

**Prevalence of sub clinical atherosclerosis among UK
South Asians and Europeans**

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Abstract

Background: South Asians demonstrate high coronary heart disease mortality, largely unexplained by conventional risk factors and unidentified by risk stratification tools. Developments in technology allow us to visualize coronary atherosclerosis non-invasively, thus providing the potential to identify presence of coronary atherosclerosis before it manifests clinically. Coronary artery calcification is closely correlated with total plaque burden and provides an assessment of coronary plaque burden. Myocardial perfusion scintigraphy provides an estimate of myocardial blood flow and thus, severity of coronary artery disease. Increased coronary artery calcification and silent myocardial ischemia predict future risk of coronary heart disease mortality, independent of conventional factors. Inflammation is a key factor in initiation and progression of atherosclerosis. High sensitivity C-reactive protein (CRP) is an important marker of active inflammation and is considered an independent predictor of future cardiovascular events. Thus, markers of subclinical atherosclerosis and inflammation could provide us with a tool for early identification of South Asians at risk of coronary events, unidentified by traditional means.

However, majority of the data for such markers is from North American and European populations, with no data evaluating the role of coronary artery calcification, myocardial perfusion scintigraphy and CRP in assessing the coronary heart disease risk in South Asians.

Methods and Results: I carried out assessments including coronary artery calcium, myocardial perfusion imaging and assessment of high sensitivity C-reactive protein for a cohort of asymptomatic South Asians and Europeans men and women, aged 35 to 75 years, who were part of the London Life Sciences Population (LOLIPOP) study. I found that: 1)

Coronary artery calcification scores were closely associated with age, male gender, cigarette smoking, hypertension, systolic blood pressure, diabetes and total cholesterol. 2) There were no differences in either coronary artery calcification prevalence or mean levels of coronary artery calcification between South Asians and Europeans, after adjustment for the measured cardiovascular risk factors. 3) Presence of diabetes and increasing coronary artery calcification were independent predictors for silent myocardial ischemia. 4) South Asian ethnicity did not influence the prevalence or the extent of silent myocardial ischemia, after adjustment for conventional risk factors. 5) C-reactive protein levels did not correlate with measures of plaque burden. 5) South Asian ethnicity was an independent predictor of inflammation as seen by levels of high sensitivity C-reactive protein. This effect was independent of, and remained significant after adjusting for conventional cardiovascular risk factors and novel factors linked to inflammation such as diabetes and indices of abdominal obesity.

Conclusions: While traditional risk factor correlate well with markers of atherosclerosis, the higher coronary heart disease risk and mortality observed in South Asians is not identified by markers of atherosclerotic burden such as coronary artery calcification and myocardial perfusion scintigraphy. South Asians have elevated levels of inflammation as seen by high sensitivity C-reactive protein levels. C-reactive protein levels are not correlated with coronary artery calcium or myocardial ischemia measured by myocardial perfusion scintigraphy. These findings suggest a role of factors such as systemic and plaque inflammation, unrelated to and unmeasured by plaque burden assessment in the higher coronary heart disease mortality observed among South Asians. The study therefore suggests a role of potential risk stratification tools reflecting the multisystem nature of CHD. These could be a combination of clinical risk factors contributing towards CHD, imaging of atherosclerotic plaque and assessment of plaque or systemic inflammation.

DECLARATION OF ORIGINALITY

I, Piyush Jain, 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.

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*This thesis is dedicated to my parents, my wife
Bela, and my daughters, Annika + Aarya. It
would not have been possible without the
unconditional support and encouragement from
them.*

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Publications

1. Prevalence of Coronary Artery Calcium Scores and Silent Myocardial Ischemia Was Similar in South Asians and Europeans in a Cross-sectional Study of Asymptomatic Subjects from a UK Population (LOLIPOP-IPC). P. Jain, J.S. Kooner, U. Raval, A. Lahiri .(Journal of Nuclear Cardiology, Volume 18, Number 3;435–42)

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List of Abbreviations

CAD	Coronary artery disease
CAC	Coronary artery calcification
CHD	Coronary heart disease
CRP	C-reactive protein
EBCT	Electron beam CT
CRP	High sensitivity C-Reactive Protein
LDL	Low density lipoprotein cholesterol
LOLIPOP	London life sciences prospective population study
Lp(a)	Lipoprotein a
MPS	Myocardial perfusion scan
SA	South Asians
UK	United Kingdom

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Chapter 1: Introduction

1.1 Coronary Heart Disease among South Asians

1.1.1 Coronary Heart Disease Epidemiology

Coronary heart disease (CHD) is the single largest contributor to global mortality, with more than 13% of the total deaths (7 million), attributed to it in 2002 (1). There are, however, distinct ethnic disparities in the incidence and mortality due to CHD. South Asians (people originating from the India, Pakistan, Bangladesh and SriLanka) demonstrate a high CHD attributed mortality. Data from the WHO (2002) demonstrated that this group contributed a quarter of the global CHD deaths, about the same number of CHD deaths as all the developed countries from Europe and America combined (1) (Figure1.1). South Asians currently constitute 22% of the global population and as a result of a high growth rate are expected to increase this proportion to 28% by 2050 (2). As a consequence of an increasing population with a high inherent CHD risk, South Asians are expected to contribute a staggering 40% of the global CHD burden by 2050 (2).

Among South Asians there appears to be a gradation of CHD risk which correlates with the level of urbanization. A study using analysis of ECG Q waves demonstrated a 4-5 fold higher prevalence of CHD in urban India compared with rural India (3) and data from countries such as Fiji, Singapore, USA, Trinidad and UK have demonstrated higher CHD mortality among South Asian migrants compared with native populations (3-10). Thus, it appears that there is increasing CHD mortality with the level of urbanization; with the greatest risk amongst South Asians living overseas.

Within the UK, South Asians formed the largest ethnic minority group and make up 4% of the total population (2001 population census). Improved healthcare and health awareness over the last 3 decades has significantly decreased CHD related mortality in the general population in the UK, with CHD mortality rates of 132.0/100,000 for the period from 1993-2003. South Asians within the UK, however, continue to show a 2-fold higher CHD mortality rate (241.0/100,000) for the same period. (4).

Due to lack of prospective data regarding CHD risk and mortality in South Asians, the reasons for this excess remain poorly understood.

1.1.2 Conventional risk factors

Large scale prospective studies such as the Framingham cohort have identified important risk factors for CHD including hypertension, hyperlipidaemia, low HDL, smoking and increasing age. One limitation of studies so far is the predominantly Caucasian base population. This potentially limits their use in other ethnic populations. However, recent global registries such as INTERHEART (5), carried out among multi ethnic populations, suggest an important role for conventional risk factors in CHD across different ethnic groups.

Existing data for migrant South Asians demonstrates that despite high CHD rates among South Asians subgroups; they do not consistently exhibit elevated levels of conventional risk factors (6,7). For example, among migrant South Asians the level of serum cholesterol, hypertension, as well as cigarette smoking rates in some cases can be equivalent to, or lower than a comparable Caucasian population (8-10). This risk factor prevalence is in contrast to the high CHD mortality, leading to the belief that conventional risk factors do not contribute to the high CHD mortality rates observed among South Asians (9).

However, observational studies performed among Asians demonstrate that serum cholesterol, blood pressure, smoking rates, and body mass index are substantially higher in

urban compared to rural Indians (11). This is consistent with their higher CHD prevalence (3). Similarly, overseas South Asians demonstrate higher prevalence of conventional risk factors as well as a higher CHD prevalence compared with their non-migrant siblings (12). Within the UK, South Asians demonstrate higher mean blood pressure levels, compared with Europeans (9,13). Cigarette smoking is observed to be twice as common in Bangladeshis (6), and the prevalence of hypertension increased two-fold in Sikhs (11), compared with Europeans.

Thus, it is clear that the conventional risk factors are relevant in pathogenesis of CHD among South Asians. However, given the lack of consistency in prevalence of risk factors among various South Asian subgroups, and indeed compared with Europeans, the question whether such risk factors explain the excess risk of CHD seen in this population remains unanswered.

1.1.3 Diabetes Mellitus and related metabolic disorders

Type 2 diabetes mellitus is a well-known risk factor for CHD, with associated accelerated atherosclerotic disease and higher rates of cardiovascular morbidity and mortality compared with non diabetics (14). The risk from diabetes is considered to be as high as that attributed to a history of previous coronary event i.e. it is considered a CAD ‘risk equivalent’ (15)

South Asians demonstrate a high prevalence of non-insulin dependent diabetes. Reported figures suggest prevalence of 2-5% in rural, 5-10% in urban, and almost 20% in UK South Asian, compared to 4% prevalence of diabetes among European men (6,16-21). Furthermore, South Asians demonstrate a higher prevalence of insulin resistance and its related metabolic abnormalities (elevated glucose levels, central obesity, glucose intolerance, elevated plasma insulin, increased triglycerides, raised PAI-1, and reduced high-density lipoprotein

cholesterol), compared with Europeans populations (6,9,11,22). The reasons for this higher prevalence in South Asians are not clear, and there are several theories postulated:

i) Genetic susceptibility and lifestyle changes

South Asians appear to have genetic predilection for diabetes (23,24), as well as metabolic abnormalities such as high triglycerides (25), abdominal obesity and insulin resistance (26). Furthermore, there appears to be positive correlation with the level of urbanization. While the prevalence of diabetes is approximately 2% in rural settings, it rises dramatically to as much as 5-10% in urban communities within India (16,18,27). Exposed to a greater level of urbanization, such as that seen among South Asian immigrants to the Western world, this increase is even more apparent. In the United Kingdom, the prevalence of diabetes in South Asians approaches 15% to 20%, markedly higher than the 4% diabetes prevalent among Europeans (6,12,20,28).

Among South Asians, urbanization and growing income has been seen to be associated with an excess calorific intake. This is in part due to excess sugar and fat consumption, coupled with decreased physical activity and sedentary way of life (28,29), and leads to obesity. The increased prevalence of obesity can potentially explain to the greater blood pressure (30) , increased prevalence of diabetes (21,31) and the increase in serum insulin, insulin secretion and decreased insulin sensitivity (32).

ii) Low birth weight

There appears to be an increased prevalence of insulin resistance and obesity observed among low birth weight babies. This is hypothesized to be due to a poor maternal nourishment causing an adaptive state in the foetus for survival under such conditions, both ante and postnatal (33). Such changes become harmful when the postnatal environment is different from that predicted antenatal, i.e. over abundant nutrients and consequent obesity.

The risk of future disease is thus greatest amongst those that subsequently become obese during adult life (34,35).

Previous studies illustrate that children of low birth weight have increased CHD mortality in later life (34,36,37). Low birth weight has been linked with an increased risk of hypertension, diabetes, insulin resistance, dyslipidaemia, with elevated hsCRP and fibrinogen levels in later life (38,39). However, the main contributing factor to the risk of CHD in this population is deemed to be insulin resistance and the risk of diabetes.

South Asian babies are small compared to other populations with an average weight of a newborn native Indian at 2.7kg, 3.1kg in UK South Asians, 3.4kg in Caucasians, and 3.7kg in North American Indians (40-43). Furthermore, previous studies have demonstrated higher insulin levels at birth for South Asians babies, compared with Europeans, after adjusting for birth weight.

Observations that the prevalence of CHD is almost 4-fold higher amongst Indians with birth-weight <2.5kg, compared a birth-weight >3.2kg (37), support the theory of low-birth weight and its sequelae contributing to excess CHD risk amongst South Asians.

Thus, the genetic predilection coupled with the change in lifestyle increases the risk for developing an insulin resistant state or the metabolic syndrome (44), with the attached high CHD risk among South Asians ethnicity.

While the prevalence of diabetes has been demonstrated to be higher among South Asians (45,46), compared with Europeans; studies from the UK and Canada have demonstrated diabetics South Asians have an almost 2 fold higher risk of cardiovascular events compared with European diabetic subjects even after adjusting for other cardiovascular risk factors (9,47). The cause of this excess is as yet completely elucidated and it therefore seems plausible that risk factors other than the previously considered ones play an important role in the excess CHD risk South Asians.

1.1.4 Novel risk factors

i) Inflammation

Atherosclerosis has often been termed as a chronic inflammatory process (48). Inflammation is one of the key factors in initiating atherosclerosis and is instrumental in pathogenesis of vulnerable plaques (49,50).

Inflammation causes damage to the endothelial membrane, resulting in increased endothelial adhesiveness and permeability allowing increased LDL entry into arterial intima. Furthermore, the endothelial damage causes release of cytokines and growth factors whose chemotactic nature stimulates monocyte-derived macrophages and T lymphocytes accumulate at the site of injury. Inflammation is thought to play a critical role in predisposing to plaque rupture by destabilization of the fibrous cap tissue, triggering majority of the episodes of coronary thrombosis (48). This is supported by studies done in animal models as well as those among human cases of sudden death, demonstrating increased macrophage activity in plaques which underwent rupture and subsequent thrombosis (51). Furthermore, recent studies in human subjects have shown markedly increased inflammation within active atherosclerotic plaques in carotid arteries (52,53).

South Asians exhibit a high prevalence of diabetes, hypertension, abdominal obesity and metabolic syndrome, with lower HDL cholesterol levels (54-57). Presence of these risk factors is associated with greater systemic inflammation (58,59). In addition, South Asians also demonstrate elevated levels of C-reactive protein (CRP) (60). This is consistent with experimental studies which suggest that abdominal adipose tissue is a major source of cytokines, including IL-6, an important determinant of hepatic CRP synthesis (61,62).

CRP is an important marker of inflammation, with elevated levels of CRP strongly associated with increased macrophage activity within plaques (63,64), and is considered an independent predictor of future cardiovascular events (51,65). Increased concentrations of CRP have been shown in both clinical and epidemiological studies to be associated with atherothrombotic events.

These observations suggest a higher level of systemic inflammation, as well as inflammatory activity within plaque, amongst South Asian compared with Europeans. Thus, it raises the possibility that inflammatory mechanisms contribute at least in part, to the increased risk of CHD amongst South Asians.

ii) Homocysteine and risk of CHD in South Asians

Homocysteine is a sulphur-containing amino acid, increasingly recognised as an independent risk factor for vascular disease (66,67). The mechanisms are not well elucidated; however, studies indicate that they could be mediated through an action on the endothelium. Studies in man show that elevated plasma homocysteine concentrations are associated with impaired endothelium-dependent vasodilatation, an early manifestation of atherosclerosis (68). Furthermore, in vitro studies demonstrate that prolonged exposure of cultured endothelial cells to homocysteine impairs nitric oxide mediated inhibition of platelet aggregation (69,70) as well as specific proinflammatory cytokines associated with migration recruitment of leukocytes across the vascular endothelium (71)

Recent studies have demonstrated higher concentrations of plasma homocysteine among South Asians compared with Europeans (9,22,72). Thus it is thought that elevated homocysteine may contribute to increased CHD mortality in South Asians.

iii) Endothelial dysfunction

The vascular endothelium is an integral part of the atherosclerotic cascade. A normal endothelium maintains homeostasis through a variety of mechanisms which include control of vascular tone, smooth muscle cell proliferation and migration, along with effects on thrombogenesis and fibrinolysis (73,74). Endothelial dysfunction is an early manifestation of atherosclerosis, preceding plaque formation, and evidence of angiographic disease.

One of the important mediators for the endothelium is nitric oxide (75), which plays an important role in maintaining vascular integrity by modulating vascular tone, enabling endothelial repair (76), inhibiting thrombosis and leukocyte adhesion, and by influencing smooth muscle cell proliferation (77). Studies have demonstrated that brachial artery flow mediated dilatation is largely mediated by nitric oxide released by the endothelium (76), and is inhibited by L-NMMA (75).

While endothelial damage and resulting dysfunction normally occurs due to damage by free radicals generated by various toxic stimuli, endothelium dependent dilatation is impaired even among healthy UK South Asians compared to Europeans (75,78). Several explanations for the impaired endothelial function among South Asians have been postulated, including the elevated levels of homocysteine and early atherosclerosis; however, the exact mechanism remains unknown and the dysfunction is unexplained by conventional atherosclerotic risk factors (78). Thus, endothelial dysfunction due to reduced activity of endothelial nitric oxide could be a significant contributor to vascular injury in South Asians.

iv) Lipoprotein (a)

Lipoprotein (a), formed from the assembly of the protein apolipoprotein (a), has been identified as an independent risk factor for vascular disease, including CHD (79,80). Though the exact mechanisms underlying this relationship are uncertain, in vitro studies suggest that lipoprotein (a) may influence cholesterol uptake and inhibit fibrinolysis (79).

Serum lipoprotein (a) concentrations are being determined at birth by variations in the *LPA* gene. Lipoprotein (a) concentrations are reported to be higher in South Asians compared to Europeans, reflecting the high CHD risk in this racial group (12)

v) Psychosocial and economic factors

Stress has been considered an important risk factor for CAD. The two most recognised components of this are socio-economic and psychosocial stress. These encompass a variety of things including low income, poor education, poor job prospects, stress at work and home, overcrowding in household, racial discrimination and social support. Such factors have been shown to be associated with CAD, a role which has been further emphasised by the INTERHEART registry (5). INTERHEART, which was a global registry, concluded that stress factors could be account for up-to 32.5% of the population attributable risk for MI. This, to put in context, was only slightly less than the population attributable risk for lifetime smoking (35.7%), and more than that for hypertension (17.9%) or obesity (20.0%). While INTERHEART was a cross sectional observational study, and the effect is yet to be proven in a prospective cohort, nevertheless the study highlights the possible importance of such factors.

There are three plausible ways in which stress can affect health. First, stress may affect health related behaviours such as smoking, diet, alcohol consumption, or physical activity, which in turn may influence the risk of coronary heart disease (81-85). This is thus by exposing to known pathogens. Second, it may influence access to and content of medical care. This is more relevant in a healthcare system which is not state funded and there is no free access to healthcare (86,87). Third, stress may cause direct pathophysiological changes i.e. direct causal effect.

While the first two concepts are fairly self explanatory, a number of mechanisms have been proposed to explain the direct pathological effect of stress in CAD. These include activation of the sympathetic nervous system, endothelial dysfunction causing release of proinflammatory cytokines and prothrombotic responses which in turn promote atherogenesis and plaque rupture (88-90). These factors may act independently or have a cumulative effect.

South Asians score poorly on measures of psychosocial stress i.e. higher levels of chronic stress, depression, lower levels of social support and demonstrated lower socio-economic status (91). South Asians are also more likely to live in areas with increased social and economic deprivation (92). In addition, studies have also suggested variability in access to healthcare among UK South Asians compare with the local European population. While it appears that South Asians might seek earlier medical advice for symptoms suggestive of angina (93), regional studies suggest that South Asians may be less likely to be referred for further investigation and referrals (94-96). The poor access to healthcare, however, is not supported by contemporary studies, at least in the UK, which suggest comparable access to healthcare for South Asians compared with Europeans (97,98). Thus within the UK, South Asians appear to exhibit at least a few if not all of the stress components related to CAD.

Stress has been demonstrated to be correlated to measures of subclinical atherosclerosis such as CAC (99) and silent myocardial ischemia (100), as demonstrated by our group among others. Therefore, in order to assess the role of such factors in CHD among South Asians, our study also conducted a comprehensive psychosocial assessment on a proportion of the participants. The psychosocial assessment was divided into measures of chronic stress, protective factors in the social environment, psychological factors and health behaviours. Univariate and multivariate regression analysis were then preformed to predict the presence and extent of calcification, using these factors.

The results from our study demonstrated that South Asian and European groups have marked differences in psychological characteristics, with higher hostility and depression scores seen among South Asian. In UK South Asian men, the only predictor for presence of CAC in univariate analysis was age of finishing education, however once behaviours were adjusted for, this was reduced to non-significance. In UK South Asian women, the indicators of socio-economic status, household consumables and social deprivation, were negatively associated with presence of CAC on univariate analysis. The association between consumables and CAC was no longer significant once social deprivation had been taken into account. Even after adjustment for health behaviours and adiposity, social deprivation was negatively related to presence of CAC.

In European men, social deprivation was positively associated with presence of CAC, independent of age and other risk factors. In European women, the work stress variables of work demands, effort-reward imbalance, and job strain were positively related to presence of CAC, even after controlling for age, health behaviours, and adiposity. This suggested that elevated levels of work stress in European women were associated with an increased risk of coronary calcification and CHD.

Analysis for the extent of CAC demonstrated that job demands and effort were positively related to the extent of CAC in South Asian men, independent of age and other confounding variables. In European men, hostility emerged as having a strong association with the extent of CAC, and this effect was not attenuated by adjustment for health behaviours such as smoking, BMI and WHR.

Thus while psychosocial factors were related to CAC, the effect was variable among Europeans and South Asian participants and not necessarily in the direction expected. While stress appears to be associated with a higher incidence of acute coronary events (101-103) and coronary ischemia (104), and while South Asians are more exposed to stress; measures of

stress are not consistently related to CAC i.e. calcified plaque. This is due to a complex interplay of factors including systemic inflammation, plaque characteristics and neuroendocrine activation which mediate the cascade of atherosclerosis. Thus the measures of plaque burden alone are poorly correlated with risk due to stress.

Thus South Asians have a combination of biological, psychosocial and lifestyle factors, the combination of which is likely to be responsible for their excess CHD.

1.2 Risk stratification/ Screening for coronary heart disease

1.2.1 Traditional risk stratification for CHD

CHD is a complex disease with a multifactorial aetiology. The effect of sum of risk factors is therefore not necessarily linear but often exponential i.e. the risk of CHD due to the presence of two risk factors is not twice that a single risk factor, but can be three, four times or higher. Thus, while it is important to assess and address individual risk factors, identification of the total burden of risk is extremely important. Risk assessment is often considered the key step in effective management of clinical risk and does so by differentiating low from high risk patients, and thereby identifying those that would benefit most from aggressive risk factor management. The rationale underlying the balance between treatment intensity and patient risk being the greater benefit of drug exposure when the patient's risk is high.

In view of the high mortality and morbidity from CHD, and the importance of early identification of subjects at high risk of cardiovascular events, several risk stratification tools have been formulated to calculating risk of CHD incidence and mortality. These include Framingham, FINRISK and SCORE among others (105,106). Majority of these equations use conventional risk factors including age, gender, hypertension, smoking and hyperlipidaemia to

calculate a medium to long term risk of CHD event. While the factors implicated in clinical coronary events as discussed in the previous section i.e. plaque burden, plaque morphology and systemic factors, are to an extent a downstream effect of the CHD risk factors, it is known that while calculating a risk proportion for the general population is a great variation of observed CHD risk within each stratum (107). Thus, there is still a large amount of risk left unaccounted for by such tools. Furthermore, while these equations have accurately predicted CHD risk in Caucasian populations, they fail to accurately estimate the risk in South Asians (108,109). One important reason for inaccurate estimation in diverse ethnic groups is that the baseline data used to create these risk stratification tools is from a predominantly Caucasian population. This has several crucial implications: first, risk equations take into account the baseline CHD risk in a population and then add the effect of each risk factor to formulate an overall risk score and therefore predictions. Since the baseline CHD risk differs among ethnic groups any calculation which does not take this into account will not provide accurate estimates. Second, as demonstrated by INTERHEART (5), the relative effect of risk factors differs among ethnic groups, i.e. there is a large variation in the relative importance of risk factors. Thus the percentage risk attributed to an individual risk factor while formulating the equations is likely to differ. Furthermore, most risk stratification tools use a categorical representation of risk factors i.e. cut offs for risk factors such as hypertension, blood sugar levels etc. While these cut off points are in themselves chosen arbitrarily even for the Caucasian population, these might be poor in assessing the risk in an ethnic population.

Third, the risk factors which were seen as significant among the baseline population i.e. Caucasians, were incorporated. These might not reflect the risk among diverse ethnic populations. While impaired vascular endothelial function(78), raised C-reactive protein (60,110), elevated homocysteine (22) and lipoprotein (a)(9,12,111) are known risk factors for

CHD, and are more prevalent amongst South Asians than Europeans, they are not accounted for in the assessment of CHD risk using these equations.

In order to accurately identify the overall risk for CHD mortality, the pathophysiology of atherosclerotic coronary plaques, as well as the pertinent local and systemic factors must be taken into account in addition to the standard risk factors.

1.3 Pathophysiology of coronary heart disease

1.3.1 Atherogenesis:

i. Initiation:

The main pathological feature in coronary artery atherosclerosis is the atherosclerotic plaque, which develops following a complex interaction between inflammation, cell necrosis and deposition of lipid within the arterial wall (48,112-115) (Figure 1.2). Endothelial dysfunction secondary to damage by free radicals generated by factors such as smoking, diabetes, hypertension and elevated LDL is often considered as the initial step in formation of plaque (48). This endothelial damage results in increased endothelial adhesiveness and permeability allowing increased LDL entry into arterial intima. In addition, the endothelial damage causes release of cytokines and growth factors whose chemotactic nature stimulates monocyte-derived macrophages and T lymphocytes accumulate at the site of injury. These macrophages engulf the LDL, and activate the hallmark foam cells of atherosclerosis.

Once the endothelium is damaged, it activates a self sustaining cascade. Activated macrophages, T lymphocytes and oxidized LDL stimulate further release of inflammatory cytokines, chemokines, and growth factors which in turn stimulate migration and proliferation of smooth-muscle cells, as well as hydrolytic enzymes. Such enzymes can induce further damage and focal necrosis, and cell death (116). The end result of this cascade of events is accumulation of foam cells, migration and proliferation of smooth-muscle cells, and the consequent formation of fibrous tissue over the necrotic lipid rich core. This leads to formation of the characteristic atherosclerotic plaque.

Atherosclerotic plaques are further categorised into early and advanced lesions, divided according to their histological characteristics.

ii. Early atherosclerosis

The density and distribution of the foam cells and the lipid particles is used to divide/separate early atherosclerosis into three stages:

1. Type I lesion: This lesion consists of the first microscopically and chemically detectable lipid deposits in the intima and the cell reactions associated with such deposits. It characteristically consists of foam cells dispersed in small groups within the arterial intima along with minimal amount of lipid deposits.
2. Type II lesion (fatty streak): It is the first grossly visible lesion in the development of atherosclerosis. The lesion characteristically consists of macrophage foam cells forming stratum, rather than isolated groups. Also present in this stage are lipid-rich intimal smooth muscle cells, and a thin layer of extracellular lipid present in the intima. As it often appears as an irregular off white to yellow-white discoloration near the luminal surface of the artery, it is also called as the “fatty streak”.
3. Type III lesion (preatheroma): This lesion is characterised by the presence of multiple extracellular lipid pools which lie below the layers of macrophages and macrophage foam cells. The lipid layer replaces intercellular matrix proteoglycans and fibres, and drive smooth muscle cells apart. As this stage lacks a well-delineated lipid core characteristic of an advanced atheroma, it is also named the “preatheroma”.

iii. Advanced atherosclerosis:

Atherosclerotic lesions are considered advanced when accumulations of lipid, cells and matrix components, occur in association with intimal disorganization and thickening, deformity of the arterial wall. Such lesions are often associated with

complications such as fissure, hematoma, and thrombosis. Advanced lesions may produce symptoms, but the lesions that precede them are clinically silent.

Histologically, this is further divided into 3 stages:

4. Type IV lesion: This lesion is characterised by a presence of a thin intima and a large lipid core. It is considered to be the first atheroma or advanced atherosclerotic plaque and often forms the “thin cap fibroatheroma”. The plaque at this stage contains macrophage foam cells and isolated smooth muscle cells between the lipid core and the lesion surface, with none or minimal fibrous tissue.

Such lesions are the most likely culprits in acute plaque rupture through disruptions of the lesion surface, haematoma or haemorrhage, and thrombotic deposits (51,117).

5. Type V lesion: These lesions are formed after repeated subclinical rupture of plaque followed by healing and formation of fibrous tissue along with calcification. While the composition of the type V lesion can vary depending on the presence or absence of calcification and size of lipid core; type V lesions are generally more fibrosed and narrowed compared with type IV lesions with fibrous connective tissue in the intimal layer.
6. Type VI lesions are further subdivided according to the culprit pathology i.e. haemorrhage, fissures or thrombosis. While type VI lesions generally have the underlying morphology of type IV or V lesions, surface disruptions, haematoma, and thrombosis may be (although less often) superimposed on any other type of lesion and even on intima without an apparent lesion.

The lesions that constitute the histopathological classification are perceived as characteristic gradations or stages that span the transition from initial minimal changes to lesions associated with clinical manifestations. The resulting classification thus reflects the temporal history of the disease. In the early stages of atherosclerosis the sequence is predictable, and uniform, but advanced lesions may progress in different sequences, resulting in several characteristic lesion types and clinical syndromes. This depends as much on plaque burden, as on plaque morphology and composition.

1.3.2 Plaque burden and morphology

While a large coronary plaque burden portends obstructive coronary artery disease, studies now show suggest that lesion composition and morphology is a better predictor of clinical outcome than severity of stenosis (117,118). It has been observed that a high percentage of acute coronary events occur in vessels with angiographically moderately stenosis (119). Furthermore, the recent studies using IVUS in subjects known to have <50% stenosis of the coronary arteries by angiography, demonstrated bulk of new coronary events associated with lesions that were eccentric and contained relatively shallow but prominent echolucent zones suggestive of large lipid collections (120). They were similar in luminal obstruction to the plaques which remained stable, i.e. did not go on to have an acute event (121).

Histopathological and IVUS examination of atherosclerotic plaques associated with acute coronary events show two-thirds of acute events result from the rupture of the thin macrophage rich fibrous cap. The rupture of this thin inflamed fibrous cap exposes the necrotic core to the luminal blood and leads to thrombotic occlusion of the coronary vessel (51,122).

These features support the concept that plaques that are prone to rupture demonstrate outward remodelling at the site of culprit lesions rather than luminal encroachment causing angiographically visible stenosis and have several important implications.

First, due to different remodelling patterns i.e. negative versus positive remodelling seen among coronary arteries with stable CAD versus acute coronary syndrome, angiography cannot necessarily risk stratify subjects well. Second, and more importantly, the thrombogenic potential of a plaque depends to a large degree on the composition of the underlying lesion or intima, as well as modifications of shear and tensile forces to which the lesion or intima is exposed, rather than presence of advanced stenosis.

1.3.2 Emerging modalities for risk assessment

As discussed in previous sections, the risk of symptomatic clinical coronary events is an amalgamation of multiple risk factors. These include a) The degree of intra luminal coronary obstruction due to plaque burden, as well as plaque surface (123,124), b) plaque characteristics/ morphology which can act as local thrombogenic agents (125), c) systemic risk factors and thrombogenicity (126).

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%. While this has been traditionally through the assessment of the coronary arteries using invasive techniques such as intravascular ultrasound; the importance of assessment of atherosclerosis burden rather than individual plaque characteristics is also being recognised by means of non-invasive techniques (127,128). Modalities such as electron beam computed tomography allow detection of calcification within the coronary arteries, and thus quantify the atherosclerosis burden therein.

Arterial disease is rarely confined to one vascular bed, thus, ultrasonography of the carotid or an accessible peripheral artery, has been used to provide an insight into disease in the cerebral or coronary vasculature; a “pan arterial” assessment that represents subclinical peripheral, cerebral and coronary vascular disease. Moreover, assessments of plaque morphology undertaken in a peripheral artery are thought to be 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 CHD (120).

While anatomical detection of atherosclerosis and quantifying plaque morphology is important, it is equally important to evaluate blood thrombogenicity and systemic inflammation, identification of the “vulnerable patients” (127), as opposed to just vulnerable plaque. 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), are thus important factors in assessing CHD risk.

However, in the absence of large scale population studies demonstrating the prognostic ability of many of the risk factors, they cannot be assimilated into population based risk stratification equations. As technology has advanced one possible improvement to such equations has been revealed in the form of ability to visualize coronary atherosclerosis and its functional significance, non-invasively before it becomes clinically manifest i.e. subclinical atherosclerosis.

1.3.3 Sub clinical atherosclerosis

Traditional epidemiological studies have been instrumental in developing the concept of cardiovascular risk factors and cardiovascular risk stratification. This is mainly through risk factors association with adverse outcomes such as death or myocardial infarction. However, there has been considerable interest in moving away from large, outcome based studies to smaller studies that use measures of subclinical atherosclerosis as their primary endpoint.

Studies based on such measures of atherosclerosis have several intrinsic advantages. It is much easier to examine the subclinical phase of atherosclerosis, when treatment or interventions have the most potential for altering the natural history of atherosclerotic disease. Treatment effects and behavioural changes are less likely to complicate interpretation of study results. Measurement of subclinical disease can enhance studies of CHD risk and prevention by allowing examination of its early stages, with other subclinical markers as well as associative factors. Finally, since measures of subclinical atherosclerosis are generally quantitative, the power to define risk associations is much improved when compared to outcome studies that use dichotomous end-points, that is, presence or absence of disease.

Thus, such studies have a potential to not only enhance the understanding of the disease process, but also risk assessments.

1.3.4 Imaging subclinical atherosclerosis

Imaging techniques can detect subclinical CHD by either directly visualising the anatomical presence of arterial disease using markers such as coronary artery calcification imaging or by measuring the effects of disease processes through functional techniques such as myocardial perfusion imaging.

Coronary artery calcification has been demonstrated to be an integral part of the atherosclerotic plaque by histopathological studies (129) and shows excellent correlation with total plaque burden (130) (Figure 1.3). Furthermore, numerous studies over the last decade have demonstrated the independent prognostic information provided by coronary artery calcium measurement in predicting CHD mortality, after adjustment for conventional risk factors. This thus identifies the actual “at risk” population and improve the predictive ability of risk stratification models i.e. subjects with high levels of coronary artery calcification have more events than those without even with the same prevalence of other CHD risk factors (131-135).

While coronary artery calcification imaging identifies presence of plaque and overall plaque burden, it does not allow quantification of obstructive coronary disease. Thus, while subjects at a higher risk of CHD are identified, the hemodynamic relevance and the resulting ischemic burden of the detected atherosclerotic lesions cannot be quantified. Myocardial perfusion imaging is a technique which has been validated for functional assessment of flow limiting lesions within coronary arteries.

Myocardial ischemia as quantified by myocardial perfusion imaging has been shown to add prognostic benefit independent to known coronary anatomy in subjects with known coronary artery disease; as well as to risk assessed by traditional risk factors and risk factor equations among asymptomatic subjects (136-140).

At a population level, however, as the prevalence of silent myocardial ischemia varies according to the characteristics of the patient population screened, the prevalence of perfusion defects is understandably low in low risk asymptomatic populations with increasing prevalence as the risk of CHD increases. Thus, the importance of perfusion imaging is in delineating presence of flow limiting disease in subjects at a high risk of CHD. This risk could be quantified either through use of risk stratification equations or direct visualization of coronary plaque through calcium imaging.

Several studies have demonstrated the use of coronary artery calcium imaging as a screening test for presence of atherosclerotic plaque, with further quantification of flow limiting coronary disease through myocardial perfusion imaging (141,142). There appears to be an increase in prevalence and extent of ischemia with increasing coronary calcium. Furthermore, these studies have demonstrated a greater mortality in subjects with higher levels of silent ischemia, with absence of inducible ischemia has been shown to identify subjects with very low (<1% per year) risk of coronary events (143) further supporting the rationale of such an approach. Thus a sequential imaging strategy using initial CAC imaging followed by selective MPI, combines the advantage of high sensitivity of CAC imaging with the specificity of MPI for predicting angiographic stenosis, thereby improving assessment of a high risk population for future CHD events (141).

1.4 Coronary Artery Calcification

1.4.1 Pathogenesis

There have been different views regarding the mechanism of calcium deposition in the atherosclerotic plaques. While calcification was initially considered a passive process of adsorption or precipitation and merely a secondary effect of advanced atherosclerotic degenerative processes; recent studies indicate that atherosclerotic calcification is an organized, active process which is mediated and regulated by various biological factors (130,144) such as apoptosis of smooth muscle and foam cells, calcification-regulating proteins and lipoproteins.

Apoptosis of smooth muscle cells and macrophages-derived foam cells is considered to be a critical trigger event in intimal vascular calcification (145), through formation of matrix vesicles (146). Apoptotic bodies along with organelle-remnants from intimal vascular smooth muscle cells serve as nucleation sites for calcification. In vitro studies have demonstrated inhibition and stimulation of vascular calcification in cell cultures of human vascular smooth muscle cells (147), to be intrinsically affected by inhibition and stimulation of apoptosis. These observations further strengthening the argument for a role of apoptosis in calcification

Calcification-regulating proteins such as osteopontin, matrix Gla protein phosphatase and bone sialoprotein appear to play an important role of in the process of atherosclerotic calcification. They are expressed by vascular smooth muscle cells, as well as by certain subsets of macrophages within plaque (148-150). Presence of osteopontin in atherosclerotic plaque and its absence in normal arteries without atherosclerosis (151), along with its co-

localization with sites of early calcification in the plaque, provide support for the role of such factors in vessel wall calcification.

Lipids and lipoproteins are considered important in the process of vascular calcification . Calcium and cholesterol crystals appear to co-localize within the lipid core (112), and oxidized lipids have been shown to induce osteoblastic differentiation and calcification of calcifying vascular cells (149). This theory is further supported by studies which demonstrated spontaneous lipid accumulation within multicellular nodules prior to calcification, and stimulation of calcification in cultures of human vascular smooth muscle cells after addition of modified lipoproteins (150).

Calcification of the intima is thus the net result of a balance of these and many other factors, promoting and inhibiting calcification. Histopathological studies have demonstrated presence of calcified nodules containing calcium hydroxyapatite within atherosclerotic plaque in the form of microscopic deposits, as early as type III or IV lesions, the “early atherosclerotic lesions”. Calcification begins as small microscopic deposits within type III or IV lesions which coalesce to form large macroscopically detectable deposits as disease advances (152).

1.4.2 Implications of plaque calcification

While the initiation and progress of coronary artery calcification is multifactorial, it is essentially linked with the process of atherosclerosis, studies have demonstrated that it exists only in arteries with an active atherosclerosis process and is absent in normal arteries (153). There is however lack of consensus regarding the role of calcium in the atherosclerotic plaque as to whether it denotes a plaque as stable or prone to rupture.

Studies which have used fluoroscopy to assess segments of the coronary tree with known plaques morphology, have indicated a wide range of histological plaque types present at segments, showing a specific pattern of calcification (154). While plaque ruptures were seen frequently in areas with speckled calcification, fragmented calcification, there was no evidence of rupture seen in segments without calcification. Healed or old ruptures were seen in areas of calcification, majority of the times associated with diffusely calcified areas (154).

Thus there are two interpretations of coronary artery calcification: First, at the individual lesion level, calcification increases with progression of the plaque, often at later stages i.e. IV or V. During these initial stages of calcification there might be increased stress near the junction of the cap and the adjacent intima. It is here, at the interface between a high and low density tissue i.e. calcified and non-calcified atherosclerotic section, that plaque rupture often occurs. Thus during early stages of plaque disease, with moderate (speckled, fragmented) calcification, it might make the plaque more prone to rupture, given that the plaque itself is vulnerable.

As the degree of calcification increases, the number of interfaces between rigid and distensible plaque initially would increase until the point at which the rigid plaques coalesce. Calcification beyond this point may be associated with decreasing risk of plaque rupture, whereas the early or intermediate stages of calcification may actually enhance plaque vulnerability, by creating more junctions.

A second interpretation could be at the level of the entire coronary tree i.e. for the patient rather than a single lesion. Thus, a heavily/ diffusely calcified plaque itself might not be prone to rupture, as supported by the stiffness and resistance to rupture demonstrated by plaques with extensively calcified plaques, compared with cellular lesions or normal vessel walls (155). However, as it is a late feature in the cascade of atherosclerotic plaque formation, its presence implies the presence of early lesions elsewhere in the coronary tree.

The potential lipid-rich and possibly unstable plaques “the thin cap fibroatheromas”, with lower amounts of detectable calcification.

Thus while the role of calcification within the plaque is not yet completely understood, in the absence of available technology that allows easy visualization of the vulnerable plaque thus limiting ability to assess the risk of rupture at the level of an individual lesion, coronary artery calcium scores are useful for detection of individuals at “high risk” of CHD (130) (156).

1.5 Coronary artery calcification imaging

Coronary artery calcification can be detected by various modalities including fluoroscopy, CT, Xray, IVUS among others. Conventional fluoroscopy was the first technique for imaging coronary artery calcification (129), followed by digital subtraction fluoroscopy. Due to the poor spatial resolution, this technique was unable to detect small plaques and was taken over by high resolution EBCT, with its superior temporal and spatial resolution (157).

The guiding principle of CT scanners is the differential attenuation of X-rays passing through various tissues of the body. EBCT scanners do not have the disadvantage of having a mechanically rotating gantry, unlike conventional CT scanners. An electron gun shoots the electrons that are then guided electro-magnetically to Tungsten target rings. The X-rays thus produced then pass through the patient and are detected on two parallel rings housed within the gantry of the scanner. It takes 100 milli-seconds (ms) for one sweep of the target rings. Considering that it requires 30-40 slices for an average 3 mm (range 1.5 – 6 mm) thickness to image the entire coronary tree, the entire heart can be imaged in 30 – 40 seconds. The radiation burden and motion artefacts are reduced by prospective gating, whereby the image acquisition is triggered at 60% - 80% of the RR interval on the ECG, corresponding to the end of diastole. The total radiation delivered during one study is approximately 0.8 – 1.3 mSv.

The main disadvantage of using conventional CT i.e. multi-slice CT, rather than EBCT scanners was the use of a mechanically rotating gantry, and thus problems with movement and acceleration, necessitating much longer radiation exposure and poor temporal resolution. The gantry speed has improved through successive scanner generations and the current state of the art scanners can complete one rotation in 330 milli- seconds. However,

they are still limited and any further improvement is unlikely due to problems in handling the huge gravitational force generated by the rapid movement of a relatively heavy gantry.

A few studies have compared the data variability between these two imaging modalities (EBCT and MSCT scanners) and they show good correlation between the scanners in terms of accuracy and reproducibility. Knez et al showed an excellent correlation between MSCT and EBCT for quantification of coronary calcium in 99 patients ($r = 0.994$, $p = 0.01$)(158). Becker et al compared the two modalities in 100 patients and found a good correlation between the two types of scanners (159).

However, for the purpose of this study an EBCT scanner as used by Agatston et al, was used for all patients.

1.5.1 Algorithms for Quantification of Coronary Calcium

The calcium scoring system was first described by Agatston et al in 1990 (157). They described a scoring algorithm, which takes into consideration the area and density of the calcified plaque. Calcified foci within the outline of epicardial coronary arteries with a threshold area of 1 mm^2 and a threshold attenuation value of 130 Hounsfield units are scored. CAC score is calculated as Maximal Computed Tomographic Number (MCTN) multiplied by area of calcification in mm^2 . The MCTN is obtained from the maximal Hounsfield intensity within the area of interest. For example, if the peak X-ray density of a calcified lesion is 400 Hounsfield Units and the total area occupied is 10 mm^2 , then the CAC score using this method is: $4 \times 10 = 40$ Agatston units (Au). The score for each lesion in a given patient is measured as shown above and all the scores are added to give the total CAC score for the patient.

While several other methods of calculating calcium mass such as volume based calcium quantification was proposed by Callister et al (160) and calculation of total calcium mass of as a product of lesion volume, average CT density of the lesion in Hounsfield units and a calibration factor (161).

However, there is no standardised reference database for the mass and volume scores as yet. Further, majority of the existing studies have used the Agatston score in relation to coronary artery calcification. Thus, it continues to be the clinical standard used in majority of the centres

1.5.2 Prognostic value of coronary artery calcification imaging

Coronary artery calcification provides us a non- invasive anatomic assessment of the coronary tree. Presence of calcium within coronary arteries is pathognomic of plaque (153) and portents extensive atherosclerotic disease with the amount/extent of calcification closely related to total atherosclerotic plaque burden (144,153), and severity of coronary disease. It is a strong predictor of coronary events and all cause mortality in subjects with and without known coronary disease.

The risk of coronary event and mortality rises with the presence of calcification (> 0 Agatston units); with worsening prognosis as the amount of calcification increases, i.e. with increasing burden of calcification (>100 Agatston units and > 400 Agatston units) (134,162). Coronary artery calcification adds independent prognostic value to that offered by conventional risk factors alone (134,163,164), supporting its role as an important risk stratification tool. More recently, a report of American College of Cardiology Foundation Clinical Expert Consensus Task Force (165) regarding the risk assessment for CHD in

asymptomatic adults has also supported the independent prognostic ability of coronary artery calcification for CHD outcomes, beyond traditional risk factors.

Several large scale studies in the last few years have evaluated the role of coronary artery calcification in different ethnic populations (134,166). While the prevalence of coronary calcification is significantly higher in subjects with traditional cardiovascular risk factors such as hypertension, diabetes, obesity, infrequent exercise, previous smoking, and hypercholesterolemia (148,166-169), the presence and burden of calcification differs among different groups (166,170,171). This difference is most striking among African American participants, who have been shown to demonstrate lower levels of calcification, despite demonstrating worse cardiovascular risk profiles, compared with North American Caucasian populations (166,170,172). However, recent prospective studies suggest similar mortality among different ethnic groups, for a given level of calcification (134,164) supporting its role as a risk stratification tool.

When compared to the vast amount of data evaluating coronary calcification among Caucasians, there is little data regarding prevalence and extent of coronary artery calcification in South Asians. Till date, only two studies have evaluated the prevalence of coronary artery calcification among South Asians. They both involved a small number of subjects, and demonstrated conflicting results. The first study was by Hatwalkar et al (173) with a cohort of 156 South Asian subjects. They demonstrated a similar prevalence of coronary artery calcification between younger South Asians and North American caucasian, but a higher prevalence and extent of calcification when compared to Hispanics, African Americans and Asians. Furthermore, the higher prevalence of demonstrated a higher prevalence of coronary artery calcification in South Asians in the older age groups. Interestingly, this study had lower rate of smoking among Caucasians, with lower rates of hypertension and diabetes among South Asians than seen in most other studies.

The second study was performed by Chaturvedi et al, with 83 South Asian subjects (174), consisting a mix of subjects with known CHD as well as asymptomatic subjects. While the risk factor prevalence was similar to that seen in the general population, it demonstrated similar coronary artery calcification among South Asians and European. While the studies have provided us with comparative data, both these studies have been small and inadequately powered to assess ethnic differences in coronary calcification.

1.6 Myocardial perfusion scintigraphy

1.6.1 Pathophysiology

The basis for myocardial perfusion imaging is the visualization of myocardial blood flow. In normal coronary circulation, the blood flow through coronary epicardial arteries is autoregulated by the arterial bed in order to maintain tissue perfusion. When a stenosis develops, the pressure across the lesion drops and consequently, the arteriolar bed progressively dilates to maintain normal blood flow to the myocardium. However, this compensatory dilatation fails to cope with increasing severity of stenosis, beyond a certain point. These autoregulatory and vasodilatory reserves are thought to reach a maximum when a stenosis beyond 90% of the coronary artery diameter is present (175). As the severity increases beyond this point the artery can no longer dilate to maintain normal perfusion flow and pressure, resulting in decreased myocardial blood flow during rest.

However, during stress or hyperaemia, this disparity of blood flow between a normal and a stenosed artery is apparent at a much lower grade of coronary artery stenosis, starting at approximately 45% diameter stenosis and progressively worsening with increasing severity of stenosis (176). Thus a normal artery will dilate to a much greater extent than a stenosed one, causing increased blood flows. The resulting differential uptake, between areas supplied by normal and stenosed epicardial arteries forms the basis for myocardial perfusion imaging (177).

The stress can be achieved through physiological means or pharmacological means i.e. through exercise using a treadmill or bicycle or through or pharmacological means i.e. using either vasodilators such as adenosine or dipyridamole or inotropes such as dobutamine.

The myocardial blood flow is visualized through use of radioactive tracers such as Thallium -201, 99m Technetium Sestamibi or 99m Technetium Tetrofosmin which are extracted up by the myocardium and the resulting gamma rays captured by the gamma camera. The resulting differential uptake of radioactive tracer secondary to regional myocardial flow disparities, downstream of coronary arteries with significant obstructive coronary artery disease during stress produces myocardial perfusion defects or “silent myocardial ischemia”.

1.6.2 Perfusion imaging

Myocardial perfusion imaging relies on the technique of single photon emission computed tomography (SPECT). The patient is injected with a radioactive isotope during stress, followed by image acquisition; and then separately undergoes “rest” or “redistribution” injection and image acquisition. The isotope enters viable myocardial cells and emits gamma or X-rays. A gamma camera on a moving gantry rotates around the patient, and detects light created when the gamma rays collide with the sodium iodide crystals in front of the camera. The machine software is programmed to know where camera head is in space and therefore can orient the heart in space. The images are then consolidated to produce a three-dimensional model of the source of activity by either filtered back projection or iterative methods. This model can then be viewed from any angle, or sliced in any plane for ease of interpretation.

Myocardial perfusion scans represent global LV perfusion; therefore the size, severity and reversibility of the perfusion defect imply the total ischemic burden. Similarly a calculation of the number of segments involved on a multislice tomographic evaluation of the SPECT study can be used to calculate both the extent and severity of the ischemic

myocardium. Using sequential short axis slices a “polar map” can be either visually or quantitatively evaluated to provide the “total ischemic burden”. Short axis slices are used to evaluate the uptake in four broad quadrants (anterior, septal, inferior and lateral). The LV is further divided into apical, mid and basal segments; the apex is assessed with the vertical long axis slice. The slices are divided into 17-20 segments. The uptake is scored in 5 grades(0, normal; 1, equivocal; 2, moderate; 3, severe reduction of radioisotope uptake; and 4, apparent absence of tracer uptake).

A representative method of scoring the total ischemic burden (178) is using summed stress, rest, and difference scores (SSS, SRS, and SDS). SSS and SRS determined by the sum of scores of each segment from the stress and rest images, respectively while summed difference scores (SDS) are determined by the sum of the difference between the SSS and the SRS. The SDS can then be converted to percent myocardium ischemia by dividing the SDS by 68—the maximum potential score (4 X 17)—and multiplying by 100(179). While as SSS > 4 is considered suggestive of presence of myocardial ischemia (180), the percentage myocardium signifies severity of ischemia (181).

1.6.2 Clinical correlation

Myocardial perfusion imaging is a well validated technique for the non-invasive diagnosis of coronary artery disease (90, 91). Presence of ischemia on myocardial perfusion scintigraphy is strongly associated with adverse cardiovascular events even in the absence of ischemic symptoms, while absence of ischemia on perfusion imaging predicts a low risk of cardiovascular events (182). Numerous studies using this technique have been performed among subjects with known coronary disease, and have demonstrated independent prognostic

value of total ischemic burden in predicting adverse clinical outcome independent of severity of coronary disease and concomitant risk factors (136-138).

More recently, the importance of flow limiting coronary disease as quantified by myocardial perfusion imaging have been assessed among asymptomatic populations including asymptomatic diabetics (139,183), those with intermediate Framingham risk scores (139) , as well in subjects with high coronary artery calcium scores (141,142). These studies have demonstrated a greater mortality in subjects with higher levels of silent ischemia, with absence of inducible ischemia has been shown to identify subjects with very low (<1% per year) risk of coronary events (143).

Previous studies have also demonstrated an increasing prevalence of stress-induced ischemia detected by MPS with increase in CAC, among patients suspected to have CHD (179,184) as well as asymptomatic healthy subjects (140,185). However, while a high prevalence of silent myocardial ischemia is observed amongst subjects with CAC scores of more than 100 Agatston units, a negligible ischemia is demonstrated among subjects with low/ absent coronary calcium as demonstrated by our group and others (140,179,184,186).

Thus, MPS is often used in conjunction with CAC imaging; with CAC providing a screening test for detecting presence of atherosclerotic coronary artery disease and MPS providing a functional assessment of flow limitation due to significant obstructive coronary artery disease.

While there are ethnic differences in atherosclerotic burden as quantified by coronary artery calcification and related mortality, there is little data assessing ethnic difference in myocardial perfusion. Shaw et al (187), in a study carried out among African-American and North American caucasian populations, demonstrated a higher prevalence of moderate to severe myocardial perfusion defects among African-American compared with North American Caucasian. However, follow up from the study identified a greater risk of events

among African- Americans for all grades of perfusion abnormalities as compared with North American Caucasian, both before and after adjustment for cardiovascular risk factors. However, there appears to be no data regarding prevalence of myocardial ischemia in relation to coronary artery calcification among South Asians.

1.7 Hypotheses

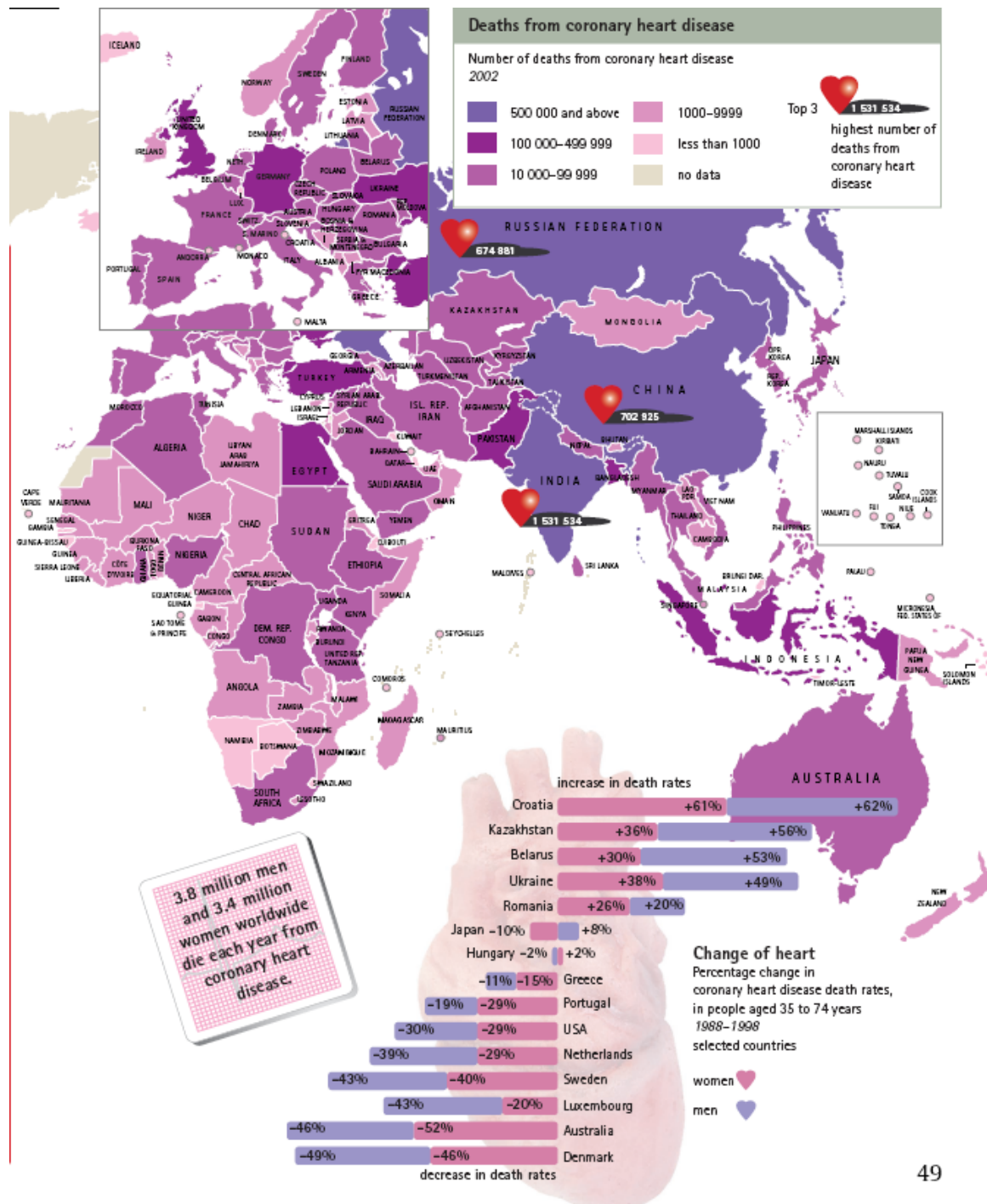
South Asians have a higher prevalence of CHD and a higher mortality attributable to CHD. The reasons for this excess are not fully known. The currently known risk factors and risk stratification equations do not accurately assess the CHD risk in this population. Thus, we have an ethnic population which forms a quarter of the global population, with one of the highest CHD rates globally, both increasing at an exponential rate (2). Despite the need for aggressive risk assessment and management in South Asians (188), there is a lack of accurate and robust risk stratification tools.

Coronary artery calcification imaging and myocardial perfusion scintigraphy are well validated techniques for the non-invasive diagnosis of coronary artery disease. While coronary calcium scores correlate strongly with the total atherosclerotic plaque burden and elevated coronary artery calcification portends advanced atherosclerotic plaque, MPS detects presence of a hemodynamically significant flow-limiting coronary stenosis. The combination of these two tests thus provides a comprehensive assessment of the coronary arterial system. It is especially useful when studying the early stages of atherosclerotic disease i.e. before it becomes clinically manifest. Along with this there is a growing body of evidence to substantiate the role of inflammation in atherosclerosis initiation and progression. This has been measured through a variety of modality from intravascular imaging, plaque imaging to assessment of systemic inflammation using molecules such as CRP.

However, majority of the studies using such techniques have been performed in predominantly European populations with little data available for South Asians.

We therefore carried out our study to test the following hypotheses:

1. Do traditional risk factors correlate with markers of subclinical atherosclerosis among South Asians?
2. Is there is higher prevalence of coronary artery calcification among in South Asians compared with Europeans?
3. Is there is elevated risk of silent myocardial ischemia in South Asians compared with Europeans?
4. Is there a greater degree of inflammation among South Asians compared with Europeans?
5. Does the inflammation correlate with measures of plaque burden?



6.

Figure 1.1 Image from World Health Report 2004: changing history, World Health Organization.

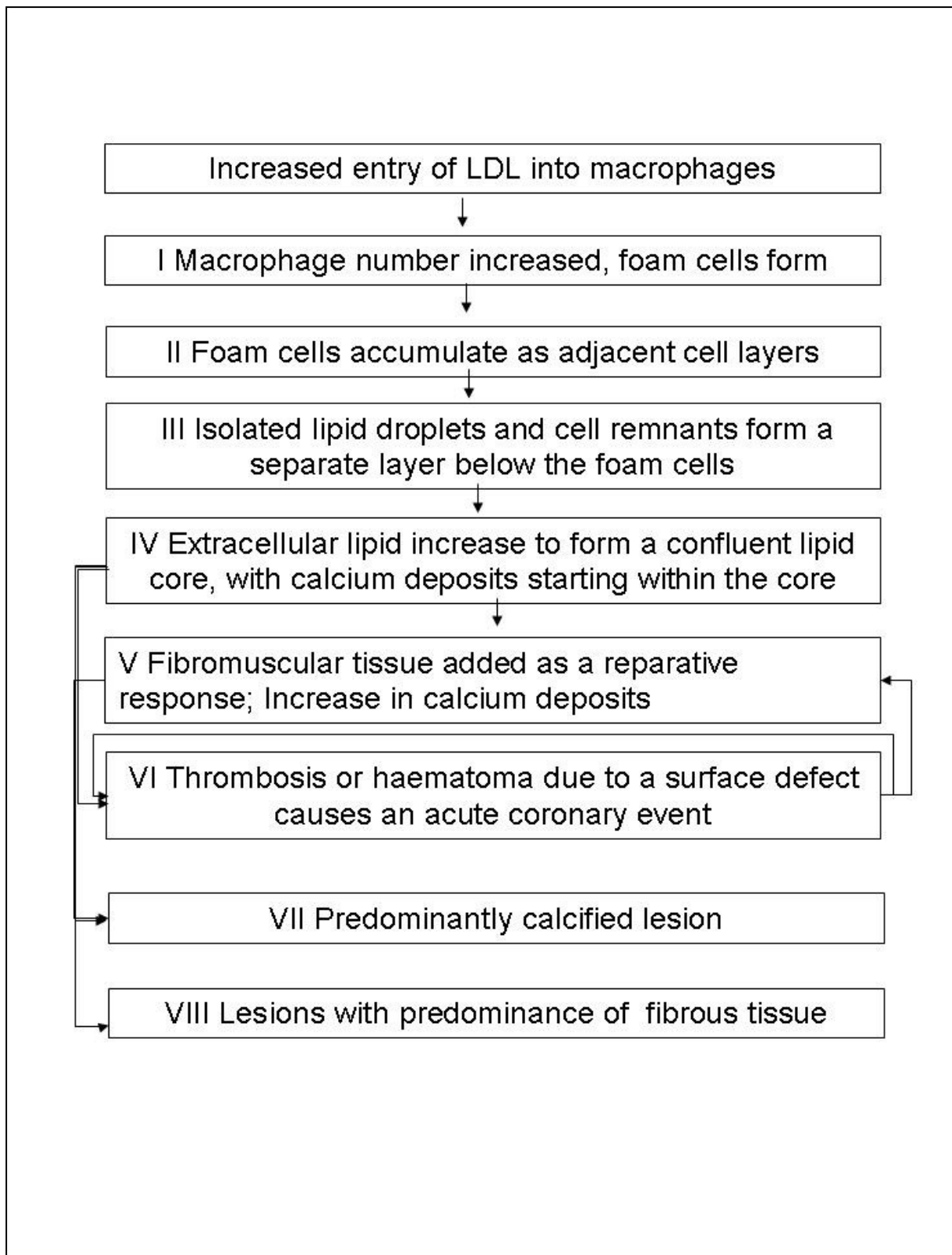


Figure 1.2 Stages in atherosclerotic plaque formation

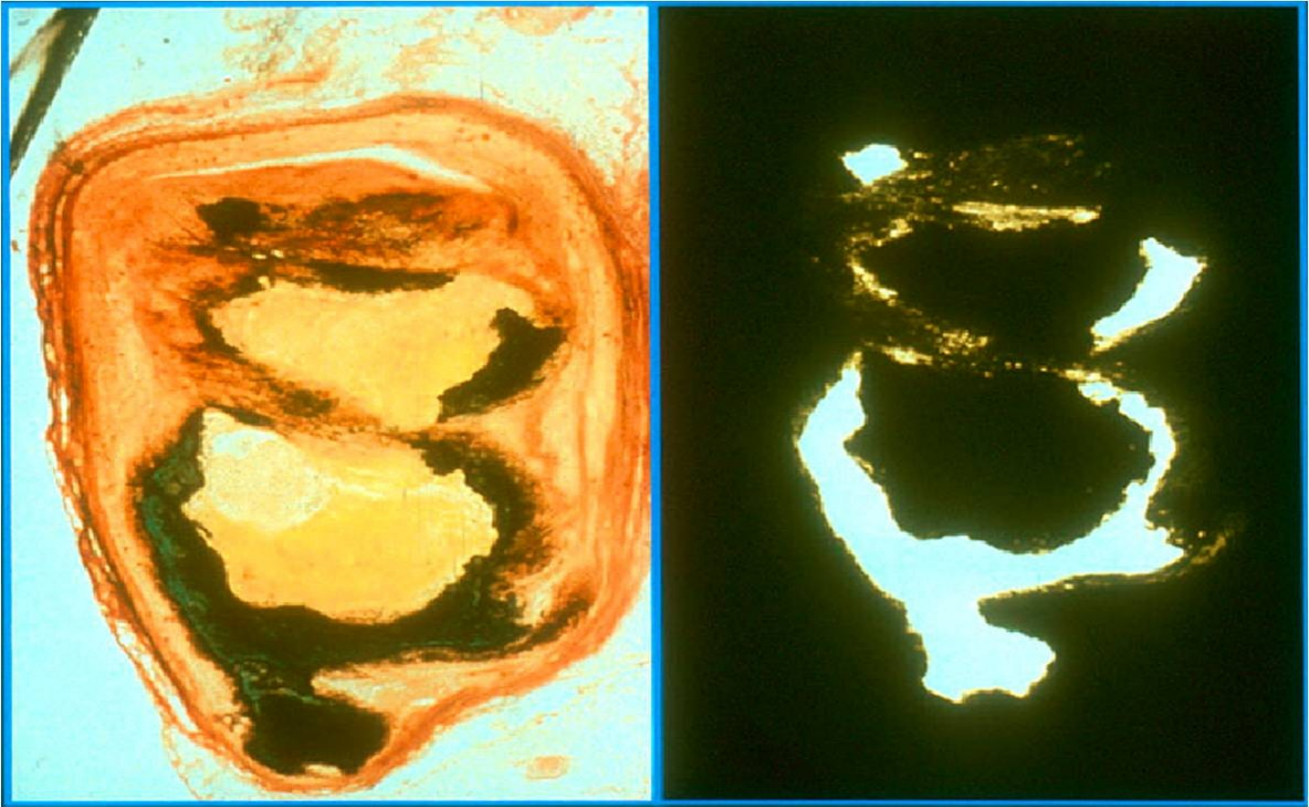


Figure 1.3 Presence of calcium in atherosclerotic plaque on histopathological and X-Ray examination of sections of the coronary tree.

(Image from Sangiorgi et al J Am Coll Cardiol 1998;31:126-33.)

Chapter2: **Methods and Materials**

2.1 Introduction

I conducted tests to compare measures of subclinical atherosclerosis, and their relationship to cardiovascular risk factors in 2369 UK South Asians and Europeans.

Written protocols were prepared for all studies. Each study and accompanying protocol was reviewed, and approved, by the Local Research Ethic committee. All subjects gave written informed consent to participate in the research projects. This chapter describes the methods used for recruitment and characterisation of subjects, tests performed, and the approach to storage and analysis of data.

2.2 Recruitment

2.2.1 ‘LOLIPOP’ study

Participants were recruited as part of the London Life Sciences Prospective Population (LOLIPOP) Study. (PI’s Prof J S Kooner and Dr J C Chambers). The LOLIPOP Study (illustrated in Figure 5.1) is a population-based investigation of cardiovascular risk among all South Asians and Europeans recruited through the age-sex lists of 58 General Practitioners (GPs) in West London, coordinated through the Cardiology Department at Ealing Hospital (Figure 2.1). It is funded by the NHS R & D, GSK and Pfizer UK. To date, 30,000 men and women aged 35-75 have been recruited in the LOLIPOP cohort. All patients of the collaborating GP surgeries, between the ages of 35-75, of all ethnicities and either sex,

were invited for a cardiovascular risk prevention assessment at their GP surgeries by trained research nurses.

Clinical data were collected by the nurses according to a standard protocol, with regular data checking and quality control. The clinical assessments of the LOLIPOP study are detailed below (Section 2.5). The map shown in Figure 2.2 illustrates the three boroughs in West London from which the participants were selected (the areas are highlighted in red).

2.2.2 LOLIPOP Intensively phenotyped cohort (IPC) sub-study

All eligible participants between the ages of 35-75 were randomly selected from the LOLIPOP study, by automated software and, stratified by age and ethnicity/ ancestry (South Asian [people with all four grandparents of South Asian origin] and European [people with all four grandparents of European origin] only). This specification was designed to ensure, as far as possible, groups of homogeneous ancestry.

Exclusion criteria included known cardiovascular disease, serious organ disease, systemic illness, serious psychiatric illness, and the presence of pathological Q waves on the ECG.

2.2.2.1 Coronary artery calcification study

7056 potential participants were identified and contacted by invitation letter, and asked to attend two different London hospitals (Ealing and Wellington Hospitals) for a range of cardiovascular tests. Out of those invited, 2600 (36.6%) subjects responded, and 2369 (90%) responders consented to undergo CAC imaging. Discussions with some non-participants revealed that most were deterred by the two separate hospital visits because of work commitments, while in a minority; anxiety about unknown hospital tests was the reason. The characteristics of responders and non responders are given in Table 2.1. There

were some significant differences between the two groups, the responders were more likely to be male, were more healthy in terms of physical activity, smoking, waist/hip ratio, BMI, blood pressure and cholesterol, and they were more likely to have a family history of heart disease. This seems to indicate that people with a stronger concern for health, with a better understanding of heart disease and implications due to a family history were more likely to be interested in, and respond to an invitation for a cardiovascular screening programme. There was no significant difference in response rates between South Asians and Europeans.

The LOLIPOP- IPC sample consisted of 1356 South Asians and 1013 Europeans aged between 35-75 years (54.7 ± 10.5), 35% female and none had documented CHD. Of the South Asian sample, 52.4% were Sikhs, 15.5% were Muslims, 25.6% were Hindus and 3.2% were Christians. The large majority (95.6%) had been born outside the UK, with average UK stay of 29.8 ± 18.4 years.

Once volunteers responded with appropriate dates, an appointment letter was sent out for Ealing Hospital. On arrival at Ealing Hospital for the first of their two appointments, the study protocol was explained to the participants and written consent was obtained. During this period, the inclusion criteria were reiterated to the participant; they confirmed that all four of their grandparents were either all Europeans or all South Asian (originated from the India, Pakistan, Bangladesh or Sri Lanka)

2.2.2.2 Myocardial perfusion imaging study

Out of the 2369 subjects who consented to undergo coronary artery calcification was measured for all participants using electron beam computed tomography. Previous work by our group and others has demonstrated negligible prevalence of silent myocardial ischemia among subjects with coronary artery calcification of less than 100 Agatston units (140,179,184). Thus, in order to minimise the number of subjects exposed to

radiation, a cut-off value of 100 Agatston units was used to select participants for myocardial perfusion imaging. 518 patients had CAC > 100 Agatston units and were invited for myocardial perfusion imaging. Of these, 256 (49%) participants consented to undergo myocardial perfusion imaging.

2.3 Clinical measures

During the cardiovascular risk assessments, clinical information was collected by study nurses at the GP surgeries and corroborated by clinical notes (**Table 2.2 a&b**). Nurses collected information about medical and family history, current prescribed medication, and smoking history. Smoking was defined as never, former smoker or current smoker along with no of cigarettes per day in order to calculate pack years. Country of birth of participants, parents, and grandparents were also recorded, together with language and religion for assignment of ethnic subgroups. A fasting blood sample was drawn to measure glycosylated haemoglobin (HbA1c) and lipid profiles. Physical assessments included anthropometric measurements, blood pressure, and 12 lead 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, 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 0.1cm.

Weight and body fat percentage were recorded using digital Tanita® 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 0.1kg. The fat percentage is calculated using bioelectrical impedance analysis. A small amount of

electric current is passed through the body and the resistance that it encounters while passing through different tissues is calculated. The basic premise is that tissue resistance differs with lean tissue and muscle offering lower resistance to the tissues, compared to fat.

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 0.1cm.

BMI was calculated by dividing the participant's weight in kilograms by the square of his/her height in meters ($BMI = \text{kg} / \text{m}^2$). Waist-hip ratio (WHR) was calculated by dividing waist by hip measurements. The metabolic syndrome was defined using the NCEP ATP III criteria (Expert Panel of NCEP) and the new International Diabetes Federation criteria (2006)

Hypertension was defined as history of hypertension or current use of antihypertensive medications

2.4.0 Imaging

2.4.1 Coronary artery calcification Imaging

Coronary artery calcification imaging was performed at the Cardiac Imaging and Research Centre (Wellington Hospital, UK), using a modified GE Imatron C-150 (San Francisco, CA, USA) electron beam computed tomography scanner equipped with high-

resolution detectors (Figure 2.3). Coronary visualization was performed without contrast. In order to include the entire coronary tree, forty contiguous 3-mm slices were obtained during a single breath-hold, starting at the carina and proceeding to the level of the diaphragm. Scan time was 100-ms per slice, synchronized to 40% of the R-R interval (189). This gating algorithm minimizes motion artefact and results in excellent inter-scan reliability. 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. Coronary artery calcification scores were calculated on an Aquarius workstation (TeraRecon, Inc., San Mateo, USA). Calcium scores between participants were adjusted with a standard calcium phantom. The output from EBCT scans was quantified into Agatston scores as described by Agatston et al(157).

In order to reduce inter-observer variability, image analysis was carried by only two research fellows trained in analysis of coronary artery calcification imaging at the same centre. For the purpose of the study, they were both blinded to the clinical and ethnic data. Midway through the study, 50 coronary artery calcification scans reported by one observer were reanalysed by the second observer and resulting coronary artery calcification scores compared to the original scores, in order to reassess and ensure low inter observer variability. There was excellent inter observer correlation with the original correlation coefficient for Agatston score between readers equal to 0.99 and the coefficient of variation equalling 2%.

Figures 2.4 and 2.5 show examples of EBCT scans performed in two people with very different levels of calcification. Figure 2.4 shows a CT of the chest with a healthy heart and no coronary calcification and Figure 2.5 shows a heart with calcification of the left main stem and left anterior descending artery.

2.4.2 Myocardial Perfusion Imaging

Myocardial perfusion scintigraphy was performed using a 2-day protocol (stress and rest) with Technitium-99m sestamibi. Stress imaging was performed by use of a symptom-limited exercise treadmill test according to the Bruce protocol and pharmacologic stress using dipyridamole (190) or dobutamine (191). Stress and rest SPECT images were acquired 60 to 120 minutes after injection of 600 MBq Tc-99m sestamibi by use of a large field-of-view, dual-headed gamma camera equipped with a high-resolution collimator (SMV DSTi; GE Medical Systems, Buc Cedex, France). Thirty-two projections (40 seconds per projection) were acquired over a 180° arc, from the 45° right anterior oblique position to the 45° left posterior oblique position. Strict quality control and motion artefact correction were used.

Image Interpretation: Stress-rest Tc-99m sestamibi SPECT scans were interpreted by semi quantitative visual analysis, and the findings were categorized for overall interpretation as normal or abnormal. Semi quantitative visual analysis was performed by use of the 17-segment model recommended by the American College of Cardiology/American Heart Association/American Society of Echocardiography/ American Society of Nuclear Cardiology (Figure 2.6 and 2.7). Tracer uptake was scored in each segment by use of a 5-point scoring system (0, normal; 1, equivocal; 2, moderate; 3, severe reduction of radioisotope uptake; and 4, apparent absence of tracer uptake). Summed stress and rest scores were categorized as low (0-4), intermediate (5-8), or high (>8). Summed difference scores (SDS) were categorized as low (0-2), intermediate (3-7) or high (>7). Gated SPECT was used to define artefacts when required. An SSS >4 was considered to be abnormal. SDS was determined by the sum of the difference between the SSS and the SRS. The SDS was converted to percent myocardium ischemia by dividing the SDS by 68—the maximum

potential score (4 X 17)—and multiplying by 100.(179) The extent of reversible myocardial ischaemia was categorized as mild (<5%), moderate (>5 and ≤10%), or large (>10%).

2.5 Statistical analysis

All analyses were performed using the statistics programme Statistical Package for the Social Sciences (SPSS) version 16.0 (Chicago, IL, USA). Categorical data are presented as number (percent), and continuous data as mean value± SD. The chi-square test was used to compare categorical variables while comparisons of continuous variables between two groups were performed using student t tests. All tests of significance were two-tailed, and significance was defined at the ≤ 0.05 level.

2.5.1 Coronary artery calcification

Due to the non-Gaussian nature of CAC and high prevalence of zero Agatston unit scores, CAC was classified into the following categories: 0 AU, 1-10 AU, 11-100 AU, 101-400 AU and >400 AU respectively. Thus, for the purpose of analysis two end points were used, one using presence of CAC defined as Agatston score >0 Au; and the other using CAC as categorical variable and classified into the above mentioned categories, in order to assess extent of plaque burden.

Prevalence and extent of CAC were compared across ethnic groups. The relations between risk factors and CAC were analyzed, both before and after adjusting for concomitant risk factors, using regression models. While a binomial logistic regression model was used to evaluate associations between risk factors and the presence of CAC, a separate ordinal

regression model was used to assess the predictive ability of risk factors for the extent of CAC.

2.5.2 Myocardial perfusion scintigraphy

Prevalence and extent of myocardial ischemia was compared across ethnic groups. The relationships between risk factors and myocardial ischemia were analysed using regression models, both before and after adjusting for concomitant risk factors. While a binomial logistic regression model was used to evaluate associations between risk factors and the presence of MPS abnormalities, an ordered logistic regression analysis was applied to identify clinical predictors of severity of myocardial ischemia. The resultant estimates (ordered log odds), were exponentiated to calculate the odds ratios of the predictors.

2.6 Power calculation

Power to detect risk associations between traditional cardiovascular risk factors and the primary endpoint of coronary calcium was calculated for the baseline study for the whole cohort and by gender, using logistic regression for a cohort study design. Estimates of the base-line prevalence of coronary calcification were obtained through published literature. The prevalence of subclinical disease is expected to range from approximately 15 % in the youngest women to almost 100 % in the oldest participants, as seen in studies performed in Northern European and American Caucasian populations (168).

Power to detect ethnicity-specific differences in coronary calcification was considered by treating ethnicity as a risk factor with 50% prevalence. A separate power calculation was obtained to detect differences in the correlation coefficient between relevant risk factors and coronary calcification, for South Asians compared to Northern Europeans. The power calculations were performed for each gender, for detecting associations of risk factors with varying prevalence levels and prevalence of coronary calcium. (Table 2.4)

For men, for an alpha error of 5 percent, the study will have more than 90 percent power to identify relations between risk factors with a prevalence of at least 10 percent in the cohort and the presence of coronary calcium, with an odds ratio of 1.5 or greater. The power to test similar hypothesis in women will be 75%.

2.7 Data storage

Data were stored in a purpose-built database written in Microsoft Access. The database had a modular design, with each component supporting specific areas of the research work. Database functions included selecting subjects for invitation, generating patient letters for invitations and appointments, collection and collation of patient data, importing biochemical results directly from the analytic laboratories, validation of data, maintaining data security and enabling data analysis. A data management committee was established that had responsibility for maintaining the database, supervising data security, validating and monitoring data collection for completeness, accuracy and timeliness. Data collection was validated in the following ways: range and logic checks during data entry, and systematic searches for incomplete datasets, duplicate patient attendances, extreme values, logical inconsistencies, digit preference, and systematic bias between observers. Once validated the record set was locked, and closed to further modification.

Table 2.1 Characteristics of responders and non responders for LOLIPOP –IPC study

	Responders	Non responders	P value
European (%)	44	44	0.35
Male Gender (%)	65	57	<0.001
Framingham score	0.09	0.12	<0.001
Family history of CAD (%)	25	20	<0.001
Ever Smoker (%)	44	56	<0.001
Waist-Hip ratio	0.93(0.082)	0.94(0.084)	0.02
Body mass index(Kg/M²)	27.3(4.4)	27.6(4.9)	0.008
Body Fat (%)	30.9(8.5)	32.0(8.9)	<0.001
PAI	1.76(0.96)	1.65(0.98)	<0.001
Total Cholesterol (mmol/l)	5.4	5.45	0.083
LDL Cholesterol (mmol/l)	3.5(0.8)	3.4(0.8)	0.1
HDL Cholesterol (mmol/l)	1.3(0.34)	1.4(0.34)	0.5
History of hypertension (%)	28%	27%	0.3
Diastolic blood pressure (mm Hg)	80.4(10.2)	81(10.8)	0.03
Systolic blood pressure (mm Hg)	132(18.9)	133(20.4)	0.003
Diabetes mellitus (%)	15	15	0.9
HbA1C (%)	6.9(1.5)	7.0(1.4)	0.3
Serum glucose (mmol/l)	5.71(1.8)	5.76(1.9)	0.4

Table 2.2a

		Measures
Anthropometric markers	Height *	
	Weight *	
	Body mass index *	
	Waist circumference *	
	Waist hip ratio *	
Haemodynamic factors	Blood pressure	Systolic BP *
		Diastolic BP *
Lipid profiles	Total cholesterol *	
	Low density lipoprotein cholesterol *	
	High density lipoprotein cholesterol *	
	Total: HDL cholesterol ratio*	
	Triglycerides *	
Fasting glucose levels	Fasting glucose *	
	HbA1c *	
Disorders	Diabetes *	

Measures

Hypertension *

Metabolic syndrome

NCEP ATPIII *

IDF *

Subclinical CHD
markers

Coronary artery calcification

Myocardial perfusion Imaging*

Table 2.2b:

Age distribution of participants

Age Cat	South	Asians	Europeans	South	Asians	Europeans
	Men		Men	Women		Women
35-44	128		82	90		57
45-54	276		204	162		72
55-64	230		223	198		101
65-75	200		198	76		72
Total	834		707	522		306

Table 2.3(a) risk factors among male participants

	South Asians Men (834)	Europeans Men(707)	P value
Age (Years)	55.5 ± 10.3	57.56 ±10.96	<0.001
Framingham score	0.08	0.11	<0.001
Physical activity index	1.51	2.14	<0.001
Body mass index (Kg/M²)	26.7	27.63(4.34)	<0.001
Waist Hip ratio	0.96 ±0.06	0.94 ±0.07	<0.001
Ever Smoker (%)	22%	57%	<0.001
Diabetes Mellitus (%)	17%	10%	<0.001
Glucose (mmol/L)	5.94 ± 1.91	5.48 ±1.41	<0.001
HbA1C	6.07 ±1.21	5.45 ±0.83	<0.001
Total Cholesterol (mmol/L)	5.28 ±1.05	5.55 ±1.04	<0.001
LDL Cholesterol (mmol/L)	3.29 ±0.88	3.5 ±0.88	<0.001
HDL Cholesterol (mmol/L)	1.2 ±1.27	1.33 ±0.33	<0.001
Triglycerides (mmol/L)	1.7 ±1.4	1.62 ±1.16	0.16
On Statins (%)	16%	7%	< 0.001
Hypertension (%)	53%	45%	<0.001
Systolic blood pressure (mmHg)	133 ± 17.26	135 ± 18	0.006
Diastolic blood pressure (mmHg)	82.4 ± 10	82.58 ± 10.0	0.343
Metabolic syndrome-IDF (%)	20	7	<0.001
Metabolic syndrome- NCEP (%)	26	20	0.02

Table 2.3 (b) risk factors in Women

	South Asian Women (522)	European Women (306)	P value
Age (Years)	54.3 ± 9	56.2 ± 10	<0.001
Framingham score	0.06	0.05	0.05
Physical activity index	1.51	2	<0.001
Body mass index (Kg/M²)	27.8 ± 4.9	26.8 ± 5.3	0.006
Waist Hip ratio	0.8 ± 0.08	0.9 ± 0.08	<0.001
Ever Smoker (%)	0.5	31	<0.001
Diabetes Mellitus (%)	23%	7%	<0.001
Serum glucose (mmol/L)	5.8 ± 2	5.3 ± 1.6	0.14
HbA1C (%)	6.1 ± 1.2	5.6 ± 1.0	<0.001
Total Cholesterol (mmol/L)	5.4 ± 1.01	5.7 ± 1.0	<0.001
LDL Cholesterol (mmol/L)	3.3 ± 0.8	3.5 ± 0.9	0.04
HDL Cholesterol (mmol/L)	1.4 ± 0.3	1.6 ± 0.4	<0.001
Triglycerides (mmol/L)	1.5 ± 0.7	1.3 ± 0.8	0.008
On Statins (%)	14%	7%	<0.001
Hypertension (%)	39%	31%	0.03
Systolic blood pressure (mmHg)	125 ± 11	124 ± 11	0.258
Diastolic blood pressure (mmHg)	77 ± 10	76 ± 10	0.109
Metabolic syndrome-IDF (%)	16	10	0.007
Metabolic syndrome-NCEP (%)	32	16	<0.001

Table 2.4 Power to Detect Cross-Sectional Risk Associations with Coronary Calcium

Prevalence of Risk Factors in Control Group	Odds Ratio	Power (%)		
		Whole Cohort (n = 2000; estimated baseline prevalence of coronary calcium = 39%)	Men only (n = 1000; estimated baseline prevalence of coronary calcium = 43%)	Women only (n = 1000; estimated baseline prevalence of coronary calcium = 25%)
10%	1.5	77	49	44
	2.0	> 95	83	87
30%	1.5	> 95	>95	76
	2.0	> 95	>95	>95
50%	1.5	> 95	>95	83
	2.0	> 95	>95	>95

Figures

Figure 2.1: Schematic representation of the LOLIPOP and LOLIPOP atherosclerosis studies

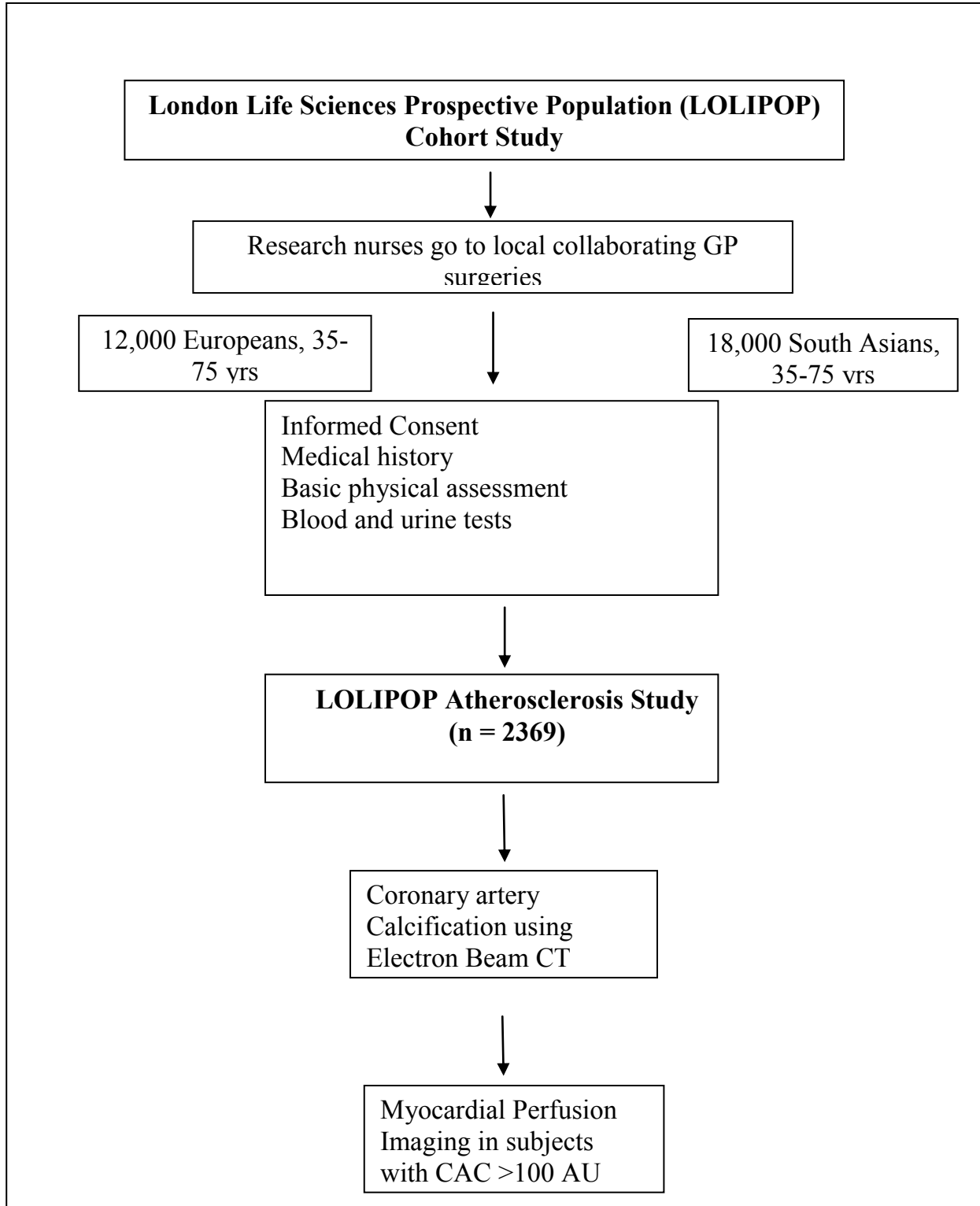


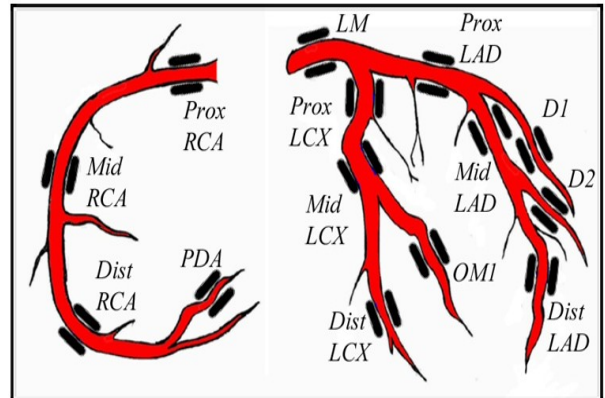
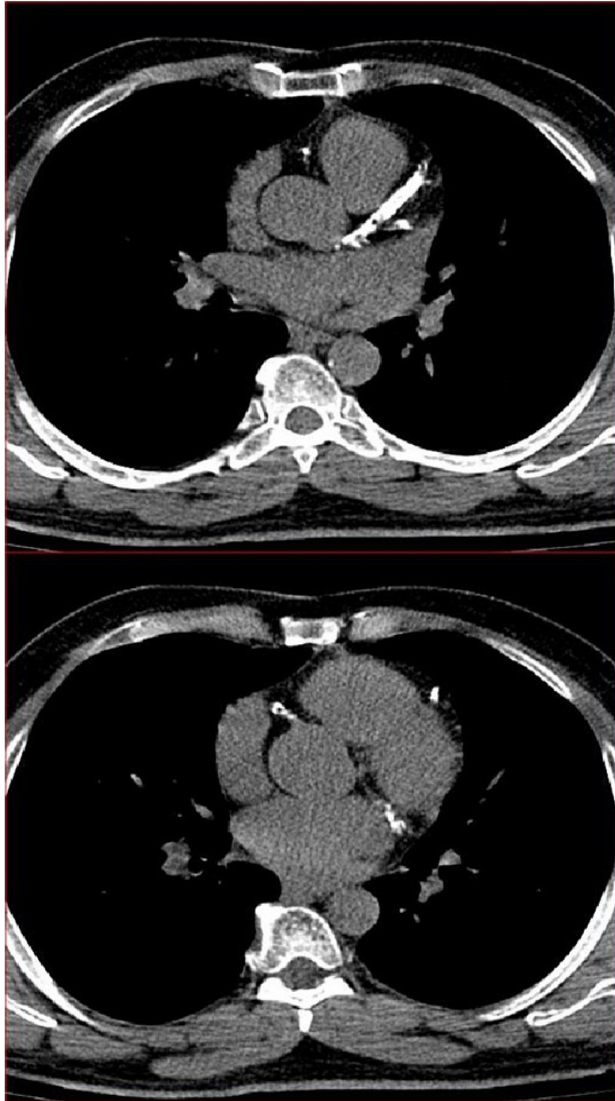
Figure 2.2: Map of London boroughs involved in the study



Figure 2.3: EBCT scan of coronary arteries with no detectable calcification



Figure 2.4: EBCT scan of a heart with coronary calcification



	Lesions		Agatston Score
Left Main	2		89.74
Left Anterior Descending	21		2209.87
Left Circumflex	24		1327.47
Right Coronary Artery	17		752.70
Total	64		4379.77

Figure 2.5a 17-segment model divided according to coronary artery territory

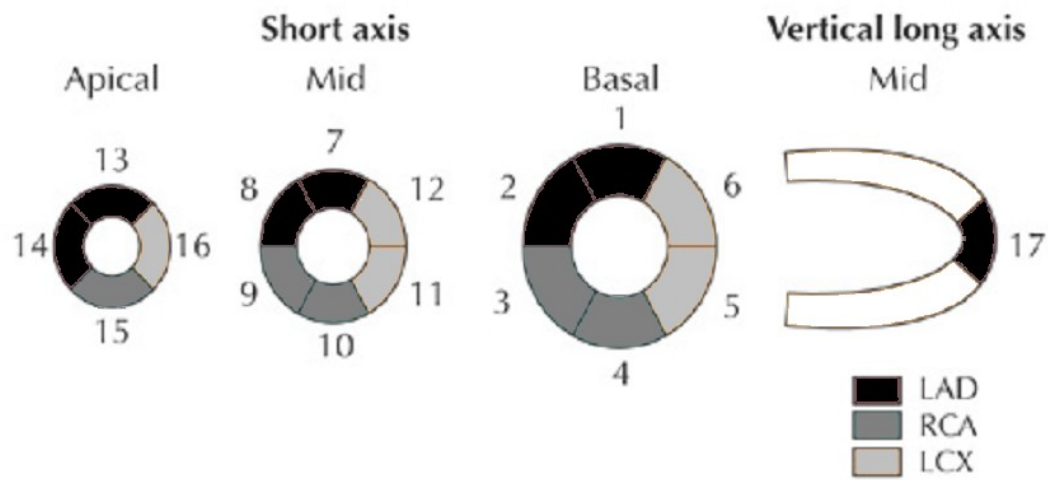


Figure 2.5b 17-segment model as polar map

- 1 = Basal anterior
- 2 = Basal anteroseptal
- 3 = Basal inferoseptal
- 4 = Basal inferior
- 5 = Basal inferolateral
- 6 = Basal anterolateral
- 7 = Mid anterior
- 8 = Mid anteroseptal
- 9 = Mid inferoseptal
- 10 = Mid inferior
- 11 = Mid inferolateral
- 12 = Mid anterolateral

- 13 = Apical anterior
- 14 = Apical septal
- 15 = Apical inferior
- 16 = Apical lateral
- 17 = Apex

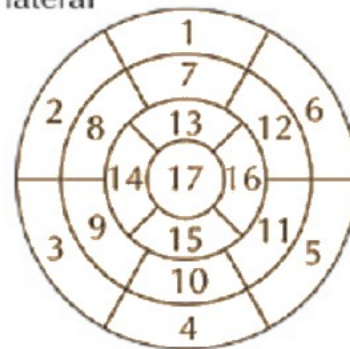
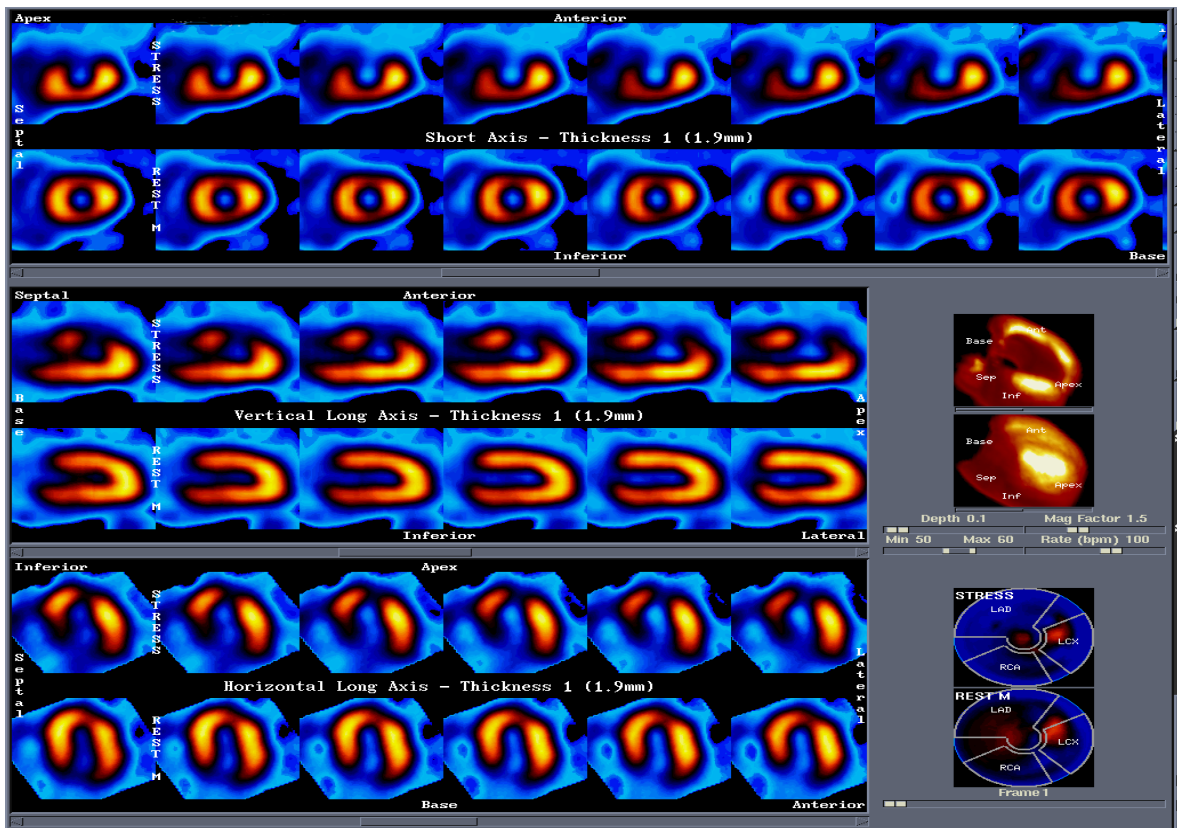


Figure 2.6 Myocardial perfusion study demonstrating inducible hypoperfusion in LAD territory



Chapter 3: Prevalence of Coronary Artery Calcium among asymptomatic South Asians and Europeans

3.1 Abstract

Background: Coronary heart disease mortality is 70% higher amongst UK South Asian compared with Europeans. Current risk stratification tools and biomarkers do not allow accurate identification of South 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 populations. We hypothesised that coronary artery calcification is increased in South Asians compared with Europeans, and may provide a non-invasive tool for assessment of increased coronary heart disease risk in South Asians.

Methods: Using electron beam computed tomography; we assessed coronary artery calcification among 2369 asymptomatic South Asians and Europeans men and women, aged 35 to 75 years, part of the London Life Sciences Population (LOLIPOP) study.

Results: Coronary artery calcification was more common in men than women, and coronary artery calcification scores were closely associated with age, cigarette smoking, hypertension, systolic blood pressure, diabetes, total cholesterol and metabolic syndrome. In contrast, there were no differences in either coronary artery calcification prevalence or mean levels of coronary artery calcification between South Asians and Europeans, after adjustment for the measured cardiovascular risk factors.

Conclusions: Coronary artery calcification is not increased in South Asians compared with Europeans, in any age group or in either gender. This contrasts with the almost 2-fold higher risk of myocardial infarction and coronary heart disease mortality observed in South Asians. Further research is needed to assess the mechanism of aggressive coronary artery disease, and resultant high mortality, among South Asians.

3.2 Introduction

South Asians (people originating from India, Pakistan, Bangladesh and Sri Lanka) account for almost a quarter of the global coronary heart disease (CHD) deaths (1). In the UK, South Asians form the largest ethnic minority group with a two-fold higher coronary heart disease mortality rate compared with the general population (4).

Global registries such as INTERHEART (5) suggest an important role for traditional risk factors in CHD in all ethnic groups. However, such factors (8,47,192) and risk scores using these factors, such as the Framingham function do not predict the higher CHD mortality in South Asians (109). Therefore, accurate and robust risk stratification is lacking for South Asians.

Intimal calcification occurs within coronary arteries in the presence of atherosclerotic plaque, and is closely correlated with histomorphometric plaque burden ($r > 0.90$)(130) and severity of coronary disease (144,153,193). Coronary artery calcification (CAC) as detected by Electron Beam Computerized Tomography (EBCT) is a strong predictor of coronary events and all cause mortality in subjects with and without known coronary disease. It also adds independent prognostic value to that offered by conventional risk factors alone (134,164,194), supporting its role as a robust risk stratification tool. However, the majority of this data is from North American and European populations, with little data existing in South Asians. The purpose of the present study was to test the hypothesis that CAC is elevated in South Asians compared with Europeans, and predicts the increased CHD risk in South Asians.

3.3 **Methods:**

I conducted coronary artery calcium imaging on 2369 subjects recruited from the LOLIOP study. While the methods for the study are described in detail in the methods and materials chapter. A brief overview follows.

Subjects: 834 male and 522 female South Asians, and 707 male and 306 female European participants between the ages of 35-75, and no history of clinical CHD underwent coronary artery calcium imaging. Exclusion criteria were history of known CVD, systemic illness, serious psychiatric illness, presence of pathological Q waves on the ECG. All participants provided informed written consent. The LOLIPOP study is approved by the Ealing and St Mary's Hospitals Research Ethics Committees.

Measurements: 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. Physical assessment included anthropometric measurements (height, weight, waist, hip) and blood pressure. For the purpose of this study, hypertension was defined as history of hypertension, a systolic pressure >140mm Hg, or a diastolic pressure >90 mm Hg and diabetes defined as history of diabetes mellitus or a fasting glucose > 7mmol/l.(195)

Imaging: CAC imaging was performed at the Cardiac Imaging and Research Centre (Wellington Hospital, UK), using a modified GE Imatron C-150 (San Francisco, CA, USA) EBCT scanner equipped with high-resolution detectors. Coronary visualization was performed without contrast, with a scan time of 100-ms per slice, synchronized to 40% of the R-R interval(189). Coronary artery calcification scores were calculated on an Aquarius workstation (TeraRecon, Inc., San Mateo, USA). Calcium scores between participants were adjusted with a standard calcium phantom.

The output from EBCT scans was quantified into Agatston scores as described by Agatston et al(157). Due to the non-Gaussian nature of CAC and high prevalence of zero Agatston unit scores, CAC was classified into the following categories: 0 AU, 1-10 AU, 11-100 AU, 101-400 AU and >400 AU respectively.

Thus, for the purpose of analysis two end points were used, one using presence of CAC defined as Agatston score >0 Au; and the other using CAC as categorical variable and classified into the above mentioned categories, in order to assess extent of plaque burden.

In order to reduce inter-observer variability, image analysis was carried by two research fellows trained in analysis of CAC imaging at the same centre. For the purpose of the study, they were both blinded to the clinical and ethnic data.

Statistics: All analyses were performed using the statistics programme Statistical Package for the Social Sciences (SPSS) version 16.0 (Chicago, IL, USA). Categorical data are presented as number (percent), and continuous data as mean value \pm SD. The chi-square test was used to compare categorical variables while comparisons of continuous variables between two groups were performed using student *t* tests. All tests of significance were two-tailed, and significance was defined at the ≤ 0.05 level.

Prevalence and extent of CAC were compared across ethnic groups. The relations between risk factors and CAC were analyzed, both before and after adjusting for concomitant risk factors, using regression models. While a binomial logistic regression model was used to evaluate associations between risk factors and the presence of CAC, a separate ordinal regression model was used to assess the predictive ability of risk factors for the extent of CAC.

3.4 Results:

Clinical and biochemical characteristics of patients

South Asians were on average three years younger than Europeans, with a higher prevalence of diabetes mellitus, hypertension, and metabolic syndrome. South Asians also had higher serum glucose and triglycerides levels with lower total cholesterol, HDL and LDL cholesterols, physical activity index and smoking rates compared with Europeans. 15% of South Asians were on cholesterol lowering medications compared with 8% of Europeans (Table 3.1).

Prevalence and extent of coronary artery calcification among UK South Asians and Europeans

The age- and gender-related burden of CAC among South Asians and Europeans is shown in Figures 3.1 (CAC prevalence) and 3.2 (CAC extent). The prevalence and extent of CAC increased with age and were higher in men compared with women ($p < 0.001$). While univariate analysis suggested a tendency towards lower prevalence and extent of coronary artery calcification among South Asians compared with Europeans (CAC prevalence, $p = 0.053$; CAC extent, $p = 0.07$), there were no significant differences among the two populations after adjusting for age and gender. Similar results were obtained when data were analysed using Agatston score of > 10 as threshold for presence of coronary artery calcification.

Using thresholds of 100 AU and 400 AU, to assess for ethnic differences in moderate and extensive atherosclerotic plaque, suggested a lower prevalence of extensive plaque among South Asians compared with Europeans (Table 3.2). However, there were no significant differences among the two populations after adjusting for age and gender.

Predictors of presence of coronary artery calcification

A multivariable logistic regression model using cardiovascular risk factors and ethnicity explained about 37% of the variation in CAC in this population. An increase in age, statin use, male gender, hypertension, current smoking and diabetes were independently associated with CAC (Table 3.3). After adjustment for cardiovascular risk factors, ethnicity was not related to prevalence of CAC, OR=1.1 (95% CI 0.8, 1.3).

Ethnic-specific analyses were performed to evaluate effect of differences in risk factor profiles on prevalence of CAC, within each ethnic group (Table 3.4). The regression model used had similar concurrent predictive ability for the two ethnic groups, explaining about 38% of the variation in South Asians and 36% in Europeans. Age, BMI, and LDL, along with male gender, and use of cholesterol lowering medication were positively associated with presence of CAC among both South Asians and Europeans. While prevalence of hypertension and diabetes were associated with presence of CAC in South Asians, an increase in triglycerides and current smoking were positively related to CAC presence in Europeans.

Predictors of extent of coronary artery calcification

A univariate ordinal regression demonstrated age, BMI, current and former smoking, statin usage, presence of hypertension or diabetes mellitus, male gender and Europeans ethnicity, as well as serum HDL and triglyceride levels were associated with elevated odds for the increasing extent of CAC. While unadjusted OR suggested higher CAC extent among Europeans, on adjusting for age and gender, using a multivariable ordinal regression model the ethnic differences no longer remained significant. Further analyses were performed adjusting for conventional cardiovascular risk factors and ethnicity, using a multivariable ordinal regression model. Age, male gender, current smoking, statin use, diabetes,

hypertension and LDL cholesterol were all positively associated with increase in extent of CAC (Table 3.5). Ethnicity appeared not to influence CAC levels, with a relative difference of 1.05 (95% CI 0.9, 1.3), for South Asians compared with Europeans participants.

Separate analyses were performed for each of the ethnic populations in order to evaluate group-specific relationships (Table 3.6). The multivariate models used to predict extent of CAC had similar concurrent predictive abilities for both ethnicities and explained 38% of the variation in CAC prevalence. Increasing age, presence of diabetes, hypertension, male gender, statin usage and current smoking were associated with an increase in extent of CAC for both populations. While BMI and LDL showed significant associations amongst the South Asians participants; former smoking and triglycerides were significant risk factors for extent of CAC in Europeans.

3.5 Discussion

In this cohort of asymptomatic men and women, aged 35-75 years, we found no ethnic group differences in either the prevalence or extent of CAC amongst South Asians and Europeans. CAC was similar in the two populations, both before and after adjustment for conventional cardiovascular risk factors. CAC did not predict or identify the excess CHD risk in South Asians in this sample.

Consistent with existing data, we found that conventional cardiovascular risk factors were associated with both prevalence and extent of CAC (166,196). The prevalence and amount of CAC among Europeans in our study was lower than reported in similar studies using asymptomatic Caucasian populations, such as the Multi Ethnic Study of Atherosclerosis (MESA) trial (166). This could be explained by the younger age of participants in our study compared with the MESA sample. The age range in our study was selected to compare subclinical atherosclerosis in younger age groups because of the high

incidence of CHD found in young South Asians (10), compared with Europeans. Our data is consistent with study done by Chaturvedi et al (174) who demonstrated similar CAC among South Asians and Europeans with, and without known coronary artery disease, but contrasts with the study by Hatwalkar et al (173) who demonstrated a similar prevalence of CAC between younger South Asians and North American Caucasian, but a higher prevalence in South Asians in the older age groups. Our study differs from the study conducted by Hatwalkar et al in that it is population-based, has a larger number of South Asians (300 subjects compared with the 30 subjects) above the age of 60 years, a broader age range of participants and includes both genders. Our results, therefore, might reflect the population distribution of CAC more accurately. To our knowledge, ours is the largest study evaluating the distribution of CAC as a marker of atherosclerosis among asymptomatic South Asians and includes a population-based sample rather than physician referred subjects. Furthermore, we have assessed both presence and burden of plaque and have used stringent ethnic group classification and standardised assessment. This greatly increases the power of the study to accurately explore the differences in coronary artery calcification between South Asians and Europeans.

Previous studies have documented ethnic group variation in distribution and prevalence of CAC. Several studies have demonstrated a lower prevalence and extent of CAC among AA participants, despite demonstrating worse cardiovascular risk profiles, compared with North American Caucasian populations (166,170,172). Histopathological evidence also suggests relatively lower levels of calcification within atherosclerotic plaques in African American (AA) populations (197). Several explanations have been put forward to explain these differences in CAC. Firstly, calcification within plaque is an active process, and is modulated by a variety of factors (148,198,199); there could therefore be an ethnicity-specific

difference in these factors (200), resulting in differing degrees of calcification within plaques across ethnic groups. Secondly, if differences in plaque composition are present, this may result in ethnic group differences in susceptibility of plaque to rupture. Thus, although worse cardiovascular risk profiles do not portend more extensive CAC, they might signify greater vulnerability of plaque and therefore a higher risk of coronary events (201) in AA compared with North American Caucasians. This argument is supported by studies which have demonstrated that similar extent of CAC does not have the same prognostic implications for all ethnic groups. Recent studies have demonstrated higher relative risk for cardiac events and higher coronary mortality (134,164,202) for AA populations compared with Europeans, despite lower prevalence and extent of CAC. Furthermore, studies using CT angiography have demonstrated a higher risk of acute coronary syndromes among subjects who exhibit plaques with evidence of positive remodelling and lower calcification burden, advancing this argument (203)

Our data do not show any differences in CAC between UK South Asians and Europeans, despite a worse cardiovascular risk profile among South Asians. This is in contrast to the excess CHD risk observed in South Asians, and has several important implications. Firstly, the plaque morphology and composition might vary between South Asians and Europeans. This could signify that either: a) There is an ethnicity-specific difference in the process of calcification, resulting in differing degrees of calcification within plaques, thus a similar extent of calcification might signify varying amounts of total atherosclerotic plaque burden and thus differences in severity of coronary artery disease among South Asians and Europeans (204), or b) While the total atherosclerotic burden is the same in the two populations; there is greater vulnerability/ inflammation of plaque, with a higher propensity to rupture or undergo thrombosis in South Asians, compared with Europeans. This inflammation could be a result of either differences in plaque morphology

and composition or a higher degree of systemic inflammation or elevated levels of prothrombotic factors in South Asians, compared to Europeans, exposing them to a higher CHD risk

Inflammation is one of the key factors in initiating atherosclerosis as well as the pathogenesis of vulnerable plaques (49). This is supported by studies done in animal models as well as those among human cases of sudden death, demonstrating increased macrophage activity in plaques which underwent rupture and subsequent thrombosis (51). Furthermore, recent studies in human subjects have shown markedly increased inflammation within active atherosclerotic plaques in carotid arteries (52,53).

South Asians exhibit a high prevalence of diabetes, hypertension, abdominal obesity and metabolic syndrome, with lower HDL cholesterol levels (54-57). Presence of these risk factors is associated with greater inflammation (58,59). Furthermore, South Asians also demonstrate elevated levels of C-reactive protein (CRP) (60), from ages as low as 9-10 year (57). CRP is an important marker of inflammation, with elevated levels of CRP strongly associated with increased macrophage activity within plaques (63,64), and is considered an independent predictor of future cardiovascular events (51,65). Results from the recently published JUPITER trial further highlight the importance of inflammation by demonstrating substantial decreases in cardiovascular events in even low risk populations following reductions in CRP levels (205). Along with this, South Asians also demonstrate elevated levels of homocysteine (22), and small oxidized LDL (206), factors known to be associated with accelerated atherogenesis and increased thrombogenicity. Recent papers by our group have also demonstrated lower CAC values, with lower risk of CAC progression in subjects with high levels of autoantibodies against oxidised LDL (207), further marking the importance of biological factors in coronary artery disease.

In addition to these biological factors, South Asians have also been shown to demonstrate high levels of psychosocial stress and chronic stress, compared with Europeans (91). Psychosocial stress has been shown to further stimulates the activation of the sympathetic nervous system, cause endothelial dysfunction and release of proinflammatory cytokines and prothrombotic responses which in turn promote atherogenesis (88). Furthermore, stress has also been found to be correlated to CAC (99), and to silent myocardial ischaemia (100), as demonstrated by our group among others. This further lends strength to the argument that systemic factors have an important role in coronary artery disease.

While these factors are known to be associated with accelerated atherogenesis and increased thrombogenicity, and their exact role in the excess CHD in Indian ascertained, they are likely to be contributory to increased vulnerability of atherosclerotic plaque in South Asians.

The increased CHD mortality in South Asians could, therefore, be a consequence of greater levels of vulnerability of the atherosclerotic plaque, as opposed to higher atherosclerotic plaque burden.

Conclusions

In conclusion, I demonstrated similar prevalence and extent of CAC among South Asians and Europeans, this contrasts with 2-fold higher risk of myocardial infarction and CHD mortality observed in South Asians. Whether the elevated coronary artery disease mortality among South Asians reflects differences in plaque composition or an increased tendency of plaque to undergo rupture/ thrombosis, compared with Europeans, is yet to be ascertained.

Table 3.1

Characteristics of Participants

	South Asians (1356)	Europeans (1013)	P value
Age (Years)	54 ±10	57± 10	<0.001
Male Gender (%)	62	70	<0.001
Physical Activity Index	1.5±0.8	2±1	<0.001
Body Mass Index (kg/m²)	27±4	27±5	0.18
Hypertension (%)	48	42	<0.001
Systolic Blood Pressure (mmHg)	131 ± 19	132 ±19	0.03
Diastolic Blood Pressure (mm Hg)	80±10	81±10	0.47
Diabetes (%)	18	7	<0.001
Serum Glucose (mmol/l)	5.8 ±1.9	5.4 ±1.4	<0.001
Statin Usage (%)	15	8	<0.001
Total Cholesterol (mmol/l)	5.3 ±1.1	5.6±1	<0.001
LDL Cholesterol (mmol/l)	3.3 ±0.9	3.5±0.9	<0.001
HDL Cholesterol (mmol/l)	1.2 ±0.3	1.4 ± 0.4	<0.001
Triglycerides (mmol/l)	1.6 ±1.2	1.5 ±1.1	<0.001
Current Smokers (%)	7	16	<0.001
Former Smokers (%)	7	37	<0.001
Metabolic Syndrome (IDF definition) (%)	19	8	<0.001
Metabolic Syndrome (NCEP) (%)	29	20	<0.001

Table 3.2: Prevalence of Coronary Artery Calcium among South Asians and Europeans

	South Asian (1356)	European (1013) %	<i>P value</i>
Coronary artery calcification > 0 Au	49 %	53 %	0.06
Coronary artery calcification >100 Au	21%	23 %	0.1
Coronary artery calcification >400 Au	8 %	10 %	0.07

Table 3.3 Estimates of Change in Odds for prevalence of CAC > 0 AU

Variable	Odds Ratio (95%CI)	P value
Model 1 Race (SA Vs E)	0.9 (0.7, 1.0)	0.06
Model 2		
Race (SA Vs E)	1.1 (0.9, 1.3)	0.3
Age (10 years)	2.8 (2.5, 3.0)	<0.001
Gender (Male Vs Female)	4.0 (3.3, 4.9)	<0.001
Model 3		
Age^a (10 Years)	2.56(2.3, 2.8)	<0.001
Diabetes	1.55 (1.1, 2.1)	0.008
Hypertension	1.49 (1.2, 1.8)	<0.001
Smoker	1	
	1.12 (0.83, 1.4)	0.49
	1.56 (1.1, 2.2)	0.014
Body mass index (kg/m²)	1.07 (1.04, 1.09)	<0.001
Statin usage	2.3 (1.6, 3.1)	<0.001
Race (SA Vs E)	1.054(0.87, 1.3)	0.68
Gender (Male Vs Female)	3.79 (2.9, 4.9)	<0.001
Triglycerides (mmol/l)	1.14 (0.98 , 1.3)	0.078
HDL cholesterol (mmol/l)	1.05 (0.74, 1.48)	0.77
LDL cholesterol (mmol/l)	1.3 (1.1, 1.47)	<0.001
R² for model	0.37	

Change in odds for presence of coronary artery calcification per unit increase in risk factor, using logistic regression

Model 1: Unadjusted OR
 Model 2: Adjusted for age and gender
 Model 3: Adjusted for standard risk factors

Table 3.4 Multivariable Estimates of Change in Odds for prevalence of CAC for Each Ethnic group

	South Asians Odds Ratio (95%CI)	P value	Europeans Odds Ratio (95%CI)	P value
Age (10 years)	2.7 (2.3, 3.1)	<0.001	2.4 (2.0, 2.8)	<0.001
Gender (Male Vs Female)	3.38 (2.8, 4.1)	<0.001	3.43 (2.7, 4.2)	<0.001
Diabetes	1.76 (1.7, 2.5)	0.006	1.2 (0.6, 2.3)	0.60
Hypertension	1.5 (1.1, 1.9)	0.003	1.45 (1.05, 2.02)	0.21
Statin use	2.1 (1.4, 3.2)	<0.001	2.9 (1.4, 5.8)	0.002
Former smoker	0.82(0.4, 1.3)	0.46	1.26(0.9, 1.7)	0.168
Current smoker	1.59 (0.89, 2.5)	0.12	1.4 (0.94, 2.23)	0.08
Body mass index (kg/m²)	1.07 (1.03, 1.11)	<0.001	1.05 (1.01, 1.09)	0.006
HDL Cholesterol (mmol/l)	0.97 (0.6, 1.5)	0.88	1.16 (0.71, 1.9)	0.539
LDL Cholesterol (mmol/l)	1.3 (1.1, 1.6)	<0.001	1.2 (1.03,1.48)	0.020
Triglycerides (mmol/l)	0.99 (0.8, 1.2)	0.9	1.4 (1.1, 1.76)	0.004
Creatinine (µmol/l)	1(0.99, 1.09)	0.76	1 (0.98, 1.01)	0.983
R² %	38		36	
Area under ROC Curve	0.81		0.81	

Change in odds for presence of coronary artery calcification, per unit increase in risk factor, adjusted for all variables listed, estimated using logistic regression

Table 3.5 Multivariable Predictors for Extent of CAC

Variable	Odds Ratio (95% Confidence Interval)	P value
Model 1		
Race (SA Vs E)	0.9 (0.74, 1.0)	0.04
Model 2		
Race (SA Vs E)	1.1 (0.5, 1.3)	0.3
Age (10 years)	3.0 (2.7, 3.3)	<0.001
Gender (Male Vs Female)	4.0 (3.8, 4.3)	<0.001
Model 3		
Age (10 Years)	2.8 (2.5, 3.1)	<0.001
Body Mass index	1.04 (1.02, 1.06)	<0.001
Current Smoker	1.8 (1.4, 2.4)	<0.001
Former Smoker	1.2 (0.9, 1.5)	0.1
Statin Use	2.3 (1.8, 3.0)	<0.001
Diabetes	1.7 (1.4, 2.2)	<0.001
Hypertension	1.7 (1.4, 2.0)	<0.001
HDL Cholesterol mmol/l	0.97 (0.9, 1.03)	0.85
LDL Cholesterol mmol/l	1.02 (1.01, 1.03)	<0.001
Triglycerides mmol/l	1.0 (0.9, 1.02)	0.2
Gender (Male vs Female)	3.8 (3.1, 4.8)	<0.001
Race (SA Vs E)	1.05 (0.9, 1.3)	0.6

Change in odds for increasing extent of CAC, per unit increase in risk factor, adjusted for other variables using ordinal regression

Model 1: Unadjusted OR
 Model 2: Adjusted for age and gender
 Model 3: Adjusted for standard risk factors

1 **Table 3.6 Multivariable Predictors of Extent of CAC in Each Ethnic Group**

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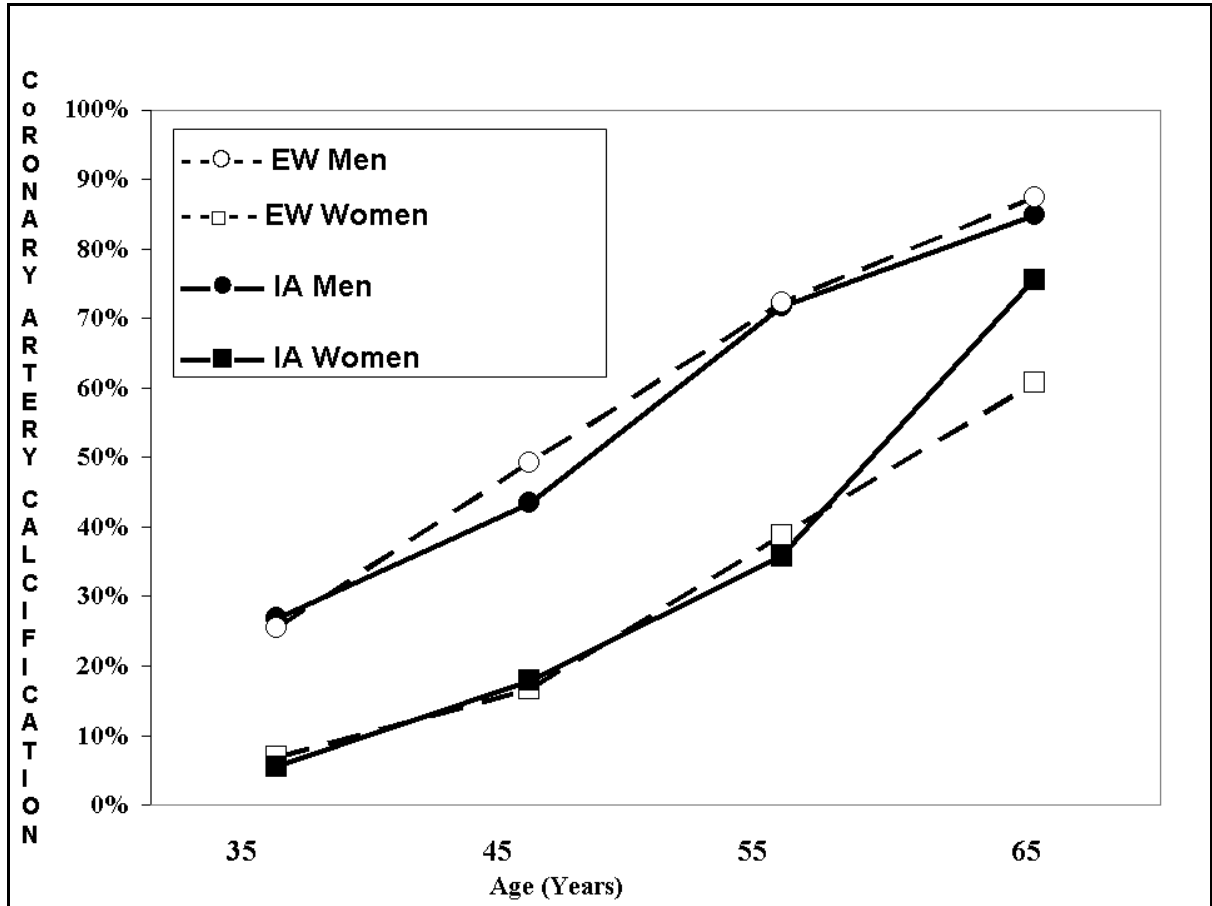
	South Asians OR (95% CI)	P	Europeans OR (95% CI)	P
Age (10 Years)	2.8 (2.4, 3.2)	<0.001	2.8 (2.4, 3.2)	<0.001
Gender (Male Vs Female)	4.2 (3.2, 5.6)	<0.001	3.8 (2.7, 5.3)	<0.001
Hypertension	1.7 (1.3, 2.2)	<0.001	1.7 (1.3,2.2)	<0.001
Diabetes	1.8 (1.4, 2.4)	<0.001	1.6 (0.96, 2.6)	0.06
Current Smoker	1.6 (1.1, 2.5)	0.03	1.9 (1.4, 2.8)	<0.001
Former Smoker	0.9 (0.6, 1.3)	0.5	1.4 (1.09, 1.9)	0.02
Statin Use	2.4 (1.7, 3.4)	<0.001	2.3 (1.5, 3.7)	<0.001
Body Mass index (kg/M²)	1.1 (1.03, 1.1)	<0.001	1.02 (0.98, 1.05)	0.2
HDL Cholesterol (mmol/l)	0.9 (0.9, 1.01)	1.0	1.01 (0.96, 1.05)	0.8
LDL Cholesterol (mmol/l)	1.03 (1.02, 1.05)	<0.001	1.02 (0.97, 1.03)	0.05
Triglycerides (mmol/l)	0.9 (0.9, 1.01)	0.7	1.02 (1.0 , 1.04)	0.02
R2%	39%		38%	

6 **Change in odds for increasing extent of CAC, per unit increase in risk factor,**
7 **adjusted for other variables using ordinal regression**

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Figure 1



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Figure 3.1 demonstrates the increasing prevalence of CAC among South Asians and Europeans Men and Women, with age

Figure 2

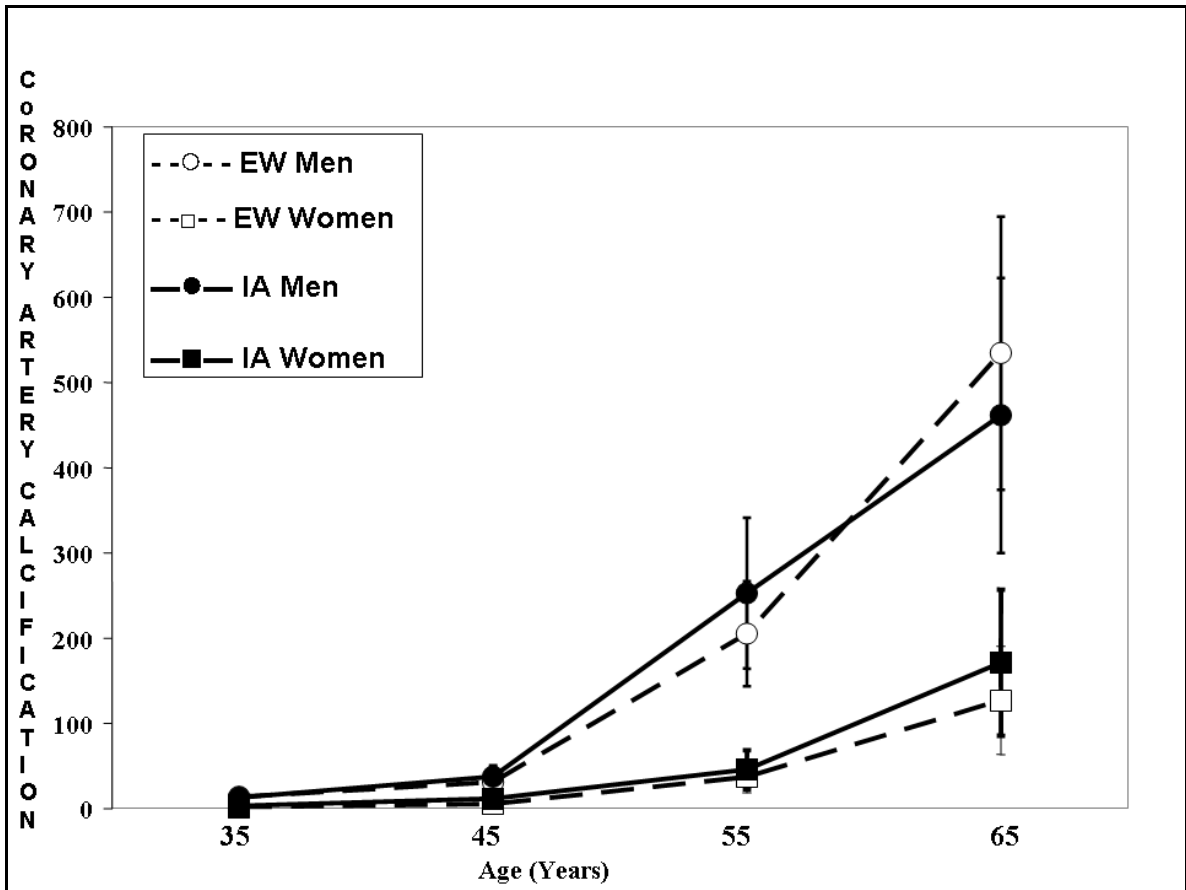


Figure 3.2 demonstrates the Mean Coronary Artery Calcification Scores in All Participants Divided By Age, Gender and Ethnicity, the error bars represent 95% confidence interval

1 **Chapter 4: Prevalence of Silent myocardial Ischemia among asymptomatic**
2 **South Asians and Europeans**

3

4 **4.1 Abstract**

5

6 **Background:** Coronary heart disease (CHD) mortality is 70% higher amongst South
7 Asians (SA) than Europeans (E), the reasons for this excess remain unexplained.
8 Coronary artery calcification (CAC) is highly correlated with coronary plaque burden
9 and silent myocardial ischemia in Europeans; but fails to identify excess risk in South
10 Asians. We hypothesised that South Asians have a higher prevalence of silent
11 myocardial ischemia compared to Europeans, despite similar CAC, and this may
12 explain their excess CHD mortality.

13

14 **Methods:** I measured CAC for 2369 asymptomatic men and women, aged 35 to
15 75 years, as part of the London Life Sciences Population (LOLIPOP) study. 518
16 subjects had CAC scores > 100 Agatston units and of these 256 (49%) patients
17 underwent Myocardial perfusion scintigraphy (MPS).

18

19 **Results:** CAC scores were similar among South Asians and Europeans, after
20 adjustment for conventional risk factors. MPS abnormalities were seen in 56 (22%)
21 subjects. Presence of diabetes (P=0.03) and increasing CAC (P< 0.001) were
22 independent predictors for severity of silent myocardial ischemia. Ethnicity did not
23 influence the prevalence or the extent of silent myocardial ischemia.

24

1 **Conclusion:** I demonstrated that MPS did not identify greater ischemia among South
2 Asians compared with Europeans. This appears incongruent with almost 2-fold higher
3 risk of CHD mortality observed in South Asians.

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4.2 Introduction:

South Asians (people originating from India, Pakistan, Bangladesh and SriLanka) account for almost a quarter of the global coronary heart disease (CHD) deaths (1). South Asians (South Asians) form the largest ethnic minority group (4% of the total population) in the UK, and continue to demonstrate a two-fold higher coronary heart disease mortality rate (4) in contrast to the decline in CHD-related mortality in the general population.

While global registries such as the INTERHEART study (5) suggest an important role of traditional cardiovascular risk factors in coronary heart disease in all ethnic groups, traditional risk factors (8,47,192) and risk scores such as the Framingham function do not predict the higher coronary heart disease mortality in South Asians (109). Therefore, it stands to reason that a high percentage of South Asians at an elevated risk for cardiovascular disease might remain undetected within the general population, due to a lack of accurate and robust risk stratification tools.

Coronary artery calcium (CAC) imaging has a strong correlation with atherosclerotic plaque disease, with amount of CAC directly related to the total atherosclerotic plaque burden and severity of coronary disease (144,153). CAC does not, however, appear to identify the excess coronary risk seen among South Asians compared with Europeans (173).

MPS is a well validated technique for the non-invasive diagnosis of coronary artery disease (180,208) and presence of stress related ischemia on MPS is strongly associated with adverse cardiovascular events(138) even in the absence of ischemic symptoms.

1 Previous studies have demonstrated an increasing prevalence of stress-induced
2 ischemia detected by MPS with CAC >100 Agatston units. This has been
3 demonstrated among patients suspected to have CHD (179,184) as well as
4 asymptomatic healthy subjects (140,185). However, there is negligible ischemia
5 demonstrated among subjects with low/ absent coronary calcium by several studies
6 including those performed by our group (140,179,184,186). Therefore, MPS is often
7 used in conjunction with CAC imaging; with CAC providing a screening test for
8 detecting presence of atherosclerotic coronary artery disease and MPS providing a
9 functional assessment of severity of stenosis.

10 While South Asians have a higher coronary heart disease risk and mortality
11 compared with Europeans(4), it has been shown that they display similar burden of
12 calcified atherosclerotic plaque, as detected by CAC imaging (173,204). We
13 hypothesised that South Asians have more obstructive coronary artery disease as
14 exemplified by a greater prevalence of reversible silent myocardial ischemia despite
15 similar burden of calcified coronary plaque, compared with Europeans. Therefore, the
16 aim of the study was to assess whether MPS helps identify the excess coronary heart
17 disease risk observed in South Asians compared with Europeans.

18

19

1 4.3 **Methods:**

2 **Participants:** All subjects were recruited from the London Life Sciences
3 Population (LOLIPOP) study. While the methods for the study have been described
4 in detail in the methods and materials chapter a brief overview follows.

5 2369 asymptomatic South Asians and Europeans men and women were
6 recruited from the LOLIPOP study, to undergo CAC imaging using electron beam
7 computed tomography (EBCT). As previous work by our group and others has
8 demonstrated negligible prevalence of silent myocardial ischemia among subjects
9 with CAC of less than 100 Agatston units (140,179,184) , in order to minimise the
10 number of subjects exposed to radiation, a cut-off value of 100 Agatston units was
11 used to select participants for MPS. 518 patients had CAC > 100 Agatston units and
12 were invited for MPS. Out of these, 256 (49%) participants consented to undergo
13 MPS. Subjects who participated in the perfusion study were comparable with those
14 who did not, on CAC scores and most standard coronary risk factors, with the
15 exception that they were 2 years younger, with a higher statin use along with lower
16 HDL cholesterol levels. The characteristics of participants and non-responders are
17 shown in Table 4.1.

18

19 **Coronary artery calcification:** CAC imaging was performed using a
20 modified GE Imatron C-150 (San Francisco, CA, USA) specially equipped with high-
21 resolution detectors, at the Cardiac Imaging and Research Centre, Wellington
22 Hospital. Coronary visualization was performed without contrast. In order to include
23 the entire coronary tree, forty contiguous 3-mm slices were obtained during a single

1 breath-hold, starting at the carina and proceeding to the level of the diaphragm. Scan
2 time was 100-ms per slice, synchronised to 40% of the R-R interval(189).

3 **Myocardial perfusion Scintigraphy:** MPS was performed using a 2-
4 day protocol (stress and rest) with Technitium-99m sestamibi. For stress imaging,
5 either a maximal stress exercise treadmill test (Bruce protocol) in conjunction with
6 pharmacologic stress using dipyridamole (190) or dobutamine stress test (191) was
7 used. This method has been validated by our laboratory and others, and has been
8 found to increase specificity compared with using only exercise/ pharmacological
9 stress. Stress and rest MPS images were acquired 60 to 120 minutes after injection of
10 600 MBq Tc-99m sestamibi by use of a large field-of-view, dual-headed gamma
11 camera equipped with a high-resolution collimator (SMV DSTi; GE Medical
12 Systems, Buc Cedex, France). Thirty-two projections (40 seconds per projection)
13 were acquired over a 180° arc, from the 45° right anterior oblique position to the 45°
14 left posterior oblique position. Strict quality control and motion artefact correction
15 were used.

16

17 **Image Interpretation:**

18 **Coronary artery calcification:** All areas of calcification within the
19 borders of a coronary artery with an optical density above 130 Hounsfield units and
20 an area greater than 1 mm² were computed. CAC scores were calculated on an
21 Aquarius workstation (TeraRecon, Inc., San Mateo, USA) and scores between
22 participants were adjusted with a standard calcium phantom. The output from EBCT
23 scans was quantified into Agatston scores as described by Agatston et al(157). Due to
24 the non-Gaussian nature of CAC, statistical analyses were carried out using Agatston

1 score transformed to their natural logarithm (\log_e). For the purpose of analysis the
2 CAC score was used as a categorical trait, defined as Agatston score 100-400 Au and
3 >400 Au.

4 **Myocardial perfusion Scintigraphy:** Stress-rest Tc-99m sestamibi
5 MPS scans were interpreted by semi-quantitative visual analysis, and the findings
6 were categorised for overall interpretation as normal or abnormal. The MPS scans
7 were reported by an independent observer (A.L.) blinded to the clinical, ethnic group
8 and coronary calcium data. Semi-quantitative visual analysis was performed by use of
9 the 17-segment model recommended by the American College of
10 Cardiology/American Heart Association/American Society of
11 Echocardiography/American Society of Nuclear Cardiology. Tracer uptake was
12 scored in each segment by use of a 5-point scoring system (0, normal; 1, equivocal; 2,
13 moderate; 3, severe reduction of radioisotope uptake; and 4, apparent absence of
14 tracer uptake). Total ischemic burden was estimated using summed stress, rest, and
15 difference scores (SSS, SRS, and SDS). SSS and SRS were determined by the sum of
16 scores of each segment from the stress and rest images, respectively.

17 A SSS >4 was considered to be abnormal. Summed difference scores (SDS)
18 were determined by the sum of the difference between the SSS and the SRS. The
19 SDS was converted to percent myocardium ischemia by dividing the SDS by 68—the
20 maximum potential score (4 X 17)—and multiplying by 100.(179) Due to the high
21 prevalence of participants with no detectable ischemia on MPS, the distribution of
22 myocardial ischemia was highly skewed and could not be transformed back to
23 normality. Hence, it was evaluated as a categorical variable, i.e. absence of reversible

1 myocardial ischemia (0%), mild defect (<5%), moderate defect (>5 and ≤10%), or
2 large defect (>10%). (209).

3 **Statistical analysis:** All analyses were performed using the statistics
4 programme Statistical Package for the Social Sciences (SPSS) version 16.0 (Chicago,
5 IL, USA). Categorical data are presented as number (percent), and continuous data as
6 mean value± SD. The chi-square test was used to compare categorical variables while
7 comparisons of continuous variables between two groups were performed using
8 student *t* tests. All tests of significance were two-tailed, and significance was defined
9 at the ≤0.05 level.

10 Prevalence and extent of myocardial ischemia was compared across ethnic
11 groups. The relationships between risk factors and myocardial ischemia were
12 analysed using regression models, both before and after adjusting for concomitant
13 risk factors. While a binomial logistic regression model was used to evaluate
14 associations between risk factors and the presence of MPS abnormalities, an ordinal
15 regression analysis was applied to identify clinical predictors of severity of
16 myocardial ischemia. The resultant estimates (ordered log odds), were exponentiated
17 to calculate the odds ratios of the predictors. From these models, an odds ratio and
18 95% confidence interval was calculated. The variance explained by the models was
19 estimated using the coefficient of determination as calculated using Nagelkerke's R^2 .

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1 4.4 Results

2 **Clinical characteristics of subjects**

3 The clinical and biochemical characteristics of the participants are described
4 in Table 4.2. South Asians were on average 2 years younger, with a higher prevalence
5 of hypertension and diabetes. They also demonstrated a higher statin usage and
6 elevated serum HbA1c levels compared with Europeans. South Asians also had a
7 lower BMI, HDL levels, and smoking rates compared with Europeans. Accordingly,
8 South Asians had a higher prevalence of metabolic syndrome as defined by the NCEP
9 criteria, however, similar Framingham risk scores. Unadjusted CAC scores suggested
10 a tendency towards lower CAC among South Asians compared with Europeans (P
11 0.06).

12 MPS was performed on 256 participants. Perfusion abnormalities were seen in
13 56 (22%) subjects, the majority (89%) of which were reversible defects. Prevalence
14 (Figure 4.1) and extent (Figure 4.2) of myocardial ischemia were compared across
15 CAC categories, and were seen to increase in parallel with CAC scores among South
16 Asians and Europeans. There was no significant difference in extent or severity of
17 myocardial ischemia among South Asians compared with Europeans.

18

19 **Predictors of presence of myocardial ischemia**

20 Univariable analysis demonstrated increasing age and higher CAC category,
21 as well as presence of diabetes to be associated with elevated odds for the presence of
22 myocardial ischemia (SSS>4). A multivariable logistic regression model, including
23 conventional cardiovascular risk factors along with ethnicity, explained about 14% of
24 the variation in myocardial perfusion defects in this population (Table 4.3a). After

1 adjustment for cardiovascular risk factors, ethnicity was not related to presence of
2 myocardial ischemia, OR= 0.9 (95% CI 0.4, 2.0).). Increase in CAC was a significant
3 predictor associated with presence of myocardial ischemia (p 0.001), with presence of
4 diabetes demonstrating borderline statistical significance (p 0.07). Multivariable
5 regression model were repeated using predictors with $p < 0.2$ (Table 4.3b). The model
6 demonstrated a good fit, with a chi square significance of 0.7, and explained about
7 8% of the variation. The results, however, remained same. CAC and DM were the
8 only predictors of perfusion defects (Table 4.3b).

9

10 **Predictors of severity of ischemia**

11 A univariable ordinal regression demonstrated increasing age, increasing
12 CAC, presence of diabetes and hypertension, as well as a history of statin usage to be
13 associated with elevated odds for the presence of myocardial ischemia. However, on
14 adjusting for conventional cardiovascular risk factors and ethnicity, using a
15 multivariable ordinal regression model, an increase in CAC and presence of diabetes
16 mellitus were independently associated with abnormal myocardial perfusion and
17 increasing severity of ischemia. After adjustment for cardiovascular risk factors,
18 ethnicity was not related to the severity of myocardial perfusion defects OR 1.08
19 (95% CI 0.6, 2.0). Multivariable regression model were repeated using predictors
20 with $p < 0.2$ (Table 4.4b). The model demonstrated a good fit, with a chi square
21 significance of 0.8, and explained about 9% of the variation. The results remained
22 consistent, and high CAC and DM were the only predictors of perfusion defects.
23 (Table 4.4b)

24

25

1 **4.5 Discussion**

2 In this cohort of asymptomatic South Asians and, aged between 35 and 75,
3 with CAC scores greater than 100 Agatston units; we found no ethnic differences in
4 either prevalence or extent of myocardial perfusion defects among South Asians and
5 Europeans. This is incongruent with almost 2-fold higher risk of myocardial
6 infarction and coronary heart disease mortality observed in South Asians. MPS did
7 not predict or identify the excess coronary heart disease risk in South Asians in this
8 sample

9 Consistent with existing data, we found a higher frequency of both present
10 and former smokers among Europeans compared with South Asians, with a lower
11 prevalence of diabetes and hypertension (7,8,10,108,174). CAC was the strongest
12 predictor of the prevalence of myocardial perfusion defects. In addition, an increase
13 in CAC was associated with concomitant elevation in the prevalence and extent of
14 myocardial ischemia (140,179,210). Diabetes mellitus was a predictor of myocardial
15 ischemia, consistent with existing data (210-212), and also corroborating previous
16 research which has demonstrated a higher prevalence of perfusion defects in diabetic
17 populations (140).

18 While the overall prevalence of perfusion defects in our population is
19 consistent with previous studies, we demonstrated a lower prevalence of severe
20 myocardial perfusion defects (184). This could be because of the asymptomatic
21 population selected for our study compared with physician-referred symptomatic
22 participants used in other studies. To our knowledge, this is the first population-
23 based study evaluating the prevalence and extent of silent myocardial ischemia using
24 MPS among asymptomatic South Asians in comparison with Europeans.

1 There is little data available which compares ethnic differences in prevalence
2 and prognostic implications of silent ischemia on MPS. Shaw et al (187), in a study
3 carried out among AA and NW populations, demonstrated a higher prevalence of
4 moderate to severe myocardial perfusion defects among AA compared with NW.
5 However, follow up from the study identified a greater risk of events among AA for
6 all grades of perfusion abnormalities as compared with NW, both before and after
7 adjustment for cardiovascular risk factors. This suggests an increased cardiovascular
8 risk among AA compared with NW, irrespective of the detected burden of obstructive
9 coronary artery disease. The lower percentage of obstructive coronary artery disease
10 in AA when compared with NW among patients referred for acute coronary
11 syndromes (213) and lower percentage of coronary stenosis despite higher CHD
12 mortality and prevalence compared with NW (170,172), lends strength to this
13 argument. It also highlights the importance of factors other than atherosclerotic
14 plaque burden in the prediction of cardiovascular events.

15 AA have higher prevalence of cardiovascular risk factors such as diabetes,
16 hypertension and smoking, and higher cardiovascular mortality compared with
17 Caucasians, however have been identified as having higher mortality for similar
18 degrees of obstructive coronary disease both on angiography and MPS. This supports
19 a greater role of physiological abnormalities, as opposed to a greater atherosclerotic
20 plaque burden, in causing adverse cardiovascular events in AA. Thus, ethnic disparity
21 in coronary heart disease risk and mortality might be better explained by differences
22 in plaque morphology and composition rather than atherosclerotic plaque burden.

23 In this cohort, South Asians exhibit higher levels of diabetes, hypertension,
24 and lower levels of smoking, compared with Europeans. They, however, demonstrate

1 similar coronary artery calcification. This lack of difference persists even after
2 adjusting for standard risk factors including smoking, diabetes and hypertension.
3 Furthermore, our data also show a similar prevalence and extent of myocardial
4 perfusion defects among UK South Asians and Europeans, for comparable levels of
5 CAC. These results contrast with the excess coronary heart disease risk observed in
6 South Asians, and have several potential implications. Firstly, this could signify that,
7 while the atherosclerotic plaque burden is similar among the two populations, there
8 are differences in plaque composition between South Asians and Europeans. There is
9 thus a greater vulnerability of plaque in South Asians, compared with Europeans and
10 results in a higher propensity of plaque to rupture or undergo thrombosis. The second
11 implication could be that a higher degree of systemic inflammation or elevated levels
12 of prothrombotic factors exist in South Asians, compared with Europeans, which
13 exposes them to higher coronary heart disease risk irrespective of atherosclerotic
14 plaque burden.

15 The differences in prevalence of risk factors could therefore portend potential
16 differences in the mechanism of plaque initiation among the two populations resulting
17 in similar burden of atherosclerosis and thus coronary calcification, but differences in
18 subsequent morphology of atherosclerotic plaque.

19 Studies in animal models, as well as those among human cases of sudden
20 death, have demonstrated increased macrophage activity in plaques which underwent
21 rupture and subsequent thrombosis (51). Moreover, studies in human subjects have
22 shown markedly increased inflammation within active atherosclerotic plaques in
23 carotid arteries (52,53). These factors support the role of inflammation as one of the
24 key factors in initiation and progression of vulnerable atherosclerotic plaques (49).

1 South Asians exhibit a high prevalence of metabolic factors such as diabetes,
2 hypertension and abdominal obesity, with lower HDL cholesterol levels (54-57). The
3 presence of these risk factors is associated with greater inflammation (58,59).
4 Furthermore, South Asians also demonstrate elevated levels of C-reactive protein
5 (CRP) (60), from ages as low as 9-10 year (57). CRP is an important marker of
6 inflammation, with elevated levels of CRP strongly associated with increased
7 macrophage activity within plaques (63,64), and is considered an independent
8 predictor of future cardiovascular events (51,65). The recently published JUPITER
9 trial further highlights the importance of inflammation by demonstrating substantial
10 decreases in cardiovascular events in low risk populations following reductions in
11 CRP levels (205).

12 In addition to these biological factors, South Asians have also been shown to
13 demonstrate high levels of psychosocial stress, as well as experiencing greater
14 chronic stress, compared with Europeans (91). Psychosocial stress has been shown to
15 further stimulates the activation of the sympathetic nervous system, cause endothelial
16 dysfunction and release of proinflammatory cytokines and prothrombotic responses
17 which in turn promote atherogenesis (88). Furthermore, stress has also been found to
18 be correlated to CAC (99), and to silent myocardial ischemia, further lending strength
19 to the argument (100).

20 While these factors are known to be associated with accelerated atherogenesis
21 and increased thrombogenicity, and their exact role in the excess coronary heart
22 disease in South Asians is not ascertained, their elevated levels are likely to be
23 contributory to increased vulnerability of atherosclerotic plaque in South Asians.

24

1 **Conclusions**

2 In conclusion, I demonstrated that ethnicity was not related to the severity or
3 extent of silent myocardial ischemia, after adjustment for cardiovascular risk factors.
4 The higher coronary heart disease risk and mortality observed in South Asians is not
5 identified by markers of atherosclerotic burden such as CAC and MPS. This suggests
6 a greater contribution of factors such as plaque morphology and inflammation in the
7 higher coronary heart disease mortality observed among South Asians, however, the
8 extent to which it explains the excess CHD risk observed among South Asians
9 requires further elucidation.

10

11

1 Table 4.1 Characteristics of responders and non responders for myocardial perfusion imaging

	Responders	Non responders	P value
Age (Years)	63±8	65±8	0.003
Agatston score(95% CI)	599±763	566±663	0.79
Body mass index	27.4±3.9	28.07±4.6	0.07
Former smoker %	28	26	0.62
Current smokers (%)	12.8	12.3	0.32
Type 2 diabetes (%)	28	24.8	0.17
Serum Glucose (mmol/L)	6.3±2.3	6.2 ±2.2	0.853
Total cholesterol (mmol/l)	5.35±1.17	5.5±1.13	0.132
Triglycerides (mmol/L)	1.65±0.88	1.72±1.14	0.476
HDL cholesterol (mmol/l)	1.26±0.29	1.33±0.35	0.016
LDL cholesterol (mmol/l)	3.3±0.9	3.4±0.0	0.291
History of Hypertension (%)	50	46	0.192
Systolic Blood Pressure (mmHg)	140±18	145±20	0.32
Diastolic Blood Pressure (mmHg)	82±10	82±11	0.74
MS-IDF %	20	20	0.71
MS-NCEP %	33	35	1.0

2

1 **Table 4.2: Characteristics of Participants**

	European (100)	South Asian (156)	P value
Age (Years)	64 ± 7	62±8	.004
Agatston score (95% CI)	696 (535-857)	530(421-639)	0.06
Hypertension (%)	38	62	<0.001
Systolic Blood Pressure	81±10	83±10	0.38
Diastolic Blood Pressure	140±18	140±17	0.9
BMI	28±4.5	27±3.3	<0.001
Type 2 diabetes (%)	18	39	<0.001
Serum Glucose (mmol/l)	5.9±2.0	6.5±2.5	0.037
HbA1c	5.7±1.2	6.4±1.4	<0.001
Current smokers (%)	21	9	0.003
Former smoker (%)	54	12.5	<0.001
On Statins (%)	21	33	0.03
Total cholesterol (mmol/l)	5.4±1.19	5.2±1.14	0.086
LDL Cholesterol (mmol/l)	3.3±0.9	3.4±1	0.15
HDL cholesterol (mmol/l)	1.3±0.32	1.19±0.25	<0.001
Triglycerides (mmol/l)	1.6 ± 0.85	1.7±0.89	0.438
Metabolic syndrome (NCEP) %	13%	25%	0.02
Framingham Risk Score	13 ±1	11 ± 1	0.2

2

1 **Table 4.3a Multivariable Predictors of Presence of Perfusion Defect**

Risk Factor	Odds ratio (95% CI)	P value
Age (10 Yrs)	1.1 (0.7, 1.8)	0.6
Log_e CAC	3.6 (1.5, 8.9)	0.004
Hypertension	1.1 (0.54, 2.2)	0.9
Body Mass Index (Kg/M²)	1.06 (0.9, 1.2)	0.2
Diabetes Mellitus	2.3 (0.9, 5.9)	0.1
HbA1c (%)	0.8 (0.6, 1.2)	0.3
Former Smoker (Vs Non smoker)	0.62 (0.3, 1.5)	0.3
Current Smoker (Vs Non smoker)	1.3 (0.5, 3.7)	0.6
HDL Cholesterol (mmol/l)	0.56 (0.2, 1.9)	0.4
Statin Usage	1.2 (0.6, 2.7)	0.5
Ethnicity (SA Vs E)	0.89 (0.4, 2.0)	0.8

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3 **Change in odds for presence of perfusion defects, per unit increase in risk factor, adjusted for**
4 **all other variables using logistic regression**
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2 **Table 4.3b Multivariable Predictors of Presence of Perfusion Defect**

<i>Risk Factor</i>	<i>Odds ratio (95% CI)</i>	<i>P value</i>
CAC category	2.6 (1.4, 4.9)	0.003
Diabetes Mellitus	1.7 (0.9, 3.3)	0.09

3

4 **Change in odds for presence of perfusion defects, per unit increase in risk factor, adjusted for**
5 **all other variables using logistic regression**

6

1 **Table 4.4a Multivariate predictors of severity of ischemia**

2

Risk Factor	Odds ratio (95% CI)	P value
Age (10 Yrs)	1.06 (0.7, 1.6)	0.8
Ethnicity (SA Vs E)	1.08 (0.6, 2.0)	0.8
Log_e CAC	4.09 (2.0, 8.02)	<0.001
History of Hypertension	0.99 (0.5, 1.8)	0.9
Type 2 Diabetes	2.2 (1.1, 4.5)	0.03
HbA1C	0.88 (0.7, 1.1)	0.2
Body Mass Index (Kg/M²)	1.06 (0.9, 1.1)	0.1
Former smoker(Vs Non Smokers)	0.68 (0.3, 1.7)	0.5
Current Smoker (Vs Non Smokers)	1.24(0.5, 2.9)	0.6
Statin usage	1.2 (0.7, 2.2)	0.5
HDL Cholesterol (mmol/l)	0.72 (0.3, 1.9)	0.5

1 **Table 4.4b Multivariate predictors of severity of ischemia**

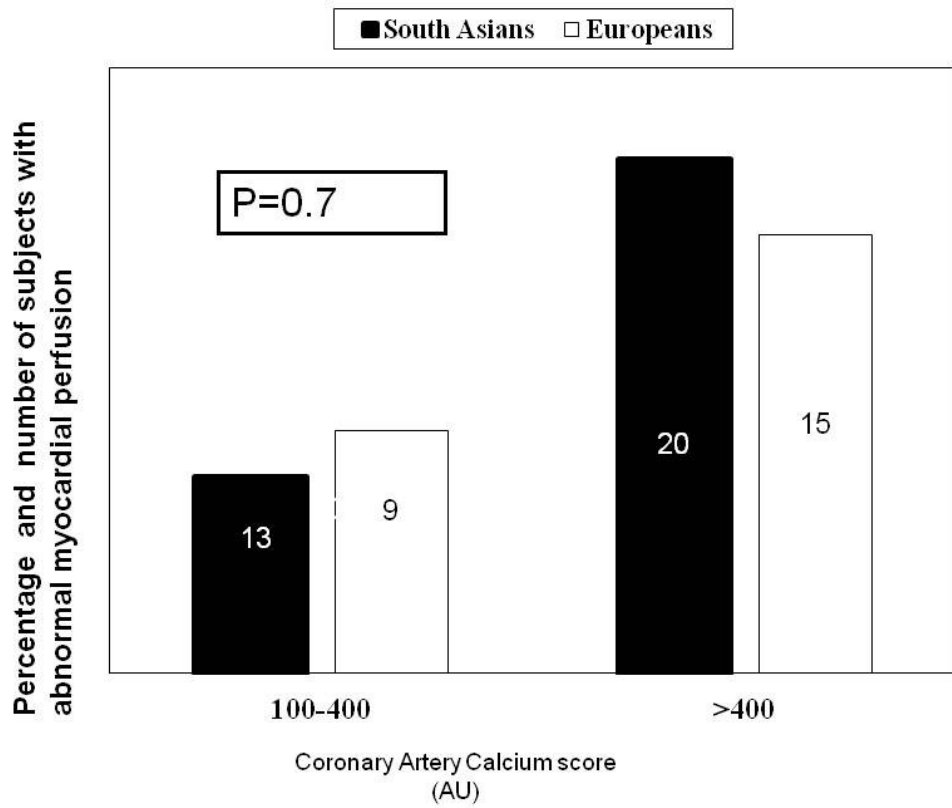
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<i>Risk Factor</i>	<i>Odds ratio (95% CI)</i>	<i>P value</i>
CAC Category	2.7 (1.6, 4.7)	0.001
Type 2 Diabetes	1.9 (1.1, 3.4)	0.02

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Change in odds for increase in severity of perfusion defects, per unit increase in risk factor, adjusted for other variables using ordinal regression

1 **Figure 4.1** Prevalence of myocardial perfusion abnormalities among South
2 **Asians and Europeans**
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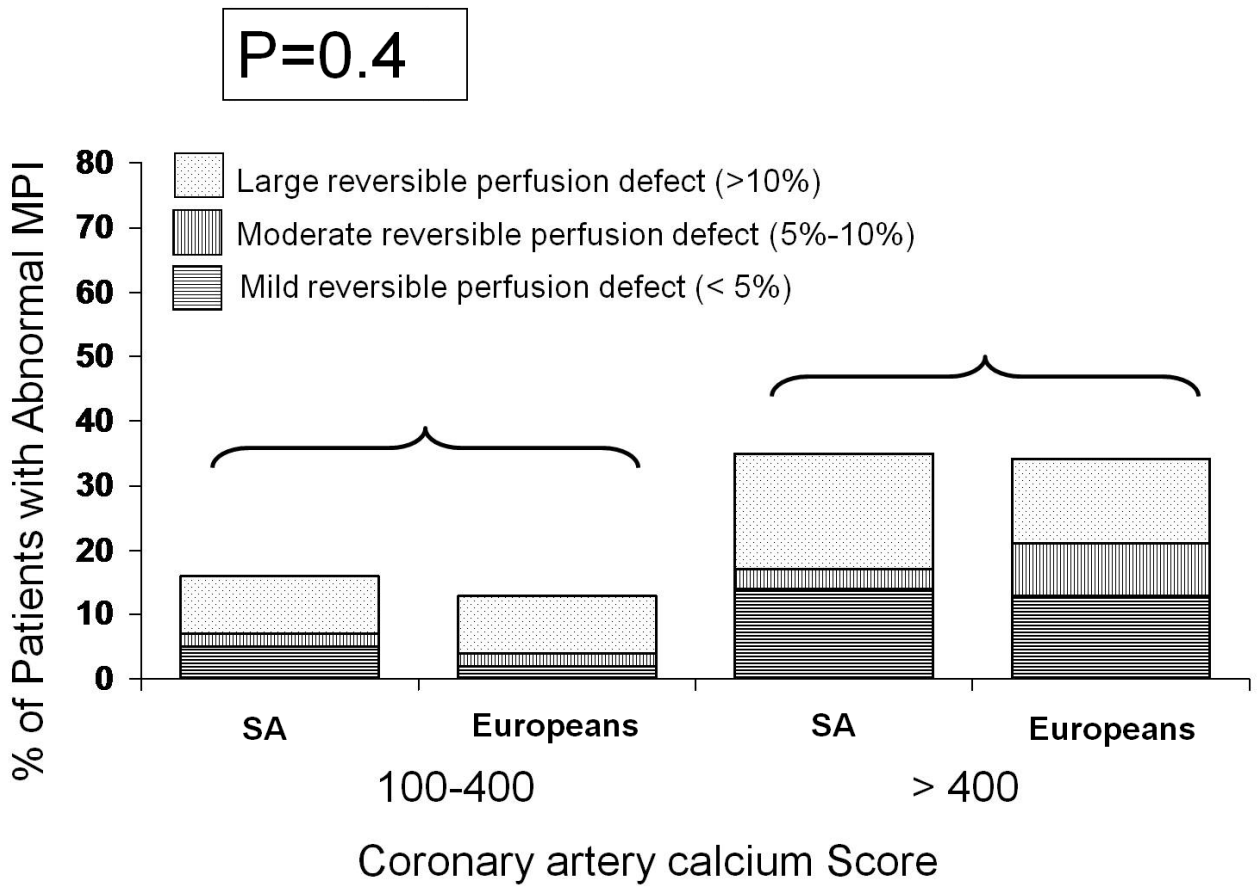


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Figure 4.2 Severity of Myocardial Perfusion Abnormalities among South Asians and Europeans



1 **Chapter 5: C reactive protein among South Asians and Europeans**
2 **and its relationship with CAC and MPS**

3
4 **5.1 Abstract**

5
6 **Background:** Coronary heart disease mortality is 70% higher amongst UK
7 South Asian compared with Europeans. Traditional risk factors do not consistently
8 predict the higher CHD risk. Markers of coronary plaque burden and stenosis, such
9 as coronary artery calcification and myocardial perfusion imaging, do not identify the
10 higher CHD risk observed among South Asians. Inflammation is a key factor in
11 initiation and progression of atherosclerosis (49,50). High sensitivity C-reactive
12 protein (CRP) is an important marker of active inflammation and is considered an
13 independent predictor of future cardiovascular events (51,65). We hypothesised that
14 South Asians have greater degree of systemic inflammation as measured by plasma
15 CRP, compared with Europeans. The greater degree of inflammation may provide an
16 explanation for their higher CHD risk despite similar coronary plaque burden.

17
18 **Methods:** The study comprised 2347 asymptomatic South Asians and
19 Europeans men and women, aged 35 to 75 years, part of the London Life Sciences
20 Population (LOLIPOP) study. Anthropometric measurements, biochemical markers
21 including CRP, and atherosclerosis imaging using coronary artery calcification were
22 performed for all subjects. 256 subjects (49% of those coronary calcification scores >
23 100 Agatston units) also underwent myocardial perfusion scanning.

24

1 **Results:** High sensitivity CRP levels were higher among men than
2 women, and were closely associated with age, cigarette smoking, hypertension,
3 HbA1C, total cholesterol and BMI. CRP levels were higher among South Asians
4 compared with Europeans, both before and after adjustment for the measured
5 cardiovascular risk factors. CRP was not associated with higher CAC levels or higher
6 degree of myocardial ischemia, for either ethnicity.

7

8 **Conclusions:** South Asians have higher levels of CRP compared with Europeans.
9 This difference remained despite adjusting for conventional risk factors. CRP levels
10 were not correlated with CAC or myocardial ischemia. While markers of
11 inflammation remain consistently elevated among South Asians, this does not
12 correlate with atherosclerotic plaque burden.

13 Though further research is needed to assess the mechanism of aggressive
14 coronary artery disease and resultant high mortality among South Asian, our study
15 suggests a role of inflammation beyond what is measured by plaque burden.

1 **5.2 Introduction:**

2 South Asians (people originating from India, Pakistan, Bangladesh and
3 SriLanka) account for almost a quarter of the global coronary heart disease (CHD)
4 deaths (1). Within the UK, they form the largest ethnic minority group (4% of the
5 total population). While improvements in health care have resulted in a decline in
6 CHD-related mortality in the general population, South Asians (SA) continue to
7 demonstrate a two-fold higher coronary heart disease mortality rate (4). A recent
8 meta-analysis including 6 studies and over 100,000 subjects assessing acute coronary
9 syndromes also suggests higher incidence (214) among this population.

10 The reasons for this excess CHD risk are not well elucidated. While global
11 registries such as the INTERHEART study suggest an important role of traditional
12 cardiovascular risk factors in coronary heart disease (5), South Asians do not
13 consistently demonstrate higher prevalence of such risk factors. Further, neither such
14 factors (8,47,192), nor risk scores comprising them such as the Framingham function,
15 accurately predict the higher coronary heart disease mortality in South Asians (109).
16 Many theories have been postulated in order to explain this unexpected higher CHD
17 risk observed among South Asians. These include possible elevated plaque burden i.e.
18 higher levels of atheroma and percentage stenosis (215), as well as excess
19 inflammation and elevated levels of prothrombotic molecules (9,12,72).

20 Over the last few decades, accurate non invasive assessment of coronary
21 plaque has become available and gained acceptance. Among such techniques,
22 coronary artery calcium (CAC) imaging and myocardial perfusion scintigraphy
23 (MPS) have been extensively used and validated.

1 CAC has a strong correlation with severity of coronary disease (144,153),
2 with increasing utilization as a measure of plaque burden. MPS assess functional
3 severity of coronary artery stenosis. There is now growing evidence to show higher
4 mortality in subjects with high CAC levels, and in those with higher levels of ischemia
5 seen on MPS, irrespective of concomitant risk factors thus increasing predictive value of
6 risk factor assessments (134,216-218). Alternately, low calcium levels and absence of
7 inducible ischemia on MPS have been shown to identify subjects with very low (<1%
8 per year) risk of coronary events (143). However, CAC and MPS do not appear to
9 identify the excess coronary risk seen among South Asians (173). While this lack of
10 difference in physical markers of CHD among Indians and Europeans is unexpected
11 in the first instance, it appears to be very appropriate given the growing body of
12 evidence which highlights the importance of inflammation in atherosclerosis and
13 plaque instability. Thus physical markers of disease will be unable to address plaque
14 morphology and composition, the presence of calcified versus inflammatory plaque,
15 and the role of inflammation in CHD (219).

16 Prospective studies in healthy subjects have demonstrated an independent
17 association between systemic inflammation and vascular events. Inflammation plays a
18 significant role in the atherothrombotic process, including the sudden rupture of
19 apparently stable plaque that is the underlying immediate cause of most acute
20 vascular events (116). Components of both the innate and acquired immune systems
21 are relevant to this progression (220). Inflammation appears to be inherent to the
22 complex interaction of lipid accumulation and immune function which promotes
23 premature atherosclerosis and accelerate plaque fissuring, a process that exposes the
24 underlying matrix to circulating thrombogenic factors and ultimately leads to platelet

1 activation and adhesion to the vessel wall causing vessel occlusion, and downstream
2 hypoxia.

3 Among such markers of inflammation, high-sensitivity CRP, an acute phase
4 reactant, has been arguably the most extensively studied of such molecules. While it
5 is as yet unclear whether the CRP is causative in the process of inflammation its
6 importance is in identification of high risk patients. Elevated levels of CRP are
7 associated with increased macrophage activity within plaques (63,64) and with a
8 higher density of high risk mixed coronary plaques (221). Presence of elevated CRP
9 levels in subjects with non calcified plaque has been associated with an increased
10 incidence of cardiovascular events (222). Increased concentrations of CRP have been
11 shown in both clinical and epidemiological studies to be associated with
12 atherothrombotic events. It is considered an independent predictor of future
13 cardiovascular events (51,65) improving risk stratification when added to
14 conventional risk factors (223,224).

15 South Asians exhibit a high prevalence of diabetes mellitus, hypertension,
16 abdominal obesity and metabolic syndrome, with lower HDL cholesterol levels (54-
17 57). Presence of these risk factors is associated with greater systemic inflammation
18 (58,59). We therefore hypothesised that South Asians have a higher degree of
19 inflammation, which can be assessed using CRP. This may explain the higher CHD
20 mortality observed in this population and form the basis for further risk stratification.

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5.3 Methods:

Participants: 2347 asymptomatic South Asians and Europeans men and women were recruited from the LOLIPOP study. The main LOLIPOP study has been described in detail in methods chapter. All subjects underwent extensive imaging including CAC imaging using electron beam computed tomography (EBCT), as well as anthropometric measurements and biochemical markers including CRP. Patients with CAC > 100 Agatston units also underwent MPS studies.

Coronary artery calcification and myocardial perfusion scintigraphy:

The methods for the imaging modalities have been described in detail in the methods and materials chapter.

High sensitivity C reactive protein: CRP was measured by automated microparticle enhanced turbidometric immunoassay run on COBAS MIRA (Roche Diagnostics GmbH).

Statistical analysis: All analyses were performed using the statistics programme Statistical Package for the Social Sciences (SPSS) version 21.0 (Chicago, IL, USA). Categorical data are presented as number (percent), and continuous data as mean value± SD. The chi-square test was used to compare categorical variables while comparisons of continuous variables between two groups were performed using student *t* tests. All tests of significance were two-tailed, and significance was defined at the ≤ 0.05 level.

1 Levels of CRP were compared across ethnic groups. The relationships
2 between conventional risk factors and CRP were analysed using regression models,
3 both before and after adjusting for concomitant risk factors. A binomial logistic
4 regression model was used to evaluate associations between risk factors and the
5 presence of high CRP levels using 2mg/L as a cutoff (205). A separate linear
6 regression model was used to assess the predictive ability of risk factors for the
7 increase in CRP. Due to the non-Gaussian nature of CRP, analyses were carried out
8 using CRP transformed to their natural logarithm (\lg_e). The resultant beta coefficients
9 were then back transformed to the original units.

10

11

1

2 **5.4 Results:**

3 **Clinical and biochemical characteristics of patients**

4 South Asians were on average two years younger than Europeans, with a
5 higher percentage of women. They demonstrated a higher prevalence of diabetes
6 mellitus, hypertension and metabolic syndrome with lower prevalence of current and
7 ex-smokers. South Asians also had higher serum glucose and triglycerides levels with
8 lower total cholesterol, HDL and LDL cholesterols, as well as physical activity index
9 compared with Europeans. South Asians had a similar BMI, though a higher waist hip
10 ratio and body fat percentage and 16% of South Asians were on cholesterol lowering
11 medications compared with 8% of Europeans. Log CRP levels were higher in South
12 Asians compared with Europeans (Table 5.1).

13

14 **Predictors of CRP**

15 Univariate linear regression was carried out in order to assess association of
16 risk factors with CRP (Table 5.2). Increasing age, LDL cholesterol and triglyceride
17 levels along with increasing WHR and BMI were associated with increasing levels of
18 CRP; as was the presence of diabetes, hypertension, and male gender. HDL levels and
19 statin usage was negatively associated with CRP. South Asian ethnicity was
20 significantly associated with increasing CRP levels.

21 Multivariable linear analysis was used to predict effect of conventional risk
22 factors and ethnicity on CRP levels, and explained 25% of the variation in CRP. It
23 demonstrated that increase in age, HbA1c, WHR, BMI, was associated with elevated
24 CRP. Presence of hypertension, male gender and current smoking were also

1 associated positively with increasing CRP. Statin usage and HDL levels were
2 negatively associated with CRP. South Asians ethnicity appeared to be independently
3 associated with higher CRP levels, even after adjusting for concomitant risk factors.
4 (Table 5.3)

5 Ethnic specific analysis showed that among Europeans, increasing age, WHR,
6 smoking and male gender were associated with increasing CRP. Among South
7 Asians, DM, WHR and Body fat were associated with elevated CRP. Statin usage and
8 increasing HDL levels was negatively associated with CRP levels among both
9 ethnicities.

10

11 **CRP as a categorical variable**

12 A multivariable logistic regression model was used to assess effect of
13 conventional cardiovascular risk factors and ethnicity on high levels of CRP using 2
14 mg/L as a cut off, and explained about 20% of the variation in CRP in this population.
15 Increasing WHR and body fat were independently associated with higher levels of
16 CRP. Statin usage was associated with lower levels of CRP. After adjustment for
17 cardiovascular risk factors, South Asian ethnicity remained an independent predictor
18 of a prevalence of elevated CRP, OR=1.4 (95% CI 1.1, 1.7).

19

20 **CRP and CAC**

21 There was a weak correlation between CRP and CAC, with a spearman's
22 coefficient of 0.06 (p 0.002). CRP levels did not predict either presence or extent of
23 coronary calcification.

24 Higher levels of CAC were associated with conventional risk factors including
25 age, male gender, diabetes and hypertension. While CRP was associated with
26 increased levels of CAC on univariate linear regression (P = 0.002), this relationship

1 was not statistically significant after adjusting for ethnicity and conventional
2 cardiovascular risk factors (Table 5.4). The results were similar when analysed
3 separately for South Asians and Europeans.

4

5 **CRP and MPS**

6 There was a no correlation between CRP and presence or extent of ischemia
7 [spearman's coefficient of 0.05 (p 0.73)]. CRP was not associated with higher
8 incidence of ischemia on MPS, before or after adjustment for conventional
9 cardiovascular risk factors.

10

1 **5.5 Discussion:**

2 In this cohort of asymptomatic men and women, aged 35-75 years, we
3 demonstrated higher levels of CRP in South Asians compared with Europeans. This
4 was independent of conventional risk factors. Furthermore CRP was not correlated
5 with coronary plaque burden as demonstrated by coronary artery calcification and
6 myocardial perfusion imaging.

7 South Asians are at a higher risk of coronary artery disease and demonstrate
8 higher CHD mortality compared to Europeans. This excess risk remains unexplained
9 and largely undetected by conventional risk stratification tools. Markers of
10 atherosclerotic plaque burden such as CAC and MPS do not identify the higher risk
11 seen among South Asians. We hypothesized that South Asians have higher degree of
12 inflammation compared to Europeans. Quantification of this inflammation using CRP
13 could provide an accurate assessment of risk in this population. We found that CRP
14 levels were higher among South Asians both before and after adjusting for
15 conventional risk factors.

16 Consistent with existing data, we demonstrated that increasing age, male
17 gender, LDL cholesterol, HbA1C levels, WHR and body fat were associated with
18 increase in CRP levels(225,226). Increasing HDL levels and statin usage found to
19 be negatively associated with CRP. CRP was not associated with CAC levels
20 (227,228).

21 Ethnic differences in CRP have been studied by our group among others.
22 High levels of CRP been documented among African Americans, Hispanics, and
23 South Asians compared with Caucasians (60,110,225,229). There are many
24 theories postulated regarding this excess including increased abdominal obesity

1 (230), higher prevalence of diabetes mellitus and insulin resistance (110) i.e. the
2 dysmetabolic profile as well as chronic stress or lower socio economic status.
3 Metabolic factors may affect the atherosclerotic process in several ways. They
4 contribute to lipid accumulation in the artery and initiate new rounds of immune-
5 cell recruitment. Also, the adipose tissue of patients with the metabolic syndrome
6 and obesity produces inflammatory cytokines, particularly tumor necrosis factor
7 and interleukin. There is however no clear answer to the question of cause and
8 effect regarding adiposity, insulin resistance and systemic inflammation as elevated
9 cytokines can cause insulin resistance; and insulin resistance with its resulting
10 abdominal obesity can cause elevation in cytokine levels.

11 A number of studies have demonstrated association of lower SES with
12 higher degree of inflammation. This is due to a mix of poor diet, chronic infections,
13 psycho social stress along with higher incidence of smoking, excess alcohol,
14 sedentary life. Thus, ethnic variations in inflammation and CRP could be due to an
15 amalgamation of such factors.

16 Irrespective of ethnic variations and differences, measures of
17 atherosclerotic plaque burden have not been associated with elevated CRP levels in
18 South Asians and Europeans (226-228,231). These findings suggest separate
19 pathophysiology in calcified plaque/ obstructive coronary disease and systemic
20 /focal inflammatory state as demonstrated by higher CRP levels.

21 Compared with Europeans, South Asians have a similar atherosclerotic
22 plaque burden but demonstrate a greater degree of systemic inflammation. The
23 difference remains significant even after adjusting for conventional risk factors,
24 diabetes and obesity. This is an extremely important finding given that

1 inflammation is a key feature of coronary atherosclerosis, and more importantly,
2 plaque instability. Thus, the unexplained high CHD mortality in South Asians
3 could be driven by unstable inflammatory plaque as opposed to a higher burden of
4 plaque, unidentified through conventional means. Assessment of inflammation
5 could thus be used to identify high risk individuals for aggressive risk factor
6 management as is been done in studies such as CANTOS (232), or further
7 invasive/non invasive investigations.

8 In conclusion, we observed that South Asians exhibit higher levels of CRP
9 compared with Europeans. This is independent of cardiovascular risk factors and
10 atherosclerotic plaque burden as assessed by CAC and MPS. While ongoing studies
11 including our own will provide data to ascertain the aetiology and prognostic
12 implications of a higher degree of inflammation among South Asians, it as an
13 important factor which needs to be addressed in risk stratification and treatment. It
14 also provides a much needed direction to guide further research into higher CHD in
15 this population.

16

1 Table 5.1: Characteristics of participants

2

	European (1004)	South Asian (1343)	P value
Age (Years)	57±10	55 ±10	<0.001
Agatston score (95% CI)	153 ± 442	123± 384	0.12
Gender (Male)	70	61	<0.001
Log CRP*	1.5 ± 3.5	2.0 ± 3.5	0.008
Hypertension (%)	19	31	<0.001
Systolic Blood Pressure (mm Hg)	79 ±10	79 ±10	0.1
Diastolic Blood Pressure (mm Hg)	129 ± 19	128 ± 19	0.7
CRP	3.7	3.97	
Body fat (%)	30 ± 8	32± 9	<0.001
Waist Hip Ratio	0.9 ± 0.09	0.94 ± 0.07	<0.001
Body Mass index	27±4.7	27±4.2	0.2
Type 2 diabetes (%)	7	18	<0.001
Serum Glucose (mmol/l)	5.4± 1.5	5.8 ± 1.9	<0.001
HbA1c	5.5 ± 0.9	6.1 ±1.2	<0.001
Current smokers (%)	17	7	<0.001
Ever smoker (%)	53	15	<0.001
Statin Usage (%)	8	16	< 0.001
Total cholesterol (mmol/l)	5.6 ±1.0	5.3 ±1.1	<0.001
LDL Cholesterol (mmol/l)	3.5 ± 0.9	3.3 ± 0.9	<0.001
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.3 ± 0.3	<0.001
Triglycerides (mmol/l)	1.5 ± 1.1	1.7 ± 1.2	<0.001
Metabolic syndrome (NCEP) %	20%	29%	<0.001

<u>Risk factor</u>	<u>Odd's ratio (95% CI)</u>	<u>P value</u>
Age (10 Years)	1.01 (1.01, 1.02)	<0.001
Race (SA vs Europeans)	1.31 (1.17, 1.46)	<0.001
Gender (Male vs Female)	1.54 (1.38, 1.72)	<0.001
Hypertension	1.43 (1.27, 1.63)	<0.001
Statin usage	0.98 (0.83, 0.87)	0.76
Type 2 Diabetes Mellitus	1.14 (1.04, 1.25)	0.006
Total cholesterol (mmol/l)	1.07 (1.02, 1.13)	0.11
HDL Cholesterol (mmol/l)	0.61 (0.55, 0.71)	<0.001
LDL cholesterol (mmol/l)	1.07 (1.01, 1.14)	0.002
Triglycerides (mmol/l)	1.14 (1.08, 1.19)	<0.001
HbA1c	1.25 (1.19, 1.31)	<0.001
Ever smoker	0.97 (0.86, 1.08)	0.6
Current smoker	CRP 1.07 (0.91, 1.28)	0.4
Waist Hip Ratio	20.49 (10.8, 36.6)	<0.001
Body mass index	1.12 (1.11, 1.13)	<0.001

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*log transformation used

Univariable linear regression, demonstrating change in CRP, for unit change in risk factor.

1 **Table 5.3 Multivariable regression for predictors of CRP levels**

<u>Risk factor</u>	<u>Odds ratio (95% CI)</u>	<u>P value</u>
Age (10 Years)	1.01 (1.0, 1.02)	.001
Race (SA vs Europeans)	1.2 (1.1, 1.4)	.002
Gender (Male vs Female)	1.2 (1.03, 1.53)	.021
Hypertension	1.2 (1.01, 1.31)	.037
Statin usage	0.7 (0.6, 0.8)	< 0.001
Type 2 Diabetes Mellitus	0.8 (0.6, 1.02)	.077
HDL Cholesterol (mmol/l)	0.7 (0.6, 0.9)	.002
LDL cholesterol (mmol/l)	1.1 (0.99, 1.13)	.054
Triglycerides (mmol/l)	0.9 (0.92, 1.07)	.959
HbA1c	1.1 (1.03, 1.25)	.011
Ever smoker	1.1 (0.94, 1.25)	.289
Current smoker	1.3 (1.04, 1.55)	.018
Waist Hip Ratio	3.0 (1.4, 6.4)	.005
Body mass index	1.06 (1.04, 1.08)	< 0.001

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Change in odds for increasing CRP per increment in risk factors estimated using linear regression, adjusted for risk factors listed.

1 **Table 5.4 Multivariable regression for predictors of CAC**

<u>Risk factor</u>	<u>Odds ratio (95% CI)</u>	<u>P value</u>
Age (10 Years)	1.1 (1.1, 1.2)	<0.001
Race (SA vs Europeans)	1.05 (0.8, 1.2)	0.6
Gender (Male vs Female)	3.9 (2.9, 5.8)	<0.001
Hypertension	1.9 (1.6, 2.4)	<0.001
Statin usage	2.1 (1.6, 2.8)	< 0.001
Type 2 Diabetes Mellitus	1.76 (1.3, 2.2)	<0.001
HDL Cholesterol (mmol/l)	0.9 (0.9, 1.01)	0.3
Total cholesterol (mmol/l)	1.02 (1.01, 1.03)	<0.001
Ever smoker	1.2 (0.9, 1.5)	0.12
Current smoker	1.5 (1.1, 2.0)	0.009
Waist Hip Ratio	0.96 (0.8, 1.1)	0.5
Body mass index	1.03 (1.01, 1.05)	0.003
Log CRP	1.04 (0.9, 1.1)	0.2

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Change in odds for increase in log CAC per increment in risk factors estimated using linear regression, adjusted for risk factors listed.

1 Chapter 6: **Limitations, Conclusions and Future Work**

2

3 **6.1 Limitations**

4 Our study has several potential limitations. Firstly, our study had a response
5 rate of 36%; while these rates are similar to those seen in population-based studies
6 such as MESA; they are low and might potentially introduce bias. However, there
7 were no differences in response rates between South Asians and Europeans.
8 Secondly, our cohort included a selected asymptomatic group, and potential
9 participants with known cardiovascular disease were excluded at entry. The study
10 thus might not accurately reflect the disease process in those with known coronary
11 artery disease. Thirdly, the study is cross-sectional, and would be affected by
12 different survival rates among ethnic groups. However, the large population-based
13 sample, stringent ethnic group classification and standardised assessment give our
14 study the potential to accurately explore the differences in sub clinical atherosclerosis
15 between South Asians and Europeans.

16

17 **6.2 Conclusions**

18 In this thesis, I have examined markers of subclinical atherosclerosis and
19 inflammation in a large cohort of unselected asymptomatic individuals of South
20 Asian and European ethnicity. I have found that: 1) Coronary artery calcification
21 scores were closely associated with age, male gender, cigarette smoking,
22 hypertension, systolic blood pressure, diabetes and total cholesterol. 2) There were no
23 differences in either coronary artery calcification prevalence or mean levels of
24 coronary artery calcification between South Asian and European, after adjustment for

1 the measured cardiovascular risk factors. 3) Presence of diabetes and increasing
2 coronary artery calcification were independent predictors for silent myocardial
3 ischemia. 4) South Asian ethnicity did not influence the prevalence or the extent of
4 silent myocardial ischemia, after adjustment for conventional risk factors. 5)
5 Conventional risk factors including obesity, hypertension and diabetes mellitus are
6 associated with elevated CRP levels. 6) South Asians have a higher degree of
7 systemic inflammation as measured by serum high sensitivity C-reactive protein
8 levels, after adjusting for conventional risk factors.

9 Coronary heart disease (CHD) is the single largest contributor to global
10 mortality, with more than 13% of all deaths in 2002 attributed to CHD(1). There are,
11 however, distinct ethnic differences in CHD incidence and mortality. South Asians
12 (people originating from India, Pakistan, Bangladesh and Sri Lanka) demonstrate
13 high levels of CHD -attributable mortality. According to figures released by World
14 Health Organization, while South Asians currently comprise 22% of the global
15 population, they accounted for almost a quarter of the global CHD deaths in 2002.
16 Furthermore, as a result of a high growth rate this proportion is expected to increase
17 to 28% by 2050 (2), and with the inherent high CHD risk, South Asians are expected
18 to contribute to almost 40% of the global CHD burden by 2050 (2).

19 This rapidly increasing population with a (2) propensity towards CHD
20 (233,234) especially at younger ages (192) is in stark contrast to the developed world
21 where slower growth rates are observed (2), alongside reductions in CHD rates
22 (4,235).

23 Among South Asians, CHD is closely related to an urbanized lifestyle, with
24 the highest rates of CHD observed among overseas South Asians (3-10). Within the

1 UK, South Asians continue to show a 2-fold higher CHD mortality rate despite
2 decreasing rates among Local Europeans for the same period (4).

3 In diseases with a high mortality, population screening has resulted in
4 decreasing mortality through early identification and treatment (236,237). For
5 coronary heart disease, screening has traditionally been through two main methods:
6 a) identification of specific risk factors and b) use of statistically created models to
7 predict risk of developing disease. Through such screening, we are provided us with
8 the estimated risk of an individual to develop clinical disease, and therefore tailor the
9 management of each component and the patient, accordingly. This presents a
10 challenge when evaluating coronary heart disease among South Asians, for two
11 important reasons:

12 First, while CHD among South Asians is not independent of conventional risk
13 factors (5,238,239), the limited data available suggests that such factors are not
14 consistently elevated among South Asians, and do not account for the increased
15 coronary heart disease (CHD) risk in South Asians (7,8,10). Type 2 diabetes mellitus
16 (T2D) and insulin resistance are more prevalent among South Asians compared with
17 Europeans, however, studies from UK and Canada have demonstrated that although
18 presence of diabetes increases mortality risk in South Asians to a greater extent than
19 in Europeans, the risk of CHD remains elevated after adjustment for traditional risk
20 factors. (8,47,192).

21 Thus, if we use risk factors to identify South Asians at higher CHD risk and
22 treat individual risk factors, it is unclear whether we address the actual risk of
23 coronary heart disease among this population, and if so, to what extent.

1 Second, risk stratification models assign a specific risk of CHD to individuals
2 within a population, based on the risk factors. This helps ascertain the population at
3 high risk for CHD, and therefore potentially able to derive maximum benefit from
4 aggressive risk factor management. However, there are limitations to such models.
5 There is substantial variation in the amount of atherosclerosis for every level of risk
6 factor exposure (240), thus it would appear intuitive that it would be so for each risk
7 category. Further, coronary events in populations deemed to be at a low risk for CHD
8 by such models further highlights limitations of such models. While such events are
9 thought to be consequent to multiple factors including duration of exposure as well as
10 interactions among risk factors and genetic susceptibility, the risk remains
11 unaccounted in the risk stratification models. Additionally, as majority of the cohort
12 studies forming the basis for scoring systems were originally from risk assessments in
13 Caucasian populations; their weaknesses are accentuated when employed in other
14 ethnic groups (241,242). Thus, the failure of risk scores such as the Framingham
15 function among South Asians (108,243) can be due to a multitude of reasons.

16 In summary, it appears that while we have a population with a high CHD
17 mortality, who would benefit from early screening and identification of CHD risk, we
18 lack the tools to do so.

19 With increasing awareness of the role of CHD in global mortality rates, there
20 has been considerable research into the identification of individuals at high risk of
21 CHD. An important development in this field has been the emergence of techniques
22 that allow the non-invasive visualization of atherosclerotic plaque.

23 Coronary artery calcification is a highly sensitive marker of coronary plaque
24 burden (130,144), and is correlated with histomorphometric plaque burden ($r >$

1 0.90)(130). Prevalence of coronary calcification is significantly higher in subjects
2 with traditional cardiovascular risk factors such as hypertension, diabetes, obesity,
3 physical inactivity, previous smoking, and hypercholesterolemia (148,166-169).
4 Coronary artery calcification provides an incremental value for predicting prognosis
5 compared with conventional risk factors alone (134,163,164), supporting its role as an
6 important risk stratification tool. However, the majority of these studies were
7 conducted in Caucasians groups, and there is scarce understanding about the
8 prevalence among South Asians.

9 In the first study, it was shown that age, BMI, current and former smoking,
10 statin usage, presence of hypertension or diabetes mellitus, male gender and European
11 ethnicity, as well as serum HDL and triglyceride levels, were associated with elevated
12 odds for the increasing extent of CAC. After adjusting for conventional
13 cardiovascular risk factors and ethnicity, age, male gender, current smoking, statin
14 use, diabetes, hypertension and LDL cholesterol were all positively associated with
15 increased levels of CAC. Ethnicity appeared not to influence prevalence or extent of
16 coronary artery calcification among South Asian compared with European
17 participants.

18 These data did not show any differences in CAC between UK South Asians
19 and Europeans, despite a worse cardiovascular risk profile among South Asians. This
20 is in contrast to the excess CHD risk observed in South Asian. This study suggests
21 that the increased prevalence of CHD amongst South Asians is not reflected by their
22 increased propensity towards atheroma formation, compared with Europeans, and that
23 other mechanisms may be responsible for their elevated susceptibility to acute CHD
24 events.

1 Myocardial perfusion imaging is a technique that has been validated for
2 functional assessment of flow limiting lesions within coronary arteries. Thus, it
3 allows quantification of the hemodynamic relevance of the atherosclerotic lesions. This
4 technique has been validated in studies using coronary angiography and coronary artery
5 calcification to quantify atherosclerotic plaque (179,244), with increasing ischemia
6 observed with higher levels of plaque. Data from our group and others has suggested
7 that when using coronary artery calcification as a marker of plaque, a cut –off of 100
8 Agatston units increases the specificity of the test. There appears to be a marked
9 difference in prevalence of ischemia noted above and below this level
10 (140,179,184,186).

11 Based on these data, and in order to assess whether similar plaque calcification
12 portents greater plaque burden among South Asians, myocardial perfusion imaging was
13 performed in a cohort of subjects with coronary artery calcification scores greater
14 than 100 Agatston units. I found that increasing age and higher CAC category, as well
15 as presence of diabetes, were associated with elevated odds for the presence of
16 myocardial ischemia among both populations. After adjustment for cardiovascular
17 risk factors, ethnicity was not related to presence of myocardial ischemia, OR= 0.9
18 (95% CI 0.4, 2.0). Prevalence and extent of myocardial ischemia increase in parallel
19 with CAC scores among South Asians and Europeans. There was no significant
20 ethnic difference in extent or severity of myocardial ischemia.

21 Anatomical presence and size of atherosclerosis lesions is only a part of the
22 aetiology of vascular disease with other equally factors involved in the development
23 of acute thrombotic events. Blood thrombogenicity is equally important in
24 determining the clinical course of an acute plaque event. To recognise the collective

1 importance of these systems it has been proposed that at risk individuals be referred
2 to as “vulnerable patients” (127). Such risk may be identified through serum markers
3 of atherosclerosis (abnormal lipoprotein profile), inflammation (high-sensitivity
4 CRP), metabolic disorders (blood glucose, triglycerides and homocysteine) and
5 through coagulation disorders (fibrinogen, factor V Leiden, increased coagulation
6 factors, decreased anticoagulant factors).

7 Inflammation is one of the key factors in initiating atherosclerosis as well as
8 the pathogenesis of vulnerable plaques (49). This is supported by studies done in
9 animal models as well as those among human cases of sudden death, demonstrating
10 increased macrophage activity in plaques which underwent rupture and subsequent
11 thrombosis (51). Furthermore, recent studies in human subjects have shown markedly
12 increased inflammation within active atherosclerotic plaques in carotid arteries
13 (52,53). Among the markers of inflammation, CRP is one of the most extensively
14 studied molecules. It is considered an independent predictor of future cardiovascular
15 events (51,65) improving risk stratification when added to conventional risk factors
16 (223,224) with increased concentrations of associated with atherothrombotic events.
17 Elevated levels of CRP are associated with increased macrophage activity within
18 plaques (63,64) and with a higher density of high risk mixed coronary plaques (221).
19 Presence of elevated CRP levels in subjects with non calcified plaque has been
20 associated with an increased incidence of cardiovascular events (222).

21 Thus, the third study evaluated systemic inflammation measured using CRP
22 levels. I demonstrated that South Asians had a worse metabolic profile with a higher
23 prevalence of diabetes mellitus, hypertension and metabolic syndrome. South Asians
24 demonstrated elevated CRP levels both before and after adjusting for metabolic

1 factors, conventional cardiovascular risk factors and measures of obesity. The CRP
2 levels were not significantly associated with markers of atherosclerosis, including
3 CAC and MPS, in either ethnicity.

4 These are vital studies for increasing understanding of CHD among South
5 Asians and have several important implications. Through the course of this project I
6 have demonstrated that South Asians have similar atherosclerotic plaque burden as
7 assessed by CAC and MPS, however, have a higher degree of systemic inflammation.
8 This is unexplained by conventional risk factors, and is does not explain or correlate
9 with atherosclerotic burden. Thus, while the total atherosclerotic burden is the same
10 in the two populations; there are higher levels of systemic inflammation and
11 prothrombotic factors in Indians Asians compared with European. The higher CHD
12 risk among South Asians could therefore be a result of a higher propensity to plaque
13 rupture or undergo thrombosis.

14 These results stress the importance of factors such as vulnerability or
15 inflammation within atherosclerotic plaque as potential explanations for the elevated
16 coronary events and CHD mortality among South Asians. They also generate several
17 important questions for future studies, and avenues of treatment.(232)

18

19 **6.3 Future work**

20

21

22 **6.3.1 Prognostic importance of coronary calcification in South Asians and** 23 **Europeans**

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26 Previous studies have suggested the addition of ethnicity based weighing
27 factors and cut offs as “correction factors” for improving tools such as metabolic

1 syndrome (245) and Framingham risk scores (241,243). The follow-up from this
2 study will allow assessment of the prognostic significance of coronary artery
3 calcification for South Asians, i.e. do South Asians have similar mortality as
4 Europeans for comparable levels of calcification or is the mortality among South
5 Asians higher or lower than Europeans for any given level of calcification. It will
6 help decide whether frequently used cut off levels for CAC such as >100, > 400
7 suggest the same prognostic significance for South Asians as for EW; or do we need
8 to define ethnic specific categories. Thus, it will guide us towards more effective use
9 of coronary artery calcification imaging as a tool for risk stratification among South
10 Asians.

11 Therefore, cohort surveillance and follow-up for clinical events will form an
12 essential component of the long term goal for this study. Study event endpoints will
13 include acute myocardial infarction and other forms of CHD, peripheral vascular
14 disease, congestive heart failure, coronary interventions and mortality. The follow-up
15 of enrolled subjects is to be undertaken at Ealing Hospital by trained research nurses.
16 The first follow-up contact commenced in January 2010 and is to be repeated in
17 2015. The NHS number and other demographic details will be used to link to health
18 records and multiple sources (Office for National Statistics, Virtual Organisation for
19 Trials and Epidemiological Studies, Hospital Episode Statistics data and clinical
20 databases in West London Hospitals) will be searched and collated to identify CHD
21 events.

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1 **6.3.2 Assessment of inflammatory or vulnerable plaque**

2 Traditionally, intravascular ultrasound has been the gold standard for
3 evaluating total atherosclerotic plaque burden of the coronary arteries. Studies
4 have used this technology to identify the morphological characteristics of coronary
5 artery plaque lesions that are responsible for acute coronary events (121).
6 However, one disadvantage with intravascular ultrasound is that it can be only
7 performed during invasive coronary angiography.

8 Recent advances in imaging, including dual source CT technology, show
9 extremely promising results, with CT coronary angiography providing non-
10 invasive information about the vessel wall and morphology of plaque lesions,
11 similar to that afforded by intravascular ultrasound (246-248). Recent prospective
12 studies using CT coronary angiography have demonstrated the role of ‘spotty
13 calcification’ (seen on CT coronary angiography) in rendering a plaque lesion
14 more prone for rupture (203). Furthermore, recent studies have been also been able
15 to characterize atherosclerotic coronary plaque lesions with a thin fibrous cap and
16 a necrotic lipid core, the “vulnerable plaque” as having distinctive ‘napkin-ring’
17 appearance on CT coronary angiography. (249). Such morphological
18 characteristics detected with non- invasive angiography will allow delineation of
19 plaque composition and thus imaging of vulnerable plaque; a step closer to the
20 identification of patients at higher risk of suffering an acute coronary event.

21 Studying South Asians with techniques such as these will help us
22 understand the pathogenesis of CHD, as well as the mechanisms behind the high
23 CHD mortality among South Asians.

24

1 **6.3.3 Evaluation of markers of systemic inflammation and pro thrombotic**
2 **factor**

3 The third important factor along with plaque burden and plaque composition
4 is the atherothrombotic state. Although not capable of conveying the actual burden of
5 atherosclerosis, there are several emerging blood biomarkers that may be useful in
6 enhancing risk stratification and prognosis of CHD.

7 Atherogenesis is characterised by the presence of local inflammation which is
8 believed to lead to plaque vulnerability, thus predisposing it to rupture causing
9 arterial thrombosis (48). Prospective studies in healthy subjects have demonstrated an
10 independent association between elevated concentrations of systemic inflammation
11 and vascular events. Among such markers of inflammation, high-sensitivity CRP, an
12 acute phase reactant, has been extensively studied. It is an extremely sensitive marker
13 of inflammation, and increased concentrations of this marker have predicted
14 cardiovascular outcomes independent of concomitant risk factors (250-252). Elevated
15 levels of CRP have been observed in a healthy population of South Asians compared
16 with Europeans while being closely related with central obesity in both populations
17 (60).

18 Markers of inflammation such as IL-6 (253,254), and pentraxin-3 (255) in the
19 plasma have also been shown to correlate with cardiovascular events. In a prospective
20 study of 510 asymptomatic diabetic patients, levels of osteoprotegerin, a cytokine of
21 the tumour necrosis factor receptor super-family, were associated with prevalence of
22 sub-clinical atherosclerosis and short-term cardiovascular events (148). The possible
23 correlation between RANKL, a member of the TNF ligand family for which

1 osteoprotegerin is a decoy receptor, and acute coronary syndromes has been reported
2 (199).

3 Lipoprotein (a) is composed of a cholesterol rich particle linked to a
4 glycoprotein of variable size called apolipoprotein (a). High levels of Lipoprotein (a)
5 have been shown to be a risk factor for CVD, with levels above 30mg/dL shown to
6 increase risk by two to three times in Caucasian populations (256). The association of
7 Lipoprotein (a) with CAD and its ability to act as a biomarker of risk appears to be
8 strongest in patients with hypercholesterolemia and particularly in young patients
9 with premature atherosclerosis.

10 Thus, evaluation of such factors could provide both an insight into
11 mechanisms of coronary disease in Indians Asians, and also provide markers to help
12 quantify risk of CHD among South Asians.

13 All subjects enrolled into the study provided blood samples which were frozen
14 and analysis has recently been completed for appropriate biomarkers of subclinical
15 CVD, relating to inflammation, lipoprotein sub particles.

16

17 **6.3.4 Potential risk stratification tools**

18 South Asians appear to be at a high risk of CHD, compared with other ethnic
19 groups. Conventional risk factors, while identifying subjects at a risk of CHD, fail to
20 identify this excess risk in South Asians. While markers of atherosclerotic plaque
21 burden do not appear to identify the high risk of coronary heart disease mortality
22 among South Asians, they do identify a group at a higher risk than the general
23 population. Bio- markers such as CRP, IL-6, osteoprotegerin and NT-pro BNP among

1 others have been demonstrated to be associated with a higher risk of acute coronary
2 events, and help identify/ stratify subjects at a high risk of CHD, and related events.

3 Thus, a potential risk stratification tool should reflect the multisystem nature
4 of CHD. This could be a combination of markers of its risk factors which contribute
5 towards CHD, imaging of atherosclerotic plaque and assessment of plaque or
6 systemic inflammation.

7

8

1 **6.4 Summary**

2 Obstructive coronary artery disease and resulting ischemia is a consequence of
3 luminal narrowing secondary to large atherosclerotic plaques. These plaques consist
4 of calcified and non calcified deposits. While coronary calcification measures
5 calcified plaque, myocardial perfusion imaging is a marker of flow limiting
6 obstruction and thus, of total plaque burden. In this thesis I have demonstrated similar
7 calcified plaque burden, along with similar extent of myocardial ischemia among
8 South Asians compared with Europeans, despite their high coronary heart disease
9 mortality. I have also demonstrated higher degree of systemic inflammation among
10 South Asians. The similar prevalence of markers of atherosclerotic plaque burden,
11 with higher degree of inflammation among South Asians compared with Europeans
12 suggest an important role of inflammation in the excess risk for coronary heart
13 disease among South Asians.

14 Future studies should therefore be aimed at evaluating the role of
15 inflammation within plaque, systemic inflammation as well as pro thrombotic factors
16 in order to understand the CHD risk among South Asians.

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