulus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sa&lt;sub&gt;septal&lt;/sub&gt; (cm/s)</td>
<td>6.4 – 10.9</td>
<td>5.9 – 11.0</td>
<td>6.0 – 10.9</td>
<td>5.2 – 9.8</td>
<td>6.0 – 10.9</td>
</tr>
<tr>
<td>Ea&lt;sub&gt;septal&lt;/sub&gt; (cm/s)</td>
<td>6.1 – 13.3</td>
<td>6.0 – 11.7</td>
<td>5.4 – 11.4</td>
<td>4.9 – 9.9</td>
<td>5.8 – 11.9</td>
</tr>
<tr>
<td>E/Ea&lt;sub&gt;septum&lt;/sub&gt; ratio</td>
<td>5.5 – 12.0</td>
<td>5.9 – 12.2</td>
<td>6.3 – 14.3</td>
<td>6.1 – 13.3</td>
<td>5.7 – 12.8</td>
</tr>
<tr>
<td><strong>Lateral annulus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sa&lt;sub&gt;lateral&lt;/sub&gt; (cm/s)</td>
<td>7.3 – 15.9</td>
<td>6.7 – 14.7</td>
<td>6.3 – 13.9</td>
<td>5.9 – 14.5</td>
<td>6.7 – 14.6</td>
</tr>
<tr>
<td>Ea&lt;sub&gt;lateral&lt;/sub&gt; (cm/s)</td>
<td>9.6 – 19.2</td>
<td>8.2 – 17.5</td>
<td>6.9 – 14.7</td>
<td>7.3 – 13.6</td>
<td>7.9 – 17.6</td>
</tr>
<tr>
<td>E/Ea&lt;sub&gt;lateral&lt;/sub&gt; ratio</td>
<td>3.2 – 8.3</td>
<td>3.9 – 9.2</td>
<td>4.3 – 11.0</td>
<td>3.9 – 11.4</td>
<td>3.8 – 9.6</td>
</tr>
<tr>
<td><strong>Average of septal and lateral annuli</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sa&lt;sub&gt;mean&lt;/sub&gt; (cm/s)</td>
<td>7.4 – 12.2</td>
<td>6.9 – 12.6</td>
<td>6.6 – 11.8</td>
<td>5.9 – 12.2</td>
<td>6.8 – 12.2</td>
</tr>
<tr>
<td>Ea&lt;sub&gt;mean&lt;/sub&gt; (cm/s)</td>
<td>8.5 – 15.2</td>
<td>7.4 – 14.0</td>
<td>6.3 – 12.4</td>
<td>6.7 – 11.3</td>
<td>7.3 – 14.1</td>
</tr>
<tr>
<td>E/Ea&lt;sub&gt;mean&lt;/sub&gt; ratio</td>
<td>4.5 – 9.7</td>
<td>5.1 – 10.4</td>
<td>5.5 – 12.0</td>
<td>5.0 – 12.0</td>
<td>5.0 – 10.8</td>
</tr>
</tbody>
</table>

Abbreviations as used in table 4.2 with Sa<sub>septal</sub> = peak systolic septal mitral annular velocity, Ea<sub>septal</sub> = peak early diastolic septal mitral annular velocity, Sa<sub>lateral</sub> = peak systolic lateral mitral annular velocity, Ea<sub>lateral</sub> = peak early diastolic lateral mitral annular velocity, Sa<sub>mean</sub> = mean mitral annular peak systolic velocity, Ea<sub>mean</sub> = mean mitral annular peak early diastolic velocity. Values represent the mean ± standard deviation and 5<sup>th</sup> - 95<sup>th</sup> percentiles.
Figures 4.1 to 4.3 illustrate the relationship between age and the tissue Doppler parameters of LV function. Age was negatively correlated with both the longitudinal systolic and diastolic velocities although the relationship was stronger with the latter (mean Sa velocity $r = -0.14$, mean Ea velocity $r = -0.50$). There was also a moderate, direct correlation between age and the E/Ea ratio ($r = 0.30$).

**Figure 4.1 - Relationship between average annular systolic velocity and age with fitted regression line and 5th - 95th percentile prediction bands for individual values**
Figure 4.2 - Relationship between average annular early diastolic velocity and age with fitted regression line and 5th - 95th percentile prediction bands for individual values
Figure 4.3 - Relationship between average E/Ea ratio and age with fitted regression line and 5th - 95th percentile prediction bands for individual values

4.5 Discussion

In this study age-specific normative reference values for the clinically important TDI parameters of LV function have been defined and presented. These values are based on a large, biethnic cohort using strict criteria for normality to ensure that left ventricles of healthy subjects with low cardiovascular risk factor and coronary artery disease burden were studied. This study has also confirmed the detrimental influence of natural aging upon longitudinal myocardial function and LV filling pressure.

Several studies have attempted to derive normative values for TDI parameters but they have tended to explore the association of these parameters with age and have consequently reported only their mean values (139-145). Although a study by Munangala et al. described percentile-based reference ranges for the diastolic TDI parameters in a large population, reference values for myocardial Sa velocity were not reported. To the best of
one’s knowledge, this is the first to study to have reported percentile-based partition values for both the systolic and diastolic TDI parameters of LV function.

Myocardial Sa velocity is an important and frequently overlooked component of systolic function that can be sensitively quantified with TDI. In patients with myocardial infarction, peak Sa velocities correspond well with overall LV EF with a measurement > 7.5 cm/s projected to be sensitive and specific in predicting normal global LV systolic function (152). Although the effects of normal aging upon diastolic TDI parameters have been well documented in the literature, the effect of age upon myocardial Sa velocity, and consequently its normative reference values, have been under-reported. The 5th percentile values for myocardial Sa velocity in this present cohort were 6 cm/s, 6.7 cm/s and 6.8 cm/s for the septal, lateral and averaged annulus respectively suggesting that cut-off values for abnormal longitudinal systolic function may be lower than previously suspected. Increasing age was observed to be associated with attenuated lateral annular systolic velocity whereas septal Sa velocity was not significantly diminished. Averaged mitral annulus Sa velocity also decreased with age just achieving statistical significance. The relative sparing of septal annular longitudinal systolic function with age has been reported previously (139,142). Although sampling at the septal mitral annulus enables a more perpendicular alignment with the ultrasound beam, velocities measured at this site may be confounded by the influence of the right ventricle. Longitudinal systolic function of the right ventricle does not appear to be attenuated by advancing age (139) and may mask any discernible influence of age upon systolic myocardial function measured at the septal mitral annulus.

The influence of age upon myocardial function was more evident in the diastolic parameters measured, extending the findings of previous studies (139-141,145,153-155). A moderate, negative correlation was evident between Ea velocity measured at both the medial
and lateral mitral annulus and age. The LV filling pressure, as estimated by E/Ea ratio, increased with normal aging. The E/Ea ratio has been widely incorporated into clinical 2D echocardiography, offering a non-invasive technique of estimating LV filling pressure (156,157). TDI performed at the lateral mitral annulus yields higher longitudinal velocities hence a smaller E/Ea ratio than when derived from the septal site. Studies have varied with regards to which annular site the E/Ea ratio is measured from, and corresponding cut-off values have been proposed to optimally detect elevated LV filling pressure (157,158). However, guidelines for diagnosing diastolic heart failure are based on an average value of the two sites, with an average E/Ea > 15 deemed specific for elevated LV filling pressure and less than 8 indicative of low/normal filling pressures (138). Intermediate values (8 < E/Ea < 15) are considered suggestive but not compelling evidence for elevated LV filling pressure, with the recommendation that ancillary, non-invasive investigations be performed to confirm or refute the diagnosis of heart failure. In this present study upper limits of normal for E/Ea, defined as values representing the 95th percentile, exceeded 8 in all age groups even when averaged E/Ea ratios derived from both septal and lateral mitral annular sites were assessed. Our data does not support the assertion that intermediate E/Ea values may be indicative of elevated LV filling pressures as healthy subjects of any age were likely to have an E/Ea ratio exceeding 8 and frequently be greater than 10. An E/Ea ratio > 15 is, however, likely to represent abnormally elevated LV filling pressures regardless of age and the mitral annular site measured.

Increased LV mass is an independent risk factor for the development of heart failure, and in this cohort LV mass increased with age, consistent with previous studies (159-161). The increase in LV mass is likely to be explained by the gradual increase in systolic BP that occurs with aging (162), a relationship confirmed in this study. The intrinsic relationship
between aging and systolic BP resulting in increased LV mass is also the likely basis for the observation of attenuated myocardial function and augmented LV filling pressures with natural aging. LA size, a morphophysiological expression of LV filling pressure, was also observed to increase with age, again reflecting the progressive impairment of diastolic function that occurs due to an increasing LV mass (148).

**Conclusion**

Reference ranges have been defined and presented for the TDI parameters of LV longitudinal function and filling pressure, based on normative percentile values derived from a large population in whom the confounding effects of cardiovascular risk factor burden and significant CAD upon myocardial function have been largely obviated. These partition values should help in the standardisation of clinical tissue Doppler echocardiography.
CHAPTER 5. STUDY II - NEW INSIGHTS INTO THE RELATIONSHIP OF LEFT VENTRICULAR GEOMETRY AND LEFT VENTRICULAR MASS WITH CARDIAC FUNCTION: A POPULATION STUDY OF HYPERTENSIVE SUBJECTS

5.1 Abstract

Background: Remodeling of the left ventricle is associated with adverse cardiovascular events but the mechanisms of these effects remain undefined. This study investigated the relationship of LV mass and geometry to LV function in a large cohort of hypertensive subjects.

Methods: 1,075 hypertensive individuals without CVD were studied from the LOLIPOP cohort study. All subjects underwent echocardiography for derivation of LVMI, measurement of transmitral filling pattern and LV EF. The tissue Doppler parameters of peak Sa velocity, Ea velocity and of LV filling pressure (E/Ea) were measured. LV function was correlated with degree of concentric remodeling, determined by RWT, and with LV geometric pattern.

Results: The presence of LVH was independently associated with significantly worse systolic function, diastolic function and E/Ea as compared to subjects with normal LV geometry or nonhypertrophic concentric remodeling. After adjustment for covariates including LVMI, peak Sa velocity and EF increased (p<0.001) whereas peak Ea velocity decreased significantly (p<0.001) with increasing degrees of concentric remodeling.

Conclusion: In hypertensives, increased LV mass is independently associated with impaired LV function and increased LV filling pressure. Increasing degrees of concentric remodeling
are associated with attenuated diastolic function but augmented systolic function, possibly representing an adaptive response to pressure overload physiology.
5.2 **Introduction**

Cardiac remodeling in the form of LVH is strongly associated with major cardiovascular events independent of BP, known risk factors and coronary artery disease (50,69-71). However, discerning the independent prognostic value afforded by alterations in LV shape in the presence or absence of LVH has proved more controversial. Concentric remodeling with normal LV mass has been associated with worse prognosis compared to normal LV geometry (76,80-82). However, classification by LV geometry has also been shown to provide little incremental prognostic information beyond that offered by LV mass alone (75,163-165). Moreover, longitudinal studies assessing the relationship of abnormal LV geometry with cardiovascular outcomes have tended to combine nonfatal cardiovascular events and death as the primary endpoint, obscuring our understanding of which patterns of LV remodeling are associated with heart failure or atherothrombotic events. Recently published follow-up data from a large cohort study suggested that LV mass, rather than concentric remodeling, is a better predictor of incident heart failure (81) yet the effect of different LV geometrical patterns upon LV systolic and diastolic function have still not been adequately defined.

Myocardial longitudinal velocities derived from echocardiography and TDI have assumed greater importance as prognosticators of cardiovascular risk (56-60). Long-axis motion of the left ventricle is an important component of LV systolic and diastolic function and the subendocardial fibres that contribute to long-axis function are particularly sensitive to various diseases and pathologies. LV filling pressure can also be estimated from TDI and conventional transmitral Doppler to derive the E/Ea ratio.
This study investigates the relative importance of cardiac geometry and mass upon the function of the left ventricle in a large population of hypertensive subjects with no prior history of CVD.

5.3 Methods

Subjects were recruited between August 2004 and November 2007 from the LOLIPOP study. Assessment of participants is performed by a trained nurse using a standard protocol including questions on medical history, family history, cardiovascular risk factors, alcohol intake, physical activity and drug history (verified from the practice computerised records). Subsequently 2,288 Indian Asian and European White subjects, aged 35-74 years and free from clinical CVD, were selected at random and enrolled into the LOLIPOP atherosclerosis cohort substudy. The study was approved by the Northwick Park and Ealing Hospitals Research Ethics Committees. Consenting subjects had a physical assessment including BP determination, anthropometric measurements and an ECG. Subjects were then invited to undergo echocardiography and provide fasting plasma and serum samples for biochemical analysis stored at -80°C.

From the LOLIPOP-ATHEROSCLEROSIS substudy, 1,075 subjects with hypertension were identified. Hypertension was defined as the presence of either a prior history of hypertension or a current prescription of anti-hypertensive medications or a mean systolic BP ≥ 140 mmHg or a mean diastolic BP ≥ 90 mmHg (BP taken from three separate measurements in a seated position).
Echocardiography

Left ventricular dimensions, geometry and ejection fraction

Transthoracic 2-D echocardiography was performed by experienced sonographers using a digital commercial harmonic imaging ultrasound system with an S3 3-MHz phased-array transducer (Philips IE33, Philips Medical Systems, Holland) at a single centre. LV dimensions were obtained in the parasternal short axis view with measurement of the interventricular septal thickness in diastole, LV dimension in diastole, LV dimension in systole and LV posterior wall thickness in diastole. LV mass was calculated using the Devereux formula (146) and indexed to height (m) to provide LVMI. RWT was used to measure the degree of concentric remodeling and was calculated as: (interventricular septal thickness in diastole + LV posterior wall thickness in diastole) / LV dimension in diastole. Subjects were stratified according to quintile of RWT and also according to LV geometrical pattern. The RWT and LVMI were used to categorise subjects as having: (1) normal geometry – normal RWT and normal LVMI; (2) concentric remodeling - increased RWT and normal LVMI; (3) eccentric hypertrophy - normal RWT and increased LVMI or (4) concentric hypertrophy - increased LVMI and increased RWT. Gender- and ethnicity-specific partition values for increased LVMI and RWT were established from data representing the 95th percentiles in the reference population without manifest CVD and without evidently modifiable risk factors (n=453). Partition values for LVMI (g/m) and RWT were: European white men - 137/0.47; European white females – 116/0.46, Indian Asian males - 118/0.50 and Indian Asian females - 107/0.47.

LV end-diastolic and end-systolic volumes indexed to body surface area were measured using Simpson’s apical biplane rule. Tracing of the LV contour was performed carefully so as to exclude papillary muscles and trabeculations, as recommended by the
American Society of Echocardiography (146). LV EF was automatically calculated following acquisition of the LV volumes using the Simpson’s method.

*Tissue Doppler imaging*

Myocardial velocities were measured on-line using a standard pulse-wave Doppler technique. The imaging angle was adjusted to ensure as near parallel alignment of the beam as possible with the myocardial segment of interest. The sample volume was placed at the junction of the LV wall with the mitral annulus of the septal and lateral myocardial segments from the apical four-chamber view and inferior and anterior myocardial segments from the apical two-chamber view. Peak velocities (cm/sec) during systole (Sa) and early diastole (Ea) were measured on-line from all four mitral annular sites segments and averaged. Estimated LV filling pressure was derived from the ratio of transmitral E velocity to Ea velocity (E/Ea ratio).

*Transmitral flow and E/Ea ratio*

The transmitral flow velocities were recorded using pulsed wave Doppler as described previously (chapter 4.3).

**Statistical analysis**

Continuous variables are summarised as the mean ± 1 standard deviation according to quintiles of relative wall thickness and also according to LV geometrical pattern. ANOVA was used to test differences of continuous variables and $\chi^2$ used to test differences amongst categorical data. Effects of increasing quintiles of RWT and different LV geometry upon parameters of LV function were evaluated by analysis of covariance (ANCOVA) in a main-effects design, using Type III sum of squares. Comparison was adjusted for relevant confounders including age, sex, race, systolic BP, diastolic BP, antihypertensive use, diabetes
and LVMI (adjustments made for LVMI when stratified by quintile of RWT). Simple contrast was used and the null hypothesis rejected at two-tailed p < 0.05. Estimated marginal means are given and displayed in figures, after adjustment for covariates.

**Interobserver variability**

Echocardiographic measurements were repeated by two sonographers in 15 subjects to assess reproducibility and interobserver variability. The coefficient of variance was 11.5%, 5.8%, 9.9% and 3.7% for LVMI, end-diastolic volume index, end-systolic volume index and EF respectively. For the TD parameters mean Sa velocity, mean Ea velocity and mean E/Ea ratio the coefficient of variance was 11.4%, 8.7% and 8.0% respectively.

### 5.4 Results

The clinical characteristics and echocardiographic features in subjects stratified by quintile of RWT and by LV geometric pattern are displayed in tables 5.1 and 5.2 respectively. Age was significantly higher in the two uppermost quintiles of RWT compared to the lowest quintile (age in fourth and fifth quintile vs. first quintile, both p<0.001). Systolic BP was significantly higher in the uppermost quintile as compared to the lowest (p=0.03), however diastolic BP did not vary significantly with RWT. Diabetes prevalence was highest in the fifth quintile (28.2 %, p=0.01). There was an even distribution of people of European white ethnicity, male gender and subjects requiring treatment for hypertension with RWT quintile. Compared to the first quintile of RWT, LVMI was significantly higher in each of the four remaining quintiles.

Normal LV geometry was present in 58.6% of the subjects studied, with concentric remodeling present in 12.4%, eccentric hypertrophy in 19.2% and concentric hypertrophy in 6.6% (table 5.2). Antihypertensive therapy was highest in subjects with eccentric hypertrophy
and lowest in those with normal LV geometry. Prevalence of diabetes was highest in subjects with concentric remodeling and eccentric hypertrophy (29.7% and 31% respectively, p<0.001).
Table 5.1 - Clinical characteristics stratified by quintile of RWT

<table>
<thead>
<tr>
<th>RWT quintile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>161</td>
<td>194</td>
<td>178</td>
<td>250</td>
<td>292</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 ± 10</td>
<td>58 ± 9</td>
<td>60 ± 8 †</td>
<td>61 ± 9 ‡</td>
<td>61 ± 8 ‡</td>
</tr>
<tr>
<td>Male (%)</td>
<td>75</td>
<td>76</td>
<td>70.8</td>
<td>71.2</td>
<td>77.1</td>
</tr>
<tr>
<td>European white (%)</td>
<td>43</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>142 ± 16</td>
<td>143 ± 16</td>
<td>144 ± 18</td>
<td>145 ± 17</td>
<td>147 ± 20 *</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>85 ± 10</td>
<td>87 ± 10</td>
<td>86 ± 10</td>
<td>86 ± 9</td>
<td>87 ± 12</td>
</tr>
<tr>
<td>Treated for HTN (%)</td>
<td>44</td>
<td>44</td>
<td>52</td>
<td>57</td>
<td>52</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>23</td>
<td>22</td>
<td>15</td>
<td>21</td>
<td>28 *</td>
</tr>
<tr>
<td>RWT</td>
<td>0.23 - 0.33</td>
<td>0.33 – 0.37</td>
<td>0.37 – 0.40</td>
<td>0.40 – 0.46</td>
<td>0.46 – 0.84</td>
</tr>
<tr>
<td>LVMI (g/m)</td>
<td>96.2 ± 25.4</td>
<td>106.8 ± 27.5 †</td>
<td>106.1 ± 32.1 *</td>
<td>111.4 ± 31.2 ‡</td>
<td>113.9 ± 33.6 ‡</td>
</tr>
</tbody>
</table>

ANOVA was used to test differences of continuous variables, \( \chi^2 \) used to test for differences in categorical variables

*p<0.05; †p<0.01; ‡ p<0.001

BP = blood pressure, HTN = hypertension, RWT = relative wall thickness, LVMI = left ventricular mass index
Table 5.2 - Clinical and echocardiographic characteristics stratified by LV geometric patterns

<table>
<thead>
<tr>
<th>LV Geometric Pattern</th>
<th>Normal</th>
<th>CR</th>
<th>EH</th>
<th>CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>651</td>
<td>138</td>
<td>213</td>
<td>73</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.6 ± 9.2</td>
<td>62.5 ± 8.5 ‡</td>
<td>62.2 ± 8.3 ‡</td>
<td>62.2 ± 8.0 †</td>
</tr>
<tr>
<td>Male (%)</td>
<td>75</td>
<td>70</td>
<td>77</td>
<td>71</td>
</tr>
<tr>
<td>European white (%)</td>
<td>40</td>
<td>44</td>
<td>46</td>
<td>41</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>143.0 ± 16.5</td>
<td>146.5 ± 20.7</td>
<td>147.6 ± 18.2 †</td>
<td>147.6 ± 20.0</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>86.2 ± 9.5</td>
<td>86.2 ± 11.8</td>
<td>85.8 ± 10.2</td>
<td>86.5 ± 12.3</td>
</tr>
<tr>
<td>Treated for HTN (%)</td>
<td>49</td>
<td>51</td>
<td>56</td>
<td>49</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>18</td>
<td>30</td>
<td>31</td>
<td>23 ‡</td>
</tr>
<tr>
<td>EDVI (ml/m²)</td>
<td>37.6 ± 9.3</td>
<td>35.5 ± 9.9</td>
<td>43.1 ± 11.5 ‡</td>
<td>37.4 ± 10.1</td>
</tr>
<tr>
<td>ESVI (ml/m²)</td>
<td>14.4 ± 4.5</td>
<td>12.9 ± 3.9 †</td>
<td>17.4 ± 7.1 ‡</td>
<td>14.5 ± 4.9</td>
</tr>
<tr>
<td>LVMI (g/m)</td>
<td>94.0 ± 19.1</td>
<td>95.0 ± 17.7</td>
<td>145.3 ± 24.1 ‡</td>
<td>149.9 ± 29.3 ‡</td>
</tr>
<tr>
<td>RWT</td>
<td>0.38 ± 0.06</td>
<td>0.55 ± 0.06 ‡</td>
<td>0.40 ± 0.05 ‡</td>
<td>0.57 ± 0.08 ‡</td>
</tr>
</tbody>
</table>

ANOVA was used to test differences of continuous variables, \( \chi^2 \) used to test for differences in categorical variables.

*\( p<0.05; \) †\( p<0.01; \) ‡\( p<0.001 \)

Abbreviations as in table 5.1 with CR= concentric remodeling, EH = eccentric hypertrophy, CH = concentric hypertrophy, EVDI = end-diastolic volume index, ESVI = end-systolic volume index
Multivariate analysis

Myocardial longitudinal function

Figure 5.1 illustrates the distribution of longitudinal peak systolic and early diastolic myocardial velocities by quintiles of RWT and by pattern of LV geometry. Compared to the first quintile, peak Sa velocity was significantly higher in each of the three uppermost quintiles of RWT (adjusted peak Sa velocity in first and fifth quintiles 8.2 cm/s and 8.8 cm/s respectively, p<0.001). Compared to normal LV geometry, both concentric and eccentric hypertrophy were associated with significantly lower adjusted mean Sa velocity (8.3 cm/s, p=0.02 and 8.3 cm/s, p=0.004 respectively vs. 8.7 cm/s). Subjects with concentric remodeling had significantly higher adjusted peak Sa velocity compared to those with concentric hypertrophy (8.8 cm/s vs. 8.3 cm/s, p = 0.02). Although peak Sa velocity was lower amongst subjects with eccentric hypertrophy compared to those with concentric hypertrophy, this difference was not statistically significant.

After adjustment for covariates, RWT in the fifth quintile was associated with impaired early diastolic myocardial velocity as compared to the first quintile (7.8 cm/s vs. 8.4 cm/s, p<0.001). Subjects with abnormal LV geometry, with or without increased LV mass, had significantly impaired Ea velocity compared to those with normal geometry (p for trend <0.001). Concentric hypertrophy was associated with a significantly lower Ea velocity when compared to eccentric hypertrophy (7.3 cm/s vs. 8 cm/s, p=0.003) and concentric remodeling (7.3 cm/s vs. 7.9 cm/s, p=0.01).

LV filling pressure (E/Ea ratio)

LV filling pressure, as estimated by the E/Ea ratio, did not vary significantly with increasing quintile of RWT (figure 5.2). However, the adjusted E/Ea ratio in subjects with normal
geometry was significantly lower compared to those with concentric hypertrophy (E/Ea = 9.7 vs. 10.8, p= 0.001) and eccentric hypertrophy (E/Ea = 10.3, p = 0.003). Concentric hypertrophy was associated with higher E/Ea as compared to concentric remodeling (10.8 vs 10, p=0.02) but no significant difference was observed when compared to eccentric hypertrophy.

*Ejection fraction*

Figure 5.3 shows that adjusted EF increased with each quintile of RWT and was significantly higher in the fifth quintile as compared to the first quintile (64.8% vs. 62.4%, p<0.001). Compared to normal LV geometry, EF was significantly higher with concentric remodeling (65.6% vs. 64%, p=0.006) and significantly lower with eccentric hypertrophy (62.5% vs. 64%, p=0.002). Subjects with concentric hypertrophy had significantly lower EF compared to those with concentric remodeling (63.6% vs 65.6%, p=0.003). Although EF was lower with eccentric hypertrophy as compared to concentric hypertrophy, the difference was not statistically significant.

*E/A ratio*

The E/A ratio decreased with each decrease in quintile of RWT (figure 5.4) and was significantly lower in the fifth quintile as compared to the first quintile (0.92 vs. 0.98, p=0.009). Subjects with concentric remodeling also had significantly lower E/A ratio compared to normal geometry (0.92 vs. 0.96, p=0.03).
Figure 5.1 - Relationship of longitudinal systolic and diastolic function to quintile of RWT and to LV geometric pattern

Velocities adjusted for age, sex, race, systolic BP, diastolic BP, treatment for hypertension, diabetes and LVMI (adjustment for LVMI made when stratification by quintile of RWT only).

*p<0.05, †p<0.01, ‡ p<0.001 vs. first quintile of RWT or vs. normal LV geometry. Significant differences also observed between CH and CR for Sa velocity (p = 0.02) and for Ea velocity (p = 0.03). LVMI=left ventricular mass index, RWT=relative wall thickness, N=normal geometry, CR = concentric remodeling, EH = eccentric hypertrophy, CH = concentric hypertrophy.
Figure 5.2 - Relationship of LV filling pressure (E/Ea ratio) to quintile of RWT and to LV geometric pattern

E/Ea ratio adjusted for age, sex, race, systolic BP, diastolic BP, treatment for hypertension, diabetes and LVMI (adjustment for LVMI made when stratification by quintile of RWT only).

* p<0.01 vs. first quintile of RWT or vs. normal LV geometry. Significant differences in E/Ea ratio also observed between CH and CR (p = 0.02). Abbreviations as for figure 5.1.
Figure 5.3 - Relationship of EF to quintile of RWT and to LV geometric pattern

Values adjusted for age, sex, race, systolic BP, diastolic BP, treatment for hypertension, diabetes and LVMI (adjustment for LVMI made when stratification by quintile of RWT only).

* p<0.01, † p<0.001 vs. first quintile of RWT or vs. normal LV geometry. Significant differences in EF also observed between CH and CR (p = 0.03). Abbreviations as for figure 5.1.
Figure 5.4 - Relationship of E/A ratio to quintile of RWT and to LV geometric patterns

Values adjusted for age, sex, race, systolic BP, diastolic BP, treatment for hypertension, diabetes and LVMI (adjustment for LVMI made when stratified by quintile of RWT only).

*p<0.05, †p<0.01 vs. first quintile of RWT or vs. normal LV geometry. Abbreviations as for figure 5.1.
5.5 **Discussion**

In this study of hypertensive subjects, LVH was independently associated with subclinical impairment of systolic and diastolic LV function as well as increased LV filling pressure, when compared to subjects with normal geometry or concentric remodeling. After adjustment for LV mass, increasing degrees of concentric remodeling, expressed as the RWT, were paradoxically associated with enhanced longitudinal and radial LV systolic function. Although impaired diastolic function was observed with concentric remodeling, there was no associated rise in LV filling pressure.

To the best of one’s knowledge, this is the first study to have examined TDI parameters of LV function amongst hypertensives in a population setting. TDI permits quantitative assessment of longitudinal myocardial function and LV filling pressure. The peak mitral annular velocities of Sa and Ea are sensitive markers of subtle impairments in LV function (166,167) with peak Ea velocity offering incremental prognostic information in hypertensive patients with LVH (58). The E/Ea ratio provides a robust method of assessing LV filling pressure compared to conventional transmitral Doppler and is also a strong prognosticator of cardiovascular risk (59,60,67).

Concentric hypertrophy is associated with the greatest risk of future cardiovascular events (76) and this pattern of remodeling is believed to ultimately progresses to LV dilatation and failure in hypertensives (77-79). An increase in LV mass can also occur in situations where wall thickness remains normal but the LV cavity dilates through myocyte elongation resulting in eccentric hypertrophy. Eccentric hypertrophy is typically associated with states of volume overload, such as mitral regurgitation, but in hypertension it may represent the early manifestation of a cardiomyopathic process without an intervening phase
of concentric hypertrophy. This alternative pathway for the development of heart failure in hypertension is supported by studies that have demonstrated eccentric hypertrophy to be associated with more severe systolic dysfunction compared to concentric hypertrophy (168,169). In this present population, longitudinal systolic function was significantly impaired in both concentric and eccentric hypertrophy compared to normal LV geometry. Subjects with eccentric hypertrophy had worse longitudinal systolic function and lower EF compared to those with concentric hypertrophy, however, the differences were not statistically significant after adjustment for covariates.

Although increased LV mass is also strongly associated with the development of diastolic heart dysfunction (170-172), the influence of concentric remodeling in the absence of LVH upon diastolic function had not been adequately defined. Previous studies have relied on transmitral Doppler assessment (13,66), a less reproducible and less capable method than TDI of identifying elevations in LV filling pressure - the hallmark of symptomatic diastolic dysfunction. Elevated LV filling pressure carries strong prognostic value in patients with heart failure, and its estimation using the E/Ea ratio is a similarly powerful prognosticator in various cardiac diseases (59,60,67). In this study, increasing degrees of concentric remodeling were associated with impaired relaxation and reduced early diastolic myocardial velocity but not with significantly increased E/Ea ratio. Concentric hypertrophy, however, impacted adversely upon both the lusitropic properties of the left ventricle and its filling pressure.

The phenomenon of enhanced longitudinal function and augmented EF with increasing degrees of concentric remodeling was observed. However, hypertrophic remodeling was associated with deteriorating LV function, accompanied by increasing LV filling pressure. The classical views of Grossman and Meerson concerning the hypertrophic
growth response of the left ventricle to pressure overload asserts that myocyte thickening is a compensatory response to normalise wall-stress (173,174). Adaptive remodeling with augmented systolic function has been demonstrated in transgenic hypertrophic rat heart models with selective activation of extracellular signal-regulated kinase (175), an important member of the signalling cascade that regulates hypertrophy. However, experimental studies have also questioned the wall-stress hypothesis with evidence that increased wall thickness can be maladaptive and attenuate systolic function (176). Evidence of LV hyperfunction associated with changes in LV geometry has not been demonstrated in a clinical study of hypertensive subjects before. The MESA study, which also employed quantitative parameters of regional myocardial function, assessed the effects of LV geometry upon systolic function in a cohort with normotensive and hypertensive individuals (177). Cardiac MRI derived peak systolic circumferential strain and LVEF decreased with increasing degrees of concentric remodeling in men. However, in women a gradual increase in function was initially observed with evidence of dysfunction occurring in the highest quintile of mass to volume ratio, with the authors concluding that a degree of concentric remodeling enhances myocardial function up to a threshold that differs in men and women.

As the design of this present study is cross-sectional, it is difficult to establish causality. Pre-existing impairments in myocardial function may have itself led to LVH/increased RWT through the activation of neurohormonal pathways. Long-term follow-up of this cohort will be able to clarify the progression of the early manifestations of hypertensive heart disease described here and whether subjects with LVH are more likely to develop heart failure compared to individuals with nonhypertrophic concentric remodeling.
Conclusion

In this bi-ethnic cohort, subclinical impairment of cardiac function was evident in hypertensive subjects with LVH. However, nonhypertrophic concentric remodeling appears to provoke a compensatory systolic response of the left ventricle to pressure load physiology.
CHAPTER 6. STUDY III - THE DISTINCT RELATIONSHIPS OF CAROTID PLAQUE DISEASE AND CAROTID INTIMA-MEDIA THICKNESS WITH LEFT VENTRICULAR FUNCTION

6.1 Abstract

Background: Subclinical carotid atherosclerosis has been associated with impaired LV function and the development of heart failure. Whether impaired LV function is related primarily to an increased IMT, or burden of plaque disease or both remains to be determined.

Methods: 2,214 subjects without clinical CVD were recruited from the LOLIPOP study. Carotid ultrasonography and transthoracic echocardiography was performed on all subjects. Carotid IMT and plaque scores were measured and their relationships with LV volumes, LV EF, myocardial LV longitudinal function (Sa and Ea velocities) and LV filling pressure (E/Ea ratio) assessed before and after adjustment for covariates.

Results: Compared to those without carotid artery disease, subjects with either increased IMT and/or presence of plaque disease had identical Sa velocity (both 9.0 cm/sec), lower Ea velocity (8.7 cm/s vs. 9.9 cm/s, p<0.001) and higher E/Ea ratio (8.4 vs 7.6, p<0.001). After multiple linear regression analysis, increasing IMT remained independently related to reduced Ea velocity (p<0.001) but not with LV EF, Sa velocity or E/Ea ratio. In a separate adjusted analysis, subjects with a severe burden of carotid plaque disease (> 5 plaques), had reduced LV EF (β -2.9, 95% C.I. 1.0 to 4.8, p=0.003), attenuated Sa velocity (β -0.79, 95% C.I. -1.2 to -0.3, p=0.003), attenuated Ea velocity (β -0.79, 95% C.I. -1.3 to -0.2, p=0.007)
and an increased E/Ea ratio (β 0.84, 95% C.I. 0.2 to 1.5, p=0.009) compared to individuals without carotid plaques.

**Conclusion:** These findings demonstrate that subclinical carotid plaque disease rather than IMT is more closely related to LV systolic function and LV filling pressure. This data supports the application of carotid ultrasonography as a tool that can potentially enhance the stratification of individuals at risk of developing heart failure syndromes.


6.2 **Introduction**

The recognition of LV dysfunction in asymptomatic individuals is of paramount importance as not only does this phase of disease foretell the development of incident congestive heart failure (178,179) but its treatment has been shown to delay the onset of overt heart failure symptoms (55). This has prompted the search for “risk factors” of incipient myocardial dysfunction, examples of which include the burden of conventional CVD risk factors, the presence of novel risk factors, abnormalities in LV geometry, increased LV mass and the degree of subclinical atherosclerosis (180-185). Increased intima-media thickness (IMT) and the presence of atheromatous plaques are forms of carotid artery disease that are both regarded as surrogate markers of atherosclerosis. Although these two phenotypes are related, the pathophysiology underlying intima-media thickening and plaque formation are not necessarily similar. Hypertrophy of the medial layer of the arterial wall can occur either as a response to hypertension or as a manifestation of normal aging, whereas plaque formation represents the maturation of the atherosclerotic process (186). Accordingly it has been suggested that these two types of arterial disease have distinct relationships to cardiac disease and vascular events (187,188).

Recent studies have suggested that increased carotid IMT is also associated with both incipient myocardial systolic dysfunction (185) and the development of clinical heart failure (189). However, these studies have been unable to discern whether impaired LV function or clinical heart failure events are related primarily to intima-media thickening, burden of plaque disease or both. Therefore, the relationships of carotid IMT and carotid plaque disease were separately assessed with echocardiographic parameters of LV systolic and diastolic function in a large cohort of subjects without clinical CVD.
6.3 Methods

Subjects were recruited between August 2004 and November 2007 from the LOLIPOP study. 2,288 Indian Asian and European White subjects, aged 35-74 years, who were free from clinical CVD, were randomly selected. Consenting subjects had a physical assessment including BP determination, anthropometric measurements (height, weight, waist-hip ratio) and an ECG. Subjects were then invited to undergo echocardiography, carotid ultrasonography and provide fasting plasma and serum samples for biochemical analysis including total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and glucose. The study was approved by the Northwick Park and Ealing Hospitals Research Ethics Committees.

Echocardiography

Transthoracic 2-D echocardiography was performed by experienced sonographers using a digital commercial harmonic imaging ultrasound system with an S3 3-MHz phased-array transducer (Philips IE33, Philips Medical Systems, Holland) at a single centre. LV dimensions were obtained in the parasternal short axis view with measurement of the interventricular septal thickness in diastole, LV dimension in diastole, LV dimension in systole and LV posterior wall thickness in diastole. LV mass was calculated using the Devereux formula (146) and indexed to height (m) to provide LVMI. LV end-diastolic and end-systolic volumes indexed to body surface area were measured using Simpson’s apical biplane rule. Tracing of the LV contour was performed carefully so as to exclude papillary muscles and trabeculations, as recommended by the American Society of Echocardiography (146). LV EF was automatically calculated following acquisition of the LV volumes using the Simpson’s method. Transmitral spectral Doppler was performed to obtain mitral inflow peak E-wave and peak A-wave velocities (cm/s). LA volume (ml) was calculated from three
measurements of left atrial dimension using the formula for an ellipse as described in chapter 4.3, with indexation to BSA.

Tissue Doppler imaging

Myocardial velocities were measured on-line using a standard pulse-wave Doppler technique from all four mitral annular sites, as described previously (chapter 5.3). Peak velocities (cm/sec) during systole (Sa) and early diastole (Ea) were measured on-line from all four mitral annular sites segments and averaged. Estimated LV filling pressure was derived from the ratio of transmitral E velocity to Ea velocity (E/Ea ratio).

Carotid ultrasonography

Carotid duplex scans were conducted using a high-resolution, non-harmonic B-mode ultrasound system (Philips IE33) with an 11- to 3-Mhz transducer. The proximal, mid and distal common carotid artery, its bifurcation and proximal portion of internal and external carotid arteries were systematically interrogated in long- and short-axis views. Gated cineloops of two cardiac cycles and still images during diastole of the common carotid artery and the bifurcation were digitally acquired for off-line analysis of IMT. Measurements of IMT were taken at the far wall of the common carotid artery and 1~cm proximal to the carotid bifurcation during end-diastole using a semi-automated edge-detection algorithm (QLab 5, Philips). A region of interest, 1cm in length, was placed parallel to the vessel wall allowing the software to detect the lumen-intimal and media-adventitial interfaces at the far wall of the vessel, enabling determination of the intima-media thickness. Values for mean IMT were derived from 3 measurements at the distal-, mid- and proximal-common carotid artery and 3 measurements from the bifurcation, for both the left and right arteries. If there was evidence of carotid plaque formation, care was taken not to include the plaque in the
IMT measurement. The common carotid artery, bifurcation, internal and external carotid arteries were then systematically interrogated in short- and long-axis views for detection of atherosclerotic plaque formation by using colour Doppler imaging. A plaque was defined as a focal structure encroaching into the arterial lumen by at least by 0.5mm, or a distinct area of intima-medial thickening that is at least 50% greater than the adjacent wall or is more than 1.5mm in thickness (190).

**Statistical analysis**

Clinical and echocardiographic parameters were primarily stratified according to those subjects with either normal carotid arteries versus those with evidence of carotid artery disease, defined as the presence of either a mean IMT measurement > 75th age-specific percentile values obtained from our healthy reference cohort and/or presence of plaque disease. In this cohort, we did not observe a relationship between ethnicity and the burden of carotid atherosclerosis, as previously published (191). Data is presented as mean ± standard deviation. Student’s t-test was performed to compare differences in continuous variables and chi-squared test to assess differences in categorical variables between the two groups. Parameters of LV volumes and function were then stratified according to: a) different patterns of carotid artery disease - namely those subjects having either no evidence of carotid disease; or increased IMT alone; or plaque disease alone or the presence of both increased IMT and plaque disease and b) carotid plaque burden - as derived from each subject’s plaque score, defined as the total number of plaques identified in both their carotid arteries. Subjects with a plaque score of 0, 1-2, 3-5 and >5 were classified as having either no, mild, moderate or severe plaque burden.

Linear regression was then performed to assess the relationship of IMT with LV volumes, LV EF, Sa velocity, Ea velocity and E/Ea ratio. The first model adjusted for the
variables of age, race, gender, presence of type 2 diabetes, systolic BP, diastolic BP, use of antihypertensive medications and LV mass. To assess whether the relationship of IMT with LV volumes and with LV function was influenced by subclinical atherosclerosis, the presence of carotid plaque disease was entered as a covariate into the second model. Further adjusted analyses were performed to assess the relationship of the severity of carotid plaque burden with LV volumes and function using ANCOVA. The effect of plaque burden upon LV volumes and function was adjusted for age, race, gender, presence of type 2 diabetes, systolic BP, diastolic BP, use of antihypertensive medications and LV mass. For post-hoc comparisons of least-square means arising in the ANCOVA models, the alpha level for rejection of the null hypothesis was adjusted after Bonferroni correction based on the number of comparisons being made (for 3 comparisons, \( p=0.05/3 = 0.0167 \), rounded up to 0.02). Given the correlation between the endpoints (measures of LV function), a full Bonferroni correction was avoided, which assumes independence of the tests being performed. Statistical analyses were performed on SPSS (version 15).

6.4 Results

Of the 2,228 subjects enrolled into the study, 2,214 had adequate data entry of carotid artery disease and LV function parameters. Of these subjects 1298 (59\%) were found to have evidence of carotid artery disease (either increased IMT and/or evidence of plaque disease). Carotid plaque disease, was present in 1027 (46\%) subjects, of which 674 (66\%) were classified as having a mild degree of plaque disease (1-2 plaques), 310 (30\%) classified as a moderate degree of plaque disease (3-5 plaques) and 43 (4\%) classified a severe degree of plaque disease (>5 plaques). The cohort consisted of 1595 (72\%) men and 1025 (46\%) European white subjects.
Compared to nondiseased carotid arteries, the presence of either increased IMT or plaque disease or both was associated with a greater burden of cardiovascular risk factors (table 6.1) such as higher prevalences of male gender, type 2 diabetes, treatment for hypertension and history of smoking. These subjects were also older, had higher systolic and diastolic BP, greater BMI, lower HDL-cholesterol, higher LDL-cholesterol and higher fasting glucose compared to controls. Structural and haemodynamic parameters reflecting LV function were also significantly associated with carotid artery disease (table 6.2). Although LV EF was slightly lower in those with carotid disease, the difference was statistically significant (p=0.03), however, Sa velocity was identical between the two groups. Abnormalities in the morphophysiological parameters of LV diastolic function such as LV mass, LA volume, Ea velocity and the E/Ea ratio were all significantly related to the presence of carotid disease. Table 6.3 illustrates the demographic and clinical characteristics according to burden of plaque disease. As expected, individuals with more plaques were older, more likely to be hypertensive, be diabetic, have higher serum glucose and have a higher LV mass. However, there was no discernible relationship between plaque burden and dyslipidaemia.
Table 6.1 - Clinical data stratified by the absence or presence of carotid artery disease

<table>
<thead>
<tr>
<th></th>
<th>Normal carotids N=916</th>
<th>Carotid disease (either IMT&gt;75th percentile and/or presence of plaque) N=1298</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54 ±9</td>
<td>59 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male/Female (%)</td>
<td>35/53</td>
<td>65/47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>European white/Indian Asian (%)</td>
<td>40/41</td>
<td>60/59</td>
<td>0.59</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 ± 4</td>
<td>28 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127 ± 17</td>
<td>136 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80 ± 10</td>
<td>81 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treated for HTN (%)</td>
<td>18</td>
<td>29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Type 2 diabetes (%)</td>
<td>9</td>
<td>17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.4 ± 1.4</td>
<td>5.8 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.4 ± 1.0</td>
<td>5.5 ± 1.1</td>
<td>0.16</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.4 ± 0.4</td>
<td>1.31 ± 0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.3 ± 0.8</td>
<td>3.4 ± 0.9</td>
<td>0.031</td>
</tr>
<tr>
<td>Ever smoker (%)</td>
<td>27</td>
<td>36</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI = body mass index, BP = blood pressure, HTN = hypertension, HDL = high-density lipoprotein, LDL = low-density lipoprotein
Table 6.2 - Echocardiographic parameters of left ventricular structure and function stratified by the absence or presence of carotid artery disease

<table>
<thead>
<tr>
<th></th>
<th>Normal carotids</th>
<th>Carotid disease (either IMT&gt;75th percentile and/or presence of plaque)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=916</td>
<td>N=1298</td>
<td></td>
</tr>
<tr>
<td>LVM (g)</td>
<td>158 ± 49</td>
<td>177 ± 54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LAVI (ml/m²)</td>
<td>16.2 ± 5.4</td>
<td>17.7 ± 6.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDVI (ml/m²)</td>
<td>38.3 ± 9.9</td>
<td>38.4 ± 10.1</td>
<td>0.17</td>
</tr>
<tr>
<td>ESVI (ml/m²)</td>
<td>14.5 ± 4.7</td>
<td>14.8 ± 5.2</td>
<td>0.81</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>62.3 ± 5.8</td>
<td>62.0 ± 6.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Sa velocity (cm/s)</td>
<td>9.0 ± 1.7</td>
<td>9.0 ± 1.7</td>
<td>0.85</td>
</tr>
<tr>
<td>Ea velocity (cm/s)</td>
<td>9.9 ± 2.3</td>
<td>8.7 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/Ea ratio</td>
<td>7.6 ± 2.1</td>
<td>8.4 ± 2.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

LVM = left ventricular mass, LAVI = left atrial volume index, EDVI = end-diastolic volume index, ESVI = end-systolic volume index, LV EF = left ventricular ejection fraction, Sa = mitral annular systolic velocity, Ea = mitral annular early diastolic velocity, E/Ea = ratio of transmitral Doppler E velocity to Ea velocity
Table 6.3 - Demographics and clinical characteristics according to burden of plaque disease

<table>
<thead>
<tr>
<th></th>
<th>None (0 plaques)</th>
<th>Mild (1-2 plaques)</th>
<th>Moderate (3-5 plaques)</th>
<th>Severe (&gt;5 plaques)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1187</td>
<td>674</td>
<td>310</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>54 ± 10</td>
<td>59 ± 9</td>
<td>64 ± 8</td>
<td>66 ± 5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>128 ± 17</td>
<td>135 ± 20</td>
<td>141 ± 19</td>
<td>144 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80 ± 10</td>
<td>82 ± 10</td>
<td>82 ± 11</td>
<td>80 ± 11</td>
<td>0.003</td>
</tr>
<tr>
<td>Treated for HTN (%)</td>
<td>18</td>
<td>27</td>
<td>43</td>
<td>60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 ± 4</td>
<td>27 ± 4</td>
<td>28 ± 4</td>
<td>27 ± 4</td>
<td>0.19</td>
</tr>
<tr>
<td>Type-2 diabetes (%)</td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.5 ± 1.5</td>
<td>5.8 ± 2.0</td>
<td>6.1 ± 1.9</td>
<td>7.0 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.4 ± 1.1</td>
<td>5.5 ± 1.0</td>
<td>5.5 ± 1.2</td>
<td>5.0 ± 1.1</td>
<td>0.045</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.4 ± 0.9</td>
<td>3.4 ± 0.9</td>
<td>3.5 ± 1.0</td>
<td>2.9 ± 0.9</td>
<td>0.007</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>162 ± 49</td>
<td>176 ± 55</td>
<td>187 ± 55</td>
<td>193 ± 49</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations as for tables 6.1 and 6.2.
The tissue Doppler parameters of LV function were further stratified according to different patterns of carotid artery disease (figure 6.1). Compared to subjects without evidence of carotid artery disease (n=916, 41%), Sa velocity was not significantly influenced by either the presence of increased IMT alone (n=319, 14%), the presence of plaque disease alone (n=575, 26%) or the presence of both an increased IMT with plaque formation (n=446, 20%). The presence of carotid artery disease, whether in the form of thickened IMT, plaque disease or both, was significantly related to impaired early diastolic function in a dose-dependent manner (9.3 cm/s, 8.8 cm/s, 8.4 cm/s respectively vs. 9.9 cm/s for controls, all comparisons p<0.001). However, LV filling pressure was only significantly elevated in those subjects with manifest plaque disease whether in the absence or presence of an increased IMT (8.4 and 8.7 respectively vs 7.6, both comparisons p<0.001). Neither LV volumes nor LV EF were influenced by an increased IMT, presence of plaque disease or both together. The effect of plaque burden severity upon LV longitudinal function, LV EF and LV filling pressure was assessed in a separate analysis (figure 6.2 and figure 6.3). Severe plaque burden was associated with significantly attenuated Sa velocity, as compared to subjects without plaque disease. The presence of plaque disease was associated with reduced Ea velocity and increased LV filling pressure in a dose-dependent manner (ANOVA p<0.001). As with longitudinal systolic function, LV EF was only reduced in those individuals with a severe plaque burden, as compared to controls (59.0% vs 62.1%, p<0.001). Increasing plaque burden was associated with greater LVESVI (p=0.01, ANOVA) and with greater LVEDVI (p=0.008, ANOVA).
A direct linear association is seen between increasing degrees of carotid artery disease and with reduced early diastolic myocardial velocity (Ea) and increased left ventricular filling pressure (E/Ea). Longitudinal systolic function is unaffected by the presence of increased intima-media thickness and/or presence of plaque disease. * p<0.001 versus subjects with IMT<75th percentile and no plaques, after Bonferroni correction for multiple comparisons. Standard error bars shown.
A linear association is seen again between increasing plaque score and with early myocardial diastolic velocity (Ea) and LV filling pressure (E/Ea). Myocardial longitudinal systolic velocity (Sa) becomes significantly attenuated in those with a severe burden of plaque disease. * p = 0.002, † <0.001 vs. subjects without plaques, after Bonferroni correction for multiple comparisons. Standard error bars shown.
Left ventricular ejection fraction becomes significantly reduced in those subjects with a severe burden of carotid artery plaques. † <0.001 vs. subjects without plaques, after Bonferroni correction for multiple comparisons. Standard error bars shown.
**Adjusted analyses**

To assess whether independent relationships existed between the degree of IMT and LV volumes or LV systolic function, multiple linear regression analyses were performed (table 6.4). In model 1, IMT was independently associated with a reduced Ea velocity (p=0.001) and increased E/Ea ratio (p=0.04) but not with LV volumes, LV EF or Sa velocity. After entering presence of carotid plaque disease into the second model, IMT remained independently associated with Ea velocity but the relationship with E/Ea ratio became weaker and borderline non-significant (p=0.05). Further adjusted analyses were performed to assess whether the severity of plaque disease was related with LV volumes and LV systolic function (table 6.5). Using ANCOVA analysis, LV EF and Sa velocity were both attenuated only in individuals with evidence of severe plaque burden which reached the alpha value for significance after Bonferroni correction for multiple comparisons of p=0.02. LV end-systolic volume index remained significantly greater in those with severe burden of plaque disease whereas LV end-diastolic volume index was no longer related to plaque burden. The Ea velocity was also significantly impaired in subjects with mild or severe plaque burden (p=0.006 and p=0.01 respectively) compared to subjects with no plaque disease. Although the E/Ea ratio increased with increasing plaque burden, the effect did not reach the adjusted statistical significance until a severe burden of plaque disease was present.
### Table 6.4 - Adjusted analysis for the relationship of carotid IMT with LV volumes, LV function and LV filling pressure

<table>
<thead>
<tr>
<th></th>
<th>Model 1 Coefficient (95% C.I.)</th>
<th>Model 1 P value</th>
<th>Model 2 Coefficient (95% C.I.)</th>
<th>Model 2 P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESVI (ml/m^2)</td>
<td>0.06 (-0.2, 0.3)</td>
<td>0.59</td>
<td>-0.30 (-0.7, 0.1)</td>
<td>0.156</td>
</tr>
<tr>
<td>EDVI (ml/m^2)</td>
<td>-0.07 (-0.5, 0.4)</td>
<td>0.76</td>
<td>-0.78 (-1.6, 0.03)</td>
<td>0.06</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>-0.16 (-0.46, 0.14)</td>
<td>0.30</td>
<td>-0.16 (-0.46, 0.15)</td>
<td>0.33</td>
</tr>
<tr>
<td>Sa velocity (cm/s)</td>
<td>0.006 (-0.08, 0.09)</td>
<td>0.88</td>
<td>-0.004 (-0.08, 0.09)</td>
<td>0.94</td>
</tr>
<tr>
<td>Ea velocity (cm/s)</td>
<td>-0.18 (-0.27, -0.07)</td>
<td>&lt;0.001</td>
<td>-0.17 (-0.26, -0.07)</td>
<td>0.001</td>
</tr>
<tr>
<td>E/Ea ratio</td>
<td>0.13 (0.03, 0.23)</td>
<td>0.014</td>
<td>0.11 (0.02, 0.21)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Abbreviations as for table 6.2

Regression coefficients represent the difference in tissue Doppler parameters per standard deviation increase (0.17 mm) in IMT.

Model 1 represents multiple linear regression analysis adjusting for age, sex, race, systolic BP, diastolic BP, type 2 diabetes, antihypertensive medications, BMI and LV mass. Presence of carotid plaque disease was entered as a covariate in model 2.
Table 6.5 - Adjusted analysis for the relationship of severity of carotid plaque disease to LV volumes, LV function and LV filling pressure

<table>
<thead>
<tr>
<th>Coefficient (95% C.I.)</th>
<th>P value</th>
<th>Coefficient (95% C.I.)</th>
<th>P value</th>
<th>Coefficient (95% C.I.)</th>
<th>P value</th>
<th>Coefficient (95% C.I.)</th>
<th>P value</th>
<th>Coefficient (95% C.I.)</th>
<th>P value</th>
<th>Coefficient (95% C.I.)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESVI (ml/m²)</td>
<td></td>
<td>EDVI (ml/m²)</td>
<td></td>
<td>Ejection fraction (%)</td>
<td></td>
<td>Mean Sa velocity (cm/s)</td>
<td></td>
<td>Mean Ea velocity (cm/s)</td>
<td></td>
<td>Mean E/Ea</td>
<td></td>
</tr>
<tr>
<td>1-2 plaques</td>
<td>-0.46(0.02,0.9)</td>
<td>0.04</td>
<td>-1.2 (-0.3,-1.9)</td>
<td>0.01</td>
<td>-0.06 (-0.5,0.6)</td>
<td>0.84</td>
<td>0.04 (-0.2, 0.1)</td>
<td>0.59</td>
<td>-0.26 (-0.4, -0.1)</td>
<td>0.004 *</td>
<td>0.22 (0.03, 0.4)</td>
</tr>
<tr>
<td>3-5 plaques</td>
<td>-0.11(-0.5,0.7)</td>
<td>0.73</td>
<td>-0.58 (-0.6, 1.8)</td>
<td>0.35</td>
<td>-0.2 (-0.6, 1.0)</td>
<td>0.63</td>
<td>0.11 (-0.4, 0.1)</td>
<td>0.35</td>
<td>-0.08 (-0.3, 0.2)</td>
<td>0.54</td>
<td>0.27(-0.5, 0.001)</td>
</tr>
<tr>
<td>&gt; 5 plaques</td>
<td>1.78(0.4,3.2)</td>
<td>0.014 *</td>
<td>1.7 (4.4, 1.1)</td>
<td>0.23</td>
<td>-2.9 (1.0, 4.8)</td>
<td>0.003*</td>
<td>-0.79 (-1.2, -0.3)</td>
<td>0.003</td>
<td>-0.79 (-1.3, -0.2)</td>
<td>0.007 *</td>
<td>0.84 (0.2, 1.5)</td>
</tr>
</tbody>
</table>

Abbrevations as for table 6.2

Regression coefficients represent difference in tissue Doppler parameters compared to subjects without carotid plaque disease.

Analysis adjusted for age, race, sex, type 2 diabetes, systolic BP, diastolic BP, antihypertensive medications, BMI and LV mass.

* denotes that p value reaches adjusted alpha value for multiple comparisons of p<0.02
6.5 Discussion

This study has demonstrated that carotid IMT and plaque disease have distinct relationships with LV systolic and diastolic function in healthy individuals. For the first time, the burden of carotid plaque disease, rather than the degree of intima-media thickening, has been demonstrated to be more closely associated with reduced systolic function, reduced diastolic function and increased LV filling pressure. An increased IMT was observed to only be independently associated with early diastolic myocardial dysfunction. These findings have important implications for the use of carotid ultrasonography as a screening tool for identifying patients vulnerable to developing heart failure syndromes.

Subclinical atherosclerosis and LV systolic function

The MESA study has shown that increased carotid IMT is correlated with attenuated myocardial systolic and diastolic function in asymptomatic individuals (185). Recently published data from the Malmo Diet and Cancer population study observed increased carotid IMT to be independently predictive of future heart failure events (189). However, by incorporating plaque dimensions into IMT measurements, neither of these studies distinguished carotid plaque as a separate disease entity from IMT. Consequently the presumed influence of IMT upon clinical parameters of LV function or incident heart failure is likely to have been exaggerated. By defining the presence and quantity of carotid plaques and by performing IMT measurements in plaque-free areas, as recommended by a recent consensus paper (190), we have been able to demonstrate that subclinical LV systolic dysfunction occurs in the presence of an increased burden of plaque disease, rather than increased intima-media thickening. Although carotid IMT is widely believed to be a surrogate marker of atherosclerosis, there is increasing evidence to suggest that this may be a
misleading analogy (192). Whereas plaque disease is synonymous with atherosclerosis, IMT is primarily determined by aging and hypertension through the hypertrophic response of the medial layer to pressure load physiology (193,194). The association between coronary heart disease is weaker for IMT than with plaque disease (187,195-197), and IMT measurement is prone to methodological heterogeneity (198) such as use of mean versus maximum values, variability in carotid artery segment sampled and the aforementioned tendency to incorporate plaque dimensions within IMT measurements.

Proposed mechanisms for subclinical arterial disease causing myocardial dysfunction include a greater likelihood of associated obstructive epicardial vessel disease (199), reduced coronary flow reserve (200,201) and the direct association of atherosclerosis with other comorbidities, such as hypertension, known to impair LV systolic function particularly when associated with hypertrophic remodeling. However, in our study the association between plaque disease and systolic function persisted after adjustment for known cardiovascular risk factors and LV mass.

**Subclinical atherosclerosis and LV diastolic dysfunction**

Both increased intima-media thickening and carotid plaque disease were strongly associated with LV diastolic function. IMT is closely correlated to LV mass (202) which itself is a major determinant of diastolic function and heart failure events. However, we have demonstrated the relationship between IMT and diastolic function to persist after adjustment for LV mass and other factors known to strongly influence diastolic function, such as age, BP and the presence of subclinical atherosclerosis. Our findings reinforce previous work that has shown LV mass not to be the primary determinant of carotid wall thickness (185,203,204). A bidirectional cause-effect relationship may also exist between ventricular function and vascular structure (193,204). Increased IMT is associated with greater collagen deposition
and increased arterial stiffness (205) which leads to an augmentation in pressure wave propagation from the peripheral circulation arriving at the aorta during systole, effectively increasing afterload, LV mass and diastolic dysfunction (206).

A correlation between the degree of arterial disease and increased LV filling pressure, as estimated by the E/Ea ratio, was also observed for the first time in a large cohort of asymptomatic subjects. Increased IMT was associated with higher E/Ea after adjustment for age, BP and LV mass. However, after adjustment for carotid plaques, this association no longer remained significant. Increasing severity of plaque disease remained positively correlated with increased LV filling pressure, which may be a reflection of the increased ventricular stiffness that occurs in patients with more severe atherosclerosis and reduced myocardial blood flow (207,208).

**Limitations**

A plaque score, or sum of plaques detected, was employed as a measure of plaque burden rather than measuring plaque dimensions which may better reflect the severity of atherosclerosis. Unless there was evidence of a flow limiting stenosis, we did not document anatomically whether a plaque was located in the common carotid artery, the bifurcation, the internal carotid artery or external carotid artery which precluded any conclusions to be drawn regarding plaque location and LV function. Although distinct relationships between different forms of carotid artery disease with LV function have been described, the prognostic importance of such classification is unknown. The cross-sectional nature of this study also makes it difficult to assign causality, in particular with respect to the relationship between subclinical vascular disease and LV diastolic function which may have a bi-directional component. Again long-term follow-up of this cohort will enable us to determine whether
increased IMT and/or plaque disease are associated with the greater risk of developing diastolic or systolic heart failure respectively.

**Conclusion**

For the first time it has been demonstrated that carotid IMT and carotid plaque disease are distinct in their relationships to clinical, echocardiographic parameters of systolic and diastolic LV function. Subjects with a severe burden of carotid plaque disease had significantly impaired LV systolic function compared to those without plaques. These findings suggest that carotid ultrasonography may help identify individuals at risk of developing heart failure.
CHAPTER 7. STUDY IV - ETHNICITY-RELATED DIFFERENCES IN LEFT VENTRICULAR FUNCTION, STRUCTURE AND GEOMETRY: A POPULATION STUDY OF UK INDIAN ASIANS AND EUROPEAN WHITES

7.1 Abstract

Objectives: We studied healthy UK Indian Asian and European white subjects to assess whether functional, structural and geometrical properties of the left heart are intrinsically related to ethnicity.

Background: Quantitative assessment of cardiac function and structure is necessary to diagnose heart failure syndromes and is validated to refine risk prediction. A better understanding of the demographic factors that influence these variables is required.

Methods: 453 healthy subjects were recruited from the LOLIPOP study. They underwent 2-D and tissue Doppler echocardiography for quantification of LV function, LV volumes, LAVI, LVMI and RWT.

Results: Indian Asians had attenuated mitral annular systolic velocity (8.9 cm/s vs. 9.5 cm/s, p<0.001), lower mitral annular early diastolic velocity (10.3 cm/s vs. 11.0 cm/s, p<0.001) and higher E/Ea ratio (7.9 vs. 7.0, p<0.001) compared to European whites. Although Indian Asians had significantly smaller left heart volumes and LVMI, they had a significantly higher RWT (0.37 vs. 0.35, p<0.001). After adjustment for covariates, these ethnicity-related differences remained highly significant (p<0.001).
**Conclusion:** Compared to European whites, Indian Asians have attenuated longitudinal LV function, higher E/Ea and demonstrate a greater degree of concentric remodeling independent of other demographic and clinical parameters.
7.2 Introduction

The echocardiographic assessment of LV function and structure has significantly enhanced CVD risk stratification. Traditional predictors of poor outcome such as LV EF and transmitral Doppler assessment of LV filling have been supplemented by TDI. Clinically relevant TDI parameters have been validated as prognosticators of CVD risk such as peak Sa velocity, peak Ea velocity and the estimate of LV filling pressure by measurement of transmitral early velocity to Ea ratio (E/Ea) (56-60). Increasing LA size is regarded as a morphophysiological expression of increased LV filling pressure and the prognostic importance of LA size parameters such as LAVI has also been confirmed more recently, particularly in higher-risk groups (83-86,209). Increased LVMI is a well established, powerful predictor of cardiovascular morbidity and mortality irrespective of aetiology (50,69) and alterations in LV geometry may also provide incremental prognostic information (76,80).

The measurement of these parameters forms the cornerstone of recent guidelines concerning the echocardiographic diagnosis of diastolic heart failure (138) but their demographic determinants are poorly understood. Importantly the influence of ethnicity upon LV function and LV filling pressure remains undefined particularly as reference data collected for LAVI and TDI parameters amongst healthy subjects has seldom been population based (150,155). Although the relationship of ethnicity with LVMI and LV geometry has been explored in large population studies, the published data has almost exclusively compared Afro-Caribbeans and European whites.

In this study we sought to evaluate the effect of ethnicity upon LV function, left heart structure and geometry using 2-D echocardiography and TDI in a healthy cohort of Indian
Asian and European white subjects free of clinical CVD, traditional cardiovascular risk factors and significant coronary artery disease.

7.3 Methods

Subjects were recruited between August 2004 and November 2007 from the LOLIPOP study. Assessment of participants was performed by a trained nurse using a standard protocol including questions on medical history, family history, cardiovascular risk factors, alcohol intake, physical activity and drug history (verified from the practice computerised records). Subsequently 2,293 Indian Asian and European White subjects, aged 35-74 years and free from clinical CVD, were selected at random and enrolled into the LOLIPOP-ATHEROSCLEROSIS substudy. Participants were defined as Indian Asian if all four grandparents were born in the Indian subcontinent (India, Pakistan or Bangladesh) and European white if all four grandparents were born in Northern Europe.

Consenting subjects had a physical assessment including BP determination, anthropometric measurements, and an ECG. Subjects were then invited to undergo echocardiography, EBCT for coronary calcium score determination (Agatston score) and provide fasting plasma and serum samples for biochemical analysis stored at -80°C. The study was approved by the Northwick Park Hospital and Ealing Hospital Research Ethics Committees.

Echocardiograms were analysed in a sub-set of 458 healthy individuals having excluded subjects with a coronary calcium score > 10 Agatston units, a prescription for cardioactive medications (antihypertensives, antianginals, hypoglycaemic agents, lipid lowering therapy, thienodipiridine anti-platelets) or any traditional cardiovascular risk factors (systolic BP > 140mmHg, diastolic BP > 90 mmHg, total cholesterol > 6.0 mmol/L, fasting glucose > 7.1 mmol/L, BMI > 30 kg/m², current smoking).
Echocardiography

Left ventricular dimensions and ejection fraction

Transthoracic 2-D echocardiography was performed by experienced sonographers using a digital commercial harmonic imaging ultrasound system with an S3 3-MHz phased-array transducer (Philips IE33, Philips Medical Systems, Holland) at a single centre. As described previously (chapter 5.3), LV dimensions were obtained allowing estimation of LV mass, with indexation performed to height. RWT was calculated as the sum of the interventricular sepum thickness in diastole and the posterior wall thickness in diastole divided by the LV dimension in diastole.

LV end-diastolic volume index and LV end-systolic volume index were measured using Simpson’s apical biplane rule as described in chapter 6.3, with indexation performed to BSA. LV EF was automatically calculated following acquisition of the LV volumes using the Simpson’s method.

Left atrial volume index

As described in chapter 4.3, LA volume was calculated from three measurements of LA dimension using the formula for an ellipse. The estimated LA volume was then indexed to BSA to obtain the LAVI.

Tissue Doppler imaging

Myocardial velocities were measured on-line using a standard pulse-wave Doppler technique from all four mitral annular sites, as described previously (chapter 5.3). Peak velocities (cm/sec) during systole (Sa) and early diastole (Ea) were measured on-line from all four
mitral annular sites segments and averaged. Estimated LV filling pressure was derived from the ratio of transmitral E velocity to Ea velocity (E/Ea ratio).

Transmitral flow and E/Ea ratio

The transmitral flow velocities were recorded using pulsed wave Doppler as described previously (chapter 4.3).

Electron beam computed tomography

Coronary calcium imaging was performed using EBCT at a single centre with a modified GE Imatron C-150 (San Francisco, CA, USA) scanner specially equipped with high-resolution detectors, as described in chapter 4.3.

Statistical analysis

Continuous variables are summarised as the mean ± 1 standard deviation. Continuous variables and their relationship to ethnicity were assessed using one-way ANOVA and categorical data by chi-squared test. Three multivariate linear regression models were applied separately to the dependent variables of LV function and structure to assess the independent effects of ethnicity, age and gender. Differences in LV function were adjusted in model 1 for age and gender, in model 2 for age, gender and ethnicity and in model 3 for age, gender, ethnicity, systolic BP, diastolic BP, BMI, LVMI and RWT. Parameters of LV structure were analysed using the same regression models with the addition of RWT in model 3 and the omission of LVMI as a covariate when LV mass was itself examined as a dependent variable. Statistical analysis was performed using SPSS version 15 with values of p < 0.05 considered statistically significant.
**Interobserver variability**

Echocardiographic measurements were repeated by two sonographers in 15 subjects to assess reproducibility and interobserver variability. The coefficient of variance was 11.7%, 11.5%, 5.8%, 9.9% and 3.7% for LAVI, LVMI, EDVI, ESVI and LV EF respectively. For the TD parameters mean Sa velocity, mean Ea velocity and mean E/Ea ratio the coefficient of variance was 11.4%, 8.7% and 8.0% respectively.

### 7.4 Results

**Risk factors**

The clinical characteristics and cardiovascular risk factor profiles of the 453 subjects are summarised by ethnicity (table 7.1). Compared to European whites, Indian Asians had significantly higher BMI, fasting triglycerides and lower HDL-cholesterol. Age, BP, total cholesterol, fasting glucose and coronary artery calcification did not differ significantly between the two groups.

**LV function**

Although LV EF and E/A ratio were identical between Indian Asians and European whites, significant differences existed in longitudinal myocardial systolic and early diastolic function (table 7.2). European whites had higher Sa and Ea velocities than Indian Asians (9.5 cm/s vs 8.9 cm/s, p<0.001). After multivariate linear regression analysis for age, gender, systolic BP, diastolic BP, BMI, LVMI and RWT, Asian race remained independently associated with lower Sa and Ea velocities (table 7.3).
### Table 7.1 - Clinical characteristics of the cohort according to ethnicity

<table>
<thead>
<tr>
<th></th>
<th>European white n = 199</th>
<th>Indian Asian n = 259</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>51.8 ± 8.5</td>
<td>50.6 ± 8.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Male (%)</td>
<td>60</td>
<td>53</td>
<td>0.15</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>119 ± 11</td>
<td>117 ± 12</td>
<td>0.18</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75 ± 8</td>
<td>75 ± 7</td>
<td>0.98</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 2.7</td>
<td>25.0 ± 2.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>53.7 ± 10.4</td>
<td>48.1 ± 9.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.3 ± 0.7</td>
<td>5.1 ± 0.8</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.5 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.2 ± 0.6</td>
<td>3.2 ± 0.7</td>
<td>0.71</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.5</td>
<td>5.1 ± 0.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.0 ± 0.7</td>
<td>1.4 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Agatston score (Au)</td>
<td>0.4 ± 1.3</td>
<td>0.4 ± 1.4</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Mean ± standard deviation shown for continuous variables

BP = blood pressure, BMI = body mass index; Au = Agatston units
Table 7.2 - Parameters of LV systolic and diastolic function according to ethnicity

<table>
<thead>
<tr>
<th></th>
<th>European white n =199</th>
<th>Indian Asian n =258</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejection fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bi-plane Simpson’s EF</td>
<td>0.62 ± 0.05</td>
<td>0.62 ± 0.06</td>
<td>0.552</td>
</tr>
<tr>
<td>Transmitral Doppler</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmitral E-wave (cm/s)</td>
<td>71.8 ± 14.0</td>
<td>74.0 ± 16.6</td>
<td>0.14</td>
</tr>
<tr>
<td>Transmitral A-wave (cm/s)</td>
<td>62.5 ± 13.8</td>
<td>66.0 ± 17.2</td>
<td>0.02</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>0.61</td>
</tr>
<tr>
<td>E-deceleration time (ms)</td>
<td>210.3 ± 49.8</td>
<td>201.8 ± 45.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Tissue Doppler</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sa (cm/s)</td>
<td>9.5 ± 1.6</td>
<td>8.9 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ea (cm/s)</td>
<td>11.0 ± 2.1</td>
<td>10.3 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/Ea ratio</td>
<td>7.0 ± 1.5</td>
<td>7.9 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are mean ± standard deviation

EF = ejection fraction, Sa = mitral annular systolic velocity, Ea = mitral annular early diastolic velocity
These differences remained significant when alternative indices of body size, such as lean body mass and height, were entered into the model instead of BMI. The E/Ea ratio was significantly higher amongst Indian Asians (table 7.2) due to their relatively lower Ea velocity but similar transmitral Doppler E velocity compared to European whites. This difference in the E/Ea ratio remained statistically significant (p<0.001) after adjustment for covariates (table 7.3).

The independent effects of age and gender upon LV function are also depicted in table 7.3. Increasing age was associated with an increase in LV EF but significantly reduced Sa velocity. Aging was associated with attenuated Ea velocity, E/A ratio and increased E/Ea ratio. Gender was not associated with LV EF, E/A ratio or Ea velocity, however, reduced Sa velocity and increased E/Ea ratio were independently associated with female sex.

**Left heart structure and geometry**

There were marked differences between European white and Indian Asian subjects with regards to left heart structure (table 7.4). European whites had significantly greater LA volumes, LV mass and LV volumes even after indexation for body size. Indian Asians had evidence of a greater degree of concentric remodeling with significantly higher RWT compared to European whites (0.37 vs. 0.35, p<0.001). Multivariate models (table 7.5) confirmed that ethnicity remained independently associated with LV volumes, LVMI, LAVI and RWT. Additional models performed with LV mass and LA volume indexed to lean body mass also confirmed that Indian Asian ethnicity remained independently associated with lower LV mass and smaller LA size.

Weak linear relationships were observed between E/Ea ratio and LAVI for European white (r = 0.2, p = 0.005) and Indian Asian (r = 0.2, p = 0.02) subjects. There was also a
positive correlation between LAVI and LVMI in both Europeans ($r=0.5$, $p<0.001$) and Indian Asians ($r=0.4$, $p<0.001$).

Advancing age was independently associated with smaller LV volumes whereas LVMI, LAVI and RWT all increased significantly with age (table 7.5). Female gender was associated with smaller LV volumes and lower LVMI compared to males, however, there was no significant effect of gender upon LAVI or RWT.
### Table 7.3 - Relationship of LV function with demographic and clinical parameters by multivariate analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LV EF (%)</th>
<th>E/A ratio</th>
<th>Sa velocity (cm/s)</th>
<th>Ea velocity (cm/s)</th>
<th>E/Ea ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter estimate</td>
<td>p value</td>
<td>Parameter estimate</td>
<td>p value</td>
<td>Parameter estimate</td>
</tr>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.70</td>
<td>0.008</td>
<td>-0.14</td>
<td>&lt;0.001</td>
<td>-0.24</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.47</td>
<td>0.38</td>
<td>0.05</td>
<td>0.14</td>
<td>-0.52</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.71</td>
<td>0.007</td>
<td>-0.14</td>
<td>&lt;0.001</td>
<td>-0.26</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.44</td>
<td>0.41</td>
<td>0.05</td>
<td>0.12</td>
<td>-0.47</td>
</tr>
<tr>
<td>Asian race</td>
<td>0.38</td>
<td>0.47</td>
<td>-0.04</td>
<td>0.22</td>
<td>-0.61</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.52</td>
<td>0.07</td>
<td>-0.13</td>
<td>&lt;0.001</td>
<td>-0.34</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.32</td>
<td>0.60</td>
<td>0.03</td>
<td>0.46</td>
<td>-0.46</td>
</tr>
<tr>
<td>Asian Race</td>
<td>-0.22</td>
<td>0.70</td>
<td>-0.02</td>
<td>0.65</td>
<td>-0.70</td>
</tr>
<tr>
<td>sBP</td>
<td>0.58</td>
<td>0.16</td>
<td>0.02</td>
<td>0.46</td>
<td>0.25</td>
</tr>
<tr>
<td>dBP</td>
<td>-0.37</td>
<td>0.33</td>
<td>-0.05</td>
<td>0.03</td>
<td>-0.21</td>
</tr>
<tr>
<td>BMI</td>
<td>0.38</td>
<td>0.21</td>
<td>-0.06</td>
<td>0.002</td>
<td>-0.06</td>
</tr>
<tr>
<td>LVMI</td>
<td>-0.71</td>
<td>0.03</td>
<td>-0.02</td>
<td>0.94</td>
<td>-0.11</td>
</tr>
<tr>
<td>RWT</td>
<td>0.79</td>
<td>0.005</td>
<td>-0.04</td>
<td>0.04</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*Change in parameter estimates Sa velocity, Ea velocity and E/Ea ratio with a 1 S.D. increment in age (8.5 years), sBP (11.4 mmHg), dBP (7.3 mmHg), BMI (2.67 kg/m²), LVMI (22.1 g/m) and RWT (0.07). sBP = systolic blood pressure, dBP = diastolic blood pressure, BMI = body mass index, LVMI = left ventricular mass index, RWT = relative wall thickness*
### Table 7.4 - Left heart structural and geometrical parameters according to ethnicity

<table>
<thead>
<tr>
<th></th>
<th>European white</th>
<th>Indian Asian</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=199</td>
<td>n=258</td>
<td></td>
</tr>
<tr>
<td>LAV (ml)</td>
<td>30.8 ± 10.4</td>
<td>25.0 ± 7.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LAV indexed to BSA (ml/m^2)</td>
<td>16.3 ± 4.8</td>
<td>14.2 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LAV indexed to LBM (g/kg)</td>
<td>5.8 ± 1.7</td>
<td>5.3 ± 1.6</td>
<td>0.004</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>159.2 ± 45.3</td>
<td>132.4 ± 33.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVM indexed to height (g/m)</td>
<td>92.1 ± 24.7</td>
<td>80.2 ± 18.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>0.35 ± 0.07</td>
<td>0.37 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDV indexed to BSA (ml/m^2)</td>
<td>43.7 ± 10.4</td>
<td>35.4 ± 8.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESV indexed to BSA (ml/m^2)</td>
<td>16.6 ± 4.8</td>
<td>13.4 ± 4.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are mean ± standard deviation. LAV = left atrial volume, LVM = left ventricular mass, ESV = end-systolic volume, EDV = end-diastolic volume.
Table 7.5 - Relationship of left heart structure and geometry with demographic and clinical parameters by multivariate analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EDVI (ml/m²)</th>
<th>ESVI (ml/m²)</th>
<th>LVMI (g/m)</th>
<th>LAVI (ml/m³)</th>
<th>RWT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.86</td>
<td>-0.55</td>
<td>3.85</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Female gender</td>
<td>-5.7</td>
<td>-2.3</td>
<td>-14.96</td>
<td>-0.57</td>
<td>0.17</td>
</tr>
<tr>
<td>Asian race</td>
<td>-8.0</td>
<td>-3.1</td>
<td>-9.95</td>
<td>-2.00</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-1.2</td>
<td>-0.66</td>
<td>3.48</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Female gender</td>
<td>-5.1</td>
<td>-2.1</td>
<td>-14.23</td>
<td>-0.42</td>
<td>0.31</td>
</tr>
<tr>
<td>Asian race</td>
<td>-8.0</td>
<td>-3.1</td>
<td>-9.95</td>
<td>-2.00</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-1.4</td>
<td>-0.76</td>
<td>2.7</td>
<td>0.78</td>
<td>0.01</td>
</tr>
<tr>
<td>Female gender</td>
<td>-4.1</td>
<td>-1.7</td>
<td>-12.5</td>
<td>-0.19</td>
<td>0.65</td>
</tr>
<tr>
<td>Asian race</td>
<td>-6.8</td>
<td>-2.5</td>
<td>-11.4</td>
<td>-2.2</td>
<td>0.02</td>
</tr>
<tr>
<td>sBP</td>
<td>-0.25</td>
<td>-0.26</td>
<td>2.4</td>
<td>0.33</td>
<td>0.28</td>
</tr>
<tr>
<td>dBP</td>
<td>-0.51</td>
<td>-0.17</td>
<td>0.56</td>
<td>-0.40</td>
<td>0.16</td>
</tr>
<tr>
<td>BMI</td>
<td>-1.3</td>
<td>-0.61</td>
<td>8.4</td>
<td>1.3</td>
<td>0.01</td>
</tr>
<tr>
<td>LVMI</td>
<td>2.4</td>
<td>1.1</td>
<td>2.05</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Change in parameter estimates LVMI, LAVI and RWT with a 1 S.D. increment in age (8.5 years), sBP (11.4 mmHg), dBP (7.3 mmHg), BMI (2.67 kg/m²) and LVMI (22.1g/m). EDVI = end-diastolic volume index, ESVI = end-systolic volume index, RWT = relative wall thickness, sBP = systolic blood pressure, dBP = diastolic blood pressure.
7.5 Discussion

This is the first population-based study demonstrating that ethnicity-related differences exist in the function, structure and geometry of the healthy left ventricle. Compared to European whites, Indian Asians were observed to have reduced longitudinal systolic and diastolic mitral annular velocities, a greater E/Ea ratio, markedly smaller left heart volumes and lower LV mass. However, the traditional measures of systolic and diastolic function such as LV EF and the E/A ratio, were unaffected by ethnicity. There was also evidence of a strong relationship between Indian Asian ethnicity and a greater degree of concentric remodeling, compared to European whites. The influence of demography upon heart function and structure was also evident when the effects of aging and gender were examined.

To the best of our knowledge, ethnicity-related differences in systolic and diastolic properties of the left ventricle have not been previously reported amongst healthy individuals. The recently published ASCOT sub-study did demonstrate significantly worse TDI parameters of diastolic function amongst hypertensive African-Caribbean compared to European whites living in the UK (132). However, the absence of a reference normotensive group and data concerning duration of hypertensive status precluded definitive conclusions to be reached regarding the independent influence of ethnicity upon myocardial function. There are no obvious explanations for the relatively attenuated longitudinal function and higher E/Ea in Indian Asians who had lower LV mass, similar BP, serum glucose and degree of coronary artery calcification compared to the European whites.

In this present study Indian Asians demonstrated a greater degree of concentric remodeling, expressed as the RWT, compared to European whites. Concentric remodeling of the left ventricle tends to manifest in situations of pressure overload resulting in radial growth and thickening of the myocyte. However, there was no significant difference in BP between
the two groups and in the multivariate analysis RWT was not significantly influenced by either systolic or diastolic BP. Ethnicity-related differences in cardiac geometry have been previously reported (210,211), again independently of standard clinical and haemodynamic variables.

Compared to European whites, migrant Indian Asians living in the UK are known to have significantly higher risk of death from CVD (96,212) and incidence rates for congestive heart failure (213). Peak Sa and Ea velocity are strong predictors of outcome in patients with established heart disease (56,57) with Ea appearing to be the superior of the two (58,214). The E/Ea ratio, which eliminates the effects of ageing and LV relaxation, correlates closely with LV filling pressure (156,157). Although E/Ea performs less well at estimating end-diastolic pressure in patients with decompensated heart failure (215,216), this ratio has proved to still be a strong predictor of outcomes in these patients and in those post-myocardial infarction (59,60,67). Concentric remodeling is associated with worse cardiovascular outcomes (76,80), as well as impairment in systolic (177) and particularly diastolic function (66). Whether the less favourable LV function and evidence of greater cardiac remodeling observed amongst Indian Asians are markers of increased CVD risk or simply representative of differing normal reference values in these ethnic groups can only be determined after long-term follow-up of this cohort.

Whereas the E/Ea ratio represents a snap-shot of LV filling pressure, an enlarged LA provides physiological and morphological evidence of a chronic elevation in end-diastolic LV pressure. The relationship between LA volume and prognosis has been confirmed in patients with pre-existing CVD such as atrial fibrillation (83), LV dysfunction (84) and myocardial infarction (85,86). The relationship of LA volume with ethnicity has not been examined sufficiently to-date. Although similar LA dimension has been reported between
African-Caribbean and Caucasian individuals (30,35) there is no published data comparing LA volumes between ethnic/racial groups in a population setting. LAVI was smaller in the Indian Asian population we studied compared to European whites. A similar correlation existed between LAVI and E/Ea ratio in both ethnicities suggesting that the smaller LA volume in Indian Asians is likely to be due to their comparatively smaller heart size rather than a reflection of chronically lower filling pressures. The disparity in LA volume was not attenuated by its indexation to lean body mass.

Increased LV mass has been shown to be a powerful independent predictor for cardiovascular morbidity and mortality in individuals previously free of clinical CVD (70). Studies assessing the independent effect of race/ethnicity upon LVMI have predominantly been conducted between African-Caribbean and Caucasian populations. However, there is very little data with respect to LV mass in Indian Asians. In a study by Kumaran et al., echocardiography was performed in 435 subjects living in Mysore, South India (131). The mean LV mass in men and women was 149g and 125g respectively, similar to the values obtained in our study (145g vs. 118g), and noted to be lower than published values in Western populations. The study also confirmed that the positive correlation between increased LV mass and risk of CVD exists also in individuals of Indian Asian ethnicity, with significantly higher LV mass observed in the 45 subjects with known CVD compared to controls. In our study, Indian Asian subjects had lower LVMI compared to European whites which, as with their smaller left atria, appears to be largely a consequence of their overall smaller heart size. Indexation to lean body mass again did not attenuate the observed differences in LV mass.

LV mass progressively was observed to increase with age and LVMI was significantly lower in females than males after adjustment for confounders including BMI.
Although LA dimension has been shown to increase with aging (217,218), studies have not yet confirmed that increasing age is associated with volumetric increases in LA size (150,219). However, in this population aging was independently associated with larger LAVI, commensurate with the higher E/Ea observed in older subjects. Advancing age attenuated Sa and Ea velocity but augmented LV EF, a documented phenomenon of aging (155) that may represent a compensatory response to impaired longitudinal function. Gender-related differences were observed in LV function with women having significantly lower Sa velocity and higher E/Ea compared to men.

Conclusion

Ethnicity-related differences exist in cardiac structure and sensitive parameters of LV function. A highly-phenotyped cohort was studied, in whom the effects of confounding factors have been largely obviated. Demographic influences need to be considered during the echocardiographic evaluation of patients, particularly in those whom the assessment of LA volume, LV volumes, LV mass, longitudinal function and LV filling pressure are essential to identify the syndrome of heart failure, whether in the presence or absence of normal systolic function.
CHAPTER 8.  STUDY V - THE INCREASED PREVALENCE OF LEFT VENTRICULAR HYPERTROPHY AND CONCENTRIC REMODELING IN UK INDIAN ASIANS COMPARED TO EUROPEAN WHITES

8.1 Abstract

Objectives: Individuals of Indian Asian ethnicity living in the UK have at least a 50% higher CVD mortality rates compared to European whites. However, there is no prospective data available regarding LVH prevalence, a powerful prognosticator of CVD events, in this ethnic group. We examined the prevalence of LVH and the degree of concentric remodeling amongst healthy UK Indian Asians compared with European whites in the LOLIPOP study.

Methods: Transthoracic echocardiography was performed in 2,232 subjects aged 35-75 years without clinical CVD. The presence of LVH was defined as an absolute increase in indexed exceeding internally derived gender- and ethnicity-specific partition values (mean + 2 s.d.) from a reference sub-group of LOLIPOP participants. RWT was calculated to provide a measure of concentric remodeling.

Results: Indian Asians had significantly higher 24-hour ambulatory BP, treatment for hypertension and prevalence of type-2 diabetes than European whites. The prevalence of LVH was significantly higher amongst Indian Asian men as compared to European white men, with an unadjusted odds ratio(OR) of 1.8 (95% CI:1.4 to 2.6). Following adjustment for clinical and haemodynamic variables, the magnitude of this effect increased (OR 2.8, 95% CI :1.9 to 4.2). Significant differences in the odds of LVH amongst Indian Asian women compared to European white women were not observed (adjusted OR 1.1, 95% CI: 0.6 to
The degree of concentric remodeling was higher amongst Indian Asian men and women as compared to their European white counterparts (adjusted RWT for men: 0.41 vs 0.39, p<0.001; for women: 0.40 vs 0.38, p<0.01).

**Conclusions:** This study demonstrates an increased prevalence of LVH amongst Indian Asian men and a greater degree of concentric remodeling amongst Indian Asian men and women as compared to their European white counterparts. Investigation of the mechanisms underlying the pathogenesis of LV remodeling and BP aetiology may help address the excess CVD mortality observed in Indian Asians.
8.2 Introduction

Migrant Indian Asians living in the United Kingdom (UK) have at least a 50% higher risk of CVD mortality than the native population (96,212), but as yet there are no validated tools capable of identifying their excess risk as compared to European whites. Indian Asians are more susceptible to metabolic syndrome hence a greater risk of developing type-2 diabetes and hypertension. However, the overall burden of “traditional” cardiovascular risk factors, tend to be lower amongst Indian Asians compared to European whites (220,221).

LVH is a form of cardiac remodeling strongly associated with major cardiovascular events independent of BP, known risk factors and coronary artery disease (50,69-71). Although ethnic disparities in this important phenotype have been demonstrated (129,130), the prevalence of LVH amongst Indian Asians remains unknown. The pattern of LV remodeling may also carry independent prognostic information to LVH (75). Hypertrophic and non-hypertrophic concentric remodeling, in particular, appear to be associated with the greatest risk of future cardiovascular events (76,80-82). However, there is again no prospective data reported on this phenotype amongst Indian Asians, either in the UK or elsewhere in the world.

We compared the prevalence of LVH, degree of concentric remodeling and quantitative measures of LV function between UK Indian Asians and European whites recruited into the LOLIPOP study.

8.3 Methods

We randomly selected 2,288 Indian Asian and European white subjects, aged 35-74 years and free from clinical CVD between August 2004 and November 2007 from the LOLIPOP study.
The study was approved by the Northwick Park and Ealing Hospitals Research Ethics Committees. Consenting subjects provided a full medical history and underwent physical assessment including BP determination, anthropometric measurements, bioimpedance for adipose and LBM measurements (Tanita Body Composition Analyser, Tanita Europe) and an ECG.

**Blood pressure assessment**

Office BP was measured in a seated position using an automated device, with the average of three separate measurements at 1 minute intervals recorded as the resting BP. 24-hour BP monitors were fitted on all subjects, providing measures of mean systolic and diastolic ambulatory BP.

**Echocardiography**

Transthoracic 2-D echocardiography was performed to obtain measurements of LV cavity and wall dimensions to obtain an estimate of LV mass as described in chapter 5.3. The presence of LVH was defined as an absolute increase in LVM exceeding internally derived gender- and ethnicity-specific partition values (mean + 2 s.d.) from a reference sub-group of LوليPOP participants (n=453), who have been previously characterised (chapter 4). Briefly, they consisted of normotensive individuals who were also free of clinical CVD, significant coronary artery disease, treatment for or evidence of other modifiable cardiovascular risk factors such as hypertension, dyslipidaemia, type-2 diabetes and smoking. In this reference subgroup, LVM indexed by LBM fully removed the correlation of this measure with weight (p=0.32) and height (p=0.06) suggesting that this indexation method, which has been validated to identify LVH compared to height-based indices (222,223), was the most appropriate for this cohort. When indexation of LV mass was performed with measures of
height or its allometric power (height$^{2.7}$), the correlation with weight remained highly significant ($p<0.001$). Indexation by BSA did not remove either the correlation with height nor weight (both $p<0.001$), but LVM and LVH indexed to BSA are still described in this study for reference and comparison.

RWT was used to assess the degree of concentric remodeling and was calculated as described in chapter 5.3. LV end-diastolic and end-systolic volumes indexed to body surface area were measured using Simpson’s apical biplane rule from which stroke volume was derived. LV EF was automatically calculated following acquisition of the LV volumes using the Simpson’s method. Fractional shortening was calculated as: (LV dimension in diastole – LV dimension in systole)/LV dimension in diastole.

Longitudinal myocardial function was quantified using on-line, pulse-wave tissue Doppler (TD) imaging, as described in chapter 5.3. Peak velocities (cm/sec) during systole (Sa) and early diastole (Ea) were measured on-line from all four mitral annular sites and then averaged. An estimate of LV filling pressure was obtained from the ratio of and the ratio of transmitral E-wave velocity and averaged Ea velocity (E/Ea).

**Statistical analysis**

Clinical and echocardiographic data were stratified according to ethnicity and gender with European white men and European white women serving as reference groups for comparison against Indian Asian men and Indian Asian women respectively. ANOVA was used to test differences of continuous variables with Bonferroni’s correction for multiple comparisons. The chi-square test was used to test differences between categorical data amongst the groups. Prevalence of LVH with and without indexation for LBM and BSA were firstly presented amongst the reference sub-group and in the entire cohort. Unadjusted and adjusted
odds ratios (OR) for LVH amongst Indian Asian men and Indian Asian women compared to their European gender equivalents were then calculated. ORs were compared for different covariates with sequential adjustment using the following models: model 1 adjusted for BP variables (mean 24-hour systolic BP, mean 24-hour diastolic BP, use of antihypertensive medications); model 2 adjusted for metabolic variables (type 2 diabetes and adipose mass) in addition to model 1 and model 3 which adjusted for haemodynamic variables (stroke volume and fractional shortening) in addition to model 2.

The relationships of ethnicity and gender with RWT and with the tissue Doppler parameters of LV function were evaluated separately by ANCOVA in a main-effects design, using Type III sum of squares and Bonferroni’s correction for multiple comparisons. Adjustments for relevant confounders were made including age, mean 24-hour systolic BP, mean 24-hour diastolic BP, use of antihypertensive medication, presence of type-2 diabetes, adipose mass and LV mass indexed to LBM.

Statistical significance was defined as P < 0.05 and all statistical analyses were performed using SPSS version 17.

8.4 Results

Clinical and demographic characteristics

Of the 2,288 subjects enrolled into the study, 56 lacked completed echocardiographic measurements for derivation of LV mass. Of the remaining 2,232 subjects, 726 (33%) were European white males, 797 (37%) were Indian Asian males, 252 (11%) were European white females and 431 (19%) were Indian Asian females. Table 8.1 summarises the demographic, clinical and anthropometric characteristics of the cohort with comparisons made between the two ethnic groups and individuals of the same gender (e.g. European white men vs Indian
Asian men and European white women vs. Indian Asian women). Although office-measured BP did not differ significantly between the two ethnic groups, mean 24-hour systolic and diastolic BP were significantly higher amongst Indian Asian men and women as compared to their European white counterparts. There was also a significantly higher prevalence of antihypertensive medication therapy and type-2 diabetes amongst Indian Asians. Indian Asian men and women generally had smaller anthropometric measures such as height, BMI, BSA and LBM, whereas weight and adipose mass were lower in Indian Asian men compared to European white men only. Waist-hip ratio was higher in both Indian Asian men and women than in their European white counterparts.

**Left ventricular mass**

Table 8.2 displays LV mass, indexed by different methods, as well as measures of LV cavity dimension and function according to ethnicity-gender subgroups. Compared to their gender equivalents, Indian Asians had lower LV mass than European whites regardless of the indexation method used. The lower LV mass amongst Indian Asians was attributable to their smaller diastolic and systolic LV cavity dimension and thinner interventricular septum compared to European whites.
Table 8.1 - Clinical and anthropometric characteristics by ethnicity-gender subgroup

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>European white men n=726</th>
<th>Indian Asian men n=797</th>
<th>European white women n=278</th>
<th>Indian Asian women n=431</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 10</td>
<td>56 ± 10</td>
<td>58 ± 10</td>
<td>56 ± 10</td>
</tr>
<tr>
<td>24hour sBP (mmHg)</td>
<td>115 ± 12</td>
<td>118 ± 12 †</td>
<td>115 ± 14</td>
<td>118 ± 14 *</td>
</tr>
<tr>
<td>24hour dBP (mmHg)</td>
<td>77 ± 7</td>
<td>78 ± 8 *</td>
<td>73 ± 7</td>
<td>75 ± 7</td>
</tr>
<tr>
<td>Office sBP (mmHg)</td>
<td>136 ± 18</td>
<td>134 ± 18</td>
<td>125 ± 17</td>
<td>127 ± 22</td>
</tr>
<tr>
<td>Office dBP (mmHg)</td>
<td>83 ± 10</td>
<td>82 ± 10</td>
<td>77 ± 9</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>Antihypertensive medication</td>
<td>18</td>
<td>32 ‡</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>9</td>
<td>21 ‡</td>
<td>5</td>
<td>16 ‡</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176 ± 7</td>
<td>170 ± 7 ‡</td>
<td>163 ± 7</td>
<td>156 ± 6 ‡</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86 ± 15</td>
<td>78 ± 12 ‡</td>
<td>71 ± 14</td>
<td>68 ± 12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28 ± 5</td>
<td>27 ± 4 ‡</td>
<td>27 ± 5</td>
<td>28 ± 5 †</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94 ± 0.08</td>
<td>0.96 ± 0.06 ‡</td>
<td>0.86 ± 0.09</td>
<td>0.91 ± 0.09 ‡</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>2.0 ± 0.2</td>
<td>1.9 ± 0.2 ‡</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.2 ‡</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>62 ± 8</td>
<td>56 ± 6 ‡</td>
<td>44 ± 5</td>
<td>41 ± 4 ‡</td>
</tr>
<tr>
<td>Adipose mass (kg)</td>
<td>24 ± 10</td>
<td>22 ± 9 ‡</td>
<td>27 ± 10</td>
<td>27 ± 8</td>
</tr>
</tbody>
</table>

*p<0.05, †p<0.01, ‡p<0.001 for European white men vs Indian Asian men and for European white women vs Indian Asian women

sBP = systolic blood pressure, dBP = diastolic blood pressure, BMI = body mass index, WHR = waist to hip ratio, BSA – body surface area, LBM = lean body mass
Table 8.2 - Echocardiographic parameters of left ventricular structure and function by ethnicity-gender subgroup

<table>
<thead>
<tr>
<th></th>
<th>European white men</th>
<th>Indian Asian men</th>
<th>European white women</th>
<th>Indian Asian women</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass (g)</td>
<td>201 ± 52</td>
<td>168 ± 46 ‡</td>
<td>151 ± 44</td>
<td>130 ± 35 ‡</td>
</tr>
<tr>
<td>LVMI to BSA (g/m²)</td>
<td>96 ± 23</td>
<td>86 ± 22 ‡</td>
<td>82 ± 21</td>
<td>74 ± 19 ‡</td>
</tr>
<tr>
<td>LVMI to LBM (g/kg)</td>
<td>3.3 ± 0.8</td>
<td>3.0 ± 0.8 ‡</td>
<td>3.4 ± 0.9</td>
<td>3.2 ± 0.8 ‡</td>
</tr>
<tr>
<td>LV diastole (cm)</td>
<td>5.2 ± 0.5</td>
<td>4.8 ± 0.5 ‡</td>
<td>4.8 ± 0.5</td>
<td>4.5 ± 0.5 ‡</td>
</tr>
<tr>
<td>LV systole (cm)</td>
<td>3.5 ± 0.5</td>
<td>3.1 ± 0.5 ‡</td>
<td>3.2 ± 0.4</td>
<td>2.9 ± 0.4 ‡</td>
</tr>
<tr>
<td>IVS thickness (cm)</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2 ‡</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2 ‡</td>
</tr>
<tr>
<td>PW thickness (cm)</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.2 ‡</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>0.39 ± 0.10</td>
<td>0.40 ± 0.09 †</td>
<td>0.37 ± 0.08</td>
<td>0.39 ± 0.08 *</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>54 ± 13</td>
<td>43 ± 11 ‡</td>
<td>42 ± 11</td>
<td>35 ± 8 ‡</td>
</tr>
<tr>
<td>Fractional shortening</td>
<td>0.34 ± 0.07</td>
<td>0.35 ± 0.08 †</td>
<td>0.34 ± 0.06</td>
<td>0.35 ± 0.09</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>61 ± 6</td>
<td>62 ± 6</td>
<td>63 ± 5</td>
<td>63 ± 6</td>
</tr>
</tbody>
</table>

* P<0.05, †p<0.01,‡p<0.001 for European white men vs Indian Asian men and for European White women vs Indian Asian women

LV = left ventricular, LVMI = left ventricular mass index, IVS = interventricular septal thickness, PW = posterior wall thickness, EF = ejection fraction
Prevalence of LVH

The internal partition values for determining the presence of LVH using different indexation methods are provided in table 8.3, with these cut-offs representing the mean + 2 s.d. values amongst the reference sub-group, free of modifiable cardiovascular risk factors and significant coronary artery disease.

Table 8.3 - Internal partition values for defining left ventricular hypertrophy

<table>
<thead>
<tr>
<th></th>
<th>European white men</th>
<th>Indian Asian men</th>
<th>European white women</th>
<th>Indian Asian women</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass (g)</td>
<td>266</td>
<td>208</td>
<td>202</td>
<td>176</td>
</tr>
<tr>
<td>LV mass/BSA (g/m²)</td>
<td>127</td>
<td>107</td>
<td>110</td>
<td>101</td>
</tr>
<tr>
<td>LV mass/LBM (g/kg)</td>
<td>4.3</td>
<td>3.7</td>
<td>4.7</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Internal partition values represent the mean + 2 s.d. derived from a reference sub-group without modifiable cardiovascular risk factors and low coronary artery calcium score

Ethnicity- and gender-specific prevalence of LVH amongst the reference sub-group, with a low cardiovascular risk factor and coronary artery disease burden, and amongst the entire cohort is illustrated in figure 8.1. Prevalent LVH was low in the reference sub-group (< 5%), with no difference in prevalence amongst Indian Asians and European whites of either gender regardless of indexation method. In the entire cohort LVH prevalence was much higher than amongst the reference group, being highest amongst Indian Asian men (LVH-BSA 17%, LVH-LBM 20%) which was significantly higher than observed amongst European white men (LVH-BSA 10%, LVH-LBM 11%, p<0.001).
Similar prevalences of left ventricular hypertrophy (LVH) are observed amongst European white and Indian men in the reference sub-group. However, in the overall cohort Indian Asian men had significantly higher prevalence of LVH compared to European white men, regardless of LVH indexation method (p<0.001). Significant differences in LVH prevalence were not observed between European white and Indian Asian women, in either the reference group nor whole cohort. BSA = body-surface area, LBM = lean body mass.

In unadjusted models, Indian Asian men had approximately twice the odds of having LVH as European white men (table 8.4). Sequential adjustment for age, BP, metabolic and haemodynamic variables further increased this effect size. However, significant differences in the ORs between European white and Indian Asian women were not observed in unadjusted or adjusted analyses.
### Table 8.4 - Odds ratios of left ventricular hypertrophy amongst Indian Asian men and women, after sequential adjustment for covariates

<table>
<thead>
<tr>
<th></th>
<th>Indian Asian men</th>
<th>Indian Asian women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVH/LBM</td>
<td>LVH/BSA</td>
</tr>
<tr>
<td><strong>Unadjusted</strong></td>
<td>1.9 (1.4-2.6)</td>
<td>1.7 (1.2-2.5)</td>
</tr>
<tr>
<td><strong>Model 1 (+age)</strong></td>
<td>2.2 (1.6-3.0)</td>
<td>1.9 (1.4-2.7)</td>
</tr>
<tr>
<td><strong>Model 2 (+ BP variables)</strong></td>
<td>1.9 (1.4-2.7)</td>
<td>1.7 (1.2-2.5)</td>
</tr>
<tr>
<td><strong>Model 3 (+ metabolic variables)</strong></td>
<td>2.2 (1.5-3.1)</td>
<td>1.7 (1.2-2.4)</td>
</tr>
<tr>
<td><strong>Model 4 (+ haemodynamic variables)</strong></td>
<td>2.8 (1.9-4.2)</td>
<td>2.5 (1.6-3.7)</td>
</tr>
</tbody>
</table>

Odds ratio of LVH for Indian Asian males vs European white males and in Indian Asian females vs. European white females. Blood pressure (BP) variables are mean 24-hour systolic BP, mean 24-hour diastolic BP and use of antihypertensive medications. Metabolic variables include presence of type 2 diabetes and adipose mass. Haemodynamic variables include fractional shortening and stroke volume.

### Relative wall thickness

Indian Asian men and women had a greater degree of concentric LV remodeling than European whites, as evidenced by their higher RWT after adjustment for covariates (table 8.5).
Table 8.5 - Adjusted relative wall thickness according to ethnicity and gender

<table>
<thead>
<tr>
<th></th>
<th>European white men</th>
<th>Indian Asian men</th>
<th>European white women</th>
<th>Indian Asian women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.39 (0.38,0.40)</td>
<td>0.41 (0.40,0.42) ‡</td>
<td>0.38 (0.36, 0.39)</td>
<td>0.40 (0.39,0.41) †</td>
</tr>
</tbody>
</table>

Vales represent estimated marginal means of relative wall thickness (RWT) with confidence intervals. †p<0.01, ‡p<0.001 for differences between Indian Asian men vs European white men and Indian Asian women vs European white women. RWT adjusted for age, mean 24-hour systolic BP, mean 24-hour diastolic BP, LVMI (BSA), use of antihypertensives, presence of type-2 diabetes and adipose mass.

**Myocardial function and LV filling pressure**

The differences in myocardial longitudinal Sa velocity, Ea velocity and the E/Ea ratio are summarised in figure 8.2. After adjustment for covariates, Indian Asian men had significantly lower Sa velocity, Ea velocity and significantly higher E/Ea than European white men. Indian Asian women also demonstrated similarly impaired longitudinal function and increased LV filling pressure compared to European white women, however, the difference in Ea velocity was of borderline statistical significance.
Myocardial longitudinal systolic (Sa) velocity, early diastolic (Ea) velocity and estimate of LV filling pressure (E/Ea) adjusted for age, type-2 diabetes, antihypertensive treatment, mean 24-hour systolic BP, mean 24-hour diastolic BP, adipose mass and LV mass indexed to lean body mass.
8.5 Discussion

This is the first study to report an increased prevalence of LVH amongst migrant Indian Asians compared to European whites. Despite having a lower indexed LV mass, Indian Asian men had a 3-fold increased odds of LVH compared to European white men. Similar LVH prevalences were observed between Indian Asian and European white women, although both Indian Asian men and women were observed to have a greater degree of LV concentric remodeling compared to European whites, a phenotype also associated with hypertensive heart disease. This study has important strengths, including population-based recruitment, use of a well-phenotyped reference cohort for definition of ethnicity and gender specific partition values for LVH and measurement of ambulatory BP.

Compared to European whites, migrant Indian Asians living in the UK are known to have significantly higher risk of death from CVD (96,212) and incidence rates for congestive heart failure (213). The burden of CVD in UK Indian Asians appears greatest in young men in whom the relative risk is at least twofold higher compared to European whites (98). LVH is an important cardiovascular phenotype known to confer an increased risk of heart failure, coronary artery disease, stroke, arrhythmia and mortality (224). Studies assessing the association of ethnicity with LVH have predominantly been conducted in American black, white and Hispanic populations. However, to the best of our knowledge, the prevalence of LVH amongst Indian Asians in the UK, or elsewhere in the world, has not been reported in the context of a population-based study.

The increased prevalence of LVH observed amongst Indian Asian men compared to European white men may be attributable to their increased BP load, an established requirement for antihypertensive medications, and an increased prevalence of type-2 diabetes.
Several studies have supported the existence of a distinct diabetic cardiomyopathic process, characterised by increased LV mass and impaired LV function independent of relevant covariates including BP (225-227). Target-organ damage in the form of end-stage renal failure is ~4-fold higher amongst UK Indian Asians compared to European whites, with hypertension and diabetes again implicated as the most likely causal factors (228). However, despite a similar burden of hypertension and diabetes amongst Indian Asian women compared to their European white counterparts, a predilection for LVH was not observed as in the men. These findings should stimulate further research into the determinants of the hypertrophic response amongst Indian Asians, namely known pro-hypertrophic mediators such as angiotensin II (229), endothelin (230), heterotrimeric G proteins (231) and genetic factors such as expression of the DD genotype of the ACE gene, which is associated with an increased risk of LVH amongst middle-aged Caucasian men (232). There has been scant research into the pathophysiology of hypertension amongst Indian Asians and this may hinder optimisation of BP control in this ethnic group. In blacks, for example, it is known that hypertension is of the low renin type with an augmented sensitivity of BP to dietary salt intake and an impaired ability to excrete ingested salt (233). Accordingly, restriction of dietary salt to treatment with diuretics and calcium channel blockers have proven to be highly efficacious strategies in BP lowering amongst blacks (234-236).

In this study Indian Asians demonstrated a greater degree of concentric remodeling, expressed as the RWT, compared to European whites. Concentric remodeling of the left ventricle tends to manifest in situations of pressure overload resulting in radial growth and thickening of the myocyte. Of the different patterns of LV remodeling, concentric hypertrophy is associated with the greatest risk of future cardiovascular events (76) and is believed to ultimately progresses to LV dilatation and failure in hypertensives (77-79). There
is also evidence that non-hypertrophic concentric remodeling is associated with worse prognosis compared to subjects with normal LV geometry (80-82).

Both hypertrophic and non-hypertrophic remodeling of the heart are associated with subclinical impairment of LV function and increases in LV filling pressure (13,66,177,237). We observed Indian Asian men and women to have significantly impaired myocardial longitudinal function and LV filling pressure compared to European white counterparts, commensurate with their increased propensity to abnormalities in cardiac structure.

Conclusion

This study demonstrates an increased prevalence of LVH, a greater degree of concentric remodeling and worse LV function amongst Indian Asian men as compared to their European white counterparts. Although Indian Asians had a higher BP burden and prevalence of type-2 diabetes, the differences in LVH observed were independent of these and other clinical covariates. Additional studies are required to better understand the hypertension aetiology and mechanism of the LV remodeling that occurs in Indian Asians. Such research may help address the striking excess of CVD mortality currently afflicting individuals of Indian Asian ethnicity.
CHAPTER 9. STUDY VI - DOES SUBCLINICAL ATHEROSCLEROSIS

BURDEN IDENTIFY THE INCREASED RISK OF CARDIOVASCULAR
DISEASE MORTALITY AMONGST UK INDIAN ASIANS?

9.1 Abstract

Background: Indian Asians living in the United Kingdom (UK) have at least a 50% higher risk of dying from CVD compared to European whites. The mechanisms underlying their excess mortality are not clear and there are no validated tools capable of identifying this increased risk. The burden and morphology of subclinical atherosclerosis detected in the carotid arteries are established prognosticators for major CVD events.

Objectives: We hypothesized that the increased prevalence of CVD amongst Indian Asians would be reflected by their having a greater burden of subclinical carotid artery atherosclerosis with evidence of vulnerable, echolucent plaque disease, compared to European whites.

Methods: We studied 2,263 healthy subjects and 148 patients with known CVD from the LOLIPOP study, who underwent carotid ultrasonography for measurement of IMT, plaque prevalence and plaque echogenicity.

Results: After adjustment for covariates, the prevalence of CVD was significantly higher amongst Indian Asians, compared to European whites (odds ration [OR] with [95% confidence interval] = 1.72 [1.2-2.3]). There were no significant differences in IMT, plaque prevalence nor plaque echogenicity between the two ethnic groups, regardless of CVD status. Age and systolic BP (adjusted β 0.43 and 0.23 respectively, both p<0.001) were the strongest
independent predictors of IMT. Lipid-lowering therapy (odds-ratio [OR] 1.72, p<0.001), antihypertensive therapy (OR 1.46, p=0.002) and smoking history (OR 1.36, p=0.004) were the strongest independent predictors of plaque prevalence.

**Conclusion:** The burden of carotid atherosclerosis does not identify the markedly increased risk of CVD amongst UK Indian Asians. Other markers and mechanisms of disease require investigation in this high-risk group.
9.2 Introduction

Indian Asians comprise one-quarter of the world’s population and are projected to account for ~40% of the global CVD burden by 2020 (110). A striking excess in CVD mortality is already apparent amongst migrant Indian Asians, particularly those who reside in the UK, where CHD and stroke mortality remains at least 50% higher, and in some subgroups over twice that of the native European white population (212). However, the excess risk amongst Indian Asians is not accounted for by “traditional” CVD risk factors, the burden of which tends to be lower than in European whites. As a result, conventional risk scoring algorithms such as the Framingham Risk Score underestimate CVD risk amongst Indian Asians (220,221).

The inability to identify high-risk individuals on the basis of classical risk factors alone has prompted the search for biomarkers of CVD risk that can detect subclinical disease at an early stage. Increased IMT and the presence of atheromatous plaques are forms of carotid artery disease that are both regarded as surrogate markers of atherosclerosis and strongly predictive of incident myocardial infarction, stroke and death (32-35). There is also growing evidence that the morphological characteristics of carotid plaques, as determined by ultrasonography, reflect their vulnerability to rupture and cause clinical events (36-40). Mature plaques which are calcified, fibrotic and more echogenic are thought to be less prone to rupture than “soft”, echolucent plaques rich in lipids, haemorrhage and elastin.

Although these biomarkers have been extensively studied in other populations, they have not yet been examined in a prospective manner in a large cohort. We hypothesised that the excess risk of CVD amongst UK Indian Asians would be reflected by evidence of an
increased burden of vulnerable, carotid atherosclerosis as compared to European whites, in a population with and without prior history of clinical CVD.

9.3 Methods

Indian Asian and European White subjects, aged 35-75 years were recruited between August 2004 and November 2007 from the LOLIPOP study. Subjects with a history of CVD (n=148) were also recruited allowing prevalence of CVD between the ethnic groups to be compared and also to validate the relationships between subclinical atherosclerosis, CVD status and ethnicity. Clinical CVD was defined as individuals with either: coronary artery disease defined as self-reported admission with myocardial infarction; or silent myocardial infarction (Minnesota criteria for Q waves); or surgical/percutaneous coronary revascularisation or self-reported/physician confirmed cerebrovascular disease. Consenting subjects had a physical assessment including BP determination, anthropometric measurements and an ECG. The assessment of participants was carried out by trained research nurses, during a 45 minute appointment, according to a standardised protocol and with regular quality-control audits. An interviewer-administered questionnaire was used to collect data on medical history, family history, current prescribed medication (verified from the practice computerised records) and cardiovascular risk factors. CVD status was confirmed by a physician through verification with GP and hospital records.

Subjects were then invited to undergo echocardiography, carotid ultrasonography and provide fasting plasma and serum samples for biochemical analysis including total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and glucose. The study was approved by the Northwick Park and Ealing Hospitals Research Ethics Committees.
**Carotid ultrasonography**

Carotid duplex scans were conducted using a high-resolution, non-harmonic B-mode ultrasound system (Philips IE33) with an 11- to 3-Mhz transducer. The protocol for performing ultrasonography for quantification of IMT and plaque disease has been described in chapter 6.3.

Each plaque was classified by a single expert reader, according to Gray-Weale (134) classification as: Type 1 (predominantly echolucent with thin echogenic cap); Type 2 (intermediate echolucent lesions with small areas of echogenicity); Type 3 (intermediate echogenic lesions with small areas of echolucency <25%) and Type 4 (uniformly echogenic lesions). An echogenicity score was derived for each patient from the product of the plaque type and plaque number, divided by the total number of plaques. For example the echogenicity score for a subject with 3 type 2 plaques and 1 type 3 plaque was calculated as: 

\[(3 \times 2 + 1 \times 3)/3 = 3.\]

**Statistics**

Data is presented as mean ± standard deviation. Student’s t-test was performed to compare differences in continuous variables and chi-squared test to assess differences in categorical variables between the two groups. The sum of plaques detected in both carotid arteries provided a plaque score for each subject, allowing subsequently categorisation into 1 of 4 groups (0, 1-2, 3-5 and >5 plaques). Subjects were also classified according to carotid artery disease status, defined as the presence of either carotid plaque disease and/or a mean IMT measurement > 75th age-specific percentile values obtained from our own healthy, reference cohort(238).
ANOVA was used to assess differences in mean IMT between ethnicity-gender grouping, using Bonferroni correction for multiple comparisons. Independent correlates of IMT were identified by multiple linear regression using stepwise procedure (p<0.05). Multiple logistic regression was used to identify independent correlates of plaque prevalence again using a stepwise method (p<0.05) and also to define odds-ratios for ethnicity and CVD prevalence, after adjustment for risk factors.

**Interobserver variability**

Interobserver variability for IMT measurement was assessed in 14 subjects between two sonographers who acquired carotid images and performed off-line analysis independently from each other. The mean IMT difference ranged from 0.00 - 0.01 mm at the common carotid artery with a coefficient of variance of 6.8 - 8.5%. At the carotid bifurcation the mean IMT difference was 0.03 - 0.05 mm with a coefficient of variance of 9.4 - 10.5%.

**9.4 Results**

Of the 2,439 subjects enrolled into the study, 2,411 had satisfactory carotid ultrasound scans permitting both IMT to be measured and plaque prevalence to be determined. 2,263 subject recruited were asymptomatic, and 143 had a prior history of clinical CVD. Of these subjects, 1058 (44%) were of European white ethnicity and 1353 (56%) were Indian Asian. Table 9.1 summarises the main demographic and clinical characteristics of the whole cohort according to ethnicity. European whites had a greater burden of traditional cardiovascular risk factors compared to their Indian Asian counterparts as reflected in their significantly higher 10-year Framingham Risk Score for coronary heart disease. European whites were older, more likely to be male, have a history of smoking and had higher total, LDL- and HDL-cholesterol. However, Indian Asians had a greater prevalence of clinical CVD, type 2 diabetes, treatment
for hypertension, treatment with lipid lowering therapy, higher waist-hip-ratio, higher HbA1c and higher triglycerides.

**Table 9.1 - Clinical characteristics of the cohort by ethnicity**

<table>
<thead>
<tr>
<th></th>
<th>European white N=1058</th>
<th>Indian Asian N= 1353</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.9 ± 9.8</td>
<td>57.1 ± 9.8</td>
<td>0.04</td>
</tr>
<tr>
<td>Male (%)</td>
<td>73</td>
<td>65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CVD (%)</td>
<td>4</td>
<td>8</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>133 ± 19</td>
<td>132 ± 19</td>
<td>0.17</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81 ± 10</td>
<td>80 ± 10</td>
<td>0.05</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>52 ± 13</td>
<td>51 ± 14</td>
<td>0.05</td>
</tr>
<tr>
<td>Antihypertensive treatment</td>
<td>19</td>
<td>30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipid lowering therapy (%)</td>
<td>10</td>
<td>19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Type 2 diabetes (%)</td>
<td>8</td>
<td>19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.5 ± 1.6</td>
<td>6.0 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.5 ± 1.0</td>
<td>6.1 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.6 ± 1.1</td>
<td>5.2 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.5 ± 0.9</td>
<td>3.2 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.6 ± 1.1</td>
<td>1.7 ± 1.0</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5 ± 4.7</td>
<td>27.2 ± 4.1</td>
<td>0.06</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92 ± 0.1</td>
<td>0.96 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ever smoker (%)</td>
<td>54</td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10 year CHD Framingham risk (%)</td>
<td>10.4 ± 7</td>
<td>8.9 ± 7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BP = blood pressure, HbA1c = glycosylated haemoglobin fraction, LDL = low-density lipoprotein, HDL = high-density lipoprotein, BMI = body mass index, WHR = waist:hip ratio, CHD = coronary heart disease
Carotid atherosclerosis amongst healthy subjects

The degree and morphology of carotid artery disease was compared between the two ethnic groups after excluding individuals with evidence of manifest CVD (table 9.2). There were no significant differences in IMT, plaque prevalence, carotid artery disease prevalence (a composite of increased IMT and/or plaque disease) or plaque echogenicity.

Table 9.2 - Ethnicity and carotid artery disease burden in healthy individuals free of clinical cardiovascular disease

<table>
<thead>
<tr>
<th></th>
<th>European white N = 1012</th>
<th>Indian Asian N = 1251</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean IMT (mm)</td>
<td>0.66 ± 0.11</td>
<td>0.65 ± 0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Carotid plaque prevalence</td>
<td>45% (469/1032)</td>
<td>45% (567/1265)</td>
<td>0.77</td>
</tr>
<tr>
<td>Carotid artery disease prevalence</td>
<td>60% (616/1032)</td>
<td>59% (741/1265)</td>
<td>0.59</td>
</tr>
<tr>
<td>Plaque echogenicity score</td>
<td>1.85</td>
<td>1.83</td>
<td>0.63</td>
</tr>
</tbody>
</table>

IMT = intima-media thickness

When individuals were stratified according to ethnicity and gender, there were no differences in IMT at any age (figure 9.1) and there were no significant differences in the burden of plaque disease either (figure 9.2). To validate the relationship between carotid atherosclerosis with risk factor burden we assessed the correlation between IMT and Framingham risk score, in both ethnic groups, excluding those with clinical CVD (figure 9.3).
A linear association is seen between age and IMT. Almost identical IMT is observed between European whites and Indian Asians of the same gender, and at all ages: European white males (n=729, mean IMT = 0.67mm) vs. Indian Asian males (n=810, mean IMT = 0.66mm), p=0.99; European white females (n=283, mean IMT = 0.64) vs Indian Asian females (n=441, mean IMT = 0.63), p=0.99. Means compared using ANOVA with Bonferroni correction for multiple comparisons.
A similar total number of carotid plaques were detected in European white versus Indian Asian subjects, ($p = 0.08$, $\chi^2$ test).
Figure 9.3 - Correlation between cardiovascular risk factor burden and carotid IMT

There is a positive, linear association between Framingham Risk Score and carotid IMT in both European white (r=0.38, p<0.001) and Indian Asian (r=0.36, p<0.001) individuals without clinical CVD.
Which demographic (age, race, gender), cardiovascular risk (systolic BP, diastolic BP, type 2 diabetes, glucose, HbA1c, total cholesterol, LDL-cholesterol, HDL-cholesterol, BMI, smoking) and risk modification (antihypertensive and lipid lowering therapy) variables were independent correlates of carotid IMT (table 9.3) and presence of carotid plaque disease (table 9.4) were then assessed. Age and systolic BP were the strongest positive correlates with IMT whereas HDL-cholesterol, diastolic BP and female gender showed weaker, negative relationships. The strongest independent predictors of plaque disease were antihypertensive treatment, lipid lowering therapy, history of smoking and LDL-cholesterol, with weaker associations observed for age, BP and gender. However, ethnicity was not independently associated with either IMT or plaque disease.

**Table 9.3 - Independent correlates of intima-media thickness as determined by step-wise linear regression amongst healthy individuals free of clinical cardiovascular disease**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised coefficient</th>
<th>Partial R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.43</td>
<td>0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.23</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-0.16</td>
<td>-0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.09</td>
<td>-0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.08</td>
<td>0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female gender</td>
<td>-0.08</td>
<td>-0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.08</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ever smoker</td>
<td>0.07</td>
<td>0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.05</td>
<td>0.06</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Abbreviations as for table 9.1
Table 9.4 - Independent predictors of plaque disease prevalence using step-wise logistic regression amongst individuals free of clinical cardiovascular disease

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>Odds ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid lowering therapy</td>
<td>0.54</td>
<td>1.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensive therapy</td>
<td>0.38</td>
<td>1.46</td>
<td>0.002</td>
</tr>
<tr>
<td>Ever smoker</td>
<td>0.31</td>
<td>1.36</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.22</td>
<td>1.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.14</td>
<td>1.15</td>
<td>0.004</td>
</tr>
<tr>
<td>Age</td>
<td>0.06</td>
<td>1.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.02</td>
<td>1.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-0.02</td>
<td>0.98</td>
<td>0.007</td>
</tr>
<tr>
<td>Female gender</td>
<td>-0.50</td>
<td>0.70</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations as for table 9.1

**Carotid atherosclerosis burden in individuals with clinical cardiovascular disease**

Prevalence of atherosclerosis was significantly higher in individuals with manifest CVD compared to asymptomatics (figure 9.4). In the overall cohort, the prevalence of established CVD was higher amongst Indian Asians compared to European whites (8% vs 4%, p=0.001). After adjustment for covariates (age, gender, systolic BP, diastolic BP, T2D, antihypertensive treatment, BMI, smoking, total cholesterol, LDL-cholesterol and HDL-cholesterol) Indian Asians had 72% higher odds of having CVD compared to European white men (OR 1.72, CI 1.2-2.3, p=0.03). However, this increased prevalence of CVD was not
reflected by an increased burden of carotid atherosclerosis amongst Indian Asians, with similarities remaining in IMT, plaque prevalence and plaque burden compared to European whites (table 5).

**Figure 9.4 - Prevalence of carotid artery disease versus CVD status**

![Bar chart showing prevalence of carotid artery disease](image)

There is a significantly higher prevalence of carotid atherosclerosis, defined as either IMT > 75\textsuperscript{th} percentile and/or presence of plaque disease, in those individuals with established CVD compared to asymptomatic individuals, in both European white and Indian Asians.
Table 9.5 - Carotid artery disease burden in individuals with prior history of cardiovascular disease

<table>
<thead>
<tr>
<th></th>
<th>European white N= 51</th>
<th>Indian Asian N= 97</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid IMT (mm)</td>
<td>0.72 ± 0.11</td>
<td>0.72 ± 0.13</td>
<td>0.74</td>
</tr>
<tr>
<td>Plaque prevalence (%)</td>
<td>67</td>
<td>71</td>
<td>0.77</td>
</tr>
<tr>
<td>Carotid atherosclerosis prevalence (%)</td>
<td>78</td>
<td>79</td>
<td>0.96</td>
</tr>
<tr>
<td>Plaque echogenicity score</td>
<td>2.0 ± 0.5</td>
<td>2.0 ± 0.5</td>
<td>0.40</td>
</tr>
</tbody>
</table>

9.5 Discussion

This is the first prospective study to have assessed the burden of carotid artery disease in an unselected cohort of UK Indian Asian and European white individuals. The findings from this study suggest a paradox, as despite their having markedly higher CVD prevalence, Indian Asians had a similar burden of subclinical atherosclerosis to European whites.

The degree of subclinical carotid artery disease, particularly in the form of plaque, has been well validated as a biomarker of CVD risk even in individuals with a low burden of conventional cardiovascular risk factors. Alterations in arterial structure predate clinical manifestations of occlusive atherosclerotic disease and tend to be widespread rather than limited to a single arterial bed (18). Increased carotid IMT is associated with cardiovascular risk factors (19,20), prevalent CVD (21,22) and the presence and extent of angiographically determined coronary atherosclerosis (23,24). Identification of such abnormalities in accessible peripheral arteries provides a means for early detection of presymptomatic vascular disease and improved cardiovascular risk stratification. However, the role of atherosclerosis imaging has not been well studied or validated in ethnic groups and
prospective data in the high-risk Indian Asian population is lacking. We demonstrated in our cohort that carotid artery disease was closely correlated with Framingham risk score and with prevalent CVD in both ethnic groups, justifying its role as a biomarker of cardiovascular risk in both ethnic groups. Subclinical atherosclerosis in the coronary arteries has previously been quantified in this cohort by electron beam computed tomography, with the results again suggesting equivalent burdens of coronary artery calcification between the two ethnic groups (136). The discord between atherosclerosis burden and apparent CVD risk in UK Indian Asians is further supported by results from a smaller, Canadian, cross-sectional study where Indian Asians were observed to have lower age- and gender-adjusted IMT than native European whites. Although IMT was slightly but significantly higher amongst European whites in our study, after adjustment for covariates, ethnicity was found neither to be an independent predictor of IMT nor presence of plaque disease. These two phenotypes of carotid artery disease were associated with different covariates/risk factors suggesting they represent distinct pathophysiology, rather than both being synonymous with subclinical atherosclerosis.

Although the burden and severity of atherosclerosis is associated with likelihood of future clinical cardiovascular events, plaque rupture is the most common plaque complication, accounting for ~70% of fatal acute myocardial infarctions and/or coronary deaths (239,240). Nonstenotic lesions are more frequent than stenotic disease and also account for the majority of culprit ruptured plaques (241-243). Therefore, biological factors known to influence, trigger or identify imminent plaque events are constantly being sought. The composition of a plaque represents the underlying pathological mechanisms at cellular and molecular levels, hence its susceptibility to rupture. Plaques exhibiting thin fibrous caps, large lipid cores, intraplaque haemorrhage and macrophage-dense inflammation are more
susceptible to future clinical, atherothrombotic events (10). Ultrasound imaging permits analysis of plaque morphology with hypoechoic plaques associated with both intraplaque haemorrhage and lipids. Hyperechoic plaques are mostly mature calcific lesions that although implicated in flow-limiting, chronic disease, are less likely to rupture spontaneously. The concept of plaque instability existing simultaneously in multiple vascular beds has been described and echolucent carotid plaques have been shown to be strongly and independently associated with future coronary events in patients with stable CVD (9,10). This is the first study to have assessed carotid plaque morphology amongst Indian Asians and European whites, but as with atherosclerosis burden, there were no discernible differences in plaque composition between the two groups.

The findings of this study suggest that other mechanisms involved in pathogenesis of clinical events warrant further investigation, with the concept of the “vulnerable patient” (8,244) assuming greater relevance in Indian Asians. Studies applying newer methods of assessing plaque morphology, such as the assessment of intra-plaque neovascularisation, are currently in progress in this group and may further identify individuals at high risk of acute thrombotic events. Attention should also turn towards other factors beyond the mere presence of plaque such as inflammation, thrombogenic tendency and myocardial susceptibility to fatal arrhythmia in order to elucidate potential mechanisms/biomarkers capable of identifying the increased CVD mortality amongst Indian Asians. Studies have shown that plasma homocysteine, C-reactive protein, fibrinogen and lipoprotein (a) concentrations are higher in Indian Asians than European whites (112,123,128). However, at present there are no outcome data supporting these factors as having a causal role for increased CVD mortality amongst Indian Asians or that the lowering of their levels will translate into a reduction in major cardiovascular end-points. The severity of clinical sequelae
to a plaque event may also depend on the electrical instability of the myocardium which can be influenced by underlying myopathic processes (244). We have previously reported healthy Indian Asians to have attenuated LV systolic function and increased E/Ea compared to European whites (245). We anticipate that long-term follow-up of this and the main LOLIPOP cohort of ~30,000 individuals will be able to draw more definitive conclusions regarding the role of systemic and myocardial factors on predicting cardiovascular outcomes, particularly amongst Indian Asians.

Limitations

As mentioned previously, current risk scoring algorithms tend to underestimate CVD risk in Indian Asians, which is evident in this cohort with Indian Asian subjects possessing lower 10-year CHD risk scores than the European whites, despite their having a greater prevalence of CVD. The lower FRS amongst Indian Asians could explain the lack of differentiation in atherosclerosis burden. However, after adjusting for Framingham and other established cardiovascular risk factors, such as type-2 diabetes which was markedly more prevalent amongst the Indian Asians, similarities in atherosclerosis burden remained. Another limitation of this study is the employment of a qualitative method for scoring plaque echogenicity which may lack sensitivity to detect subtle differences in plaque morphology compared to semi-quantitative techniques such as determination of plaque grey-scale medians (246).

Conclusions
The burden of subclinical atherosclerosis amongst healthy UK Indian Asians does not identify their markedly higher risk of CVD, as compared to European whites. Factors beyond the presence and degree of atherosclerosis merit further investigation in this high-risk group.
PART III – CONCLUSIONS, STUDY LIMITATIONS & FUTURE WORK

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CHAPTER 10. CONCLUSIONS

This thesis has examined biomarkers of subclinical CVD using imaging-based techniques, in a large cohort of unselected, asymptomatic individuals of Indian Asian and European white ethnicity. Using novel and established echocardiographic indices, this thesis has explored two broad themes:

i) *The association of imaging biomarkers and demography with myocardial function* – novel relationships between the pattern of LV remodeling, the pattern of subclinical atherosclerosis and the intrinsic influence of ethnicity with the quantitative parameters of LV function have been defined.

ii) *The imaging biomarkers that may identify the increased risk of CVD mortality amongst Indian Asians* – for the first time the prevalences of subclinical atherosclerosis and LVH amongst UK Indian Asians have been documented.

By defining a reference subgroup of individuals free of modifiable cardiovascular risk factors and significant coronary artery disease, the first study reported normative reference values for TDI parameters of longitudinal systolic function and LV filling pressure, as well as examining the effects on the ageing process upon these indices. Although TDI has significantly enhanced the echocardiographic assessment of cardiac function, the technique suffers from a lack of standardisation due to the absence of normative reference range values. This study has helped to address this problem by describing percentile-based values for the parameters of longitudinal function and LV filling pressure in a highly-phenotyped subgroup free from clinical CVD, conventional cardiovascular risk factors and significant coronary artery disease as defined by coronary calcium scoring. This study also observed attenuated
myocardial systolic and diastolic function to be associated with the normal ageing process. This data can be applied routinely in the clinical echocardiography laboratory, providing ethnicity- and age-specific reference values for the TDI parameters of longitudinal function and LV filling pressure. This data does not support the assertion that intermediate E/Ea values (8<E/Ea< 15) may be indicative of elevated LV filling pressures as healthy subjects of any age were likely to have an E/Ea ratio exceeding 8 and frequently be greater than 10. An E/Ea ratio > 15 is, however, likely to represent abnormally elevated LV filling pressures regardless of age and the mitral annular site measured. By defining a reference subgroup, gender- and ethnicity- specific partition values for LV mass, required for defining the presence of LVH, were also derived.

The second study advances the understanding of mechanisms potentially responsible for the increase in cardiovascular events associated with different patterns of LV remodeling. Previous studies assessing the relationship between different patterns of LV remodeling and cardiac function had utilised relatively insensitive indices of LV systolic and diastolic function. This study is one of the first to employ TDI in a large population setting, allowing subtle relationships between cardiac function and LV geometry to be detected. Increasing degrees of LV concentric remodeling were initially observed to be associated with augmented LV systolic function until hypertrophic remodeling had manifested, resulting in systolic dysfunction. This data suggests that non-hypertrophic concentric remodeling may represent an adaptive response to pressure overload physiology, as had been observed in the classical animal experiments on the 1960’s but, until this study, had yet to be reproduced in humans. Hypertrophic remodeling was also associated with increased LV filling pressure suggesting that these individuals may be at greater risk from developing heart failure syndromes than those with the non-hypertrophic form of concentric remodeling.
Although carotid artery disease is associated with cardiovascular outcomes, the performance of carotid IMT alone as a prognosticator has been inconsistent, with evidence that plaque disease maybe a better reflection of atheroma burden. Although these two phenotypes are related, the pathophysiology underlying intima-media thickening and plaque formation are not necessarily similar. Hypertrophy of the medial layer of the arterial wall can occur either as a response to hypertension or as a manifestation of normal aging, whereas plaque formation represents the maturation of the atherosclerotic process. In the third study, the relationships of IMT and plaque disease were separately explored with qualitative echocardiographic parameters of LV myocardial function and filling pressure. The data demonstrates that incipient myocardial systolic dysfunction is related to the burden of carotid plaque disease rather than the degree intima-medial thickening. This is the first study to have demonstrated that the main two phenotypes of arterial disease possess distinct relationships with LV function and has implications for future outcomes-based studies. The application of carotid ultrasonography in the risk stratification of healthy individuals could potentially be expanded beyond the prediction of atherosclerotic vascular events and help tailor primary preventative interventions. For example, commencing an angiotensin converting enzyme-inhibitor could be considered as first-line treatment in individuals with evidence of carotid plaque disease and hypertension in order to slow the progression of incipient LV dysfunction.

Echocardiographic assessment of LV function and structure is increasingly being sought to diagnose heart failure syndromes, particularly in the presence of preserved LV EF. The recognition of LV dysfunction in asymptomatic individuals is of paramount importance as not only does this phase of disease foretell the development of incident congestive heart failure but its treatment has been shown to delay the onset of overt heart failure symptoms. This has prompted the search for “risk factors” of incipient myocardial systolic and diastolic
dysfunction, examples of which include the burden of conventional CVD risk factors, abnormalities in LV geometry, increased LV mass, female gender and the degree of atherosclerosis. However, the influence of demographic variables, in particular ethnicity, upon LV function is poorly understood. The fourth study explored the relationship between ethnicity and the quantitative tissue Doppler and 2-D echocardiographic measures of LV function, structure and geometry. Indian Asians were observed to intrinsically have attenuated longitudinal LV systolic function, diastolic function and higher end-diastolic LV pressures than European whites. Whether the less favourable LV function and greater cardiac remodeling observed amongst Indian Asians identifies a more vulnerable myocardium with an increased susceptibility to acute ischaemic and arrhythmogenic events, or is simply representative of distinct but normal cardiac structure and physiology, can only be determined after long-term follow-up of this cohort for primary cardiac events.

LVH is strongly associated with major cardiovascular events and mortality, independent of other cardiovascular parameters. Although the increased prevalence of this important phenotype has been well defined in other high-risk, ethnic groups, the true prevalence of LVH amongst Indian Asian had been hitherto unknown. After adjustment for relevant covariates, the fifth study demonstrated Indian Asian men to have an almost threefold higher odds of LVH, again with a significantly higher degree of concentric remodeling. To the best of one’s knowledge, this is the first description of an established biomarker of cardiovascular risk being more prevalent amongst Indian Asians, which may be commensurate with their increased risk of CVD mortality.

This study has been adequately powered to allow comparison between the two ethnic groups for the prevalence of subclinical carotid atherosclerosis. Indian Asians are reported as having at least a 50% greater risk of CVD mortality compared to European whites, and in the
fifth study, the prevalence of clinical CVD in the “disease” subgroup was 50% higher amongst Indian Asians than European whites. Surprisingly, the degree of subclinical atherosclerosis measured in the carotid arteries did not identify this elevated risk, as both carotid IMT and plaque prevalence were similar to that in European whites. Increased IMT and the presence of atheromatous plaques are forms of carotid artery disease that are both regarded as surrogate markers of atherosclerosis and strongly predictive of incident myocardial infarction, stroke and death. This study suggests that the increased prevalence of CVD amongst Indian Asians is not reflected by their increased propensity to atheroma formation, compared to European whites, and that other mechanisms may be responsible for their increased susceptibility to acute CVD events. This findings of this study are buttressed by those of colleagues, who undertook analysis of coronary artery calcification data derived from the EBCT scans performed on this cohort which again showed no difference in the degree of coronary atherosclerosis between the two ethnic groups (Appendix 1).

With data presented demonstrating a higher prevalence of LVH, abnormal LV geometry and impaired LV function compared to European whites, this thesis should encourage further research into pro-hypertrophic mechanisms, and their impact upon maladaptive cardiac remodeling amongst individuals of Indian Asian ethnicity. Candidate mechanisms based on the observations of this thesis would include 24-hour BP load, prevalence of pre-existing hypertension and prevalence of type-2 diabetes, which were all greater amongst Indian Asian individuals compared to European whites.

10.1 Limitations

Although the limitations of each study have been acknowledged throughout the thesis, important issues affecting the study as a whole are restated here. Although there are advantages to studying biomarkers of subclinical disease (chapter 1.2), the lack of clear,
“hard” CVD end-points permits only associations between covariates and dependent variables to be observed, with implicit conclusion drawn. The first phase of follow-up for this cohort is scheduled to be completed by 2014, which will allow validation of the observations described in this thesis and the discovery of potentially new mechanisms of clinical disease events. Another limitation was the lack of data provided regarding prescription medication usage, differences in which could have confounded the ethnicity-related differences in LV function that were observed, for example beta blocker or angiotensin converting enzyme inhibitors which may affect the diastolic properties of the heart. However, in chapter 7 (“Ethnicity-Related Differences in Left Ventricular Function, Structure and Geometry: A Population Study of UK Indian Asians and European Whites”) individuals taking cardioactive medications (antihypertensives, antianginals, hypoglycaemic agents, lipid lowering therapy, thienodipyridine anti-platelets) were excluded from the analysis. This suggests that the differences observed in LV function between the two ethnic groups in LV function were unlikely to be secondary to discrepancies in the prescription of cardiovascular disease modifying agents.
10.2 Future work

Cohort surveillance and follow-up for clinical events

Study event endpoints will include acute myocardial infarction and other forms of CHD, stroke, peripheral vascular disease, congestive heart failure, CVD interventions and mortality. Follow-up of enrolled subjects will be undertaken at Ealing Hospital by trained research nurses. The first follow-up contact commenced in January 2010 and is to be repeated in 2015. The NHS number and other demographic details will be used to link to health records and multiple sources (Office for National Statistics, Virtual Organisation for Trials and Epidemiological Studies, Hospital Episode Statistics data and clinical databases in West London Hospitals) will be searched and collated to identify CVD events.

Quantification of adventitial vasa vasorum

Despite the similar burden of subclinical atherosclerosis evident amongst Indian Asians and European whites recruited in this cohort, further research is required to explore whether the excess of CVD mortality amongst Indian Asians may be attributable to an enhanced vulnerability to undergo atherothrombotic plaque events. Although no differences in the morphological appearances of the plaques were detected between the two groups, a qualitative, visual method was employed. As discussed earlier (chapter 1.3.1.4), the detection of neovessels in the arterial wall in the form of prominent adventitial vasa vasorum is correlated with plaque instability and clinical events, and can be detected using contrast-enhanced carotid ultrasonography (CECU). Therefore, we have recently performed CECU on ~200 Indian Asian and European white subjects previously identified as having carotid plaque disease during carotid ultrasonography from the original LOLIPOP-
ATHEROSCLEROSIS cohort. Analysis of the data is currently being conducted, using computer software as well as visual estimation, to assess the degree of adventitial vasa vasorum contrast enhancement.

**Analysis of blood biomarkers**

As discussed earlier (chapter 1.1), CVD risk stratification can be enhanced by measuring various blood biomarkers that are directly or indirectly related to the atherothrombotic state. All subjects enrolled into the study provided blood samples which were frozen and analysis has recently been completed for appropriate biomarkers of subclinical CVD, relating to inflammation, cardiac function, lipoprotein sub particles and renal function.

Atherogenesis is believed to be characterised by a local inflammatory process which can lead to destabilisation of the plaque and predispose it to rupture which triggers most episodes of arterial thrombosis (247,248). A number of prospective studies in initially healthy subjects have demonstrated an independent association between slightly elevated concentrations of systemic inflammation and vascular events. The largest database exists for high-sensitivity CRP, an acute phase reactant that is an extremely sensitive marker of inflammation, increased concentrations of which reflect underlying atherosclerotic burden and also predict cardiovascular outcomes (249-253). Elevated levels of CRP have been observed in a healthy population of Indian Asians compared to European whites whilst being closely related with central obesity in both populations (112).

Measurement of the natriuretic peptides BNP and NT-proBNP do not only serve as markers of LV systolic dysfunction but are also elevated in the setting of cardiac ischaemia (254-256) and are associated with worse outcomes in patients with stable coronary artery disease. Recent data suggests levels of NT-proBNP are also independently associated with
the burden of atherosclerosis even after adjusting for LV function (257,258), suggesting that atherosclerosis itself may stimulate natriuretic peptide synthesis and release.

Although not capable of conveying the actual burden of atherosclerosis, there are several emerging blood biomarkers that are useful in risk stratification and prognosis of CVD. Lipoprotein (a), or Lp (a), is composed of a cholesterol rich particle linked to a glycoprotein of variable size called apolipoprotein (a). High levels of Lp (a) have been shown to be a risk factor for CVD, with levels above 30mg/dL shown to increase risk by two to three times in Caucasian populations (259). The association of Lp (a) with CAD and its ability to act as a biomarker of risk appears to be strongest in patients with hypercholesterolaemia and particularly in young patients with premature atherosclerosis.

Impaired renal function portends a less favourable prognosis in patients with confirmed acute coronary syndromes (260,261). The incidence of end-stage renal failure requiring dialysis is also 3 times higher amongst UK Indian Asians compared to European whites (262). Cystatin C is a cysteine protease inhibitor involved in the catabolism of proteins that is produced in all nucleated cells at a constant rate and is freely filtered by the glomerulus without secretion or subsequent reabsorption to the blood flow (263). Cystatin C has been shown to be a better endogenous marker of glomerular filtration rate than serum creatinine (264) and its promise as a risk marker has been confirmed in patients with heart failure (265) and acute coronary syndromes (266) but it has yet to be assessed prospectively in an asymptomatic cohort.

Analysis of 3-D volumetric data

LV datasets using 3-D, real-time TTE imaging have been analysed using semi-automated software to provide LV end-diastolic and end-systolic volumes. Demonstration of an
incremental benefit in measuring 3-D volume data compared to standard 2-D estimates of LV volumes necessitates longitudinal follow-up of the cohort for clinical cardiovascular events, particularly incident heart failure episodes.
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APPENDIX 1

Investigation of Coronary Artery Calcification as a Marker of Increased Cardiovascular Risk in UK Indian Asians and European whites (136)

Piyush Jain, John C Chambers, Paul Elliott, Emily D Williams, Barbara Kraly, Stanley Muscat, Avijit Lahiri, Jaspal S Kooner

Background: Coronary heart disease mortality is 70% higher amongst UK Indian Asian compared with European whites. The risk stratification tools and biomarkers that are currently used do not allow accurate identification of Indian Asians at increased risk of coronary heart disease. Coronary artery calcification is highly correlated with coronary plaque burden, and is an independent predictor for future coronary heart disease events in North American and European white populations. We hypothesised that coronary artery calcification is increased in Indian Asians compared with European whites, and may provide a non-invasive tool for assessment of increased coronary heart disease risk in Indian Asians.

Methods: We investigated 2369 Indian Asian and European white men and women, aged 35 to 75 years, for coronary artery calcification using electron beam computed tomography. Participants were recruited from the practice lists of 58 family physicians in West London, as part of the LOLIPOP study, and all were free from clinical cardiovascular disease.

Results: In comparison to Europeans, Indian Asians had a higher prevalence of hypertension, type-2 diabetes and metabolic syndrome, higher waist-hip ratio, body fat, triglycerides and serum glucose levels with lower HDL cholesterol. Cigarette smoking and total cholesterol levels were lower in Indian Asians compared with European whites. Coronary artery calcification was more common in men than women, and coronary artery calcification scores were closely associated with cardiovascular risk factors including age, cigarette smoking,
hypertension, systolic blood pressure, diabetes, total cholesterol and metabolic syndrome (all statistically significant with \( p < 0.05 \)). In contrast, there were no differences in either coronary artery calcification prevalence or mean levels of coronary artery calcification between Indian Asians and Europeans, after adjustment for the measured cardiovascular risk factors.

**Summary:** Coronary artery calcification is not increased in Indian Asians compared with European whites, in any age group, or in either gender. Similar coronary artery calcification in Indian Asians and Europeans contrasts almost 2-fold higher risk of myocardial infarction and coronary heart disease mortality observed in Indian Asians. Coronary artery calcification does not predict or identify the excess coronary heart disease risk in Indian Asians in this population.