The echocardiographic manifestations of an urban, working class community with a high cardiovascular risk profile.

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in the College of Health Sciences, Nelson R Mandela School of Medicine, Department of Medicine, University of Kwazulu-Natal

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DECLARATION

By submitting this dissertation, I declare that the entirety of the work contained herein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously, in its entirety or in part, submitted it for obtaining any qualification to the University of Kwazulu-Natal or any other institution of Higher Education.

_____ _____

D R Prakaschandra **Date**

This thesis is dedicated to my late grandmother Lutchmiamma Chetty, and the fulfilment of a promise…

PUBLICATIONS AND PRESENTATIONS

1. The risk factor profile of a local community in KwaZulu-Natal: a preliminary analysis. DR Prakaschandra. South African Heart Association Congress, 2009 (oral presentation)
2. Cardiovascular risk factors and risk factor clustering in the Phoenix community. Prakaschandra DR. Bayer GP Update, 2009 (Guest speaker).
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ABBREVIATIONS

Aa	Myocardial velocity associated with atrial contraction
ASE	American Society of Echocardiography
ATP III	Adult Treatment Panel III
Am	Transmitral A – wave associated with atrial contraction
Aa	Myocardial velocity during atrial contraction
BMI	Body mass index
BP	Blood pressure
CDL	Chronic diseases of lifestyle
CVD	Cardiovascular disease
DHF	Diastolic Heart failure
DM	Type 2 Diabetes Mellitus
DNA	Deoxyribose nucleic acid
DT	Deceleration time
Ea	Early myocardial velocity using tissue Doppler
EF	Ejection fraction
Em	Transmitral early diastolic wave
FRET	fluorescence energy transfer
FS	Fractional shortening
HDL	High-density lipoprotein
HFNEF	Heart Failure with normal ejection fraction
HOMA	Homeostatic model
IDF	International Diabetes Federation
IR	Insulin resistance
IVRT	Isovolumic relaxation time
LA	Left atrium
LAV	Left atrial volume

LAVI	Left atrial volume index
LPL	Lipoprotein lipase gene
LV	Left ventricle
LVMI	Left ventricular mass index
LVEDP	Left ventricular end diastolic pressure
LVH	Left Ventricular Hypertrophy
LVM	Left ventricular mass
MRI	Magnetic resonance imaging
MS	Metabolic syndrome
NCD	Non-communicable disease
NCEP	National Cholesterol Adult Panel
PCR	Polymerase chain reaction
PON-1	Human Paraoxonase-1 gene
PW	Pulsed-wave
RWMA	Regional wall motion abnormality
Sa	Systolic wave on tissue Doppler
SEAT	Sub-epicardial adipose tissue thickness
SHF	Systolic Heart Failure
SNP	Single nucleotide polymorphisms
TDI	Tissue Doppler imaging
TGL	Triglyceride
VAT	Visceral adipose tissue
VHD	Valvular heart disease
WC	Waist circumference
WHO	World Health Organisation
WT	Wild-type

ABSTRACT

Background:

The metabolic syndrome (MS), consequent upon the pandemic of obesity and diabetes, is associated with an increased risk for cardiovascular (CV) disease. Development of sub-clinical cardiac structural and functional changes associated with CV disease risk factors may be detected on echocardiography. The extent to which these structural changes and CV risk factors are dependent on genetic factors is not clearly established. This project was designed to investigate the relationship between CV disease risk factors, cardiac structural and functional changes and underlying genetic abnormalities. Specifically, the risk factor profile and the presence of the MS were determined. This was then correlated with the echocardiographic findings and gene polymorphisms.

Method:

A randomly selected cohort of 1428 subjects from the Phoenix community was studied. Demographic data was collected using the WHO STEPS instrument. Blood samples for biochemistry and genetic analysis, together with anthropometric measurements, were collected. Blood pressure and echocardiography was performed on all subjects. The metabolic syndrome was classified according to the National Cholesterol Education Panel (NECP) Adult Treatment Panel III (ATP III) and International Diabetes Federation (IDF) criteria. The Lipoprotein Lipase and Human Paraoxonase-1 genes were genotyped on a Light Cycler 480 Real-Time PCR instrument, using allele-specific probes and sequencing.

Results:

There was a high prevalence of CV risk factors in this sample; particularly increased waist circumference (79%), obesity (64%) insulin resistance (58%) and hypertension (50%) across the age groups. This translated into a high prevalence of MS (38% using NCEP ATPIII and 46% using IDF criteria). There were significant echocardiographic differences between subjects with and without MS for chamber dimensions ($p<0.001$), left ventricular wall thickness ($p<0.001$) and mass ($p<0.001$), diastolic indices (E-wave {$p<0.001$}, trans-mitral ratio {$p=0.017$}) and sub-epicardial adipose tissue (SEAT) thickness ($p<0.001$). Stepwise multivariate analysis identified age (95% CI 0.975; 0.998), gender (95%CI 0.48; 0.9) and hypertension (95% CI 0.53; 0.99) as independent risk factors for diastolic abnormalities. Logistic regression identified age as the most significant contributor to diastolic abnormalities (OR=1.02; 95%CI 1.009; 1.03; Wald=13.4), followed by the waist circumference (OR=1.025; 95%CI 1.014; 1.037) and BMI (OR=1.075; 95% CI 1.035; 1.117). Genetic analysis showed significant associations between the heterozygous variant of Q192R genotype (PON-1 gene) and elevated HDL levels and also between this variant and obese women ($p= <0.05$).

Conclusion:

The high prevalence of CV risk factors and MS in this community has reached epidemic proportions. Although the MS was associated with significant remodelling of cardiac structure, alteration of diastolic indices and increased sub-epicardial adipose tissue thickness, BMI and waist circumference were stronger promoters of altered cardiac

physiology. This augurs poorly for this population group unless intervention is introduced to address the markedly high prevalence of these culprit drivers.

CHAPTER ONE

Introduction and the Literature review

1.1 Problem identification and motivation for this study

The prevalence of cardiovascular disease (CVD) in developing countries is reaching epidemic proportions (Yusuf *et al.*, 2001). This has been attributed to the change in disease profile, with a transition from poverty-driven, communicable diseases to chronic, non-communicable diseases (Beaglehole & Bonita, 2008), owing to the exposure to risk factors for CVD in these populations. Traditional risk factor clustering (hyperinsulinaemia and hyperglycaemia associated with insulin resistance, together with adipokines {adipocyte cytokines}) have been well documented to lead to vascular endothelial dysfunction, abnormal lipid profile, hypertension (Lindsay *et al.*, 2004), and vascular inflammation. These factors all contribute to the development of CVD (Koh *et al.*, 2005), including coronary artery disease (CAD) and stroke, and have been associated with high cardiovascular mortality and morbidity (de Las Fuentes, 2007).

Coronary artery disease is characterised by complex pathophysiological mechanisms, ending in atherosclerosis (Lusis *et al.*, 2004). The prevalence of this disease has reached alarming proportions in both developed and in developing countries. In addition, marked changes in disease profiles have been documented in migrant populations, especially in urbanised groups, as a result of exposure to multiple risk factors linked to lifestyle and behavioural adaptations (Yusuf *et al.*, 2001). Since CVD risk factors are heterogeneous and complex in nature, individual susceptibility to CVD is determined by the interplay of traditional risk factors, together with biochemical and environmental factors (Lusis,

2004), as well as heritable components (Damani, 2007), operating differentially in different populations.

Each of these risk factors increases the risk of CAD to varying degrees and, in combination, significantly increase risk. Risk factor clustering has long been recognised to contribute to CVD risk and has been developed into syndromic entities such as the metabolic syndrome (MS). To what extent the metabolic syndrome is independently associated with increased risk is not clear, nor is it well established whether risk factors confer added risk through structural changes in the cardiovascular (CV) system. The worldwide pandemic of obesity and other CV risk factors has rekindled interest in the identification of high-risk, asymptomatic subjects, with the view to implement intensive, medical interventions that would reduce CVD risk.

Although risk factors like hypertension, Type 2 Diabetes mellitus (DM) (Liu *et al.*, 2001) and obesity (Peterson *et al.*, 2004) have been shown to alter cardiac structure, few studies have examined the effect of individual or clustering of MS components on cardiac structure and function, particularly in defining pre-clinical cardiac disease. Echocardiography is a non-invasive modality that permits accurate assessment of the cardiac phenotype and function in the presence or absence of these risk factors. The measurements derived would allow extrapolation regarding the degree of pathophysiological transition, and hence timeous intervention. Whether these structural changes are mediated through the risk factors alone, or whether the changes occur

through genetic signalling via pathophysiological pathways contributing to CVD, has recently become very topical.

Insulin resistance and obesity have been reported as the main components of the metabolic syndrome (MS), where there appears to be a continuum between the two conditions. Insulin resistance, which is related to obesity and DM, is characterised by defective signalling at many levels (Ginsberg *et al.*, 2005). With ensuing insulin resistance, the expression of lipoprotein lipase (LPL), a lipolytic enzyme that is essential for the hydrolysis of chylomicrons, is reduced with subsequent reduction of LPL activity (Mead & Ramji, 2002).

LPL is a key regulator of lipoprotein metabolism, with LPL regulation being subject to insulin regulation. Therefore low LPL activity could result in significant metabolic consequences. This has been shown by studies which implicate LPL in conditions characterised by hypertriglyceridemia (like MS), in addition to tissue lipid accumulation which is associated with obesity-related insulin resistance (Lim *et al.*, 2009).

It is known that genetic factors may affect insulin sensitivity profoundly, with those genes involved with DM being polygenic, involving single nucleotide polymorphisms (SNPs) in multiple genes.

Since insulin resistance is reported as one of the central components of MS (Grundy, 2006), and LPL activity reported to be subject to both insulin regulation (Pollare, 1991), and to the influence of genetics (Weissglas-Volkov & Pajukanta, 2010), we believed that it

was important to study genes involved in lipoprotein metabolism to determine whether genetic variations could account for individual susceptibility to MS.

There is strong evidence that links oxidative stress with insulin resistance, impaired glucose tolerance and DM (Meigs *et al.*, 2003; Ceriello & Motz, 2004), where oxidative stress is reported to impair insulin action. In fact, Reaven (2007) reported a correlation between MS and endothelial dysfunction in individuals with susceptibility to oxidative stress. This hypothesis is based on the evidence that suggest that prolonged exposure to high glucose results in the generation of mitochondrial NADH by substrate-induced increase in the citric acid cycle activity (Maddux *et al.*, 2001). This process culminates in the formation of free radicals, superoxide in particular, which causes toxic effects to the endothelium, in addition to impaired endothelium-dependant vasodilation; all of which is mediated by oxidative stress.

A reduced intracellular antioxidant defence system in humans is associated with insulin resistance (Ceriello & Motz, 2004), with the deficiency in the reactive oxygen species (ROS)-scavenging ability conferring a genetic predisposition to insulin resistance and its advancing complications (Ceriello & Motz, 2004) in some individuals. Several genetic polymorphisms have been described that are associated with insulin resistance and oxidative stress. Serum paraoxonase-1 (PON-1), in particular, which is an antioxidant and a high-density lipoprotein (HDL)-associated enzyme encoded by the PON-1 gene, has significant anti-atherogenic properties (Shih *et al.*, 1998). PON-1 activity has been

reported to decrease in the presence of insulin resistance due to oxidative stress, with reduced serum PON-1 activity being associated with DM and CVD (Barbieri, *et al.*, 2002). It is also well established that PON-1 gene polymorphisms (Q192R, L55M) alter both the level and activity of the enzyme (Clendenning *et al.*, 1996; Blatter-Garin *et al.*, 1994; Mackness *et al.*, 1993; Aviram *et al.*, 2000).

Since MS increases risk for CVD and is associated with high levels of lipid peroxides (Reaven, 2007), and PON-1 is reported to contribute to the prevention of low-density lipoprotein (LDL) oxidation and lipid peroxidation (Mackness *et al.,* 1993), we felt it was important to explore the interaction between MS and PON-1 polymorphisms as mechanisms of atherogenesis, particularly in ethnic groups with a strong predisposition for developing premature CAD. There are limited descriptions in the literature linking the risk factor profile and the genetic variations to the development of the metabolic syndrome in the South African Asian Indian community (Ranjith *et al.*, 2002), which has a high prevalence of CV risk factors, particularly DM and hypertension. In this community, we searched for a possible link between common SNPs in the Lipoprotein lipase (LPL) and Paraoxonase 1 (PON-1) gene and CV risk factors and the resultant susceptibility to the development of the MS.

1.2 *Aims and objectives*

The main aim of this study was to determine the prevalence of cardiovascular risk factors in the Phoenix community and their relationship to cardiac structural changes and genetic patterns. The specific objectives were**:**

1.2.1 To determine the major risk factors for cardiovascular disease in the Phoenix community

1.2.2 To identify the prevalence of metabolic syndrome in this community

1.2.3 To define the echocardiographic parameters in normal individuals and establish what changes, if any, occur with identifiable cardiovascular risk factors

1.2.4 To identify echocardiographic variables that may be associated with metabolic syndrome

1.2.5 To identify genetic patterns associated with metabolic syndrome

1.3 *The Literature Review*

Cardiovascular disease (CVD) has reached epidemic proportions worldwide and accounts for the main cause of death in low and middle-income countries (WHO, 2009e; Poole-Wilson, 2005). This was highlighted by the INTERHEART study, which derived data from individuals in 52 countries, ranging in socio-economic status (Yusuf *et al.*, 2004). Recent studies have identified several major risk factors which contribute to the rising burden of CVD (Yusuf *et al.*, 2001) and to the development of myocardial infarction (MI). Yusuf *et al.* (2004) reported nine potentially modifiable risk factors comprising behavioural, biological and psychosocial factors for the development of MI worldwide. These were smoking, history of hypertension or DM, waist-hip ratio, dietary patterns, physical activity, and consumption of alcohol, blood apolipoproteins and psychosocial factors.

Coronary artery disease and MI are the most common form of CVD (Gazino, 2005) caused most commonly by atherosclerotic lesions (McPhee & Hammer, 2010). Atherosclerosis has been, until recently, viewed as a lipid-based disease in which the initiation and advancement of the disease is related to a low-grade systemic inflammatory state (Libby, 2002). When atherosclerosis progresses to flow-limiting disease, namely ischaemia, clinical and symptomatic CVD ensues (Virmani *et al.*, 2006). The earliest manifestation of atherosclerosis is characterised by the appearance of fatty streaks in medium-sized and large arteries, which begins in adolescence, and, in the absence of accelerating factors, develops slowly through to old age. Urbanisation, industrialisation, automation and affluence of a society that arise out of epidemiological and socio-economic transition

have been associated with acceleration in the development of the disease. This is further exacerbated by the effects of unhealthy lifestyle changes which result in a sedentary activity profile and nutritional changes with the consumption of a cholesterol-and-salt-rich diet, which is low in fresh fruit and vegetables.

This global transition has also resulted in the changing of diseases patterns, which is now dominated by chronic diseases like CVD and DM. In addition, progress in modern medicine, improvements in childhood nutrition and pharmaceuticals have resulted in a decline in death from infectious diseases. The subsequent increase in life-expectancy of the population, especially the elderly, exposes them to CV risk factors to an age where cardiovascular disease manifests clinically. This phenomenon is now contributing to the rising burden of healthcare, with the burden further increased by the disproportionately higher prevalence of traditional CV risk factors in migrant ethnic minorities (Wu *et al.*, 2011) like the South Asian Indians in the United States and the United Kingdom, and more worryingly, in the young.

1.3.1 The Epidemiology of Cardiovascular disease

The epidemiology of CV disease has been linked to the accumulation of risk factors related to biological factors, and activity and consumption patterns throughout the course of an individual's life (Fuster & Kelly, 2010), with recent studies pointing to the contributory effects of nutrition in early life, on the cardiovascular profile later in life

(Victora *et al.*, 2008). The recent increase in CVD prevalence has been attributed to the rise of the presence of abnormal blood lipid profiles, the rise in unhealthy lifestyle and behavioural traits (like smoking, inactivity), and the transition to "westernised" diets. The increasing prevalence of smoking and hypertension, which, consequently, have been reported as the most common CV risk factors in the world (including in middle- and high-income countries) (WHO, 2009b), further increase this burden. The manifestation of hyperinsulinaemia then results from the unhealthy combination of hypertension, obesity, and impaired glucose tolerance (Modan *et al.*, 1985; Ruderman *et al.*, 1998).

Cardiovascular risk has also been reported in foetal life and infancy, accumulating throughout childhood through to adolescence and adulthood (Ong & Loos, 2007). This was particularly evident in populations undergoing a transition from chronic under-nutrition to adequate nutrition (Hales & Barker, 2001), where foetal under-nutrition was suggested to cause insulin resistance. The WHO still positions childhood malnutrition as the most common risk factor in low-income countries (WHO, 2009e). The thrifty phenotype hypothesis was suggested by Neel in 1962 as the possible underlying mechanism for the development of CV risk and disease progression based on poor postnatal nutritional resources (Hales & Barker, 2001).

Neel (1962) proposed that genes which allowed the conservation of glucose and efficient storage of energy as fat during periods of food abundance, aided survival during times of food shortage. With the development of agriculture and affluence, the pressures of caloric deficit gradually waned, to the point where these "thrifty genes" were no longer

needed in some societies. This is most evident in those societies which are undergoing socio-economic changes from poverty, where the effect of these thrifty genes may manifest with the subsequent development of DM and the metabolic syndrome (Hales & Barker, 2001).

This hypothesis was further supported by a recent study that followed up babies from birth to adulthood in five low and middle-income countries (Victora *et al.*, 2008). The authors showed that under-nutrition and lower birth weight in childhood were risk factors for elevated blood pressure, high glucose concentrations and abnormal lipid profiles once adjusted for adult body mass index and height. They suggested that the rapid weight gained in the postnatal period was linked to the development of these CV risk factors.

Inasmuch as these mechanisms may explain CV risk in low income and developing countries, it appears to be limited to certain population groups or ethnicities, since it does not explain the excessively high incidence of risk factor clustering that manifest in, for example, Asian Indians who are born at normal weight (Dhawan, 1995).

1.3.2 Cardiovascular risk in South Asian Indians

The population of South Asian Indians number approximately 20% of the world's population (Gupta *et al.*, 2004). With immigration and subsequent adoption of lifestyle and dietary habits (particularly a high-fat diet leading to obesity) from western countries,

their CV risk profile has greatly increased. It is well reported that Asian Indians are ethnically more susceptible to developing CVD than other ethnic groups (Yusuf S *et al.*, 2001; UKPDS, 1998; Sandeep *et al.*, 2011), including other groups of Asians. This excess of CV risk has been partly explained by Mukhopadhya *et al.* (2005) who reported that Asian Indians had an increased predisposition for development of insulin resistance, and has been found to occur more commonly in young Indians than in young Chinese (Pan *et al.*, 2004). Insulin resistance in turn, has been suggested to confer a greater prothrombotic risk (Kain *et al.*, 2003) and has been reported to be significantly associated with the presence of CV risk factors, even in subjects with normal glucose tolerance (Sandeep *et al.*, 2011). Insulin resistance has also been reported more frequently in new-borns in subjects of Asian Indian descent, which persists through to childhood (Krishnaveni *et al.*, 2005). Other studies (Anand *et al.*, 2000; Hoogeveen *et al.*, 2001) have shown that homocysteine, lipoprotein (a) and plasminogen activator inhibitor-1 levels are higher in the Asian Indian than in the Caucasian population, and may account for the prothrombotic state documented in Asian Indians.

Asian Indians also have a more adverse metabolic and cardiovascular profile when compared to other population groups. This was reported in a study of South Asian, Afro-Caribbean and European subjects aged 40 – 69 years (McKeige *et al.*, 1991) where, with the exception of total cholesterol, South Asian Indian men showed lower HDL levels, higher fasting insulin and triglycerides levels, higher waist-to-hip ratios and a higher

prevalence of DM, as compared to the Afro-Caribbean and European subjects. In addition, the SHARE study, involving subjects from South Asian, European and Chinese subjects showed that Asian Indians carried 4.5 higher odds of developing CAD as compared to European subjects (Anand *et al.*, 2000), as well as a higher prevalence of CVD for similar degrees of atherosclerosis in the three populations.

Furthermore, Asian Indians are known to have a higher percentage of subcutaneous and abdominal fat in spite of lower muscle mass and body mass index (BMI) (Misra, 2003). This phenotype, known as the "thin-fat" phenotype has been reported to be an important contributor to insulin resistance (Misra *et al.*, 2004). Insulin resistance related to obesity, therefore, occurs frequently at a lower BMI (Misra & Khurana, 2008) in Asian Indians, even at traditional 'normal' levels of less than 25kg/m^2 (Snehalatha *et al.*, 2003). Thus, the BMI may underestimate the true CVD risk in Asians, especially when it is used alone as an indicator of obesity or for CV risk scoring. This was further evidenced in two studies, Conus *et al.* (2004) and St-Onge *et al.* (2004), where vascular endothelial dysfunction, abnormal lipid profiles and hypertension, which are traditionally associated with metabolically obese subjects, was reported in individuals who did not have an excess of body fat. This rationale has been instrumental for the World Health Organisation (WHO) revising the cut-offs for obesity from 30 kg/m^2 to BMI to 25 kg/m^2 in 2004 (WHO Expert Consultation) for Asian Indians. In addition, since BMI is a measure of general adiposity (Lemieux, 2007), some large-scale studies have rather recommended the waist-to-hip ratio together with waist circumference as better measures of abdominal obesity

(Grundy, 2005; Yusuf *et al.*, 2004). Relative adiposity present in native South Indian children at birth has been found to be due to in-uteri programming of insulin resistance, promoted through genetic mechanisms (Yajnik *et al.,* 2002).

The genetic contribution for CAD was also promulgated by Yusuf *et al.* (2001), in an attempt to elucidate the cause for variations in risk factor prevalence, as well as possibly explain the increased risk in some individuals in a population for CAD, and not others. Inasmuch as South Asian Indians have been identified as having an increased propensity for CVD, the disease prevalence is on a rampant increase in other ethnic groups in developing countries as well. The development of premature CAD (Enas *et al.*, 2007) and higher mortality rates from CVD in Asian Indians (Harding, 2003) further exacerbates the situation as the extent and severity of CAD in Asian Indians has been reported as being more aggressive and malignant when compared to other population groups (Murray *et al.*, 1994), in spite of traditional CVD risk factors being similar or lower than other populations groups (Patel *et al.*, 2006). These risk factors are projected to rise to epidemic proportions, in the near future (Gupta *et al.*, 2007), further stimulating the increased incidence of premature CAD. However, a major cause for concern is that the prevalence of CV risk factors is even higher in Asian Indians in the diaspora, reportedly a 3-to-5 fold increased risk of a CV cause of death (McKeigue et al., 1991) than other ethnic groups in their adopted countries.

1.3.3 Cardiovascular risk in South Africans

Africans are presently living in a period of immense change, consequent on the transformation in the socioeconomic status and patterns of living, and a marked increase in urbanisation (Mayosi *et al.*, 2009). This socio-economic transition has now exposed many individuals to the changes in diet and lifestyle which accompany urbanisation. This transition is also believed to be responsible for the increase in DM and other non-communicable diseases (Motala *et al.*, 2011) which are leading to the rise in the prevalence of CV disease, alarmingly in the younger population. This poses a major health concern with both epidemiological and economic implications (Morcos *et al.*, 2009), especially in the vulnerable low-middle-income countries of the world, such as South Africa (Tibazarwa *et al.*, 2009), where the rate of transition has escalated following the abolishment of apartheid policies in 1994.

There is evidence to indicate that, as leading causes of death in sub-Saharan Africa, CV disease is a close second to HIV, tuberculosis and malaria (Opie & Mayosi, 2005). In South Africa, combined with poverty-related diseases, the HIV/AIDs pandemic and high injury rates, the emerging increase in chronic diseases of lifestyle (CDL), now quadruples the country's burden of disease (Leeder *et al.*, 2004; Steyn *et al.*, 2004). The South African National Burden of Disease reported in 2000 that CDL were the leading cause of death, followed by HIV/AIDS, infectious diseases and injuries (Bradshaw *et al.*, 2007). The escalation of CDL to epidemic proportions have also been attributed, in part, to the focus of more human resources and infrastructure on the treatment of communicable diseases,

and low prioritisation of prevention strategies (Stewart *et al.*, 2011) for CVD. However, it appears that the overburdened and underfunded Healthcare system still struggles to cope even with HIV/AIDs and poverty-related diseases, and is cause for concern as mortality rates amongst South Africans for DM and CVD are also on the rise (Mayosi *et al.*, 2009). Since it is well established that CVD is largely preventable (Pearson *et al.*, 2003) if population-wide interventions are initiated, it is logical that attempts to investigate the prevalence of CVD in these high-risk populations should be renewed, with a view to implement strategies that will stem the tide of the CVD epidemic.

Several studies have been done in South Africa, looking at the CV risk factor profile of Blacks and Caucasians, where a general trend of increasing prevalence of obesity has been documented (Mbanya *et al.*, 2010; Mayosi *et al.*, 2009; Puoane *et al.*, 2002). A study by Kalke & Joffe (2007) showed that the prevalence of CAD in Africans was 4%, attributed to the contribution of low total cholesterol and the effects of insulin resistance, as compared to 23% prevalence in the White population. They projected that this prevalence was on the increasing trend, as the effects of urbanisation and a sedentary lifestyle translated into high-cholesterol diets and insulin resistance. This was also evident in an urban community in Mamre, near Cape Town, where subjects were reported to be at a high probability of suffering a CVD event in the next 10 years (Steyn *et al.*, 2004). Although the Asian Indian population is known to be high-risk for CVD, there is very limited data available on community-based evaluations of traditional CVD risk factors.

1.3.3.1 CARDIOVASCULAR RISK IN SOUTH AFRICAN ASIAN INDIANS

There are approximately 1.2 million Asian Indians currently living in South Africa (Stats SA P0302, 2011), who make up approximately 2.5 % of the total population. It has been established that the leading causes of morbidity and mortality in the Asian Indian community is related to CAD and other CV complications (Seedat *et al.*, 1990). This community-based survey in the Durban metropolitan area documented the prevalence of CV risk factors in 778 subjects between 15 and 69 years of age, and found that the prominent risk factors were hypercholesterolemia, hypertriglyceridemia, DM, and smoking in men. A later study by Motala *et al.* (1993) reported that there was a high risk for the progression of impaired glucose tolerance to overt DM in Indian subjects in South Africa. The high prevalence of risk factors and the severe nature of coronary heart disease (CHD) in the South African Indian population lead these researchers to recommend an immediate and intensive primary prevention programme of CHD risk factors. To date, as far as we are aware, no such programme has been initiated.

The initial epidemiological study on hypertension in the South African Indian community was performed in 1978 by Seedat *et al.*, who reported a prevalence of 19% (higher in females than males). A later study in 1988 by Omar *et al.* documented the prevalence of hypertension as 14.2% and DM as 9%. In yet a later study, these researchers documented an increase in the prevalence of DM in the South African Indian community (11%), with glucose intolerance found in 5.8% of the sample in Chatsworth, Durban (Omar *et al.*, 1993). These two risk factors (hypertension and DM), coupled with urban

lifestyle patterns and sedentary habits, have consequently been suggested as the reasons for the excess in coronary heart disease in the South African Indian community (Seedat, 1994), although Sewdarsen *et al.* (1987) found that dyslipidaemia and obesity were also major contributory factors. This large study by Sewdarsen *et al.* (1987) compared fasting serum lipid and lipoprotein levels in 620 consecutive male survivors of myocardial infarction with those of 524 healthy male volunteer controls. Hypercholesterolemia was reported as the most common abnormality (25%), with obesity being significantly more common in patients with hypertriglyceridemia. Type 2 Diabetes Mellitus and hypertension were observed more commonly in patients with combined hypercholesterolemia and hypertriglyceridemia, illustrating a pattern of clustered risk factors. Later studies of subjects in this population group identified smoking and a positive family history for CAD (Ranjith *et al.*, 2002) as common risk factors for CAD. More recent evidence from Ranjith *et al.* (2008) suggests that genetic polymorphisms may have an impact on the phenotypic expression of the metabolic syndrome in young Asian Indian males presenting with acute myocardial infarction. Since the interplay between genetic abnormalities and the response to environmental factors could not be clearly established, the study authors strongly recommended further studies of other genes involved in lipid metabolism and insulin resistance. Since then, further evaluations have not been performed on community subjects in the Asian Indian population in Durban.

The high prevalence of hypertension and DM, coupled with their comorbid complications in all the communities in South Africa places a high burden on the resources of the public

health sector. In fact, risk factor clustering has been reported to increase an individual's risk for the development of CVD, and is becoming a frequent clinical finding as the young and ageing population becomes exposed to the effects of the socio-economic transition.

1.3.4 The Metabolic Syndrome

The metabolic syndrome (MS) has been described as a heterogeneous (Zimmet *et al.*, 2001) clustering of several risk factors (hypertension, dyslipidaemia, glucose intolerance, abdominal obesity). The syndrome is believed, through mechanisms that remain unclear, to greatly increase the risk of CVD in an individual with an increased number of risk factors.

Unlike DM and obesity, which have clear diagnostic criteria, there have been many eponyms of MS before the evolution of the current definitions. The first observations were reported in 1923 by Kylin, where he described the combination of hyperglycaemia and gout as a syndrome. This was further extended by Himsworth (1936), when he proposed the delineation of DM into the insulin-resistant and the insulin-sensitive variety, where insulin resistance was associated with obesity. This idea was systematically re-introduced by Reaven in 1988 in his Banting Lecture, when he coined the term "Syndrome X" and proposed a pathophysiological link between the CV risk factors. He suggested that insulin resistance was the common etiological factor for a cluster of metabolic risk factors, hence the term 'syndrome' (Reaven, 1988). At that time, there appeared to be some contention as to what the main determinant of Syndrome X

was: the researchers in the DM field (sharing a similar view to Reaven) maintained that insulin resistance was the dominant determinant, while others, between 1998 and 2000, replaced Syndrome X with "the Metabolic Syndrome", which described the clustering of metabolic risk factors (Alberti *et al.*, 1998; Groop, 2001). In keeping with the evolution of this syndrome, some authors (Groop *et al.*, 2001) coined the concept of the 'dysmetabolic syndrome', in order to better describe the abnormalities that are suggested by the definitions.

In 1998, the WHO (Alberti *et al.*) proposed unifying criteria for the diagnosis of MS, as there were marked differences in the prevalence of MS in different studies, which some attributed to unavailability of clearly defined criteria (Isomaa *et al.*, 2001). The WHO criteria comprised of clinically evident insulin resistance, that is, impaired glucose tolerance, impaired fasting glucose or DM (Huang, 2009). Furthermore, two additional risk factors were necessary to fit the criteria, those being elevated blood pressure, elevated triglycerides, low HDL, obesity and microalbuminuria (Alberti, 1998). These criteria were simplified by the National Cholesterol Adult Panel Adult Treatment Panel III (NCEP ATP III) in 2001, by restricting the diagnosis of MS to three out of the five risk factors, for simpler recognition of the components, and for easier clinical practice. The NCEP ATP III criteria were further recently supported by those of the American Heart Association and National Heart, Lung, and Blood Institute (with minor modifications) (Grundy *et al.*, 2005). The International Diabetes Federation (IDF) replaced the WHO

criteria for the diagnosis of MS, with the mandatory inclusion of ethnic-specific waist circumference cut-offs (Alberti *et al.*, 2005).

Most recently, in a joint statement from the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity (Alberti et al., 2009) proposed unifying criteria for the definition for MS. This definition is identical to the NECP ATP III, but included the IDF waist circumference ethnic and gender cut-offs, and was called the 'Harmonizing criteria'.

The specific criteria for each of the 3 definitions are detailed in the *Methods* section in Chapter 2.

1.3.4.1 THE PATHOPHYSIOLOGY OF THE METABOLIC SYNDROME

The underlying pathophysiology unifying the individual components of MS remains unclear, but has been related to many factors: obesity and insulin resistance, in addition to chronic stress, increased cellular oxidative stress, micro RNA molecules, intrinsic glucocorticoid actions, but to name a few (Kassi *et al.*, 2011). The current literature reports that obesity and insulin resistance are the central components responsible for the pathogenesis of MS, as these have been reported to contribute to the development of metabolic risk factors (Phillips, 2008). Atherogenic dyslipidaemia, elevated blood pressure and elevated plasma glucose are the well-recognised metabolic risk factors, which are

associated with a prothrombotic and proinflammatory state (Alberti *et al.*, 2009). Atherogenic dyslipidaemia comprises lipoprotein abnormalities, which include elevated serum triglycerides and apolipoprotein B, reduced HDL levels and increased small LDL particles (Grundy, 2007). The metabolic syndrome appears to initiate atherogenesis by the promotion of apoB-containing lipoproteins elevation, which in turn, advances the development of atherosclerotic lesions. The development of the atherosclerotic plaque is accelerated by low levels of HDL, elevated glucose levels and inflammatory cytokines (Grundy, 2008).

An inflammatory milieu has been proposed to explain the link between obesity, insulin resistance and DM, constituting the MS, while it has been suggested (Das, 2002) that the underlying pathophysiology of MS may also be related to low-grade systemic inflammation. These observations were established on the presence of raised plasma levels of C-reactive protein (CRP), tumour necrosis factor (TNF-α) and IL (inter-leukin)-6 in hypertensive, obese, insulin resistant and diabetic individuals. Consequently, these levels were also observed in non-diabetic subjects (IRAS study), and correlated significantly with an increasing number CV risk factors (Festa *et al.*, 2000), as well as in patients with CAD, before and after the onset of these diseases (Das 2001; Das 2002). Another more recent study (Samaras *et al.*, 2010) showed that visceral adipose tissue in diabetics express higher levels of adipokines involved in inflammation, which was related to fasting glucose and insulin action, further strengthening the link between visceral obesity, inflammation and DM.

The sudden and dramatic rise in the prevalence of MS has been reported in many population and ethnic groups without marked change in the human genome (Phillips, 2008), and is suggestive of the important role that is modulated by environmental, lifestyle and nutritional, as well as genetic factors in susceptible individuals. Furthermore, a high heritability for each of the components of the syndrome (Groop *et al.*, 2001), has been reported in twin and familial aggregation studies. Together with the large variation in terms of age and susceptibility in subjects with similar CV risk factor profiles, a case for the possible interplay between genetics and environmental factors (Onat *et al.*, 2009) becomes increasingly more plausible.

1.3.4.1.1 Insulin resistance

Glucose is the primary signal for secretion of insulin by the pancreatic islets beta cells. The main effects of insulin are to decrease plasma glucose and free fatty acid concentrations, as well as to stimulate protein synthesis (McPhee & Hammer, 2010). Circulating insulin allows for the diffusion and utilisation of glucose into the adipocytes and muscle cells. In individuals who are metabolically normal i.e. insulin sensitive, changes in insulin sensitivity are accompanied by compensatory alterations in the response of the beta (ß)-cell to glucose. In subjects who are insulin resistant, a compensatory increase in insulin secretion from the pancreas occurs in order to overcome impaired insulin action in the peripheral tissues, resulting in a compensatory increase in ß –cell mass to maintain normoglycaemia (McPhee & Hammer, 2010). Insulin resistance itself is believed to be mediated by glucose signalling as well as genetic factors

(Goodarzi *et al.*, 2007).

- *The signalling cascade*

At the cellular level, most of the metabolic effects of insulin are mediated by the signalling cascade involving the insulin receptor substrate (IRS) proteins, phosphorylation, and activation of the enzyme phosphatidylinositol (PI) 3-kinase, through which insulin produces most of its metabolic actions (Cheatham *et al.*, 1994). The response in subjects with normal sensitivity to insulin, where insulin itself is sufficient to suppress lipolysis, results in a reduction of very low-density lipoprotein (VLDL), triglyceride and apolipoprotein-B production and secretion, thereby preventing the pathophysiological cascade that leads to insulin resistance (Ginsberg *et al.*, 2005). The response in subjects who are obese with DM is very different, and is characterised by defects at many levels (Cusi *et al.*, 2000). An inadequate strength of insulin signalling from the insulin receptor downstream to the final substrates of insulin action via IRS-1 /PI 3-kinase pathway (Ginsberg *et al.*, 2005) results in diminished glucose uptake and utilisation in insulin target tissues (Jiang *et al.*, 1999; Cusi *et al.*, 2000).

- *Adipocytokines*

Certain adipocytokines are also implicated as a cause for impaired signalling by means of the insulin receptor, thereby increasing lipolysis, the excess of which is secreted into the plasma (Mazurek *et al.*, 2003). This release of non-esterified fatty acids, particularly those from visceral adipocytes (as these drain directly into the portal system), bathes the liver

in fatty acids, resulting in the liver becoming insulin resistant (Bergman *et al.*, 2007). The increase in free-fatty acids (FFA) has also been directly implicated as a cause for dyslipidaemia associated with insulin resistance, as triglyceride synthesis and storage is increased, with the excess being secreted as VLDL (Sniderman *et al.*, 2007). This then results in alterations to lipoprotein lipase.

- *Oxidation of free-fatty acids and lipotoxicity*

Another mechanism for insulin resistance in obese individuals arises from the increase in the oxidation of fatty acids. When the storage capacity of adipose tissue is saturated, unoxidized long-chain fatty acids are driven into non-adipose tissues, such as the liver, muscle, heart, and pancreatic-islets. The consequent formation of reactive lipid moieties then produces adverse effects by promoting metabolically relevant cellular dysfunction (lipotoxicity) and lipoapoptosis (Kusminski *et al*., 2009). The excess oxidised FFAs are then esterified into triglyceride, which, in excess amounts results in an increase in the mitochondrial acetyl coenzyme A (COA) and NADH: NAD ratios, inactivating pyruvate dehydrogenase. The subsequent rise in the levels of intracellular citrate levels, leads to the inhibition of phosphofructokinase, and G6P accumulation. This then results in diminished glucose uptake and the inhibition of glycolysis, since G6P inhibits hexokinase activity (Haring, 1991; Cheatham *et al.*, 1994).

Insulin resistance in peripheral tissues, like the muscle, arises from disruption of the glucose-fatty acid cycle. In the skeletal muscle, however, it occurs because of the

inhibitory effect of increased plasma FFAs on insulin-mediated glucose transports (Cornier *et al.*, 2008). The continuum between insulin resistance and obesity is further highlighted by its association with general obesity, central obesity, hypertension, and abnormal lipid levels, which are each components of MS. In fact, Grundy *et al.* (2005) reported that obesity, coupled with physical inactivity and a diet high in fat, was responsible for insulin resistance. This was further supported by Roche (2005), who suggested a progressive phenotype that inter-linked insulin resistance with obesity, DM and MS.

1.3.4.1.2 Adiposity

Eckel and co-researchers (2005) suggested that the dramatic rise in the prevalence of obesity was one of the main reasons for the promulgation of the metabolic syndrome definitions. Many experts believe that obesity is a dominant driver in insulin resistance (Cornier *et al.*, 2007), as it has been established that body fat is a strong predictor of insulin resistance and CV risk (Misra *et al.* 2004,). The literature also documents a stronger correlation observed between central or abdominal obesity or "android or male-type obesity," as reported by Vague (1947) and increased CV risk, insulin resistance, DM, hypertension and mortality as compared to the "gynoid or female-type of fat distribution" in the lower body or gluteo-femoral or peripheral depot (Wajchenberg, 2000).

Abdominal fat is composed of two different anatomic depots, namely, subcutaneous and intra-abdominal fat. Subcutaneous fat can be separated into superficial and deep layers, while intra-abdominal fat can be divided into intra-peritoneal and retro-peritoneal sites. Intra-peritoneal fat, also termed visceral fat, is composed of mesenteric and omental fat masses (Märin *et al.*, 1992).

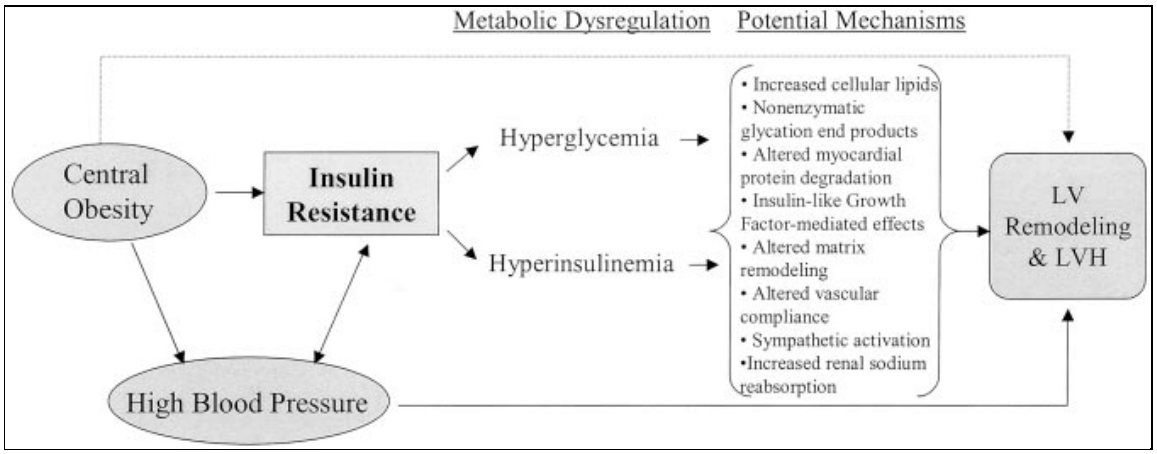
The expanded mass of adipose tissue, as seen in obesity, results in an increased production of FFAs (Ginsberg *et al.*, 2005). Insulin resistance accelerates FFA lipolysis from adipose tissue triglyceride stores, and vice versa, with the visceral adipose store being known to contribute to insulin insensitivity and increased FFA lipolysis (Bergman *et al.*, 2007). Visceral adipose tissue, which was initially thought of as an inert storage organ, is currently viewed as a depot for pathogenic fat (Mahabadi *et al.,* 2009), with some researchers (Juhan-Vague *et al.,* 2003; Kershaw *et al.*, 2004; Wellen & Hotamisligil, 2003) regarding it as an endocrine organ because of the bioactive molecules that are produced. Recent literature provides a strong role for the systemic effects of adipose tissue on atherosclerosis, as it correlates significantly with inflammatory markers (Pou *et al.*, 2007), CV factors (Rosito *et al.*, 2008) and the metabolic syndrome (Fox *et al.*, 2007). This may possibly be explained when surplus adipose tissue produces a disproportionately high amount of non-esterified acids and proinflammatory adipocytokines which hamper systemic responsiveness to insulin (Schinner, 2005; Ghanim, 2004). Furthermore, insulin action may be modified locally within the adipose tissue, through the paracrine effect of the adipocytokines. Once these adipocytokines reach the portal circulation, they are

drained into the liver where they are responsible for modulation of the synthesis of hepatic proteins and trigger the development of an inflammatory milieu (Tremblay *et al.*, 2011). This process is also believed to result in impaired glucose action, compensatory hyperinsulinaemia and glucose intolerance.

1.3.4.1.3 Cardiac structural alterations related to insulin resistance

In the normal cardiac myocyte, glucose serves many crucial functions like serving as fuel supply, gene expression and sarcomeric changes (Young *et al.*, 2002). Loss of regulation of glucose therefore results in disruption of these regulatory functions in the heart. With ensuing insulin resistance (Figure 1.1), cardiac remodelling occurs through the contributory effects of lipotoxicity, sympathetic up-regulation, inflammation, oxidative stress, and fibrosis (Taegtmeyer *et al.*, 2002). Other documented changes related to insulin resistance are an increased size of the left atrium (LA), as well as an increase in the left ventricular (LV) wall thickness and mass (LVM) (Rutter *et al.*, 2003).

Figure 1.1: Possible mechanisms which underlie cardiac remodelling in insulin resistance



Rutter et al., 2003

Experimental, pathological, epidemiological and clinical studies have shown that overt DM causes changes within the cardiac structure and function in the absence of coronary atherosclerosis, hypertension or any other known cardiac disease. The elevation of plasma nonesterified fatty acid levels observed in subjects with DM results in the activation of PPARα (van de Weijer *et al.*, 2011). With ensuing pressure overload and/or prolonged exposure to hyperglycaemia and/or hyperlipidaemia, a decrease in PPARα expression results, limiting the fatty acid oxidation capacity of the heart. The excess of accumulation of intramyocardial lipids then results in lipotoxicity, which plays a critical role in the development of contractile dysfunction and the diabetic cardiomyopathy (Cosson & Kevorkian, 2003). Left ventricular diastolic dysfunction is perceived as the earliest preclinical manifestation of the diabetic cardiomyopathy, and these changes may

be detected non-invasively, using echocardiography. Echocardiography may also be used to evaluate the accumulation of visceral fat around the heart, which recently, has been found to be associated with the presence of MS and CAD.

- *Sub-epicardial adipose tissue*

The role of sub-epicardial adipose tissue, which is also called the visceral or pericardial fat around the heart, its relationship to normal cardiac structure and its role in cardiovascular risk has until only recently, been very limited (Iacobellis, 2003). Epicardial fat is more closely related to abdominal visceral fat (which makes up the largest deposition of visceral fat [Rosito *et al.,* 2008]), than total fat, which, during embryogenesis, originates from brown fat tissue, and later differentiates into white adipose tissue (Marchington *et al.*, 1989). Iacobellis *et al.* (2005) described the local interactions between the adipose and muscular components of the heart as they shared the same blood supply, with no fascia separating the myocardial and epicardial layers. Other functions of sub-epicardial adipose tissue (SEAT) were to act as a buffer against toxic levels of free-fatty acids between the myocardium and the vascular bed (Marchington et al., 1989), and to provide a steady supply of free fatty acids to meet energy demands of the myocardium. However, SEAT may also exert a harmful effect on the myocardium, since pericardial fat has also been associated with metabolic risk factors and CVD (Rosito *et al.*, 2008), and is believed to alter, via the expression of several biomarkers (Baker, 2006), cardiac function and promote atherogenesis.

There are two mechanisms that have been hypothesised to potentially explain the interrelationship between SEAT and CAD. Chaowalit *et al.* (2006) summarised the first as one which was associated with conventional CV risk factors and MS. The second, related to the possible paracrine function of SEAT, emanates from the local interaction of adipose tissue locally with coronary arteries (Baker *et al.*, 2006).

The paracrine release of cytokines from the periadventitial epicardial fat diffuses into the coronary walls, interacting with cells in each of its layers. This, in turn, leads to the amplification of vascular inflammation and plaque instability (Mazurek *et al.*, 2003).

Furthermore, periadventitial applications of inflammatory cytokines and adipokines have been known to induce inflammatory cell influx into the arterial wall, suggesting that these molecules from pericoronary tissues may be responsible for the alteration of arteriolar homeostasis (Miyata *et al.*, 2000). Current research also proposes a link between visceral adiposity, inflammation and the risk of DM, based on the increased release of inflammatory markers from visceral fat in diabetics (Samaras *et al.*, 2010; Van de Weijer *et al.*, 2011). SEAT thickness, in particular, has also recently been associated with the clinical parameters of the MS (Chaowalit et al., 2006): with the epidemic rise of MS, evaluation of SEAT may well be a useful composite marker for the detection of the syndrome.

1.3.4.2 THE PREVALENCE OF THE METABOLIC SYNDROME

The prevalence of the MS is increasing worldwide (Grundy, 2008), with prevalence estimates dependent upon the definition which is used. The age-adjusted prevalence of

MS in the United States has been reported as 23.7% (Ford *et al.*, 2002), with approximately 13% of adolescents having the syndrome (Ford *et al.*, 2003). This prevalence has been reported in similar proportions in Europe, with 15% -23% of Europeans being diagnosed with the MS (Hu *et al.*, 2004).

The sex, age, race, ethnicity and the level of socio-economic transition of the population being studied are also determinants of MS prevalence (Ford et al., 2003), with a concurrent increase in prevalence being documented with obesity (Cornier et al., 2008). South Asians have a 20 - 25% prevalence of MS, with a substantially higher prevalence observed in Asian Indians (Eapen *et al.*, 2009). The increasing prevalence of insulin resistance and DM have been purported as one of the main drivers of MS in Asian Indians, with the Chennai Urban Population study (Mohan *et al.*, 2003) reporting the frequency of MS varying from 41.1 to 49.2% (Wasir *et al.*, 2008) in urban Asian Indian adults. The prevalence of the MS in migrant Asian Indians has been reported to vary from 31.6% (Misra *et al.*, 2005) to 38.2% (Misra *et al.*, 2010). These current estimates of prevalence are a cause for concern, with the projected escalation even more worrying, as the conglomeration of CV risk factors (like obesity, insulin resistance and hypertension) become increasingly common. There is general agreement that adverse outcomes are more likely when risk factors occur simultaneously (Kahn *et al.*, 2005), although the usefulness of the syndromic nature of the MS in predicting CV risk has become a topic of much debate in recent years.

1.3.4.3 CURRENT CONTROVERSIES REVOLVING AROUND THE METABOLIC SYNDROME

The American Diabetes Association and the European Association for the Study of Diabetes published a joint statement in 2005 questioning whether the components of MS, as was currently defined, implied any unique pathophysiology by calling it a 'syndrome' (Kahn *et al.*, 2005). They also questioned whether additional risk was conferred beyond its individual components, and if there was any medical value in diagnosing these risk factor components as a 'syndrome'. The main points that were raised in the joint statement (Kahn *et al.*, 2005) were:

a. Lack of a common definition which has resulted in discrepant results on its association with CVD morbidity and mortality,
b. The criteria for inclusion is ambiguous and the rationale for the cut-offs are not clearly defined
c. The unifying role of insulin resistance as the main aetiological basis for the syndrome is unsure, and the value of including DM in the definition is questioned
d. CVD risk is based on the presence of specific risk factors only, with no clear rationale for including or excluding these factors
e. The CVD risk associated with MS appears to be the sum of its components and treatment options are geared towards treating individual risk factors, and not the 'syndrome' per se

In 2010, the WHO Expert Consultation report (Simmons *et al.*) echoed similar opinions, questioning the practical utility of MS as a clinical tool for diagnosis or disease

management. A corroboration of two prominent studies (PROSPER and BHRS) showed that MS was not associated with vascular risk in the elderly (Sattar *et al.*, 2008), nor was CV risk greater in MS as compared to the sum of its individual components (Stern *et al.*, 2004). In fact, a recent article documenting the 'rise and fall of the Metabolic Syndrome' (Borch-Johnsen & Wareham, 2009) claims that in spite of all the research done on MS, clear reasons underlying the susceptibility to developing CVD or DM still remain vague.

In spite of the lack of a unified definition of MS, a large body of literature supports an association between MS and CVD. Irrespective of the diagnostic criteria used, there have been many recent epidemiological studies that have supported the increased risk for CVD in subjects with MS (Bayturan et al., 2010; Cabre *et al.*, 2008; Hu *et al.*, 2004; McNeil *et al.*, 2005; Ford, 2004; Alexander *et al.*, 2003). A meta-analysis by Ford *et al.* (2005) which examined the relative-risk for all-cause mortality, CVD and DM from the general population using the WHO and NCEP ATP III MS definitions found that the population-attributable fraction for MS was 12-17% for CVD and 20-52% for DM. Similarly, in Asian Indians patients, Lakka *et al.* (2002) showed that the presence of MS predicted CVD in middle-aged men, and was associated with a two-fold risk for developing cardiovascular mortality. More recently, a large, internal, multi-ethnic study from INTERHEART reported a >2.5-fold increase in the risk for acute MI in subjects with MS (Mente *et al.,* 2010). An increased all-cause mortality (RR=1.35; 95% CI) for CVD, CAD and stroke was also reported in a meta-analysis (Galassi *et al.,* 2006), where the authors highlighted the importance of detecting, preventing and treating the underlying risk factors which make

up MS in the general population. A similar recommendation was made by the authors of another meta-analysis of 37 longitudinal studies comprising 43 cohorts (Gami *et al.,* 2007). They showed that individuals with MS were at an increased risk of cardiovascular events, particularly when compared with those studies which followed up subjects without CAD. Such was the strength of their findings that they recommended primary intervention programmes to address MS as a single entity, in addition to treating individual CV risk factors.

The metabolic syndrome is also an important risk factor for subsequent development of DM and/or CVD. This was borne out from a study by the Third National Health and Nutrition Examination Survey (NHANES III, 2003) data, which graded subjects for CVD risk based on the presence of MS and DM. Subjects without MS were found to have the lowest risk for CVD events, with those having MS being at intermediate risk, while diabetic subjects had the highest level of risk for CVD events. Similarly, Meigs *et al.* (2003), when using the Framingham cohort, found that irrespective of the criteria used, those individuals with MS were at a higher risk of CVD. These authors, like Galassi *et al.* (2006) further advocated the importance of early detection, prevention and therapy for MS.

More recently, these associations have been supported by the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International

Atherosclerosis Society; and International Association for the Study of Obesity (2009). They found that subjects with the MS were twice as likely to develop CVD over 5-20 years, and had a 5-fold increased risk for developing DM, as compared to those without the MS. A joint statement from the International Diabetes Federation proposed a new set of unifying criteria for MS in 2009. These were cognisant of the limitations associated with the current definitions mentioned above, with the new definition being called the "Harmonizing criteria". In spite of the disagreements amongst the different expert panels regarding the definitions and the criteria, there emerged a consensus from this group that the term "metabolic syndrome" was acceptable for the condition describing clustering of metabolic risk factors for DM and CVD (Alberti *et al.*, 2009). This group also proposed that central obesity, as measured by waist circumference measurement, was no longer obligatory (as per IDF definition), but formed one of the risk factors for the definition. In an attempt to unify the criteria, they proposed the 'Harmonized criteria' in which 3 out of the 5 risk factors was needed for the diagnosis of MS (see *Methods* for elaboration).

Therefore, a diagnosis of the metabolic syndrome appears to be valuable when attempting to delay the development of DM and atherosclerosis. This would involve timeously identifying a patient who needs aggressive lifestyle modification focused on weight reduction and increased physical activity (Rozenzweig *et al.*, 2008; Eckel *et al.*, 2005), and hence a reduction of multiple risk factors (Alberti *et al.*, 2009). For this strategy to be most effective, it would be particularly important to ascertain the frequency of risk factor clustering and the most influential components, particularly in

ethnic groups with a traditionally high CV risk factor prevalence. One such group in whom this would be especially relevant is the Asian Indian group in whom a high CV risk factor profile, with a consequently high predisposition for premature CAD, has been well established.

1.3.4.4 THE METABOLIC SYNDROME IN SOUTH ASIAN INDIANS

The metabolic syndrome has been extensively researched in the South Asian Indian population in India, and in some parts of the diaspora. Cardiovascular risk factor clustering has been reported to be on the increase in Asian Indians (Enas *et al.*, 2007), with Misra & Khurana (2009) recently reporting an MS prevalence of 38.2% in urban Asian Indians. The SHARE study (Anand *et al.*, 2000) reported a 41.1% prevalence of MS in urban Indians when applying the ATP III criteria with the modified waist circumference cut-off, with MS being present in 27.9% of participants with normal blood glucose profiles. Using the ATP III criteria only, Gupta *et al.* (2004) reported a 31.6% prevalence of MS in urban Indians, to name a few studies. The exact reason for this excess in Asian Indians still remains unclear, hence the existence of many possible mechanisms currently being postulated.

As discussed above, there is a high incidence of CV risk factors and CAD found in Asian Indians, particularly, insulin resistance, essential hypertension and DM (McKeigue *et al.*, 1994) as compared to other Western population groups. The exact mechanism for this excess risk is not known, but one, which has been reaffirmed by Misra *et al.* (2004), is

that Asian Indians tend to gain fat in the abdominal area, with consequent development of central obesity rather than generalised obesity. At the biochemical level, Das (2003) suggested that the increased expression of 11ß-HSD-1, which is triggered by high levels of TNF-α and IL-6 (which are elevated even in healthy Asian Indians) may be responsible for the development of abdominal obesity.

Additionally, the initial trigger for the development of CV risk factors and MS may be present even during the foetal phase (Das, 2002), as studies on children of Asian Indian origin have shown that insulin resistance occurs early in childhood (Whincup *et al.*, 2002) or infanthood, with a degree of heritability. The components of MS are highly heritable in Asian Indians, with recent studies showing a heritability (h^2) value of between 0.27 and 0.53 (Zabaneh *et al.*, 2009). It has been suggested that the risk factor profile of migrant Asian Indians might be higher than the native populations of their adopted countries, even higher still than subjects in India (McKeigue *et al.*, 1989).

1.3.4.5 THE METABOLIC SYNDROME IN SOUTH AFRICANS

Currently, there is no data available on the national prevalence of insulin resistance, DM or MS. What is known from data collected in 1990's, is that the prevalence of DM is the highest in the Indian population (13%), and followed by a lower prevalence in the Black (5-8%) and in the White (4%) populations (Huddle & Kalk, 1994). In South Africa, particularly, there is limited information regarding MS in Black subjects. A recent study, looking at randomly selected rural Black South Africans of Zulu descent (Motala *et al.*,

2011) reported an overall prevalence of 22% when using the 2009 Joint Interim Statement (JIS) criteria. A lower prevalence of 10% and 13% was reported by Jennings *et al.* (2009), who studied 209 normal and overweight Black female subjects without known disease, using ATP III and IDF criteria, respectively. Another study looked at a group of Black and White corporate executives and reported a MS prevalence of 31% (Ker *et al.*, 2007) for both population groups. The only recent local report on MS in Asian Indians is a study by Ranjith *et al.* (2008) who found that the MS was prevalent in 60% of subjects in their cohort of young patients, hospitalised with myocardial infarction.

Although the MS has become increasingly common as part of an epidemic of obesity, hypertension and insulin resistance, few studies have examined the effects of the MS on early changes in cardiac structure and function. There is evidence that the MS is associated with an increased LV mass as well as early changes in LV diastolic function, but little local data is available on how these risk factors together increase CV risk, or how they interplay to alter cardiac structure and function.

1.3.5 The use of echocardiography as a diagnostic tool for early detection of cardiac structural and functional abnormalities

The detection of preclinical changes in cardiac structure in response to the presence of CV risk factors may allow timeous intervention before permanent, irreversible changes occur (Mogelvang et al., 2009). The STRONG Heart Study (Drukteinis *et al.*, 2007) analysed clinical characteristics and left ventricular structure and functions in 1940

participants of American Indian heritage in the 14 to 39 age group. There was a 35% prevalence of prehypertension with concomitant greater LV wall thickness and LV mass. They reported that these prehypertensive subjects were more likely to develop left ventricular hypertrophy (LVH) than normotensive subjects were. Prehypertension has also been associated with higher rates of CVD in older subjects (Devereux *et al.*, 2000). Early detection of prehypertension would permit appropriate intervention and prevent left ventricular hypertrophy with its sequelae of ischaemic stroke (Fox *et al.*, 2007) and CV events (Levy *et al.*, 1990).

Early changes in myocardial function which are associated with CV risk factors are characterized by a normal ejection fraction and subtle changes in the diastolic parameters. Diastolic abnormalities have also been associated with the presence of insulin resistance, with overt changes being documented in the presence of DM. A decrease in ventricle filling during the initial phase of diastole (Nagueh *et al.*, 2001), prolongation of the isovolumic myocardial relaxation time (measured by the isovolumic relaxation time [IVRT]) and an increase in the left atrial (LA) filling pressure confirm a worsening degree of diastolic filling, eventually deteriorating into diastolic dysfunction. Common structural changes that occur in the presence of overt DM are characterised by such diastolic abnormalities and significant increases in the LV mass, independent of the effects of other traditional risk factors (de Marco et al., 2012). For example, Shapiro, *et al.* (1981) reported that left ventricular dysfunction was a common finding in asymptomatic diabetic subjects, which was related to the degree of microvascular

complications with myocardial involvement in diabetes. The STRONG heart study (Devereux *et al.*, 2000) also reported similar findings, with diabetic American Indian subjects having significantly increased LV mass, left ventricular wall thickness, as well as reduced myocardial function, as compared to subjects with normal glucose tolerance.

Diastolic dysfunction (DD) does not always present with symptoms of heart failure, yet it is currently regarded as one of the first markers of imminent CVD (Paulus *et al.*, 2007). The prevalence of diastolic heart failure (DHF) has risen dramatically to over 50% (Owan *et al.*, 2006), and accounts largely for heart failure admissions in the elderly (Graves *et al.*, 1994). DHF is also referred to as heart failure with normal left ventricular (LV) ejection fraction (HFNEF)(Zile *et al.*, 2004), with older subjects, females, hypertensive subjects, diabetic subjects, obese and subjects with LV hypertrophy being at increased risk. Diastolic dysfunction is also believed to be the first stage of the diabetic cardiomyopathy (Cosson & Kevorkian, 2003), and is related to the duration of DM (Kim *et al.*, 2011). However, the subtle changes that occur during the progression of diastolic dysfunction to diastolic failure, which, in its normal course precedes systolic dysfunction, may not be detected with conventional echocardiography. The loss of longitudinal motion, one of the most common functional changes that occur with ageing and diastolic dysfunction, is associated with subendocardial interstitial fibrosis, increased conduit arterial pressures and increased central arterial pressures (Fraser, 2009). These subtle changes may be detected by myocardial velocity measurements, using Tissue Doppler imaging (TDI), and

has been used to diagnose DD with greater sensitivity and specificity than 2-D and M-Mode echocardiography (Poirier *et al.*, 2001).

Although TDI indices are a reflection of diastolic filling pressures at an instant, an increased LA size has been reported to better reflect the cumulative effect of filling pressures over time (Prichett *et al.*, 2003). Enlargement of the LA is a concurrent finding in systolic and diastolic dysfunction and in overt CVD (Tsang *et al.*, 2002) with LA remodelling being predictive of CV events. Since the LA expands in three dimensions and eventually assumes a more spherical shape, Prichett *et al.* (2003) proposed that the left atrial volume index (LAVI) was a better indicator of LA remodelling than the LA size. Furthermore, it was recently reported that the LAVI measurement is a more accurate clinical prognosticator for cardiac disease (Kedia *et al.*, 2008), with a similar predictability as the EF for heart failure hospitalisation and mortality in ambulatory adults with CAD (Ristow *et al.*, 2008). In the absence of conclusive TDI findings (i.e. diastolic filling ratios Em/Ea ≥ 15) a LAVI measurement > 40 ml/m^2 is indicative of LV diastolic dysfunction (Paulus *et al.,* 2007).

Since there are limited community-based studies providing information regarding cardiac structural and functional changes in subjects with preclinical disease, particularly in the young, this project sought to determine a non-invasive assessment of these parameters using echocardiography. The degree of structural alteration associated with the risk factors profile, as well as risk factor clustering, was ascertained.

1.3.6 The Metabolic syndrome and cardiac structure

The growing incidence and prevalence of obesity in South Africa and worldwide has led to the metabolic syndrome becoming an increasing public health concern. Population-based studies exploring the effect of MS on cardiac structural and functional abnormalities are limited, and few studies have included quantitative assessment of LV diastolic function. A recent study by Aijaz *et al.* (2008) found that metabolic syndrome was associated with an increased LV mass index and LV diastolic dysfunction in American Caucasian women. Also, it has been observed that obese adolescents with MS often have increased LV mass suggesting that this is a response not only to increased haemodynamic load but also to possible neurohormonal effects relating to clustering of metabolic risk factors (de Marco *et al.,* 2012)**.** Similar findings were described by Sundström *et al.* (2006), who reported an increase in the LV mass in subjects with MS in a large group of elderly subjects, with these changes related to the contributory effects of insulin resistance. The STRONG Heart study (Chinali *et al.*, 2006) reported on a group of young Native American Indian subjects (24% prevalence of MS in this cohort), and found that MS was associated with LA dilation, LV hypertrophy, reduced systolic and impaired diastolic function.

What emerges from these studies is that there are clear structural changes that occur in the presence of MS. According to our knowledge, no such study has been performed on subjects in South Africa, particularly in a population group with a traditionally high CV risk factor profile. Furthermore, the cardiac structural and functional changes in MS subjects

with milder forms of obesity, insulin resistance and hyperlipidaemia, especially in this community setting, remain unclear. This study therefore planned to explore the association of MS with changes in cardiac structure and function across genders in a broad range of age groups in a community setting, using Doppler imaging as well as an estimation of epicardial fat. The measurement of the sub-epicardial adipose tissue thickness was also made to determine its value as a composite marker of the metabolic syndrome.

1.3.7 Echocardiography and SEAT thickness

Obesity, particularly visceral obesity, is regarded as one of the main determinants of MS (Bosello *et al.*, 2000). Although waist circumference is a surrogate measurement of visceral fat, its limitation lies in its inability to delineate subcutaneous from visceral fat. The detection of sub-epicardial adipose tissue [SEAT] using echocardiography, has been demonstrated and validated using MRI (Iacobellis, 2003) and CT (Ahn *et al.*, 2008), where echocardiographically-derived measures of epicardial fat correlated strongly with visceral abdominal fat. SEAT thickness has also been associated with the clinical parameters of the MS (Chaowalit *et al.*, 2006), as well as anthropometric (body mass index and waist circumference) [Ding *et al.*, 2009], insulin resistance and obesity (Iacobellis & Leonetti, 2005). Pericardial fat is also predictive of incident CAD, independent of conventional risk factors, after adjustment for BMI and other CV risk factors (Baik *et al.*, 2007). SEAT is significantly thicker in patients with unstable angina in comparison to those with stable

or atypical angina (Ahn et al., 2008), with recent studies (Park et al., 2010; Eroglu et al., 2009) reporting similar findings in subjects with MS and CAD, as compared to those with normal coronary arteries. A Turkish study (Kaan *et al.*, 2008), confirmed a close relationship between subepicardial adipose tissue and the MS in a small cohort of 123 patients, with these authors suggesting that an assessment of SEAT thickness might be valuable in the routine echocardiographic examination to predict the presence of MS.

In this study, we used Doppler echocardiography to evaluate cardiac structural and functional changes in all subjects, together with two-dimensional echocardiography for the visualising and assessment of visceral adipose tissue. We also studied selected gene polymorphisms in these subjects to ascertain a possible genetic predisposition.

1.3.8 Single Nucleotide Polymorphisms and the Metabolic syndrome

There is a substantial body of evidence that supports the concept of a genetic basis for CVD and DM (Motulsky & Bruznell, 2002), as has been shown in family studies (Levy, 2003). Although large genetic epidemiological studies have identified gene polymorphisms that confer increased susceptibility to CVD, few studies have examined these polymorphisms in 'normal' subjects to determine their propensity to metabolic alterations (such as MS) and to atherosclerosis. Furthermore, although varying associations between cardiovascular disease and genetic polymorphisms in different

populations have been documented, the general emphasis still remains on the need and importance of ethnic-specific investigations (UKPDS, 1998). Most common diseases, like DM and atherosclerosis, are accompanied by disturbances in the biochemical traits. The inheritance of biochemical traits, which may serve in risk factor stratification, may be more influenced by genetic variation (Wallace *et al.,* 2008), rather than their related diseases.

Corella (2002) suggested that the population-attributable risk for CVD was determined by the high allelic frequencies of a mutation or single nucleotide polymorphism (SNP), which manifested significantly only at population-level. Kathiseran *et al.* (2008) further emphasized the value of SNPs that were able to influence the variation in (for example) lipid levels amongst individuals in a population, providing information about CV risk beyond the lipid level itself. Therefore, one of the fundamental questions underlying this aspect of the project was to determine whether environmental or genetic factors are associated with particular biochemistries or phenotypes. Furthermore, it was important to know the extent to which these phenotypes were modulated by CV risk factors. As this analysis forms part of a larger genetic component related to the Phoenix Lifestyle Project, this study focusses on the genetic associations in relation to CV risk factors related to the metabolic syndrome only.

Although the expression of each of the major component of MS is known to be caused by the complex interaction of environmental and genetic factors, understanding the role of

the genetic factors still remains unclear, since the literature provides limited description of the genetic mechanisms underlying the pathophysiology of MS (Nugent, 2004). A large number of genetic association and linkage studies reviewed by Joy *et al.* (2008) and Zabaneh *et al.* (2010) could not provide any confirmed associations with MS. Since adipose tissue has been reported to play a central role in the pathogenesis of MS, by interacting with genetic variants at candidate genes for dyslipidaemia, hypertension and insulin resistance, candidate gene polymorphisms that were related to these conditions were studied. Since oxidative stress has been implicated as a cause of endothelial injury, which in turn, has been proposed as the aetiology for DM, candidate gene polymorphisms related to endothelial injury were also studied. On this basis, common polymorphisms in the Lipoprotein Lipase (LPL) and the Human Paraoxonase-1 (PON-1) genes were chosen. The rationale for choosing these genes is explained below.

1.3.8.1 LIPOPROTEIN LIPASE

Circulating lipids are carried in lipoproteins, which consist of esterified and unesterified cholesterol, triglycerides, phospholipids and protein (Preiss-Landi *et al.*, 2002). Lipoprotein lipase (LPL) is the major rate-limiting enzyme that catalyses the hydrolysis of triglyceride from circulating triglyceride-rich lipoproteins, very low density proteins and chylomicrons (Wang, 2009). During this process, surface-free cholesterol and phospholipids are transferred to HDL particles, increasing the concentration of HDL

(Preiss-Landi *et al.*, 2002). The products from these catalytic reactions, like fatty acids, are taken up by the tissues and processed differentially; hence LPL acts as a bridging protein which mediates the cellular binding and up-take of lipoproteins (Stein, 2003).

Lipoprotein lipase is found throughout the body, with the highest activity and mRNA levels found in the heart, skeletal muscle, adipose tissue, and to a lesser degree, in the lungs and brain (Zechner, 1997). It is expressed in the adipocytes, macrophages, smooth muscle cells, and most importantly, on the luminal surface of the capillary endothelium (Zechner, 1997). Since LPL plays a pivotal role in overall lipoprotein metabolism, it is involved in forward cholesterol transport and contributes to the maturation of high-density lipoprotein (HDL) precursors, which are themselves involved in reverse cholesterol transport (Pillarisetti, 2003). Lipoprotein lipase also controls the delivery of triglyceride-derived free-fatty acids to muscle, adipose tissue, and vascular wall macrophages, where lipid uptake influences peripheral insulin sensitivity, central obesity, and foam cell formation (Mead, 2002).

Most of the identified SNPs with functional effects have been reported to lead to loss of enzymatic function, and hence, higher triglyceride and lowered HDL levels (Kathiresan *et al.,* 2008). A reduction in LPL activity could result in adverse metabolic consequences and contribute to the clinical phenotype (Zhang *et al.*, 1996), which is supported by the strong association that has been reported between reduced LPL activity, major cardiovascular risk factors (Mead, 2002) and MS (Aguilera *et al.*, 2008). Therefore, diminished LPL activity is suggested to be one mechanism that results in impaired clearance of circulating

lipoproteins, with subsequent hypertriglyceridemia (Wang *et al.*, 2009). An increase in LPL activity, however, is associated with a favourable lipid profile, namely, a lower triglyceride and higher HDL levels (Preiss-Landi *et al.*, 2002). Hayden *et al.* (1991) reported that lipid abnormalities have genetic determinants, with other researchers postulating that the metabolic parameters that determine serum lipid and lipoprotein levels are modulated by multiple gene-gene and gene-environmental interactions (Corella *et al.*, 2002; Lee *et al.*, 2004).

1.3.8.1.1 The Lipoprotein lipase gene

The gene that codes for LPL is located on chromosome 8p22, spans close to 35 kb, and contains 10 exons (Oka *et al.*, 1990). Most of the over 100 identified LPL gene mutations are rare, with 20% occurring in the non-coding regions (Murthy *et al.*, 1996). Three common exonic variants, namely, D9N, N291S and S447X have been identified (Van Bockxmeer, 2001).

Abnormalities of LPL function are associated with adverse pathophysiological conditions like hyperlipidaemia. Prospective case-control studies have shown a strong and consistent association of LPL polymorphisms with raised triglyceride levels and CAD (Sagoo *et al.*, 2008). A recent cross-sectional and longitudinal study of 2045 African Americans and 2116 Europeans was undertaken by Tang *et al.* (2010) to determine the association of eight LPL polymorphisms with the lipid profile. Apart from the population-

related heterogeneity that was observed over the 20 year follow-up, they reported that lipid variations were influenced by the effect of ageing on the LPL polymorphisms. LPL variants have also been associated with obesity in South Indian Asians (Radha *et al.*, 2007).

Since raised triglyceride levels and lowered HDL levels make up two of the defining features of MS the study of the LPL gene was considered important in this project. Furthermore, since there are clearly mechanistically opposing effects of LPL polymorphisms on lipid profile, the S447X polymorphism (which has been found to have a favourable effect on lipid profile), and the N291S polymorphism (which has been reported as having an adverse association with HDL and triglyceride levels) were studied.

1.3.8.1.2 Polymorphisms in the LPL gene

The S447X polymorphism that is located on exon 9 is reported as being one of the most common polymorphisms, with a frequency of 5.6% to 21.1 % (Sagoo *et al.*, 2008; Humphries *et al.*, 1998). This SNP is characterized by a C-G transversion, where the X-allele encodes a prematurely truncated LPL protein, Serine, converting the 447 codon prematurely to a termination codon (Rip *et al.*, 2006). In contrast to other LPL SNPs, the S447X variant is an exception within the coding region, and has been linked to increased lipolytic activity (Ross *et al.*, 2005). Hokanson (1999) suggests that the functional properties of this truncated protein results in an enhanced bridging function, which leads

to increased clearance of triglyceride-rich lipoproteins. In a case-control study of subjects with combined hyperlipidaemia, Wung *et al.* (2006) showed that individuals with this mutation had significantly lower triglyceride, low-density lipoprotein cholesterol and TG/HDL-C ratio, which are established risk factors for CVD (Hokanson *et al.*, 1996) in contrast to individuals without the SNP. This was also shown in a recent meta-analysis (Kathiresan *et al.*, 2008), and has been supported in a large cohort of 1577 Chinese Canadian subjects (McGladdery, 2001), and in other twin and candidate gene association studies (Huang *et al.*, 2006; Groop, 2001).

There have been subsequent studies which show that carriers of the X447 allele have a higher degree of protection against developing MS (Jensen *et al.,* 2009). For example, in the Turkish Adult Risk Factor (TARF) study, Komurcu-Bayrak *et al.* (2007) evaluated the relationship of the S447X variant with serum lipid levels and MS. They found that individuals with this variant had higher levels of HDL and lower fasting glucose when compared to those with the wild-type. Furthermore, in a study of two hundred Egyptian patients with acute myocardial infarction, S447X was found to be associated with a favourable lipid profile. Conversely, this profile was reversed with the addition of DM (Tarek *et al.*, 2011) as a risk factor. Another study examining the association between the S447X variant, hypertension-induced LV growth and CAD risk, showed that the hypertensive carriers of the variant were at an increased risk of LVH and risk for CAD. This was in contrast to the protective trend observed in normotensive subjects with the variant (Talmud *et al.*, 2007).

Since considerable variation has been reported between study populations (Kathiresan *et al.*, 2008) in the association of the S447X polymorphism with plasma lipids, its association with risk of CHD, in addition to genetic factors (Corella *et al.*, 2002) and environmental exposures like smoking (Lee *et al.*, 2004), we investigated the association between S447X with MS and its components. Furthermore, the influence of this SNP on the lipid profile or on the MS has not been studied in a community-based cohort of randomised men and women. This is particularly pertinent to the Phoenix community, in South Africa, whose inhabitants, being of predominantly Asian Indian origin, are known to have a high propensity for adverse lipid profiles and DM (Seedat *et al.*, 1990).

In contrast to the cardio-protection conferred by the S447X mutation, the N291S polymorphism has been associated with low lipoprotein lipase enzymatic activity, and hence, an unfavourable lipid profile (Zhang*et al.*, 1996; Hayden *et al.,* 1991), since identified in 1994. This may be due to the location of the N291S SNP in a heparin-binding cluster, which is thought to affect the interaction of LPL with the cell wall glycosaminoglycan, as well as the result of the substitutions of the amino acid in the N-terminal domain of LPL which is responsible for catalytic function (Mead *et al.*, 2002).

A large meta-analysis comprising 17 630 subjects (Hokanson *et al.*, 1996) showed that this mutation was associated with a marginal risk for CVD, with stronger associations reported in certain populations. Another meta-analysis of 13 studies by Wittrup *et al.* (1999) reported that this mutation was associated with a significant increase in triglyceride levels and low HDL, with Souverein *et al.* (2005) reporting a significant

association between this mutation and triglyceride levels in a study of 512 males with CAD. This was also shown in EARS study (Humphries *et al.*, 1998) where younger subjects with N291S mutation were more likely to have elevated TG and decreased HDL concentrations, potentiated by moderate obesity. The N291S SNP has also been associated with increased atherosclerotic risk, as reported by the Framingham Offspring study (Kastelein, *et al.*, 1999). A meta-analysis by Yaomin *et al.* (2006) found that this mutation conferred additional risk for a dyslipidaemia profile and was associated with DM and CAD, with López-Ruiz *et al.* (2009) and Sagoo *et al.* (2008) reporting that N291S could identify subjects with a high CV risk and was associated with adverse lipid profiles.

Therefore, in view of the high propensity of Asian Indians for dyslipidaemia and DM, the study of the associations between this polymorphism, DM and dyslipidaemia in this sample was determined to ascertain if additional CV risk for MS was conferred by the presence of the N291S polymorphism.

1.3.8.2 THE HUMAN PARAOXONASE (PON) AND THE PON-1 GENE

The generation of excess reactive oxygen species (ROS) with a loss of reduction-oxidation (redox) homeostasis has been reported to play an important role in the pathogenesis of DM, hypertension, and consequent CVD (Whaley-Connell *et al.*, 2011). The generation of excess free radicals has also been reported to contribute to proinflammatory and profibrotic pathways, which promote insulin-signalling impairment, reduced endothelial-

mediated vaso-relaxation, and associated CV and renal structural and functional abnormalities (Martinelli *et al.*, 2005). Clinical and experimental studies have suggested that oxidative stress with a concurrent decline in anti-oxidant defence mechanisms, could lead to cell organelle and enzyme damage, with a resultant increase in lipid peroxidation and insulin resistance (Maritim, 2003). Oxidative stress and higher concentrations of lipid peroxides have also been reported in prediabetic subjects (Senti *et al.,* 2003), linking oxidative stress to insulin resistance when endothelial dysfunction was found to be an underlying cause for insulin resistance. It has also been suggested that high oxidative stress promotes impaired insulin action, which further increases the degree of oxidation, resulting in subsequent impairment of insulin action on adipocytes (Rudich *et al.*, 1997). There appears, therefore, to be a close relationship between insulin resistance, obesity and oxidative stress, which is more apparent by the elevated markers of systemic oxidative stress which have been documented in subjects with insulin resistance and DM (Meigs *et al.,* 2007). Elevated levels of oxidative stress are also accompanied by a decrease in the expression of PON-1, an enzyme which is reported to have considerable anti-oxidant properties.

Human serum paraoxonase (PON-1) is a calcium-dependant esterase, and a polymorph enzyme, which is a 45-kDa molecular weight enzyme, composed of 355 amino acids, synthesized and expressed by the liver, and bound to HDL. High density lipoprotein (HDL) is the serum transport vector for PON-1, and is essential for its metabolism as it is involved with the stimulation, secretion and stabilisation of PON-1. Impairment of the

anti-oxidative activity of HDL (Hansel et al., 2004) increases oxidative stress, which, in turn, contributes to the development of atherosclerosis.

One of the main functions of PON-1 is to decrease lipid peroxidase generation during LDL oxidation by destroying the biologically active phospholipids in oxidatively modified LDL (Li *et al.*, 2003). PON-1 is also involved with the reduction of the ability of oxidized LDL to trigger monocyte-endothelial cell interactions and hydrolysis of oxidised LDL-associated compounds (Watson *et al.*, 1995), which triggers the inflammatory response and promotes foam cell formation (Aviram *et al.*, 1999). This process, due mainly to the anti-atherogenic activity and the anti-oxidative effects of HDL (Getz, 2004), negates the effects of oxidised lipids which are responsible for the pathogenesis of atherosclerosis (Florentin *et al.*, 2008; Mackness *et al.*, 1993.)

PON-1 is also associated with the high-density lipoprotein sub-fraction that contains apolipoprotein A1 (Mackness *et al.*, 1993), which is believed to have an important role in protection and preservation of cell membrane integrity (Durrington *et al.*, 2001). It has also been reported to have an important additional protective function on LDL, by preventing the transformation of LDL into biologically active atherogenic particles, and thus limiting LDL peroxidation (Mackness *et al.*, 1993).

1.3.8.2.1 Polymorphisms in the Human Paraoxonase-1 gene

The PON-1 gene has been identified as a potentially important candidate gene as it appears to have an important role in promoting or protecting against oxidative stress (Levy, 2003), with a direct effect on the pathophysiology and acceleration of CVD. PON-1 activity is thought to be modulated by CV risk factors, as well as by environmental and lifestyle factors (Balcerzyk *et al.*, 2007). Decreased PON-1 activity is associated with a greater risk of disease that involves lipid peroxidation and oxidative damage (Veiga *et al.*, 2011), for example, DM, renal disease, liver cirrhosis and CVD. Leviev *et al.* (2001) found lower PON-1 activity in non-diabetic subjects with abnormal fasting glucose, as compared to higher PON-1 activity in those subjects with normal glucose levels. Furthermore, initial studies examining PON-1 activity on transgenic mice (Tward *et al.*, 2002) found an overexpression of PON-1, which was related to a decrease in atherosclerotic lesion formation as compared to PON-1 knock-out mice, strongly suggesting that PON-1 is a major determinant of atherosclerosis. There is clear evidence which indicates that PON-1 activity regulation is strongly influenced by genetics. This is further supported by the fact that there is wide variation in terms of the enzymatic activity of PON-1 in humans, which has been explained partly by the genetic variation (genetic polymorphisms) in the coding region of the PON-1 gene.

The PON-1gene is part of a paraoxonase family of genes, located on chromosome 7q21.3-22.1 (Primo-Parmo, 1996). The PON-1 gene has 9 exons spanning 26 kb, with two common missense polymorphisms being identified, namely, the Gln192Arg (Q192R) and

Leu55Met (L55M). These variants have been reported to confer risk to CAD in varying degrees (Watzinger *et al,* 2002). PON-1 enzymatic activity for paraoxon is modulated at the PON-1 locus by the polymorphism Q192R on codon 192, which creates an amino acid substitution of arginine (R) for glutamine (Q), resulting in the mutation known as the 192R allele. This variant displays opposing effects on PON-1 enzymatic activity, which has been reported to be determined by alleles: low paraoxonase activity is determined by the Gln allele (Q192), whereas high paraoxonase activity is determined by the Arg allele (Adkins *et al.*, 1993). What this translates into is that the HDL particles from the Gln/Gln192 variant appear more effective in protecting LDL from oxidative changes (Mackness *et al.*, 1999).

The Q192R SNP (Arg192) has been linked with an increased risk for DM (Flekak *et al.*, 2008), CAD (Watzinger *et al.*, 2002) and with ischaemic stroke (Dahabreh *et al.*, 2010) and has been associated with lower PON-1 serum activity (Kim *et al.*, 2007). The Arg192 mutation has been reported as being less effective in protection from the effects of oxidative stress (Aubo *et al.*, 2000). This is possibly attributed to the decreased affinity of this SNP to HDL, leading to decreased protein stability and activity (Gaidukov *et al.*, 2006), and has been linked with coronary artery spasm in Japanese subjects (Fujihara *et al.*, 2011). This was also shown in a study of 156 Spanish patients, where diabetic subjects with the 192R allele demonstrated a 2.5 fold increase in the odds ratio (OR) for myocardial infarction (Aubo *et al.,* 2000), with the 192R allele reported as being an independent risk factor for CAD in non-diabetic subjects as well (Imai *et al.*, 2000). Low PON-1 activity and the 192 RR genotype has also been associated with the severity of

CAD (Kerkeni *et al.*, 2006) in a Tunisian population, and with conventional CV risk factors (Balcerzyk *et al.*, 2007). Studies by Pati & Pati (1998) and Jian Ping *et al.* (2005) reported higher frequencies of the Arg- allele in patients with CAD and MI, as compared to a higher frequency of the Gln- allele in controls, with increased risk of MI in subjects that were diabetic and/or obese. They suggested that these conditions which were associated with high oxidative stress had a modifying effect on the association, as Q192R was not independently associated with MI.

In contrast, a recent case-control study of 650 subjects, of whom 350 had severe (>70%) coronary artery stenosis, from North-west Punjab, showed that the Q192R SNP was independently associated with CAD (Gupta *et al.*, 2011). Additionally, there was a significant association reported between the R allele and the development of CAD in a group of Egyptian subjects (Mohamed *et al.*, 2010).

The second common PON-1 polymorphism, the L55M mutation, results from the nucleotide substitution on codon 55. This substitution produces an amino acid substitution of methionine for leucine, resulting in the mutation known as the 55M allele. The L55M mutation has been reported to be of importance to paraoxonase function because of its association with paraoxonase serum concentrations, but with small effects on enzyme activity (Blatter-Garin *et al.*, 1994), although Mouhamed *et al.* (2011) reported low PON- activity associated with this polymorphism.

Previous studies have associated this allele with less protection from oxidative stress, and hence increased risk of CVD (Imai *et al.*, 2000, Watsinger *et al.*, 2002). The L55M polymorphism has also been associated, although to a lesser degree, with ischaemic stroke (Banerjee, 2010). In a study of 222 case-controlled subjects, Taşkiran *et al.* (2009) reported that the frequency of PON-1 55M allele was significantly higher in subjects with premature CAD than normal subjects, with a significant relationship demonstrated between the PON-1 M/L55 polymorphism and premature CAD (p=0.017).

What emerges is that the PON-1 polymorphisms are associated , with varying degrees with CV risk factors and disease, since Leu55 and Arg 192 have been associated with CAD in some, but not all, clinical studies. These inconsistencies were highlighted in a recent meta-analysis (Christiansen *et al.*, 2004), who showed a weak, but significant association with CAD in the Gln192Arg variant. Martinelli *et al.* (2005), in their study of PON-1 polymorphisms, pointed out the inherent limitations of this meta-analysis, which were related to high heterogeneity of the population groups and clinical outcomes, to name a few. In spite of these controversies, it appears that the studies which show the strongest association with CV risk were the ones with high-risk populations, like diabetics (Martinelli, *et al.*, 2005), where the effects of oxidative stress and risk factor clustering (like the metabolic syndrome) are more apparent.

The two prominent components of the MS, namely, glucose intolerance and obesity, are contributors to oxidative stress. This may be explained by the role of oxidative stress pathways and a reduction in antioxidant defences, with consequent impairment of insulin

signalling, and resultant insulin resistance (Martinelli *et al.*, 2011). Another hypothesis, proposed by Grundy (2007) is that the MS manifests in subjects with a specific metabolic susceptibility, most likely insulin resistance, and with an accumulation of excess of body fat. The MS itself has also been reported to contribute to elevated levels of oxidative stress, as it is considered to be associated with a proinflammatory and prothrombotic state, thus contributing to the acceleration of CVD and atherosclerosis (Kim & Younossi, 2008).

Increased oxidative stress and lower PON-1 activity has been associated with a greater severity of risk factor clustering (Senti *et al.*, 2003). This association was strengthened by findings from the Verona Heart Project (Martinelli *et al.*, 2005), where a significant association was documented between the Leu55 and Arg 192 alleles, in subjects with MS and CAD. They also reported that subjects with MS and both alleles had significantly increased risk of CVD, as compared to subjects without MS, but with the 55Met/Met and 192Gln/Gln genotype. This was also borne out by Oliviera *et al.* (2004) who reported that the Met/Met55 allele, like the Gln/Gln192 allele had protective effects against oxidative stress, with the MM genotype being associated with lower triglyceride levels, hence, conferring protection against CAD.

Recent genetic analysis in young South African Indian males (≤ 45 years old) have reported gene-gene and gene-environment interactions (Ranjith *et al.*, 2008) and found that the interplay between environmental risk factors and genetic factors may be more important in contributing to the development of MI in young Indians. Although such

ethnic-specific data on the contribution of LPL and PON-1 activity and polymorphisms to the development of CVD is steadily growing, there have been few data on these polymorphisms on communities with high risk factor profiles. Hence the objective was to study these polymorphisms in a sample with known elevated CV risks, to determine whether these polymorphisms conferred increased risk for the development of the MS.

1.4 *Rationale*

It is understandable that the metabolic syndrome causes an exponential increase in the risk of cardiovascular disease (de Las Fuentes, 2007) because of the multiplicity of risk factors that together constitute the syndrome. However, very few studies (none to our knowledge in the Asian Indian community, to date) have evaluated the relationship between MS and the associated changes in LV structure and functions (Chinali *et al.*, 2006; Aijaz, 2008). In this study, the objective was to investigate whether MS was associated with changes in cardiac structure and/ or function. In particular, it was important to know whether early functional changes, as assessed by diastolic indices, worsened progressively with increasing burden of MS. Furthermore, the presence and implications of SEAT thickness in these subjects and its value as an early diagnostic marker in the development of MS, using echocardiography, was evaluated.

The LPL gene has emerged as an important candidate gene for the components of the MS (Goodarzi *et al.*, 2004). Triglyceride-rich lipoprotein metabolism is impaired in diabetics, and LPL has been reported as the culprit for the alterations found in these patients (Carr, 2004). We investigated the associations between polymorphisms and risk factors, as well as the allele frequency in normal subjects with MS in this cohort.

Furthermore, since MS is associated with increased oxidative stress, which appears to be modulated by PON-1 polymorphisms, it was important to define the genetic patterns relating to risk factor profile in those individuals with the MS.

1.5 Outline of the thesis

Chapter 1 incorporates the Introduction, followed by the review of the current Literature (above), which touches briefly on conventional cardiovascular risk factors. The focus is then turned to cardiovascular risk factors in Asian Indians, particularly in South Africa.

Risk factor clustering, in particular, the metabolic syndrome, is then discussed. The current controversies surrounding the relevance of the metabolic syndrome are then briefly explored, with some motivation to support the decision to study this syndrome in this population group. A brief pathophysiology of the metabolic syndrome, the atherogenic phenotype in Asian Indians, and its varying prevalence world-wide is then discussed.

The next portion of the review of the literature is allocated to the importance and utility of echocardiography as a screening tool for the detection of early structural and functional changes in the heart, associated with cardiovascular disease. Of particular importance would be the acquisition of echocardiography values in normal subjects, and comparison of these with subjects with the metabolic syndrome or its individual components. The role of novel echocardiography measurements, like the use of tissue Doppler Imaging for the assessment of diastolic dysfunction and the measurement of sub-epicardial adipose tissue (SEAT) thickness is then discussed.

The genetic portion of this project aims to describe the frequency, association and patterns of single nucleotide polymorphisms in subgroups of participants based on age,

gender, anthropometry and certain lifestyle habits, as well as presence or absence of the metabolic syndrome or its individual components. This includes an in-depth discussion of each of the gene polymorphisms investigated and their intermediate expressions in this study.

Chapter 2 consists of a detailed description of the study design, methodology and experimental procedures. This is followed by the results (Chapter 3), which include the risk factor prevalence, the prevalence of metabolic syndrome and echocardiographic changes, and finally the genetic analysis of the PON-1 and lipoprotein lipase polymorphisms studied. The discussion and conclusion are found in Chapter 4 and focus on the molecular mechanisms for the development of the metabolic syndrome, as well as the possible mechanisms underlying cardiac structural changes.

CHAPTER TWO

Materials and Method

2.1 Study design

This was an analytical, cross-sectional study where a random sample of participants from the Phoenix community was selected and sub-divided into two categories based on the presence or the absence of the metabolic syndrome (outcome). The various exposures such as echocardiography and genotyping were then compared between the two outcome groups.

2.1.1 Study population and location

The study was carried out on subjects residing in the Phoenix area (EThekwini Municipality, Durban, Kwazulu-Natal), made up of predominantly South African Asian Indians.

The latest census data indicate that Indians make up 2.5% (Stats SA P0302, 2011) of South Africa's population. The history of this population has been documented as being descendants of indentured sugar-cane workers, who were brought to Kwazulu-Natal between 1860 and 1911 (Seedat, 2005). The community of Phoenix is a culturally heterogeneous one, with inhabitants of varying socio-economic status. Although the prevalence and incidence of Type 2 Diabetes Mellitus (DM) has been studied on limited occasions in the Indian population, there has been no recent community-based evaluation of the inhabitants of the community of Phoenix. It is believed that the

transition in the socio-economic status of the community has deleteriously contributed to the acceleration of atherosclerotic disease.

2.1.2 Classification of normal controls

Subjects who were not documented with cardiovascular or metabolic risk factors were classified as normal.

2.1.3 Study period

Data collection began in 2007 as part of the Phoenix Lifestyle Project (Ethics number: E336/05). D R Prakaschandra was responsible for the major portion of the data collection, including the performance of the echocardiography examination and the genetic analysis (Nucleic acid extraction, Allele-specific Polymerase Chain Reaction, sequencing and analysis).

2.1.4 Sampling strategy

A map of Phoenix which showed the households was used to select these by using simple random sampling. Individuals within the target age group were then selected using the Kish method (Appendix 1) of sampling. Participants were stratified by gender and age: the rationale being that the risk factors and disease prevalence differ among age groups

and genders. Participants (age 15 to 64 years) were divided into each of the 10 strata, stratified for age (15-24, 25 – 34, 35 – 44, 45 – 54, 55 - 64,) and gender (male and female).

2.1.4.1 SAMPLING

Only one member from each household was included in the study to improve generalizability to the Phoenix population. In order to obtain adequate numbers in all strata without enrolling more than one individual in the household, the field-worker followed a prescribed guideline (Appendix 1) when visiting a randomly selected household.

2.2 Statistical planning

2.2.1 Sample size

The sample size calculation was performed by Mrs T Esterhuizen, Biostatistician from the University of Kwazulu-Natal. The sample size calculation was adjusted for sub-group analysis (taking into account the smallest subgroup size) with sufficient power (80%) to detect clinically significant differences based on the findings of de Las Fuentes (2007). Based on this, and for the genetic analysis, a sample size of 724 subjects was required to detect significant differences between groups.

2.2.2 Exclusion criteria:

I. Bedridden or physically disabled

II. Pregnant and lactating women

III. Inability to give informed consent

IV. Cancer patients having received treatment within the last year

2.3 Data collection methods and tools

2.3.1 Recruitment process and administration of the STEPS Questionnaire

Advertising for this project inviting interested participants was done in the local newspaper and radio station. The recruitment was done by trained personnel. Informed consent was obtained from subjects who agreed to participate in the study, after detailed information about the study was given by trained field workers (Appendix 2). A questionnaire tool, validated by the World Health Organisation (WHO) STEPS was used to record the demographic information and behavioural measurements at the subjects' homes (Appendix 3), this being a modified STEPS Instrument for Non-communicable Disease (NCD) Risk Factors, Version 1.3a questionnaire. The subjects were then telephonically given an appointment for the next phase of the study. On the stipulated date and time, subjects were collected from their homes and taken to the Lifestyle Centre at the Inkosi Albert Luthuli Central Hospital (IALCH) in Cator Manor, Durban.

2.3.2 Questionnaire parameters

The demographic information included age, gender, education, income, number of people with living in the household activity profiles, as well as dietary patterns. Other information included a history of DM, hypertension or an immediate family history of cardiovascular risk factors. These responses were entered into Step 1 of the STEPS questionnaire.

2.4 The Clinical Examination

At the Lifestyle Centre, the blood pressure and heart rate was recorded, and thereafter, anthropometric measurements (height, weight, mid-upper-arm, waist and neck circumferences and triceps skin fold thickness) measurements were made. Blood and urine samples were taken for biochemical analysis. An electrocardiogram was recorded and a comprehensive echocardiogram was performed. The following methods for measurements that were recorded are explained below.

2.4.1 Blood pressure and heart rate

Subjects were seated for at least 5 minutes prior to recording the blood pressure using a mercury manometer connected to a standard 12.5 x 23 cm cuff (a larger cuff (15.5 x 32.5 cm was used for those with a mid-upper arm circumference above 33 cm). The blood pressure was taken on 3 occasions at 1-minute intervals. The mean diastolic and systolic measurements were used for analyses.

2.4.2 The diagnosis of systemic hypertension and pre-hypertension

Systemic hypertension was defined according to Joint National Committee VII (JNC VII) criteria as a BP ≥140/≥90 mmHg and/or current antihypertensive therapy (Chobanian *et al.,* 2003), or a self-report of previously diagnosed hypertension.

Pre-hypertension was defined using the JNC VII guidelines as a systolic blood pressure of 120-139 mm Hg systolic or a diastolic blood pressure of 80- 89 mm Hg (Chobanian *et al*., 2003).

2.4.3 Anthropometry measurements

2.4.3.1 HEIGHT

This was measured to the nearest 0.1 cm using a metal measuring tape applied to a wall and a flat headboard at right angles to the wall to ensure correct reading. The subjects were measured without shoes, heels against the wall and the angle of the eye level with the external auditory meatus.

2.4.3.2 WEIGHT

This was determined to the nearest 0.5kg on a balance scale with the subject in light clothing and without shoes. The scale was standardised at the beginning of the study.

2.4.3.3 WAIST CIRCUMFERENCE

This was measured according to standardized guidelines with the subjects standing comfortably. The smallest circumference between the xiphisternum and the umbilicus on expiration was taken as the waist circumference.

2.4.3.4 BODY MASS INDEX

The body mass index (BMI) was calculated automatically by computer software according to the WHO (http://www.who.int/bmi/index.jsp) as weight (kg)/ height (m) ^{2}, and was further classified according to the WHO guidelines:

a. BMI : 18.50 kg/m^2 – 24.99 kg/m^2= normal weight
b. BMI ≥25 kg/m^2 -29.99 kg/m^2 = overweight
c. BMI ≥30 kg/m^2 = obese

In response to evidence that Asian populations had have different associations between BMI, distribution and percentage of body fat and CV risks than European populations, the WHO Expert Consultation revised the cut-offs for obesity from 25 kg/m^2 to 23 kg/m^2 for Asian Indians (WHO Expert Consultation, 2004). The classification for adult Asians was as follows:

a. <23.0= normal weight
b. 23.0 to 24.9 kg/m^2 = overweight
c. ≥25.0 kg/m^2 = obesity

These cut-offs were also used together with the current international WHO guidelines in our analysis.

2.5 Biochemical measurements

After a ten hour overnight fast, whole- blood samples were collected, drawn from the cubital vein with minimal stasis in all subjects by trained phlebotomists or the attending physician. Plasma was assayed for total cholesterol, HDL-cholesterol, and triglycerides. Fasting blood glucose was measured using spectrophotometry and plasma insulin was measured by radioimmunoassay. A whole-blood sample was also collected for genetic analyses.

In subjects who did not self-report DM, a 75g glucose monohydrate in 250ml water was ingested over two minutes. An additional blood sample for glucose and insulin estimation was drawn two hours later. Blood samples were delivered within one hour of collection to the Chemical Pathology Laboratory at IALCH for estimation of the lipid profile, glucose and insulin levels.

2.5.1 Definition of Impaired fasting glucose concentration, prediabetes, insulin resistance and Type 2 Diabetes Mellitus

The impaired fasting glucose (IFG) concentration was defined using guidelines published by the American Diabetes Association (2010) by fasting plasma glucose concentration > 5.6mmol/l -6.9mmol/l. Impaired glucose tolerance (IGT) was defined by fasting plasma

glucose concentration after the 2-hour glucose tolerance test being in the range of 7.8mmol/l – 11.0mmol/l.

Subjects with IFG and IGT, without a prior history of DM, were classified as those in the prediabetic stage, according to the guidelines from the International Expert Committee Report on the role of the A1C assay in the diagnosis of DM (2009). The summarised guidelines (Buysschaert & Bergman, 2011) classified those with FBG: 5.6–6.9 mmol/L or IGT: 7.8–11.0 mmol/L as having prediabetes.

The diagnosis of DM was made from a self-report of previously diagnosed DM. Subjects who did not have a previous history of DM but had a fasting plasma glucose concentration ≥7.0 mmol/L (Genuth *et al*; 2003), a 2-hour fasting glucose concentration of ≥11.1mmol/l and/or current medical therapy with an oral hypoglycaemic agent and/or insulin, were classified, according to the American Diabetes Association's (2006) set criteria for the definition of DM, as diabetic.

2.5.1.1 THE DIAGNOSIS OF INSULIN RESISTANCE

The Homeostatic model of Insulin resistance (HOMA-IR) has been reported to serve as a surrogate measure of the insulin resistance phenotype (Cheal *et al*, 2004). Although the HOMA-IR index is inferior to the clamp technique in terms of accuracy, the utility of this index makes it possible to study a large number of subjects in a fasting state, with a single

glucose and insulin measurement. We calculated the HOMA-IR values as follows (Matthews *et al*., 1995):

$$\text{HOMA-IR} = \frac{\text{fasting insulin } (\mu U/m) \times \text{fasting glucose (mmol/l)}}{22.5}$$

A HOMA-IR value > 2.6 was used as a diagnosis for insulin resistance (Ascaso *et al*., 2003).

2.5.1.2 DEFINITION OF DYSLIPIDAEMIA

Using the National Cholesterol Adult Panel (NCEP) guidelines (Grundy *et al*., 2001), the following cut-offs were used to define elevated total cholesterol, triglyceride and decreased HDL levels:

a. Elevated total cholesterol: > 5.17mmol/l
b. Elevated triglycerides: > 1.69mmol/l
c. Decreased HDL: < 1.04mmol/l

2.6 The diagnosis of the Metabolic Syndrome

There are several definitions that propose criteria for the diagnosis of the metabolic syndrome (MS), namely that recommended by the WHO (Balkau *et al.,* 1999), whose criteria were simplified by the National Cholesterol Adult Panel (NCEP) Adult Treatment Panel III (ATP III) (Adult Treatment Panel, 2002). Later, the International Diabetes Federation (IDF) replaced the WHO criteria for the diagnosis of MS, with the mandatory inclusion of ethnic-specific waist circumference cut-offs (Alberti *et al.,* 2005). More recently, Alberti *et al*. (2009) proposed unifying criteria for the definition for MS, which is

a composite of the ATP III and IDF criteria: this wass aptly named the 'Harmonizing criteria'.We therefore decided to use the three common definitions of MS in our study, as the previous definitions have either been modified or replaced.

2.6.1 Diagnosis using the NCEP ATP III criteria

The presence of metabolic syndrome as stipulated by the NCEP APTIII criteria was defined as 3 or more of the following 5 risk factors in Table 2-1.

Table 2-1: NCEP criteria for diagnosis of the Metabolic Syndrome

	NCEP ATP III criteria
Waist circumference measurement	
Men	> 102 cm
Women	> 88 cm
Triglycerides	≥1.7 mmol/L
HDL cholesterol	
Men	<1.03 mmol/L
Women	<1.29 mmol/L
Blood pressure	≥130 / ≥85 mmHg
Fasting glucose	≥6.1 mmol/L

2.6.2 Diagnosis using IDF criteria

The IDF proposed ethnic-specific cut-offs for waist measurement and defined the metabolic syndrome as the presence of central obesity plus any two other risk factors. The criteria are shown in Table 2-2. Subjects who were on medical therapy for

hypertension or DM were included, irrespective of the values obtained during the clinical examination or biochemistry.

Table 2-2: IDF criteria for diagnosis of the Metabolic Syndrome

	IDF criteria
Waist circumference measurement for South Asians:	
Men	Waist ≥ 90cm
Women	Waist ≥ 80cm
Raised triglyceride	>1.7mmol/L
Reduced HDL cholesterol*	
Men	<1.03mmol/L,
Women	<1.29mmol/L
Blood pressure*	≥130 / ≥85mmHg
Raised fasting plasma glucose*	≥5.6mmol/L

2.6.3 Diagnosis using the "Harmonized" criteria

In 2009, the joint statement from the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity (Alberti *et al*) proposed world-wide unifying criteria for the clinical diagnosis of the metabolic syndrome, calling it the

'Harmonized definition'. This definition is identical to that of the NCEP ATP III definition, except that ethnic-specific cut-points (IDF waist circumference) for the waist circumference measurements are included. The definition comprises a minimum of 3 risk factors and is summarised in Table 2-3.

Table 2-3: Harmonized criteria for diagnosis of the Metabolic Syndrome

	Harmonized criteria
Waist circumference measurements for South Asians	
Men	Waist ≥ 90cm
Women	Waist ≥ 80cm
Triglyceride	≥1.7 mmol/L
HDL cholesterol	
Men	<1.03 mmol/L
Women	<1.29 mmol/L
Blood pressure	≥130 and/ ≥85 mmHg
Fasting glucose	≥6.1 mmol/L

2.7 Echocardiography

Each subject underwent a transthoracic two-dimensional (2D) guided M-mode echocardiogram, and a comprehensive Doppler echocardiogram. Echocardiograms were performed with a Siemens CV70 instrument (Siemens, New York, NY) using a 3.5 MHz transducer with subjects lying in the left lateral decubitus position.

2.7.1 Two-dimensional and M-Mode measurements

M-Mode measurements were performed for the measurements of the aortic root and left atrial (LA) size according to published guidelines (Schiller *et al*., 1989). The left atrial volume (LAV) was calculated according to the American Society of Echocardiography (Sahn *et al*., 1978; Pritchett *et al*., 2003) as an ellipse using the formula: $\tau/6(SA1\ x\ SA2\ x\ LA)$ where SA1 = the M-mode LA dimension and SA2 and LA are measurements of short- and long-axis in the apical four-chamber view (Figure 2.1).

The LAV was then indexed to BSA to calculate the left atrial volume index (LAVI). The LAVI was considered to be dilated if the index was more than 29ml/m^2 (22±6ml/m)^2 (Lang *et al*., 2006).

Figure 2.1: Measurement of LA dimension using M-Mode and 2-D

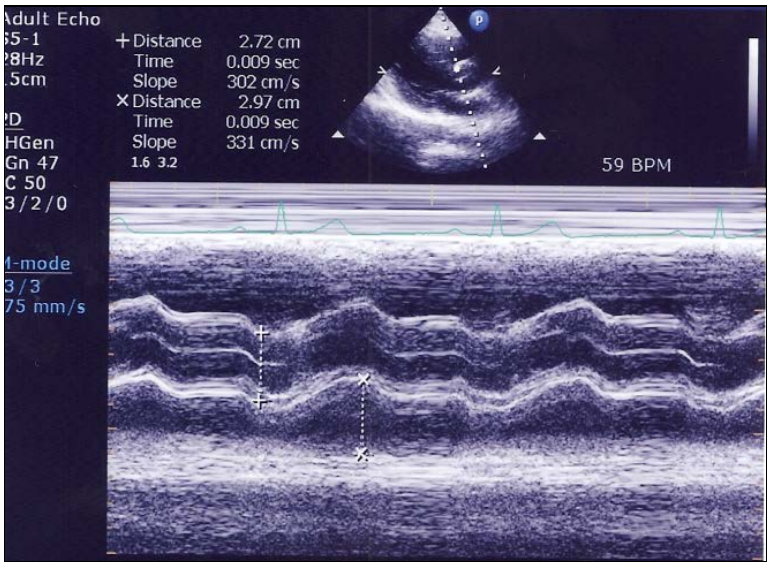


Figure 2.1.(a) Measurement of SA1: the widest LA diameter in the parasternal long axis view by 2-D from the inter-atrial septum and the lateral boundary of the left atrium

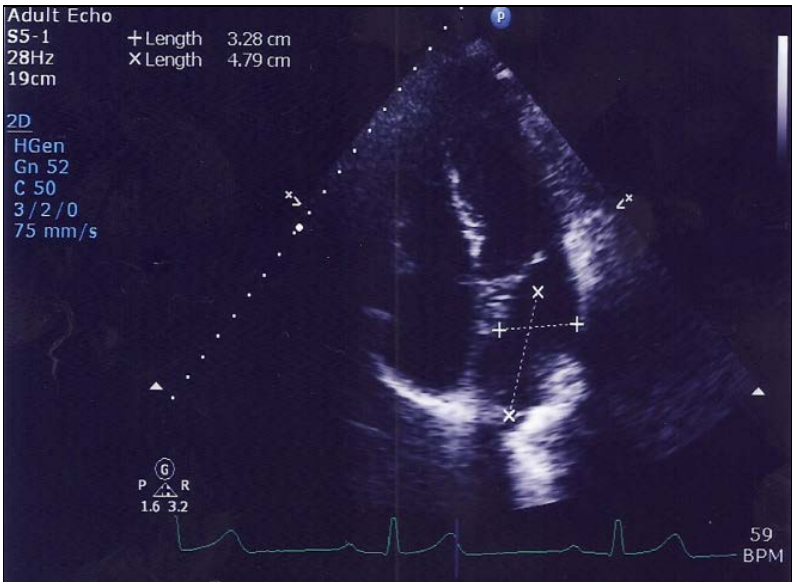


Figure 2.1. (b): Measurement of SA2 and LA: distance of the perpendicular line measured from the middle of the plane of the mitral annulus

The left ventricular (LV) internal dimensions and the thickness of the posterior and septal wall were measured according to published guidelines (Schiller *et al*., 1989). The left ventricular mass (LVM) was estimated by using by the M-mode-derived cubed method and divided by height$^{2.7}$ to correct for body habitus (Devereaux *et al*., 1986). This was termed the left ventricular mass index (LVMI).

The Fractional Shortening (FS) was calculated using the modified Simpson's formula. The ejection fraction (EF) was measured using a monoplane image in the 4-chamber view, by tracing the contours of the LV internal diameter during peak systole and peak diastole. The ejection fraction was calculated automatically by computer software using the Teichholz formula. The ejection fraction was further confirmed by eye-balling, and was used as the measure of LV systolic function.

2.7.2 Doppler Echocardiography

Trans-mitral inflow velocities were used to detect and quantify diastolic dysfunction and were obtained using Pulsed-wave (PW) Doppler in the apical 4-chamber view with the sample volume placed between the tips of the mitral valve leaflets (Quinones *et al*., 2002). The trans-mitral early diastolic (Em) and atrial (Am) velocities were measured and were used to calculate the trans-mitral E/A ratio. The isovolumic relaxation time (IVRT) was also measured from the cessation of LV outflow to the onset of LV inflow.

Tissue Doppler imaging (TDI) was used to obtain left ventricular (LV) myocardial velocities in the apical chamber views with a 2 mm sample volume placed at the septal and lateral

mitral annulus (Oh, 2006). The early myocardial velocity (Ea), myocardial velocity associated with atrial contraction (Aa) and the systolic (Sa) velocities were measured (Dumesnil *et al*., 2002). In subjects where regional wall motion abnormality was not detected, the mitral annular velocity was measured on the lateral side. All echocardiographic measurements were averaged over three consecutive cardiac cycles, measured by a single investigator (DRP) blinded to all other variables.

2.7.2.1 CALCULATION OF THE LEFT VENTRICULAR END DIASTOLIC PRESSURE USING TDI

The calculation of the LV end diastolic pressure (LVEDP) was done according to the recommendations by Nageuh *et al* (1997) and Ommen *et al* (2000), who reported a strong correlation between the LVEDP and the diastolic filling ratios, in simultaneous cardiac catheterization and echocardiographic studies i.e. the ratio of the mitral inflow E wave to the tissue Doppler Ea wave (Em/Ea) [Figure 2.2]. We classified the estimation of the LVEDP using their criteria, as follows:

a. Em/ Ea (lateral) >10 or Em/Ea (septal) >15 is correlated with an elevated LV end-diastolic pressure
b. Em/Ea<8 is correlated with a normal LV end-diastolic pressure.

Figure 2.2. Calculation of TDI Em/Ea (using pulsed-wave and tissue Doppler)

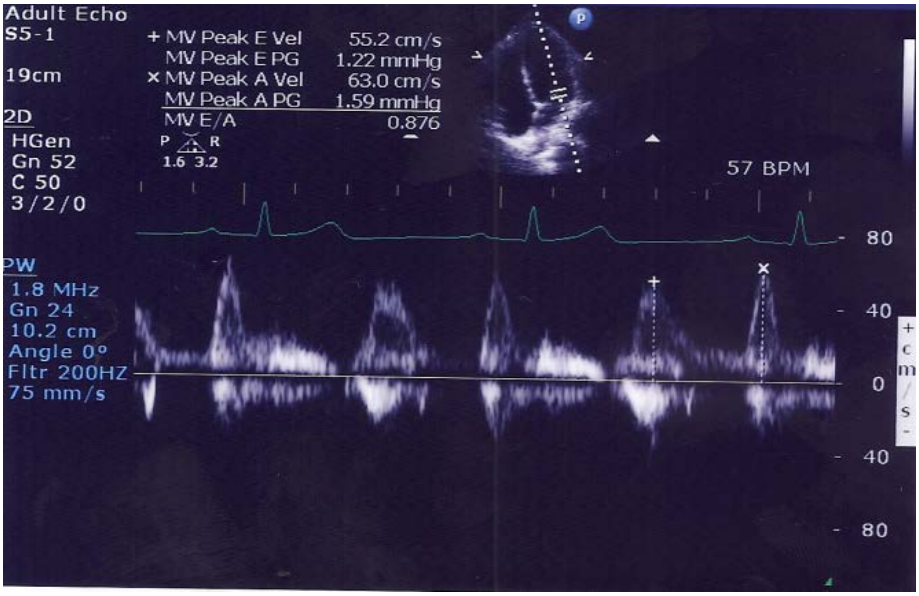


Figure 2.2 (A): Pulsed-wave Dopller across mitral valve leaflets

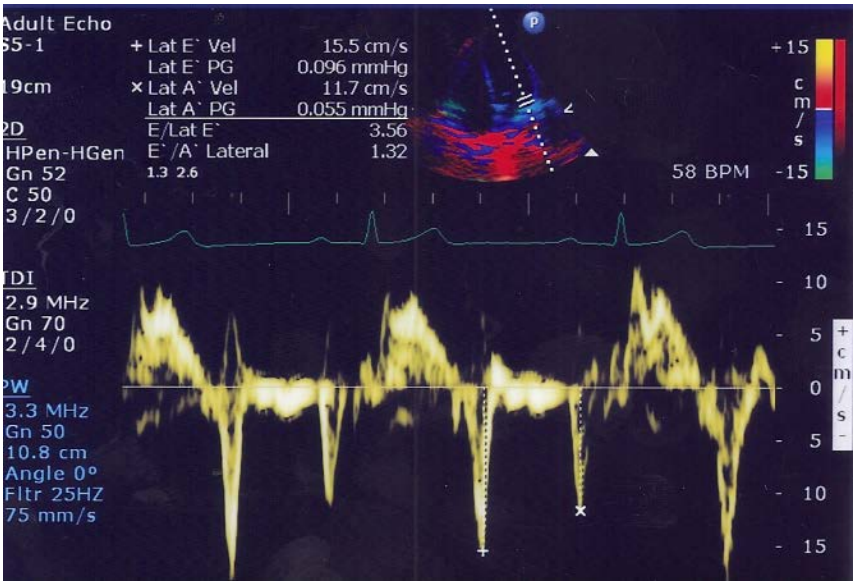


Figure 2.2 (B): Tissue Doppler tracing on the lateral mitral annulus

2.7.3 Definition of diastolic abnormalities and diastolic dysfunction

Diastolic abnormalities were identified using the guidelines proposed by the European Study Group on Diastolic Heart Failure (1998). In the presence of preserved ejection fraction, diastolic abnormalities were identified using the criteria tabulated below in Table 2.1. The presence of any Doppler abnormality was considered a feature of diastolic abnormality.

Table 2-4: Age-specific diastolic abnormality criteria

Age	**<30 years**	**30-50 years**	**>50 years**
E/A ratio	<1	<1	<0.5
IVRT (ms)	>92	>100	>105

The diagnosis of diastolic dysfunction (DD) was made when the subject had echocardiographically-derived diastolic abnormalities (Table 2-4) and was on current diuretic therapy and/or exhibited features of left atrial dilation. Left atrial enlargement was classified as the mean LA diameter of more than 45 mm.

The diagnosis of DD has been recently revised and according to Nagueh *et al* (2009) are summarised as follows:

a. PW Doppler criteria: E/A ratio <1 if age <55 or <0.8 if age >55, and/or Deceleration time (DT) >240ms

b. TDI criteria: Ea ≤12.9 cm/s if age <40; Ea ≤10.2 cm/s age 40–59; and Ea ≤7.2 cm/s if age ≥60 (Alam *et al*; 1999)

c. Em/ lateral Ea>10 or Em/ septal Ea>15 (suggests elevated LV end-diastolic pressure; Nagueh *et al*., 2009).

However the recent guidelines from the Heart Failure and Echocardiography Associations of the European Society of Cardiology (Paulus *et al*., 2007) report that a diagnosis of LV diastolic dysfunction is 'preferably derived from myocardial TDI (Em/Ea ≥ 15).' In the absence of these tissue Doppler findings, i.e., if the Em/Ea was ≥8≤ 15, they suggested that concentric LV remodelling as a potential surrogate for direct evidence of diastolic LV dysfunction, and recommended that, an LV wall mass index of 122 g/m^2 in females or an LV wall mass index of 149 g/m^2 in males be used as evidence for the diagnosis of HFNEF. They also recommended that a measurement of the LAVI be used with the cut-off, according to the Heart Failure and Echocardiography Associations of the European Society of Cardiology (Paulus *et al*., 2007) guidelines, with a LAVI > 40 ml/m^2 being indicative of LV diastolic dysfunction.

Therefore, in this study, the diagnosis of LV diastolic dysfunction was made if the criteria according to European Study Group on Diastolic Heart Failure (1998) was satisfied i.e. 1 or more diastolic abnormalities and current diuretic therapy and/or LA enlargement. This was then compared to the newer criteria as proposed by the European Society of Cardiology (Paulus *et al*., 2007):

a. E/lateral Ea>10 or E/septal Ea>15 or,

b. If Em/Ea was ≥8≤ 15,and LVMI 122 g/m^2 in females or an LV wall mass index of 149 g/m^2 in males or,

c. If Em/Ea was ≥8≤ 15 and LAVI > 40 ml/m^2

2.7.4 Sub-epicardial adipose tissue thickness measurement

The thickness of the epicardial fat was measured in the free wall of the right ventricle in the parasternal long and short axis as proposed by Iacobellis *et al* (2003)[Figure 2.3]. Epicardial adipose tissue appears as an echo-free or hyper-echoic space, and has been validated as an indicator of visceral fat (Iacobellis, 2003). The measurement of epicardial fat on the right ventricle was chosen, as this point is recognised as having the highest absolute thickness of epicardial fat (Schejba, 1989). The parasternal long-axis and short-axis views allow the most accurate measurement of epicardial adipose tissue on the right ventricle, with the best cursor-beam orientation in each view (Iacobellis, 2003).

2.7.5 Intra-observer variability

It is established that intra-observer variability is low, when the echocardiograms are performed by 1 experienced echocardiographer (Ogah *et al*., 2006), as is the case in our study. Furthermore, each measurement was performed according to the ASE guidelines to ensure standardization. At least 3 cycles were recorded, and the average was documented. Measurements of SEAT thickness was made at two sites, and an average of 3 cycles at these 2 sites were taken.

Measurement of intra-observer variability was calculated from samples recorded on the same subject at different intervals. A coefficient of variance of 4%, 5%, 4%, 4%, 2% and 9% was found for the mean LA, LVM, EDD, ESD and EF, SEAT measurements, respectively. The Doppler parameters yielded an intra-user variability of 4% for transmitral filling velocities (Em/Am), 7% for the mean Ea velocity and 11% for the mean Em/Ea ratio on tissue Doppler imaging, respectively. All measurements were stored on computer, and printed as hard copies. The images were reviewed off-line by two experienced observers (DPN and DRP) for analysis.

Figure 2.3: Measurement of Sub-epicardial Adipose Tissue

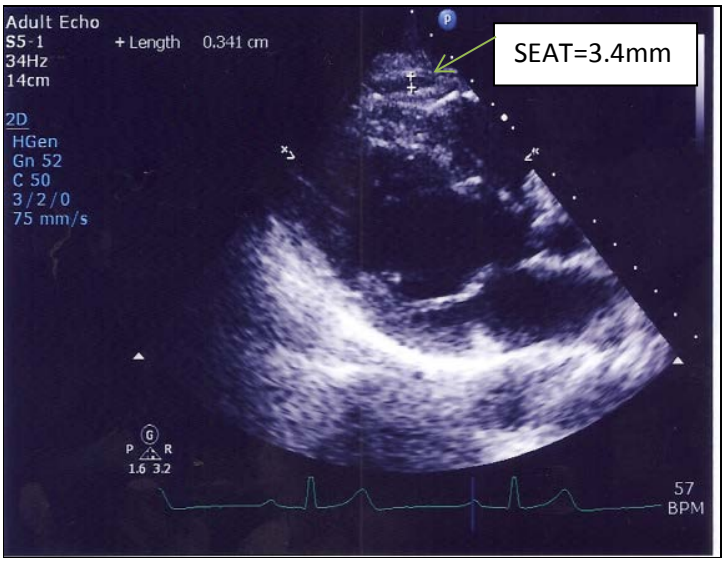


Figure 2.3.(A): SEAT measurement in parasternal long axis

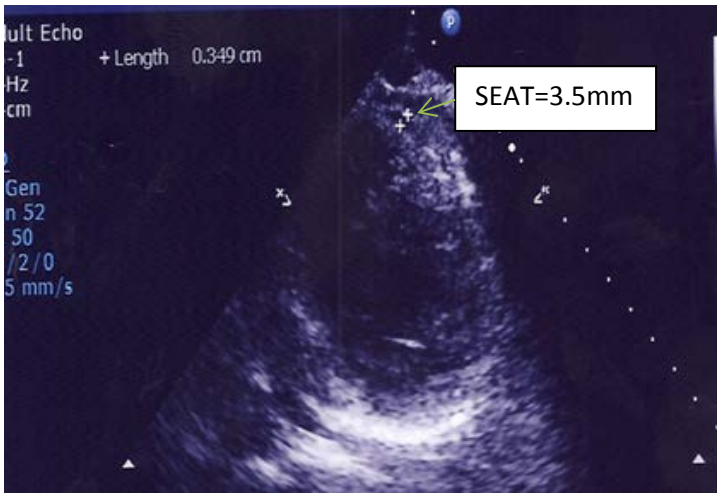


Figure 2.3. (B): SEAT measurement in parasternal short axis

2.8 The Genetic analysis

2.8.1 Sample collection

The samples for the genetic analysis were collected in ethylenediaminetetraacetic acid (EDTA) tubes and sent to the Chemical pathology Department for centrifuging. They were stored as aliquots in 500 µl cryo-tubes and frozen at -80°C until DNA isolation.

2.8.2 Selection of genes and SNPs for analysis

The genes selected for this study forms part of a larger investigation of the same cohort of South African Asian Indians in Kwazulu-Natal. The candidate was responsible for performing most of the DNA isolation and all of the genotyping, gel electrophoresis and sequencing techniques described and analysed in this study.

The criteria for selection for the genes and SNPS being studied have been discussed under the literature review and rationale.

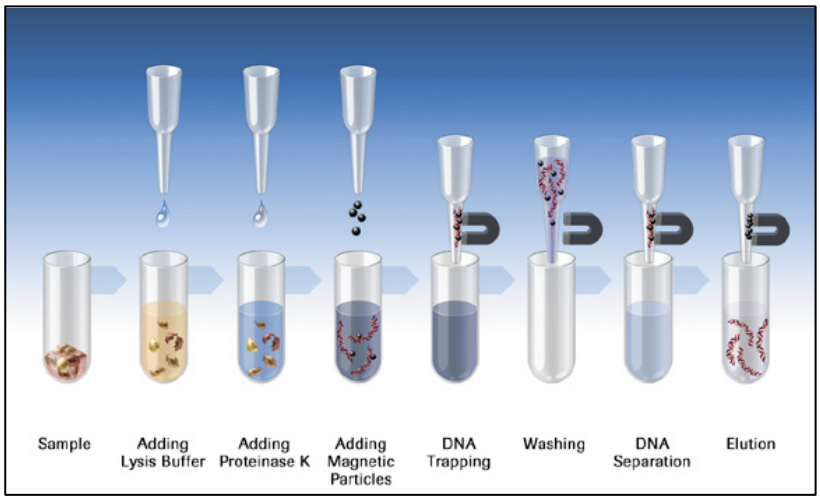
2.8.3 DNA isolation

Total nucleic acid (TNA) was isolated from whole blood using the automated MagNA Pure Instrument (Roche Applied Science) according to the manufacturer's instructions. A brief description of the procedure, which is based on magnetic bead technology, is as follows:

- The MagNA Pure instrument was programmed to extract total nucleic acid from whole blood.

- The extraction involved loading of the sample into the Sample Cartridge.
- In order for cell lysis to occur, the MagNA Pure instrument (using the automated program) added the Lysis/Binding Buffer to each sample, releasing all nucleic acids.
- The nucleases were denatured and the addition of Proteinase K digested the proteins.
- Due to the chaotropic salt conditions, isopropanol, and the high ionic strength of the Lysis/Binding Buffer, the TNA bound to the silica surface of the added magnetic glass particles (MGPs).
- The MGPs with bound TNA were magnetically separated from the lysed sample, and then washed with Wash Buffer to remove unbound substances (such as proteins, cell membranes, PCR inhibitors) and to reduce the chaotropic salt concentration.

Figure 2.4: Extraction of total nucleic acid



The purified TNA was then eluted at 70°C into the Elution Cartridge, while the MGPs were retained in the reaction tip and discarded.

The isolated TNA was made up into aliquots and stored in 2ml screw-in cryo-tubes and stored at -80°C until the DNA quantification measurements were done.

2.8.3.1. DNA QUANTIFICATION

The determination of DNA concentration and purity was performed using the NanoDrop 2000 Spectrophotometer (Thermo Fischer Scientific, Inc) by measuring the absorbance of the diluted DNA at 260 and 280 nm. The generally accepted extinction coefficients for nucleic acids are:

- Double-stranded DNA: 50 ng-cm/µL
- Single-stranded DNA: 33 ng-cm/µL
- RNA: 40 ng-cm/µL (NanoDrop 2000/2000c Spectrophotometer; V1.0 User Manual)

The extracted total nucleic acid was made up into 25µl aliquots using a 1:5 ratio of distilled water to sample, in labelled PCR tubes and stored and stored at -80 °C until used for PCR.

2.8.4 Genotyping

2.8.4.1 RATIONALE FOR CHOICE OF THE GENOTYPING METHOD

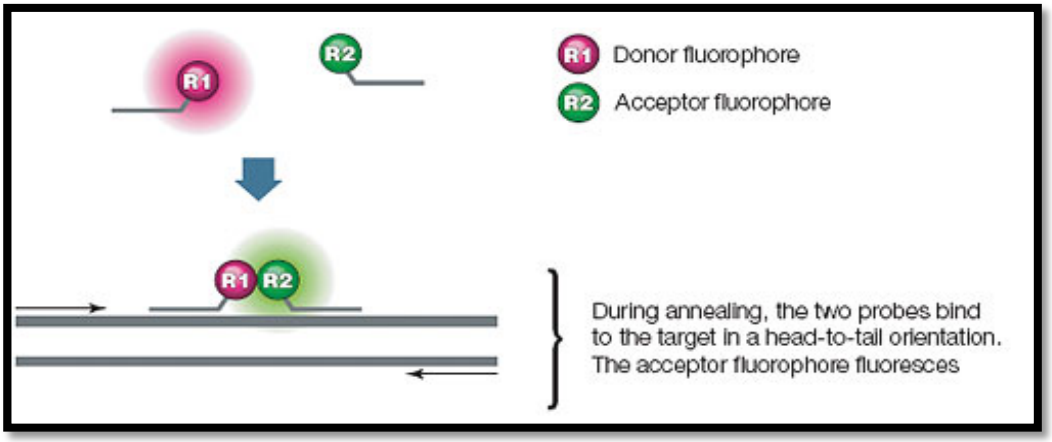
Sequence-non-specific methods of genotyping (based on the capture, cleavage or mobility change during electrophoresis or liquid chromatography) are commonly used in the detection of polymorphisms. However, these methods have been reported as being suboptimal due to the uncertainties that exist regarding the inferred genotype being the true genotype, as the reaction is not sequence-specific (Kwok *et al*., 2003). Sequence-specific methods are able to reliably discriminate between alleles, using allele-specific hybridisation, nucleotide incorporation, oligonucleotide ligation and invasive cleavage (Kwok, 2003). This method, which is known for its simplicity and accuracy for SNP genotyping, was used in this study.

2.8.4.2 PRINCIPLES FOR HOMOGENOUS REAL-TIME SNP GENOTYPING USING HYBRIDIZATION PROBES

Fluorescence monitoring using hybridization probes employs the use of allele-specific probes that are designed to hybridise to the target sequence only when there is a perfect match between probe and target DNA (Kwok 2003). Two probes, each labelled with a different reporter fluorophore are used in the same reaction, and this allows both SNP alleles to be detected in a single tube (Syvänen, 2001). The one base mismatch (Figure 2.5) is able to destabilise the hybridization to prevent the allelic probe from annealing to the target sequence.

In this study, hybridization probes and the principle of fluorescence energy transfer (FRET) was used, where a fluorescence signal is generated if fluorescence resonance energy transfer (FRET) occurs between two adjacent fluorophores (Didenko, 2001). The first hybridization probe (Figure 2.5), which is labelled with fluorescein as donor fluorophore (R1) on its 3' end, can hybridize in close proximity to a second hybridization probe that is labelled with the acceptor fluorophore, labelled with LC Red 640 at its 5' end (R2). The fluorescein becomes excited by the LED light source from the LightCycler system, and transfers energy to the acceptor fluorophore. The acceptor fluorophore then emits light of a longer wavelength that can be measured with a photodiode. This detection strategy allows monitoring of the amplification process on a per-cycle basis because the intensity of the FRET signal depends on the amount of specific PCR product generated (Nauck, 1999). Continuous monitoring of the fluorescence as the temperature is raised from annealing to denaturation produces a sharp decrease in fluorescence when the detection probe dissociates from the template.

Figure 2.5: The principle of fluorescence energy transfer (FRET)



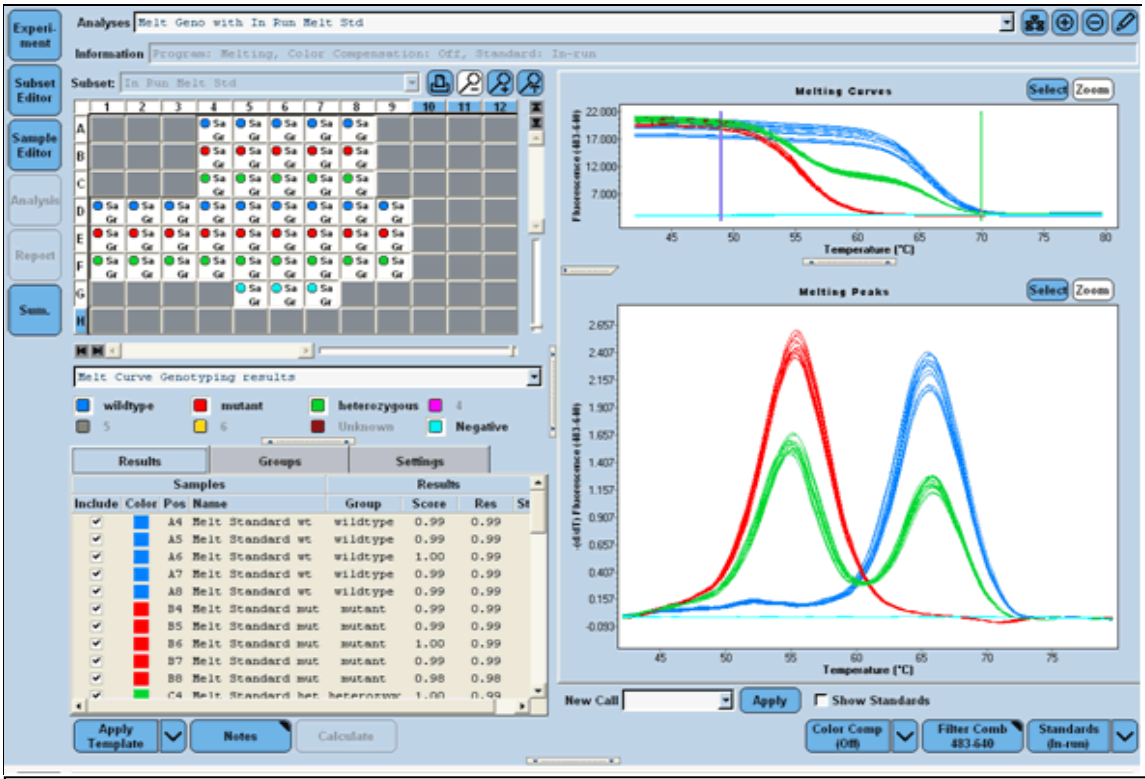
Downloaded from: http://www3.bio-rad.com/B2B/vanity/gexp/content

FRET only occurs when hybridization probes (R1 and R2) bind to their target sequences i.e. when there is a match

2.8.4.3 PRINCIPLES FOR AND MELTING CURVE ANALYSIS

When heated during the Melting Curve stage, the probes melt away from the template sequence, resulting in the loss of fluorescence. The melting temperature (Tm) at which probes melt is specific to the particular mutation that it was designed for (Pont-Kingdon & Lyon, 2005). The fluorescence signal (F) is plotted in real- time against temperature (T), producing melting curves for each sample (F vs T). Melting curves are then converted to derivative melting curves by plotting the negative derivative of the fluorescence with respect to temperature against temperature [-(dF/dT) vs T] (Gordon *et al*., 1998). Therefore, wild-type, heterozygote and /or variants are observed by the fluorescence pattern observed at increasing temperatures, as different curves (Figure 2.6).

Figure 2.6: The principle of Melting curve analysis



2.8.4.4 REAL-TIME POLYMERASE CHAIN REACTION AND MELTING CURVE ANALYSIS

The real-time polymerase Chain reaction (PCR) was performed according to the manufacturer's instructions using the Roche LightCycler 480 System. A summary of the primer and probes used for the PCR amplification and the melting curve analysis is shown in Table 2.5 below. All primer and probe sets were manufactured by Roche Applied Science and the melting temperature (Tm) for each SNP was calculated as shown in Table 2-5. The melting temperature would have been calculated as a function product length, GC content and sequence composition so subtle differences in the sequence composition will result in a shift in the Tm. The different variants under the probe will then result in

different probe stabilities, with specific alleles being distinguished by their Tm_s (Ririe *et al*., 1997). The LightCycler 480 machine that was used bases its Genotyping algorithm on grouping samples with similar melting curve shape either by auto-calling.

Table 2-5: Primer and Probe sequences for the selected candidate genes and polymorphisms

Gene: SNP	Primer sequences	Probe sequences	Mutation
PON-1:L55M	(f):AAAGAAAACACTCACAGAGCTA	LC Red 640-CTGGCTCTGAAGACATGGAGATACTG-PH	A to T
	(r):TCAAGTGAGGTGTGATAAAGAAAT	ACCTGTACTTTCTGTTCTCTTTTCTGGCAGA-FL	
PON-1:Q192R	(f):TATTGTTGCTGTGGGACCT	LC Red 640-CCCAAATACATCTCCCAGGATCGTAAGTA-PH	A to G
	(r):ACATACTTGCCATCGGG	CTTGGACTATAGTAGACAACATACGACCACGCTA-FL	
LPL:S447X	(f):TTCTGTTCTAGGGAGAAAGTGT	LC Red 640ATTCAGAGACTTGTCATGGCATTTCACAAATACCG-PH	C to G
	(r):CATGAAGCTGCCTCCCTTA	AATGCTCACCAGCCTCACTTC-FL	
LPL:N291S	(f):TGAGTTGTAGAAAGAACCGC	LC Red 640-TTTGGCTCTGACTTTACTGATCTCATAGC-PH	A to G
	(r): GGACTCCTTGGTTTCCTTATT	GAACGAGTCTTCAGGTACATTTTGCTGCTT-FL	

Pon1: Human Paraoxonase Gene 1; LPL: Lipoprotein lipase gene; FL: Fluorescein; PH: Phosphate end; f: forward primer; r: reverse primer

Table 2-6: Melting curve temperature calculation for selected candidate genes and polymorphisms

Gene: SNP	Amplicon length (bp)	GC %	Mutation position	WT Tm(°C)	Mutation Tm(°C)
PON-1:L55M	152	40.3	301	61.1	64.9
PON-1:Q192R	198	42.4	301	58.7	66.1
LPL:S447X	164	48.8	401	64.7	55.1
LPL:N291S	152	44.7	301	60.7	64.9

Tm: melting curve temperature; WT: wild-type

Polymerase chain reaction was performed by rapid cycling in a reaction volume of 10 µL with 0.5 µmol/L each primer, 0.2 µmol/L anchor and detection probes, 50 ng of genomic DNA and LightCycler 480 Genotyping Master. This is a ready-to-use hot-start reaction master mix designed specifically for single nucleotide polymorphisms detection with Melting Curve analysis, using allele-specific hybridization probes. The master mix is supplied as a 10-times concentrated stock solution containing nucleotides, *Thermus aquaticus* DNA polymerase, and 10 mmol/L Mg^{2+}. The PCR mix was pipetted into a 96-well Lightcycler Multiwell PCR reaction plate, to which were added 2, 5 µl of DNA template. The Multiwell plate was then sealed, centrifuged and then transferred to the plate holder of the Roche Light Cycler 480 Instrument, and the real-time PCR reaction was initiated.

After pre-incubation at 95 °C for 10 minutes, amplification was performed using 35 cycles of denaturation (95°C for 10 seconds), annealing (55°C for 10 seconds), and

extension (72 °C for 10 seconds). Observation of real-time amplification was possible as fluorescence was measured at the end of the annealing period of each cycle. When target amplification was completed, a melting curve stage was recorded by cooling the reaction mixture to 45°C, holding it at 45°C for 2 minutes and then slowly reheating it to 95°C. Fluorescence was measured continuously during the slow temperature ramp to monitor the dissociation of the fluorescein-labelled detection probe. A final cooling stage cooled the reaction mixture to 40°C in 30 seconds. The entire run took approximately 75 minutes in a homogenous setting.

2.8.4.5 NEGATIVE CONTROLS

At least two negative controls were run with each plate, by replacing the DNA template with PCR-grade water. This was done to detect contamination, if it was present.

2.8.5 Gel electrophoresis

In order to investigate whether PCR amplification was successful, 2µl of each PCR product was mixed with 5µl Gel red loading buffer (2 mg/ml Gel red and 35% (w/v) sucrose). The solution was loaded onto a 3% (w/v) horizontal agarose gel [6g agarose in 100 ml 1TBE]. A 100 base pair (bp) ladder was also loaded onto the gel to confirm PCR amplification of the correct fragment size. Electrophoresis was performed at 80 V for 90 minutes in 1TBE buffer solution. The bands were then visualised by ultraviolet light transillumination (GeneSnap MultiGenius Bio Imaging System, Syngene).

2.8.6 Genotyping by sequencing

2.8.6.1 PURIFICATION OF PCR PRODUCTS

PCR products were purified using the Qiagen Gel extraction Kit, according to the manufacturer's guidelines following gel electrophoresis, which was performed using a low DNA mass ladder, and run at 100 V for 60 minutes. The DNA fragment was then excised from the agarose gel with a scalpel, using the SynGene (Vacutech) transilluminator. The excised gel was weighed, to which was added 3 volumes of Buffer QG, and incubated at 50°C for 10 minutes until the gel dissolved. After following the manufacturer's protocol, the DNA was aliquoted into Eppindorf tubes and stored at -20°C until the sequencing reaction.

2.8.6.2 SEQUENCE REACTION

The SOP that was referred to, was designed by the Hasso Plattner Laboratory at the University of Kwazulu Natal (SEQ 002; Version 001).The method is summarized briefly as follows:

A master mix was prepared using the Terminator Ready Reaction Mix (Applied Biosystems) (0.4µl), 5 x concentration sequencing buffer (2.0 µl), primers for the specific SNPs (3.2pmol) and deionised water (up to 10 µl). The master mix was centrifuged and aliquoted into the 96-well plate. The purified PCR template was then added to the wells, centrifuged and placed in the thermal cycler under the conditions in Table 2-7.

Table 2-7: Stages for the sequence reaction

No of cycles	Temperature	Time	Process
1	96°C	60 seconds	Initial denaturation
25	96°C	10 seconds	DNA denaturation
	50°C	5 seconds	Primer annealing
	60°C	4 minutes	Primer extension
	4°C	HOLD	-

The PCR products were stored at 4°C, protected from light. Purification of the sequencing products were performed on the same day as the sequencing reaction.

2.8.6.3 PURIFICATION OF SEQUENCING PRODUCTS (PLATE CLEAN-UP)

The SOP that was referred to was designed by the Hasso Plattner Laboratory at the University of Kwazulu Natal (SEQ 003; Version 001), and was added to each well, as shown in Table 2-8.

Table 2-8: Purification of sequence products

Reagent	Per well
125mM EDTA pH8.0	1 µl
3M NaOAc Ph5.2	1 µl
100% Ethanol	25 µl

The plate was securely sealed with adhesive foil, vortexed briefly and centrifuged for 20 minutes at 3000 x g. The plate was then inverted onto a paper towel and centrifuged at 150 x g for 1 minute, to which 35 µl of 70% fresh cold ethanol was added to each well. Centrifuging, inverting and re-centrifuging were repeated as above, and samples were dried in the thermal cycler for 1 – 5 minutes at 50 °C. The plate was then sealed with an adhesive cover, wrapped in foil and stored at -20°C until sequencing.

2.9 Statistical analysis

The data was captured onto Microsoft Excel spread sheets, cleaned and transferred into SPSS Version 17 software package.

The distributional properties for all variables were investigated, and those that were not normally distributed were log-transformed, or analysed using non-parametric testing. Means and standard deviations (Mean±SD) and median values and inter-quartile ranges (IQR) (non-Gaussian distributed variables) were calculated for continuous variables and frequencies for categorical variables for descriptive purposes.

Categorical data was summarized as a percentage of the group total with corresponding 95% confidence intervals (CIs). Comparison among groups was made by the Chi-square test. Comparisons among groups were based on analysis of variance models. Post hoc analysis of variance comparisons of continuous variables were based on the t- test.

Frequency analysis was performed to determine the prevalence of risk factors in this population, as well as their relationships with each other and with echocardiographic

parameters. Echocardiographic parameters and genetic patterns were analysed in the entire sample, and further sub-group analyses were performed between the genders and the age groups. Subjects were then classified into those with and without the MS based on the ATP III, IDF and Harmonized criteria. Statistical differences were sought from clinical parameters, echocardiographic parameters and polymorphisms in all subjects, and then in sub-groups depending on age, gender, presence or absence of MS or each of its components. Differences were considered statistically significant when $p < 0.05$.

Logistic regression analysis, stratified for age and sex, was performed for prevalence of echocardiographic abnormalities among subjects with isolated MS and compared with those without the MS. Spearman's Rho was used to determine the strength of the correlation.

Linear models were constructed in order to investigate the association between each MS trait, and echocardiography variables. The Analysis of covariance (ANCOVA) was used to compare levels of echocardiographic variables (LVMI, LVM, LVPW, LVS, LVEDD, Em/Am and SEAT) between subjects with and without MS. SEAT values (in mm) were used in logistic regression to determine its contribution to the diagnosis of MS.

Receiver operator characteristic (ROC) curve analysis was used to determine the discriminating capacity of biochemical, anthropometry measurements, genotypes and echocardiography parameters in determining the presence of MS. The area under the curve (AUC) was used determine the predictive efficiency of each of these variables.

The genetic analysis was performed on coded subjects identified by study number. The analysis was performed without prior knowledge of the grouping of subjects into those with and without MS.Gene counting was used for the estimation of genotype and allele frequencies and was expressed as percentages of the totals.

Cross-tabulations were constructed with MS and the MS components with the genotype category. Non-parametric variables were expressed as mean ranks and the Kruskal-Wallis test was used to determine the significance. Median values for MS components as well as echocardiography variables were compared between the three genotype groups. Hardy Weinberg equilibrium was calculated once the allele frequencies in the population were known.

2.10 Ethical considerations

The applicant received full ethical clearance for this study from BREC for echocardiography and blood storage for genetic studies, and this was received on 23rd October 2009 (BE 172/09). Recertification of ethics for the Phoenix Lifestyle Project has been granted by the University of KwaZulu-Natal Bioethics committee. Ethics number: E336/05.

CHAPTER THREE

Results

3.1 Demographic data

The study comprised 1428 randomly selected subjects from the suburb of Phoenix, which is situation in the EThekwini Municipality of KwaZulu-Natal. The sample was dominated by females, making up 72% of the sample, whilst the male component comprised 28%. The mean age (±SD) of the sample was 43 (±15) for males and 46 (± 12) for females. The mean age of the total sample was 45.5 (±13). The highest number of subjects within the sample was in the 45-54 year age group (Table 3-1).

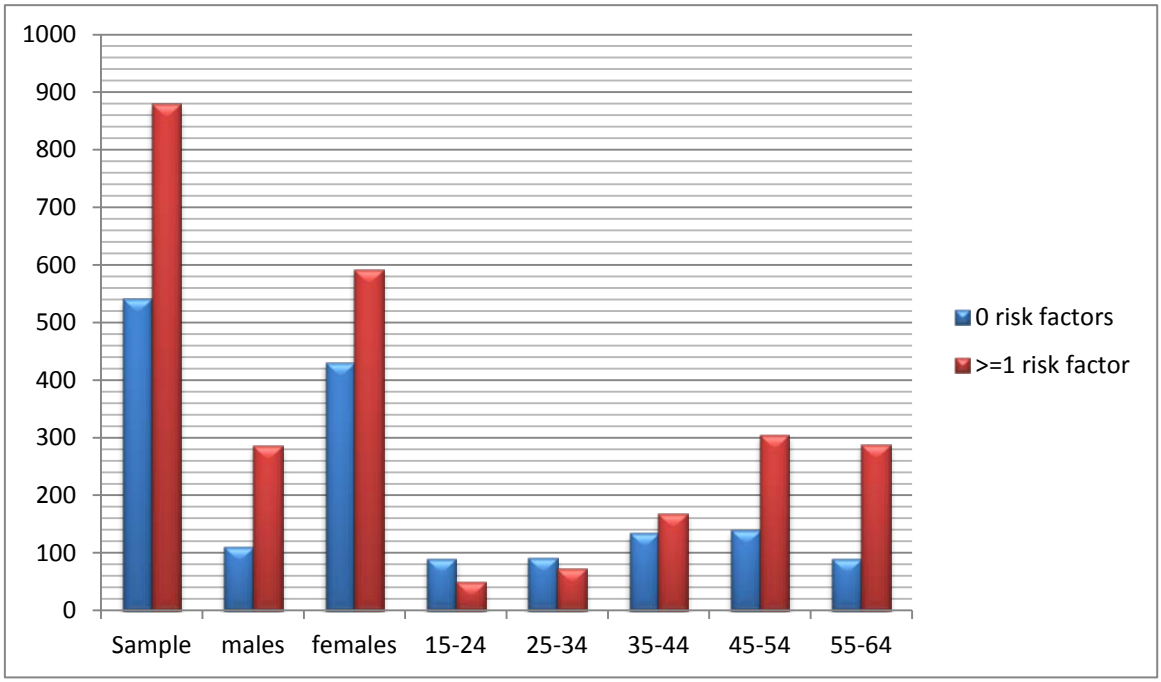
Table 3-1: Distribution of subjects

Age group	n	Males (n %)	Females (n %)
15-24	140 (10%)	58 (41%)	82(59%)
25-34	165 (12%)	63(38%)	102(62%)
35-44	304 (21%)	76(25%)	228(75%)
45-54	442 (31%)	89(20%)	353(80%)
55-64	377 (26%)	116(31%)	261(69%)
TOTAL	1428	402(28%)	1026 (72%)
Mean age	45.5 ± 13	43.43(±15)	46(± 12)

3.2 Prevalence of cardiovascular risk factors

There were 543 (38%) subjects who did not exhibit any of the 5 common cardiovascular (CV) risk factors (elevated total cholesterol and triglyceride levels, decreased high-density lipoprotein [HDL], impaired fasting glucose and hypertension) that were tested for. The remaining 62% of subjects in the sample presented with 1 or more CV risk factors (Figure 3.1). There was an increase in the proportion of subjects with CV risk factors with advancing age. The median and interquartile range (IQR) values for the major risk factors for cardiovascular disease in this sample are shown in Table 3-2.

FIGURE 3.1: Distribution of subjects with and without CV risk factors

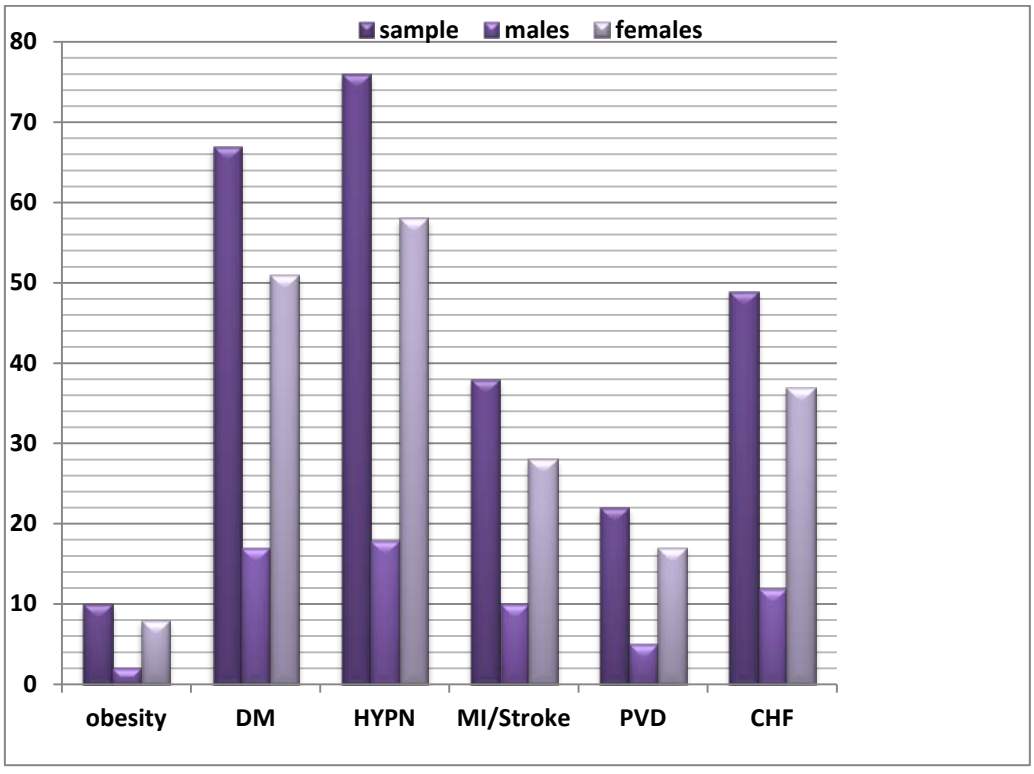


The gender and age distribution of subjects with CV risk factors: subjects with no risk factors are shown in blue. The highest proportion of subjects with >1 risk factor is in the 45-54 age group.

3.2.1 Family History of Cardiovascular risk factors

A positive family history for CV risk factors (obesity, Type 2 Diabetes Mellitus [DM], hypertension, myocardial infarction/stroke and heart failure[CHF]) was recorded in all subjects. The main disease reported in this sample was a positive family history of hypertension (76%) in both males (68%) and females (79%). This was followed DM (67**%**) in both genders (Figure 3.2). Almost half of all subjects (48 %) reported a positive family history for heart failure (Figure 3.2).

FIGURE 3.2: Frequencies of positive family history for CV risk factors



The gender-wise distribution of subjects with a positive family history for CVD: the most frequent positive history reported is hypertension.

3.2.2 Anthropometric parameters

There was a high prevalence of raised BMI (66%) in this population (Table 3-7a). The frequency of raised BMI increased from the 1st to the 4th age groups (19% to 39%), and decreased by the 5th age group (Table 3-7a). Using the standard guidelines of interpretation, 32% of subjects were classified as being overweight (BMI >25<30 kg/m^2), and remaining 34% were classified as being obese (≥ 30kg/m^2) (Table 3-7a). Using the ethnic-specific cut-off for BMI for Asian Indians (≥23 kg/ m^2) (WHO Expert Consultation, 2004), the prevalence of obesity (≥25 kg/m^2) increased to 64% (Table 3-7a). There were significant differences between the genders (p = 0.000), as a raised BMI was more prevalent amongst the females (84%).

The median and inter-quartile range (IQR) waist circumference measurement for males was 89 cm (77, 98) and 95 cm (85,105) in females (Table 3-2). Using ethnic-specific criteria as specified by the International Diabetes Federation (IDF) [90cm in males and 80 cm in females], the waist circumference was increased in 51% of males and in 82% of females (Table 3-7a).

3.2.3 Lifestyle and behavioural characteristics

Smoking was reported by 26% of subjects, with the largest number of smokers observed in the youngest (29%) and oldest (28%) age groups, respectively (Table 3-7a). There were significantly more male than female smokers (p = 0.001).

3.2.4 Clinical observations

The median (IQR) systolic blood pressure (SBP) was 130 mmHg (118,144) and the median (IQR) diastolic blood pressure (DBP) was 79 mmHg (77, 98) in males. In the female group, the median (IQR) SBP was 131 mmHg (118, 146) and the median (IQR) DBP being 81 mmHg (74, 90) mmHg. There were no significant differences observed between the genders. There was a gradual increase in both the mean systolic and diastolic blood pressure accompanying advancing age (Table 3-2).

Hypertension was documented in 49% of subjects (Table 3-7a). There was a marked increase in the prevalence of hypertension observed from the 1st (6%) to the 5th (71%) age groups. There were 211 (15%) subjects who were previously undiagnosed with hypertension (Table 3-3).

Prehypertension (*SBP/DBP 120-139 mm Hg/ 80- 89 mm Hg*) was found in 676 subjects (49%), with the prevalence distributed evenly between males and females. However, there was an increase in the frequency of prehypertension observed with increasing age.

A positive family history for hypertension was recorded in 78% (1072) of participants, of whom 40% (551) were hypertensive already. This translated into a doubling of risk for hypertension (OR= 2.08; 95% CI=1.6, 2.7) when a positive family history was present (Table 3-4).

Table 3-2: Median and inter-quartile ranges (25th-75th) for physiological, anthropometry and biochemical measurements

Parameter	Males	females	P	15-24	25-34	35-44	45-54	55-64
BMI (kg/m2)	24.6(21.2,27.8)	28.6(25,33)	**0.001***	21.7(18.3,26.6)	27(22,32)	28(24,32)	28.2(25,32)	27.15(23.8,31)
WC (cm)	89(77, 98)	95(86,105)	**0.001***	77(70, 88)	90(79,104)	94(84,104)	96(88,105)	95(88,103)
SBP (mmHg)	130(118,144)	131(118,146)	0.83	117(108,125)	122(113,136)	125(114,139)	135(123,148)	140(127,155)
DBP(mmHg)	79(71,89)	81(74,90)	0.253	70(64,78)	79(72,87)	81(73,90)	82(75,92)	82(75,91)
FBG(mmol/l)	5.1(4.7,5.9)	5.3(4.8,6.5)	**0.008***	4.7(4.5,5.0)	4.9(4.5,5.2)	5.0(4.7,5.7)	5.5(5.0, 6.7)	5.8(5.1,8.1)
GTT(mmol/l)	6.3(5.2,8.4)	7.2(5.9,9.5)	**0.001***	5.5(4.7,6.5)	6.1(5.3,7.7)	6.8(5.9,8.6)	8.0(6.2,10)	7.7(5.9, 10)
TC (mmol/l)	5.3(4.5,6.1)	5.4(4.6,6.1)	0.219	4.4(3.9,5.0)	5.0(4.5, 5.6)	5.3(4.6,6.0)	5.6(4.9, 6.3)	5.7(5.0, 6.4)
TG (mmol/l)	1.5(1.1,2.2)	1.5(1.1,2.0)	0.644	0.9(0.7,1.1)	1.4(1.0, 1.8)	1.5(1.1, 2.0)	1.8(1.2,2.1)	1.7(1.3,2.3)
HDL(mmol/	1.17(1.0, 1.34)	1.3(1.1, 1.5)	**0.001***	1.3(1.09,1.5)	1.2(1.0,1.4)	1.2(1.02,1.4)	1.3(1.1,1.5)	1.3(1.1.,1.5)
HOMA-IR (*iu*)	2.09 (1.2,4.1)	3.3(1.9,5.4)	0.50	1.9(1.3,3.3)	2.6(1.7, 4.2)	3.0(1.6, 4.87)	3.2(1.9,5.4)	3.3(1.7,6.0)

BMI: Body mass index, WC: Waist circumference; TC: Total cholesterol, HDL: high-density lipoprotein; TG: triglyceride, FBG: fasting blood glucose; GTT: 2-hour fasting plasma glucose concentration, SBP: Systolic blood pressure; DBP: diastolic blood pressure

***Significant differences between males and females**

Table 3-3: The crude prevalence of Hypertension and prehypertension

n	Males 377	Females 1001	Prevalence 1378	15-24 128	25-34 161	35-44 298	45-54 430	55-64 374
Systemic hypertension	157 (42%)	512 (51%)	669 (49%)	8(6%)	39 (24%)	114 (38%)	246 (57%)	266 (71%)
Self-reported	91(24%)	367(37%)	458 (68%)	5(4%)	12(7%)	61(20%)	168(39%)	214(57%)
Previously unknown	66(18%)	145 (14%)	211 (32%)	3(2%)	27(17%)	53 (18%)	78 (18%)	52 (14%)
Prehypertension	189(50%)	487(49%)	676 (49%)	51(40%)	79(49%)	137(46%)	223(52%)	191(51%)

Table 3-4: Positive family history of Hypertension

n	Males (n) 377	Females(n) 1001	Prevalence 1378	15-24 128	25-34 161	35-44 298	45-54 430	55-64 374
Family history	268(71%)	804(80%)	78%	99(77%)	124(77%)	226(76%)	332(77%)	296(79%)
Prehypertensive#	130(34%)	384(38%)	37%	35(27%)	61(39%)	106(36%)	167(39%)	149(40%)
Hypertensive*	119(44%)	432(54%)	40%	7(7%)	32(26%)	91(40%)	203(61%)	222(75%)

[#]p (trend) =0.847; OR (95%CI) = 1.03(0.8; 1.3)
**** p (trend) =<0.001; OR (95%CI) = 2.08(1.6; 2.7)***

3.2.5 Biochemical parameters

3.2.5.1. BLOOD GLUCOSE AND INSULIN

The median fasting blood glucose levels in males was 5.1 mmol/l and significantly higher (5.3 mmol/l) in females (p= 0.008) (Table 3-2). There was a steady increase in the fasting blood glucose levels observed with increasing age.

There was a total number of 455 (32%) subjects who were classified as diabetic, with the highest prevalence (49%) observed in the 55-64 year age group. Of the diabetic subjects, 16 % of males and 22 % of females self-reported their status, leaving over one third of diabetic subjects who did not know their status (Table 3-5).

The prevalence of DM increased with advancing age and was significantly higher in females (p = 0.001). Of note, there was a high prevalence of DM (38%) in subjects who had a first-degree diabetic family member, and this was associated with an almost 3-fold likelihood (OR= 2.53; 95% CI=1.94, 3.3) of subjects with positive family history developing DM themselves (Table 3-6).

Using the guidelines proposed by the American Diabetes Association (2012) for the classification of prediabetes, we found a prevalence of 20% in male subjects and of 25% in female subjects with prediabetes, at the time of testing (Table 3-5).

There was also a high frequency of insulin resistance observed in this sample (58%), which increased progressively with advancing age. The highest prevalence was observed in the 4th and 5th age groups, with 61% of subjects in each age group being classified as insulin resistant, as assessed by the HOMA-IR index (Table 3.7a).

Table 3-5: The crude prevalence of Diabetes and Prediabetes

n	Males 377	Females 1001	Prevalence 1378	15-24 128	25-34 161	35-44 298	45-54 430	55-64 374
DM prevalence	96 (25%)	349 (35%)	445(32%)	8(6%)	20(12%)	80(27%)	155(36%)	183(49%)
Self-reported	60(16%)	221(22%)	281(63%)	3 (1%)	5 (3%)	52 (17%)	98 (23%)	123 (33%)
Previously unknown	36 (10%)	128 (13%)	164(37%)	5 (4%)	15 (9)%	28(9%)	57(13%)	60(16%)
Prediabetes[#]	76(20%)	250(25%)	326(24%)	9 (7%)	34 (21%)	74 (25%)	154(36%)	106(28%)

 # using IFG and IGT criteria

Table 3-6: Positive family history of Diabetes Mellitus

N	Males 377	Females 1001	15-24 128	25-34 161	35-44 298	45-54 430	55-64 374
Family history	245(65%)	703(70%)	92(72%)	95(59%)	209(70%)	297(69%)	260(70%)
Median FBG (mmol/l)	6.6	6.7	5.0	5.3	6.3	7.0	7.2
Prediabetic #	67 (27%)	225 (32%)	7(8%)	16 (17%)	60(28%)	112(38%)	88(34%)
DM*	73 (19%)	287 (29%)	8(9%)	25(26%)	66(22%)	130(44%)	142(55%)

[#]p (trend) =0.21; OR (95%CI) = 1.062(0.83; 1.36)

**** p (trend) =<0.001; OR (95%CI) = 2.53(1.94; 3.3)***

3.2.5.2 LIPID PROFILES

The median (IQR) value of total cholesterol (TC) was recorded as 5.3 mmol/l (4.5, 6.1) (Table 3-2). There was an 18% (cf 17%) prevalence of elevated total cholesterol (≥ 6.5 mmol/l), when applying the cut-offs used by Seedat *et al* (1990). This was most prevalent in the 55-64 year age groups (Table 3-7b). This prevalence tripled to 60% in both genders when the NCEP cut-off (5.1 mmol/l) was applied. There was a steady increase in the prevalence of raised TC levels with advancing age, with 70% of subjects in the 55-64 age group recording elevated levels of total cholesterol.

The median (IQR) triglyceride value (Table 3-2) was 1.5 mmol/l (1.1, 2.1), with elevated triglyceride levels (> 2.83 mmol/l) being detected in 11% of the sample. The highest prevalence was recorded in the 25-34 year age groups, with 16% of subjects presenting with hypertriglyceridemia. A higher prevalence of hypertriglyceridemia was observed when the new NCEP (Grundy *et al*., 2001) cut-off (>1.69 mmol/l) was applied, quadrupling the prevalence to 44%, with an increasing trend noted with advancing age (Table 3-7b).

The median HDL levels were within normal limits across all age groups, with 14% of the sample presenting with levels lower than <1.0 mmol/l (Table 3-7b). There was a similar dyslipidaemia pattern in the 25-34 year age groups, which recorded the highest prevalence of abnormal HDL levels (29%), with males presenting more frequently with decreased HDL levels. Using the NCEP cut-off for HDL (< 1.04 mmol/), the prevalence of

decreased HDL levels increased to 23% (Table 3-7b).

3.2.6 Overall risk factor profile

There were significant differences between males and females for six out of the nine risk factors that were studied (Table 3-7a; 3-7b). Females were more prone to obesity, insulin resistance, DM and hypertension (Table 3-7a). There was a higher prevalence of low HDL cholesterol in men (24%) compared to women (10%). As mentioned earlier more than half the men were smokers.

The most common risk factor in this sample was an increased waist circumference (using the IDF criteria), which was found in 79% of subjects, followed by general obesity in 64% of subjects (Table 3-7a). The most common biochemical abnormality was hypercholesterolemia, found in 60% of subjects (Table 3-7b). Both men and women were insulin resistant (58%) with a higher prevalence documented in women (63%).

Table 3-7a: Distribution and frequency of selected major cardiovascular risk factors: Clinical characteristics, BMI and waist circumference

n= 1378#	Males 377	Females 1001	p	15-24 128	25-34 161	35-44 298	45-54 430	55-64 374	Prevalence
BMI < 25kg/m^2	146 (39%)	323 (32%)	ns	86(67%)	58(36%)	78(26%)	80(19%)	103(27%)	**469(34%)**
>25<30 kg/m^2	128 (35%)	318 (33%)	ns	22(17%)	38(24%)	94(32%)	166(39%)	126(33%)	**446(32%)**
>30 kg/m^2	51 (14%)	419 (42%)	**0.000***	24(19%)	59 (37%)	112 (38%)	160 (39%)	119(30%)	**474(34%)**
<23 kg/m^2**	155 (43%)	161 (16%)	**0.000***	82 (64%)	47 (29%)	62(20%)	52(12%)	73(20%)	**317 (23%)**
23.0 to 24.9 kg/m^2	60 (16%)	116 (12%)	ns	10 (1%)	20 (11%)	33 (11%)	55(12%)	59(15%)	**177(13%)**
>25 kg/m^2**	179 (47%)	702 (70%)	**0.000***	46 (36%)	97(60%)	208(69%)	331(76%)	246(65%)	**881(64%)**
Waist*(cm)**	192(51%)	856 (82%)	**0.000***	47(37%)	103(64%)	233(78%)	368(86%)	297(79%)	**1048(79%)**
Diabetes									
HOMA- IR (>2.6)	165 (44%)	626 (63%)	**0.000***	52 (41%)	84 (52%)	171 (55%)	261(61%)	227(61%)	**795(58%)**
DM (%)	96 (25%)	349(35%)	**0.001***	8(6%)	20(12%)	80(27%)	155(37%)	183(49%)	**445 (32%)**
Hypertension and smoking									
BP$^{\&}$:≥140/≥90	157 (42%)	512 (51%)	**0.001***	8(6%)	39 (24%)	114 (38%)	246 (57%)	266 (71%)	**669 (49.5%)**
Smokers (%)	208 (53%)	144 (14%)	**0.001***	37(29%)	33(20%)	69 (23%)	93(22%)	103(28%)	**235(26%)**

***indicates statistical significance; #n due to missing values; BMI: Body mass index, FBG: fasting blood glucose; SBP: Systolic blood pressure; DBP: diastolic blood pressure; **Modified criteria for Asian Indians (WHO Expert Consultation, 2004); ***IDF criteria**

Table 3-7b: Distribution and frequency of major cardiovascular risk factors: Lipids

n= 1378#	Males 377	Females 1001	p	15-24 128	25-34 161	35-44 298	45-54 430	55-64 374	Prevalence
TC > 6.5mmol/l^	67 (18%)	183(18%)	ns	6(5%)	12(7%)	46(16%)	95(22%)	91(24%)	**250(18%)**
TC > 5.17mmol/l!	227 (60%)	588 (59%)	ns	25(20%)	73(45%)	168(56%)	292(68%)	263(70%)	**821(60%)**
HDL (< 1.0mmol/)^	91(24%)	96(10%)	**0.000***	17 (13%)	47 (29%)	47(16%)	46(11%)	29(8%)	**187(14%)**
HDL (< 1.04mmol/)!	133(35%)	183(18%)	**0.000***	32(25%)	46(29%)	80(27%)	84(20%)	77(21%)	**319(23%)**
TGL(>2.83mmol/l)^	51 (14%)	100(10%)	ns	3 (2.1%)	26(16%)	26(9%)	48(11%)	46(12%)	**149(11%)**
TG (>1.69mmol/l)!	175(46%)	424(42%)	ns	18(14%)	45(28%)	118(40%)	217(50%)	202(54%)	**600(44%)**

***indicates statistical significance**

TC: Total cholesterol, HDL: high-density lipoprotein; TGL: triglyceride, ^ (Seedat et al., 1990) ;!(NCEP guidelines, 2001); BP&: JNC criteria

3.2.4 Correlation between cardiovascular risk factors

The correlation between individual risk factors is shown in Table 3-8. The analysis, using Spearman's Rho showed a strong correlation (r_s > 0.500), shown in darkest purple between the BMI, waist circumference, fasting blood glucose and HOMA-IR values. A moderate correlation (r_s > 0.300 < 0.500), coded dark purple (Table 3 - 8) was observed between triglyceride levels and the waist circumference, FBG, glucose tolerance test values, total cholesterol and HOMA-IR values. A moderate inverse correlation existed between triglyceride and HDL levels. The remaining associations were weak, but statistically significant (r_s > 0.100 < 0.300, shown in lightest purple). All correlations with HDL were inversely orientated, where a lower level of HDL was moderately correlated with higher levels of triglycerides, and weakly correlated with fasting blood glucose, total cholesterol, HOMA-IR, BMI, waist circumference and blood pressure.

Table 3-8: Spearman's Rho correlation between risk factor variables

Parameter	BMI	WC	FBG	2-H	TC	TGL	HDL	SBP	DBP	HOMA-IR
BMI	1	+++	+	++	+	+	-	+	++	+++
WC	+++	1	++	++	+	++	-	++	+	+++
FBG	+	++	1	+++	+	++	-	+	+	+++
2-Hr GTT	++	++	+++	1	+	++	-	+	+	++
TC	+	+	+	+	1	++	-	+	+	+
TGL	+	++	++	++	++	1	--	+	+	++
HDL	-	-	-	-	-	--	1	NS	-	-
SBP	+	++	++	+	+	+	NS	1	+++	+
DBP	++	++	+	+	+	+	-	+++	1	+
HOMA-IR	+++	+++	+++	++	+	++	-	+	+	1

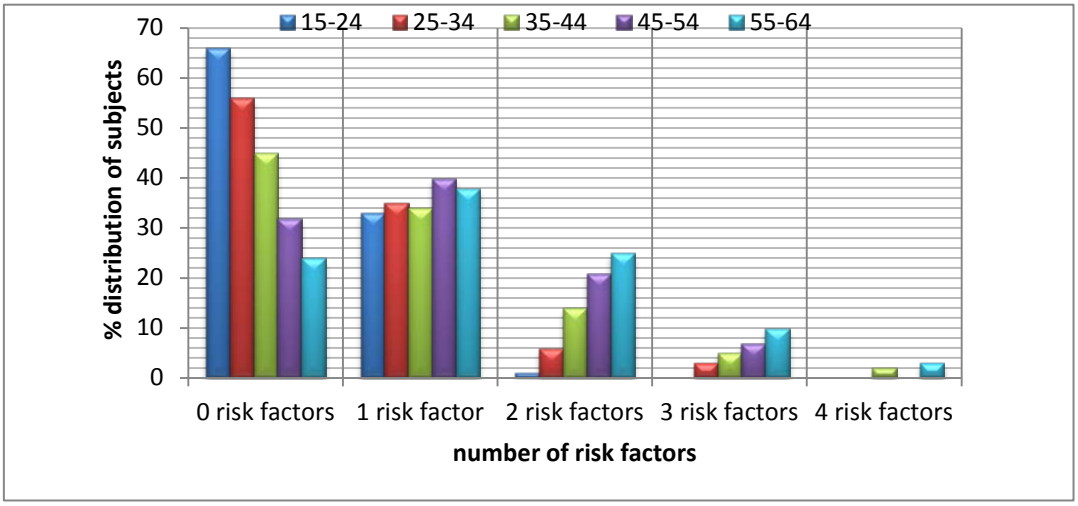
BMI: Body mass index, WC: waist circumference; FBG: fasting blood glucose, 2-H: 2-hour GTT; TC: Total cholesterol, TGL: triglyceride; HDL: high-density lipoprotein; SBP: Systolic blood pressure (mean); DBP: diastolic blood pressure (mean); HOMA-IR: values as measured by HOMA-IR index.

KEY: + (weak); ++ (moderate);+++ (strong) ; -(weak inverse); -- (moderate inverse);--- (strong inverse)

3.2.5 The age distribution of cardiovascular risk factors

The distribution of CV risk factor clustering (elevated total cholesterol and triglyceride levels, decreased HDL, impaired fasting glucose and hypertension: WHO cut-off) is shown in Figure 3.3 below. There was a proportional inverse relationship between the absence of CV risk factors and age, where the proportion of subjects without CV risk factors decreased with advancing age. The majority (66%) of subjects in the 15-24 year age group presented with no risk factors, as compared to 24% in the 55-64 year age group. Of note, we documented 33% of subjects in the 15-24 year age group who presented with a single risk factor, with under 2% who presented with 2 CV risk factors. A slightly higher percentage was observed in the 25-24 year age group (35% with 1 CV risk factor, 6% with 2 CV risk factors) and a smaller number (3%) with 3 CV risk factors. The 35-44 year age group contained 30% of subjects with 0 risk factors, 40% with 1 risk factor, 21% with 2 risk factors and 7% with 3 risk factors, with approximately 2% of subjects who had 4 risk factors. There were approximately 3% of subjects in the 55-65 year age group who also had 4 risk factors. There were no subjects who tested positive for all 5 risk factors.

FIGURE 3.3: Distribution of traditional risk factor clustering according to age



The highest number of subjects without conventional CV risk factors was in the 15-24 age groups. This proportion decreased with advancing age.

3.3 The Metabolic Syndrome

3.3.1 The prevalence of the Metabolic Syndrome

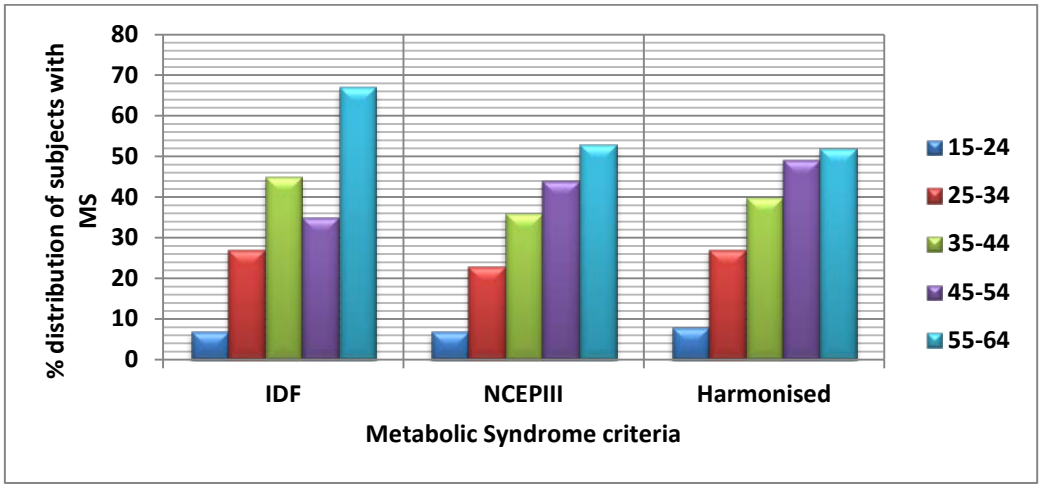
The crude prevalence of the metabolic syndrome (MS) was 38% (518 subjects) using the criteria proposed by the Adult Expert Panel (ATP) III panel, which increased to 46% when the International Diabetes Federation (IDF) criteria was applied (Figure 3.4). According to the Harmonized criteria (Alberti *et al*., 2009), the prevalence of MS was 41% (Table 3-9).

Table 3-9: Gender-wise prevalence of the Metabolic Syndrome according to IDF, NCEP ATP III and Harmonized criteria

	IDF	NCEP ATP III	Harmonized
Males	131/398 (33%)	90(23%)	128/398 (32%)
Females	536/1024(52%)	427(43%)	462/1026 (45%)
Prevalence	**667/1422 (46%)**	**518/1374 (38%)**	**590/1424 (41%)**

The general trend across all 3 criteria was a higher prevalence of the MS in females, with these differences being significant between males and females ($p < 0.005$).

FIGURE 3.4: Age-wise prevalence of the Metabolic Syndrome according to IDF, NCEP ATP III and Harmonized criteria



The age-wise pattern of the metabolic syndrome prevalence: apart from the IDF definition, there was a distinct linear increase in prevalence with increasing age, with the lowest prevalence in the youngest age groups.

The age-wise prevalence showed a corresponding increase in the prevalence of MS with increasing age (Figure 3.4), where the 55-64 year age group had the highest prevalence, subject to the definitions used [67% (IDF), 53% (NCEP) and 50% (Harmonized)]. There was a sharp increase in the prevalence of MS from the 25-34 year age group (27%) to the 35-44 year age groups (45%) when the IDF definition was applied, a pattern which was not seen with the other criteria.

3.3.2 The Kappa statistic

Of the total 1422 participants, 619 (43%) had a concordant positive diagnosis according to both the IDF and Harmonized definitions of MS, and 720 (51%) were negative according to both definitions (Table 3-10). The total percentage of observed agreements was in 1339 (94%) of subjects, with 83 (6%) subjects being discordant. Thus the kappa statistic indicated a strong level of agreement (almost perfect [Landis and Koch, 1997]) between the two tests (Kappa = 0.883).

The agreement between the NCEP ATP III and Harmonized definitions yielded a Kappa of 0.812. There was therefore a stronger agreement between the IDF and Harmonized than the NCEP ATP III and Harmonized definitions (Table 3-10).

Table 3-10: Degree of agreement between the Harmonized, NCEPA III and IDF criteria

		Harmonized criteria		
		No	**yes**	**Total**
IDF	**no**	720	35	755
	yes	48	619	667
Total		768	654	1422
Kappa= 0.883				

		Harmonized criteria		
		No	**yes**	**Total**
NCEP III	**no**	729	0	**729**
	Yes	127	518	**645**
Total		**856**	**518**	**1374**
Kappa= 0.812				

3.3.3 The frequency and clustering of MS components

Figure 3.5 below shows the distribution and clustering of MS components in subjects according to age. These subjects were classified with MS using the IDF criteria, which, in addition to using ethnic-specific cut-offs, was able to identify the highest number of individuals with MS.

There were 35% of subjects in the youngest age groups having no components for MS (Figure 3.5). The 45-54 year age groups prominently displayed the highest percentage of subjects with a single MS component and subsequent clustering of MS components. Furthermore, a third of subjects in this age group had all 5 components of MS, exceeded

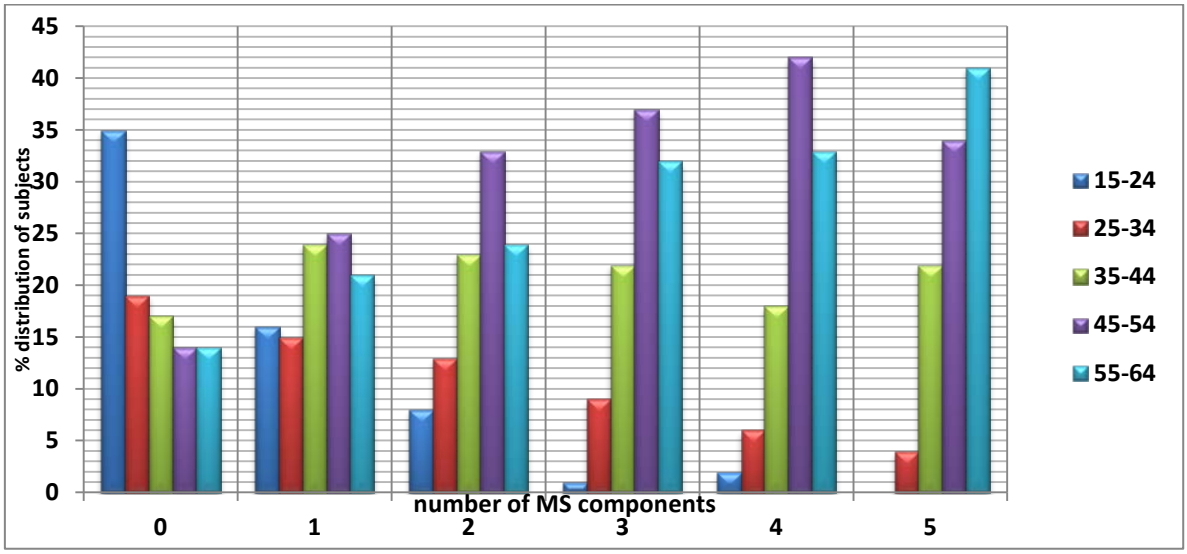
only by the 55-64 year age groups, where 41% of subjects in this age group showed clustering of all 5 risk factors.

The most frequent components contributing to the diagnosis of MS using the NCEP criteria was an increased waist circumference ([males >102 cm] [females >88 cm) in 84.2% of subjects, followed by elevated triglycerides levels (> 1.7 mmol/l) in 78% of subjects (Figure 3.7). There was an increase in the frequency of abdominal obesity in all subjects (100%) with MS as defined by the IDF criteria ([males ≥ 90 cm][females ≥ 80cm]), followed by elevated triglycerides levels (> 1.7 mmol/l) which was observed less frequently, but significantly, in 67% of subjects with MS (Table 3-11).

A similar trend was observed when the Harmonized criteria was applied, with an increased waist circumference ([males ≥ 90 cm] [females ≥ 80cm]) seen in 84.2% of subjects, followed by elevated triglycerides levels (> 1.7 mmol/l) in 78% of subjects with MS (Table 3-11).

Impaired fasting glucose and hypertension were the least common abnormalities observed across all three criteria. As a result of the application of the IDF criteria, the greatest number of subjects with MS was identified. Hence, we present subsequent analysis for the IDF criteria only in this population with a high propensity for CVD.

FIGURE 3.5: Clustering of Metabolic Syndrome components according to age groups (IDF criteria)



The clustering of MS components according to age: the youngest age group had the largest proportion of subjects with no MS components, while the oldest group had the largest proportion with all 5 MS components.

Table 3-11: Distribution of MS components in subjects with and without MS

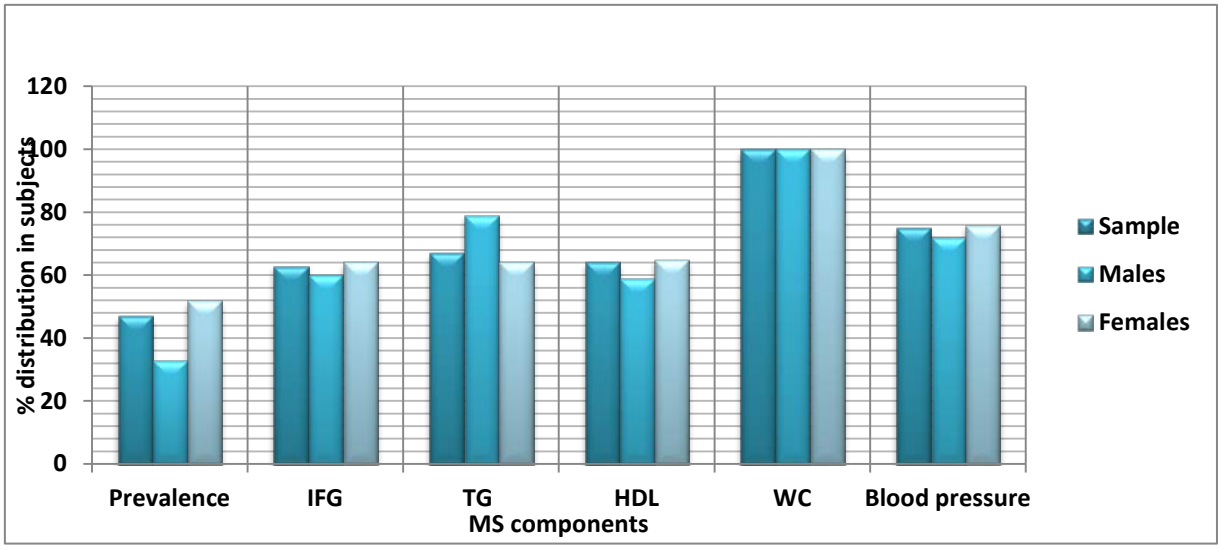
Variables	NCEP ATP III	
	Yes (n = 518)	**No (n = 856)**
Fasting Glucose ≥ 6.1 mmol/L	310 (60%)	86 (10%)
TG ≥ 1.7 mmol/L	396 (78%)	186 (21.7%)
HDL [males <1.03] [females <1.29] mmol/L	376 (72.6%)	230 (26.9%)
Waist Circumference[males >102cm][females >88cm]	436 (84.2%)	317 (37%)
Blood Pressure ≥130 / ≥85 mmHg	301 (60.1%)	143 (16.7%)
	International Diabetes Federation	
	Yes (n = 667)	**No (n = 755)**
Waist Circumference[males ≥ 90 cm][females ≥ 80cm]	667 (100%)	381 (51%)
Fasting Glucose ≥ 5.6 mmol/l	423 (63.4%)	111 (14.7%)
TG > 1.7mmol/l	447 (67%)	140 (19%)
HDL [males <1.03] [females <1.29]	427 (64%)	188 (25%)
Blood Pressure ≥ 130 / ≥ 85 mmHg	325 (51%)	118 (16.3%)
	Harmonized Criteria	
	Yes (n = 518)	**No (n = 856)**
Fasting Glucose ≥ 6.1 mmol/L	329 (56%)	256 (43%)
TG ≥ 1.7 mmol/L	446 (76%)	140 (24%)
HDL [males <1.03] [females <1.29] mmol/L	417 71%)	170 (29%)
Waist Circumference[males ≥ 90 cm][females ≥ 80cm]	565 (96%)	25 (4%)
Blood Pressure ≥130 / ≥85 mmHg	327 55%)	244(75%)

3.3.4 The frequency of individual MS components in males and females

Analysis of the frequency of the MS components between the genders in Figure 3.6, using the IDF criteria indicated that there was a significantly higher prevalence of MS in females, as compared to males ($p < 0.05$); with females more likely to present with abdominal obesity ($p < 0.05$). There were also significant differences between males and females for the prevalence of impaired fasting glucose levels ($p = 0.000$), with females having a higher frequency of elevated triglycerides, elevated blood pressure and decreased HDL levels ($p= 0.000$).

Analysis of males and females showed differing trends in terms of the most common MS component. In male subjects with MS, the most common MS component was an elevated triglyceride level, present in 79% of subjects (Figure 3.6). In addition, all of them had an increase in waist circumference measurements in accordance with the IDF criteria. In females, hypertension (76%) was the most common components of MS, followed by decreased HDL levels (65%).

FIGURE 3.6: Gender-wise distribution of Metabolic Syndrome components in subjects with MS using the IDF criteria



Although the prevalence of MS was significantly higher in females, distribution of the MS components was almost equally distributed in both genders, aside from the elevated triglyceride levels, which was more frequent in males.

3.3.5 Clinical and metabolic criteria in relation to the glycaemic profile

The prevalence of CVD risk factors and the MS (IDF criteria) are described in males and females, as well as in subjects with normal blood glucose, impaired fasting glucose (IFG) and DM (Table 3-12). There were significant inter-gender differences, as a significantly higher proportion of females were diagnosed with MS ($p < 0.05$), with the median FBG ($p = 0.013$), BMI and waist circumference measurements ($p = 0.000$) being significantly higher in females than males.

In the total sample, there were 600 subjects with a normal glycaemic profile, 328 with prediabetes and 456 subjects with DM. The frequency of MS was highest in subjects with

DM (53%), with 25% of subjects with MS being prediabetic and 20% normoglycaemic (Table 3-12).

There was a worsening of the lipid profile as the glycaemic profile changed from normal to prediabetic, and then to DM. The median systolic and diastolic blood pressures were also higher in diabetics as compared to those with normal and prediabetic blood glucose profile. Similarly median BMI and waist circumference increased with the transition from normoglycaemia to prediabetes and DM (Table 3-12).

Table 3-12: IDF criteria for Metabolic Syndrome (median IQR) according to glycaemic levels (median plasma glucose levels)

	Gender			Blood glucose profile(n=667)		
Parameter	**Males(n=398)**	**Females(n=1024)**	**p-value**	**Normal**	**Prediabetic**	**Diabetic**
Prevalence of MS (n=667)	131 (33%)	536(52%)	**<0.05***	131(20%)	166(25%)	370 (55%)
Total cholesterol (mmol/l)	5.5(4.8,6.2)	6.1(5.2,8.6)	0.355	5.1(4.4,6.8)	5.4(4.7,6.1)	5.6(4.9,6.4)
Fasting Glucose (mmol/l)	6(5, 9.1)	6.1(5.2,8.6)	**0.013***	4.9(4.6,5.1)	5.8(5.4,6.2)	14.6(9,21)
Triglycerides (mmol/l)	2.3(1.8,3.0)	1.9(1.5,2.4)	0.79	1.3(0.9,1.7)	1.6(1.2,2.1)	1.9(1.4,2.6)
HDL (mmol/l)	1(0.9,1.15)	1.2(1.04,1.33)	**0.000***	1.3(1.1,1.5)	1.24(1.1,1.4)	1.2(1.02,1.4)
Systolic Blood Pressure (mmHg)	142(128,155)	139(127,153)	0.63	123(114,137)	135(123,149)	138(127,153)
Diastolic BP (mmHg)	89(80,97)	86(77,93)	0.12	77(69,86)	82(75,91)	85(76,92)
HOMA-IR	4.2(2.6,6.6)	4.7(2.9,7.5)	0.356	1.9(1.2,2.99)	3.5(2.2,5)	5.3(3.2,9.2)
Waist Circumference (cm)	99.5(94,105)	100.5(93,109)	**0.000***	88(77,98)	97(89,105)	97(89,106)
BMI (kg/m^2)	27.8(26,30.1)	30(26.7,34.8)	**0.000***	25(21,30)	29(26,33)	28.5(25,33)

indicates statistical significance: p < 0.05

3.4 The Echocardiographic analysis

There were 1420 subjects who yielded optimal image quality for the evaluation of two-dimensional measurements, and for pulsed-wave (PW)-Doppler evaluation of diastolic indices. Tissue Doppler indices (TDI) and measurement of the sub-epicardial adipose tissue (SEAT) thickness were taken from a subset of 582 subjects.

Table 3 -13 shows the median values (IQR) in the population for chamber size, systolic and diastolic parameters, and the prevalence of contractile and valvular abnormalities. The ejection fraction (EF) was within the normal range for the cohort, but 24 subjects (<2%) presented with an EF < 50%. Of these subjects, five showed characteristics of dilated cardiomyopathy, while the remaining subjects had normal ventricular dimensions, but with contractile dysfunction.

Using the gender-specific ranges recommended by Lang *et al* (2006, Appendix 4), the median values for the chamber dimensions and wall thickness were found to be within normal limits**.** The median values as well as the inter-quartile ranges in men [138g (112,171)] and in women [119g (95,152)] were well within in the normal ranges for LVM. The LVMI (LVM indexed to BSA) was also in the normal range for men [78.4g/m^2 (64, 93)] and women [69.5 g/m^2 (58, 87] [Appendix 4 for normal values range].The median LVMI, indexed to height$^{2.7}$ was higher in women (35.5$m^{2.7}$[29, 46]) than in men (34 $m^{2.7}$ [27, 42]) and were in the normal range, with the 75th percentile being slightly higher than prescribed by Lang *et al* (Appendix 4). This median value increased proportionally with increasing age.

Table 3-13: Echocardiographic chamber dimensions and wall thickness (median [IQR])

Variables n	Males 397	Females 1023	15-24 165	25-34 303	35-44 444	45-54 372	55-64 397
LA mean (mm)	30(24,33)	28(26,30)	31(29,34)	34(30,37)	34(31,37)	35(31,38)	35(32,39)
LAVI(ml/m^2)	14.5(1,18)	16(12,18)	12(16,14)	14(11,16)	15(11,17)	16(12,18)	17(12,19)
LVEDD(mm)	49(45, 52)	46(43, 50)	46(43,50)	47(47,51)	47(44,50)	47(44,50)	47(43,51)
LVESD(mm)	30(27,32)	27(24,30)	28(25,31)	29(27,32)	28(25,30)	28(25,31)	28(24,31)
PW (mm)	6 (6,8)	6 (6,8)	6 (5,6)	6 (5,7)	6 (6,8)	7 (6,8)	7 (6,8)
IVS(mm)	8 (8,9)	7 (6,9)	6 (6,8)	7 (6,8)	7 (6,8)	8 (6,9)	8 (6,9)
LV M (g)	138 (112,171)	119 (95,152)	104 (85,131)	117 (97,149)	118 (95,148)	129 (104,164)	135 (105,176)
LVMI(g/m^2)	78 (64,93)	69.5 (58,87)	64 (54,77)	66 (57,82)	67 (56,82)	73 (63,92)	78 (64,98)
LVMI (h$^{2.7}$)	34 (27,42)	35.5 (29,46)	27.8 (23,35)	31.5 (26,39)	33 (27.5,42)	37.6 (31,47)	38.6 (30.7,50)
SEAT (mm)	3.9 (3.5,4)	4 (3.5,4)	3.5 (3.0,3.5)	3.5 (3.0,4.0)	4 (3.0,4.0)	4 (3.5,4.5)	4 (3.5,4.5)

LA: left atrial; LAVI: left atrial volume index; LVEDD: left ventricular end-diastolic dimension; LVESD: left ventricular end-systolic dimension; PW: posterior wall; IVS: interventricular septum; LVM: left ventricular mass; LVMI: left ventricular mass index; SEAT: sub-epicardial adipose tissue

Table 3-13: Echocardiographic chamber dimensions and wall thickness (median [IQR]) ..*continued*

Median echocardiography variable values in the sample: systolic and diastolic parameters							
	Males	**Females**	**15-24**	**25-34**	**35-44**	**45-54**	**55-64**
EF (median)	67(63,71)	70(64,75)	68(64,73)	68(64,72)	69(65,74)	69(64,75)	68(63,74)
≥ 50 % (n)	387 (97%)	1010 (99%)	139(99%)	164(99%)	301(99%)	436(98%)	366(98%)
< 50% (n)	10 (3%)	13 (1%)	1(0.7%)	1(0.6%)	2(0.6%)	7(2%)	12(2%)
Em	1.22 (0.96, 16)	1.1(0.88, 1.4)	1.7(1.4, 2.1)	1.5(1.2,1.7)	1.2(1.02,1.4)	1.06(0.84,1.25)	0.9(0.75,1.0)
Em/Am	1.0(0.8, 1.3)	1.0(0.8, 1.24)	1.3(1.1, 1.6)	1.04(0.9,1.3)	1(0.8,1.3)	1(0.8,1.2)	1(0.8,1.2)
IVRT	104(88,128)	104(88,120)	96(80,112)	99(88,112)	104(88,120)	109(88,120)	111(96,128)
TDI: Em/Ea	7(6,8)	7(6,9)	7(6,9)	7(6,8)	7(6,8)	7(6,8)	7(5,9)
EF: ejection fraction; Em: early transmitral inflow velocity; Am: transmitral atrial contraction wave; Ea: early myocardial velocity; IVRT: isovolumic relaxation time; TDI: Tissue Doppler indices; RWMA: regional wall motion abnormality; VHD: valvular hear disease							
Prevalence of cardiac disease in the sample							
RWMA (n/%)	8 (2%)	16 (2%)	0%	0%	4(1%)	5 (1%)	15(4%)
VHD (n/%)	1(0.3%)	5 (0.5%)	0%	0%	0%	0%	6 (2%)
Other. (n/%)	5(1%)	3(0.2%)	0%	0%	2(0.6%)	3 (0.7%)	3 (0.8%)

The Doppler Em measurements decreased with advancing age, while a corresponding attenuation of the trans-mitral ratio (Em/Am) was observed. The isovolumic relaxation time (IVRT) increased with advancing age, while the tissue Doppler diastolic ratio (Em/Ea), remained fairly consistent across the age groups.

There was an increase in SEAT thickness, with advancing age, mostly evident from the increases in the 25^{th} and 75^{th} percentile values. The median values were increased in females as compared to males.

3.4.1 Echocardiography and Cardiovascular risk factor profile

Since 62 % of the subjects in this sample presented with 1 or more CV risk factors, we compared the echocardiographic parameters in subjects with, versus those without CV risk factors. Table 3-14 shows the median (IQR) values in subjects with and without CV risk factors. In the presence of CV risk factors, mean left atrial (LA) dimension, left atrial volume index (LAVI), left ventricular mass (LVM), left ventricular mass index (LVMI), and SEAT thickness were significantly higher ($p < 0.05$). Similarly the transmitral flow velocities (Em and Em/Am) were lower in subjects with CV risk factors. The tissue Doppler diastolic filling ratio (Em/Ea) was higher in males with CV risk factors, while the IVRT was longer in females with CV risk factors.

Significant differences were observed between those with and without CV risk factors in the thickness of the posterior wall, the LV mass and LVMI in subjects from 25-64 years of age. The SEAT thickness was significantly higher in the 55-64 year age groups ($p= 0.003$).

Table 3-14: Comparison of median echocardiographic variables in subjects with and without CV risk factors

Echo parameter	No CV risk factors		≥ 1 CV risk factor	
	Males	Females	Males	Females
LA (mm)	36(32,40)	36(32,39)	37(34,40)	37(34,40)*
LAVI (ml/m^2)	13(10,17)	14(11,17)	14(11,18)	15(12,19)*
LVEDD (mm)	49(45,52)	45(43,48)	48(45,52)	46(43,50)
LVESD (mm)	30(28,32)	27(24,30)	29(27,32)	27(24,30)
LVS (mm)	8(6,9)	7(6,8	8(8,9)	8(6,9)*
LVPW (mm)	6(6,8)	6(5,7)	6(6,8)	7(6,8)*
EF (%)	68(64,72)	69(64,75)	67(71,82)	70(64,75)
LVM (g)	135(114,159)	109(89,138)	139(110,177)	125(102,163)*
LVMI (g/m^2)	77(65,90)	65(55,78)	79(64,95)	73(61,94)
Em	1.34(0.11,1.8)	1.2(1.0,1.5)	1.2(0.9,1.5)*	1(0.8,1.2)*
Em/Am	0.14(0.1,0.16)	0.12(0.1,0.16)	0.11(0.1,0.14)	0.1(0.08,0.14)*
IVRT (ms)	112(96,120)	96(88,112)	104(88,128)	104(88,120)*
SEAT (mm)	3.5(3,4)	3.5(3,4)	4(4,3.5)*	4(3.5,4.5)*
TDI (Em/Ea)	5(1.0,7.0)	4(1.0,7.0)	6(1.4,7.0)	4(1.3,7.0)*

***indicates statistical significance between groups i.e. subjects with and without CV risk factors**
Em: early transmitral inflow velocity; Am: transmitral atrial contraction wave; Ea: early myocardial velocity; Aa: myocardial velocity associated with atrial contraction; LA: left atrium; LVEDD: left ventricular end-diastolic dimension; LVESD: left ventricular end-systolic dimension; LVPW: left ventricular posterior wall; SEAT: sub-epicardial adipose tissue thickness; LVMI: left ventricular mass index; LAVI: left atrial volume index

3.4.2 Correlation co-efficients between echocardiography variables

When echocardiography variables were compared to each other (Table 3-15), the strongest associations using Spearman's rho (r_s) (indicated by $r_s > 0.500$), were observed between the LVEDD and LVESD ($r_s = 0.654$), the EF and LVESD ($r_s = -0.698$), LV septal thickness and LV Mass ($r_s = 0.713$) and between the posterior wall (PW) and LV mass ($r_s = 0.640$). There were moderate relationships ($r_s > 0.300 < 0.500$) between SEAT and the following parameters: LV septal thickness ($r_s = 0.356$), posterior wall thickness ($r_s = 0.360$), LV Mass ($r_s = 0.372$), and an inverse relationship with the early transmitral flow velocity (Em) ($r_s = -0.385$). Statistically significant ($r_s > 0.100 < 0.300$), albeit weaker relationships, existed between SEAT and the following parameters: presence of CV risk factors ($r_s = 0.207$), LA size ($r_s = 0.287$), and an inverse relationship with the trans-mitral ratio (Em/Am) ($r_s = -0.206$) (Table 3-15).

Table 3-15: Correlation co-efficient of echocardiography variables and their association with each other (rho-values)

Parameter	LVEDD	LVESD	LA	LAVI	EF	LVS	PW	LVM	LVMI	Em	E/A	Em/Ea	SEAT
Risk										-			+
LVEDD		++						++					
LVESD					___			++					
LA						+		+		-	-		-
LAVI		+	+++		-	++	+	++	++		-	-	+
EF		___											
LVS							+		++		+		+
PW						+			++				
LVM	++	++									+		+
LVMI							++						+
Em	-												
Em/Am	-					+		+				++	-
TDI:Em/Ea											++		
SEAT			-			+		+	+		-		

KEY: + (weak); ++ (moderate);+++ (strong) ; -(weak inverse); -- (moderate inverse);--- (strong inverse)

3.4.3. Diastolic abnormalities and dysfunction in the sample

Diastolic abnormalities were identified using the criteria set by the European Study Group on Diastolic Heart Failure (1998). The diagnosis of diastolic dysfunction (DD) was made according to the guidelines set by the Heart Failure and Echocardiography Associations of the European Society of Cardiology (Paulus *et al*., 2007). Based on these guidelines (see *Methods*), 436 subjects (31%) were classified as having diastolic abnormalities. Only five subjects (< 1%) fulfilled the diagnosis for diastolic dysfunction (Table 3-16).

Subjects with diastolic abnormalities were predominantly female (78%). They also presented with higher waist circumference measurements and an increased prevalence of DM. These subjects were also more likely to be smokers.

Echocardiography showed an increase in the LVMI, diminution of the trans-mitral ratio, a prolonged IVRT and an increase in Tissue Doppler diastolic indices, consistent with the deviation from normality.

The diagnosis of DD was made when the subject had echocardiographically-derived diastolic abnormalities (Table 2-1 in *Methods*) and exhibited features of left atrial dilation (> 45 mm). In the absence of these criteria, subjects with TDI diastolic filling ratio (Em/Ea) ≥ 15 were classified as having diastolic dysfunction. If the Em/Ea was ≥8≤ 15, then the LVMI > 122 g/m^2 in females or > 149 g/m^2 in males was used to diagnose DD. In this manner, there were 5 (< 0.5%) subjects who were classified with DD in this sample. These subjects presented with a higher BMI and a larger waist circumference (Table 3-16).

The accompanying echocardiographic changes shown in the table below (Table 3-16) showed an elevated LAVI and LVMI, with the transition from diastolic filling abnormalities to the entity of diastolic dysfunction. Subjects with DD also had an increased IVRT, and a decreased trans-mitral Doppler ratio (Em/Am). Further subgroup analysis revealed elevated TDI diastolic filling ratio (Em/Ea) (7 in subjects with diastolic abnormalities to 8 in subjects with DD).

Table 3-16: Clinical, echocardiographic and anthropometric median values (IQR): subjects with and without diastolic abnormalities

n*	Normal : n =(988)	Abnormalities (n= 436)	Dysfunction (n=5)
Age	48(38,55)	45(35, 54)	55(54, 59)
Women (%)	695 (70%)	331(76%)	4 (80%)
BMI (kg/m^2)	27(24,32)	27(23,32)	37(31,39)
Hypertension	481(49%)	192(44%)	3(60%)
WC	92(83,103)	95(84,105)	115(102,120)
DM	316 (22%)	140(32%)	2 (40%)
Smokers	241(17%)	114(26%)	0
LVMI(g/m^2)	64(53, 78)	75(63,92)	85(75, 100)
LAVI(ml/m^2)	15,4(12,19)	12(10,15)	30(23,38)
LVPW(mm)	6(6,8)	6(6, 8)	7(7,9)
LVS(mm)	8(8,9)	6(6, 8)	7(6,10)
IVRT(mm)	68(64, 88)	104(88, 120)	96(96,96)
Em/Am	2.5(0.6,)	0.11(0.8,0.15,)	0.1(0.1,0.2)
TDI: Em/Ea	1.4(1.1,1.8)	7(6,8)	8(5,11)

***varying n due to missing values**

Independent determinants of diastolic abnormalities on univariate analysis were age, BMI, LVMI and LAVI (Table 3-17). Multivariate step-wise logistic regression analysis indicated that the odds of having diastolic abnormalities using the echocardiographic

criteria was 0.658 in females, and the odds increased by 0.986 per year of increasing age. Furthermore, the likelihood of diastolic abnormalities increased by 0.73 for every mmHg increase in the blood pressure, and by 0.882 for every ml/m^2 increase in the LAVI.

Table 3-17: Univariate and Multivariate independent risk factors for diastolic abnormalities

Risk factor	Univariate	Multivariate	
	p-value	Odds ratio	p-value
Age	**0.000***	0.986(0.975;0.998)	**0.025***
Gender (female)	0.544	0.658(0.48,0.900)	**0.009***
Hypertension	0.072	0.73(0.533;0.999)	**0.049***
DM	0.065	0.782(0.577;1.06)	0.114
BMI	**0.03***	0.760(0.534; 1.08)	0.129
LVMI	**0.001***	0.992(0.981;0.975)	0.137
LAVI	**0.000***	0.882(0.882;0.854)	**0.000***

indicates statistical significance

BMI: Body mass index; LVMI: Left ventricular mass index; LAVI: left atrial mass index

3.4.4 Echocardiography and the Metabolic Syndrome

The LVEDD, LVESD and the EF were within the normal limits for both subjects with and without MS, with significant differences observed in the LVEDD and EF between the two groups (Table 3-18). The LA dimension, LAVI, LVPW and LVS were significantly increased in subjects with MS. This was accompanied by an increase in the LVM and LVMI in these subjects ($p= 0.001$). The sub-epicardial adipose tissue thickness was also significantly higher in subjects with MS ($p< 0.005$), and this was evident in both males and females.

Trans-mitral Doppler flow velocities showed that the Em (early mitral filling velocity) as well as the Em/Am (trans-mitral filling ratio) was significantly increased ($p< 0.005$) in subjects with MS as compared to those without. Further sub-group analysis and interrogation using TDI also showed significant differences between the 2 groups ($p= 0.001$), where subjects with MS had higher TDI diastolic filling ratio than those without MS.

Since hypertension by itself is a major determinant of LV mass, adjustments were made for blood pressure. The significant differences in echocardiographic parameters between those with and those without MS persisted (Table 3-18). However, these differences were abolished for LVEDD and TDI diastolic filling ratio between groups.

Table 3-18: Median echocardiographic values by presence or absence of the Metabolic Syndrome (IDF criteria)

	Metabolic Syndrome (IDF criteria)			
	MS present	**MS absent**	**p**	**p#(adjusted)**
LA	35(39,32)	34(37,30)	**<0.005***	**0.000***
LAVI	17(19,12)	14(17,10)	**0.001***	**0.027***
LVEDD	47(51,44)	46(50,43)	**0.004***	0.074
LVESD	28(31,25)	28(31,25)	0.651	0.363
LVPW	7(8,6)	6(7,5)	**0.001***	**0.000***
EF	70(75,64)	69(73,64)	**<0.005***	**0.015***
LVS	8(9,6)	7(8,6)	**<0.005***	**0.026***
LV Mass	138(176,110)	114(144,91)	**0.001***	**0.000***
LVMI	39.4(50,32)	32(39,26)	**0.001***	**0.000***
Em	0.99(1.2, 0.8)	1.27(1.6, 1.02)	**0.001***	
Em/Am	0.11(0.12, 0.08)	0.12	**0.023***	**0.000***
IVRT	104(120,88)	120	0.539	0.274
SEAT	4.0(4.5,3.5)	3.5(4,3)	**<0.005***	**0.000***
TDI Em/Ea	5.26(7, 1.2)	3.2(6.36,1,2)	**0.001***	0.424

***indicates statistical significance p < 0.05**
 # adjusted for hypertension

Table 3-19: Relationship of echocardiography variables with the Metabolic Syndrome and its components*

	Metabolic Syndrome components					
	Fasting Glucose	TG ≥ 1.7 mmol/L	HDL	WC	BP	MS
LA dimension	+	+		+	+	+
LAVI(ml/m^2)	+	+	+	+	+	+
LVEDD (mm)	+				+	
LVESD (mm)						
LVPW (mm)	+	+	+	+	+	+
LVS (mm)	+	+			+	+
LV Mass (g)	+	+		+	+	+
LVMI(g/m^2)	+	+	+	+	++	++
SEAT (mm)	++	+		+	++	++
Em/Am	--	-		-	--	+
IVRT(ms)					+	
TDI:Em/Ea	-	-	-	-	-	+

*only correlation co-efficient with statistically significant p-values shown

KEY:+ (weak); ++ (moderate);+++ (strong) ; -(weak inverse); -- (moderate inverse);--- (strong inverse)

The strength of the relationship between the echocardiographic parameters and the diagnosis of MS, as well as its individual components were determined. Statistically significant relationships are shown in Table 3-19. The strongest correlations were observed between the LV mass and hypertension, and the LV mass and the presence of MS (r_s =0.337 and 0.319, respectively). Another significant relationship was observed between SEAT, the fasting blood glucose levels, hypertension and the presence of MS (r_s = 0.318, 0.301 and 0.355 respectively). There was also an inverse relationship between the fasting blood glucose and the Em/Am ratio (r_s = - 0.324). The TDI diastolic filling ratio showed a weaker, but significant correlation (r_s =0.206) with the presence of MS.

Further comparisons within subgroups (Table 3-20) showed that there was a significant increase in the SEAT thickness in the presence of insulin resistance, DM, hypertension, smoking, as well as general and abdominal obesity (p = 0.000).

Bivariate analysis between SEAT and clinical, biochemical and echocardiography parameters showed that the strongest correlations existed between SEAT and other measures of adiposity, i.e. the BMI (r_s = 0.416) and the waist circumference (r_s = 0.415). There were also moderate correlations (r_s = 0.301) between SEAT and the presence of MS and hypertension using the IDF cut-off (Table 3-20). Weaker, but statistically significant relationships were observed between SEAT thickness and DM (r_s = 0.249) and insulin resistance (r_s = 0.287).

Table 3-20: Comparison of SEAT thickness (means +/- SD) in sub-groups

Variable	Group	SEAT (mean)	r_s	p
Gender	Males	3.8±0.8	0.059	0.350
	Females	3.9±0.8		
Hypertension	No	3.6±0.7	0.301	**0.000***
	yes	4.1±0.8		
Diabetes Mellitus	No	3.7±0.7	0.249	**0.000***
	yes	4.1±0.8		
Smoking	No	3.7±0.8	-0.052	0.208
	yes	3.9±0.8		
Abdominal obesity	No	3.5±0.7	0.415	**0.000***
	yes	3.9±0.8		
General obesity	No	3.4±0.7	0.416	**0.000***
	yes	4±0.8		
Insulin resistance	No	3.6±0.7	0.287	**0.000***
	yes	4.0±0.8		

***indicates statistical significance**

Table 3-21: Univariate and multivariate biochemistry, anthropometry and echocardiography prediction models for detection of MS (IDF criteria)

	Univariate analysis			Multivariate analysis		
	OR	**95% CI**	**p**	**OR**	**95% CI**	**p**
IDF BP	10.6	8.2,13.6	**0.000***	16.1	8.2,31.2	**0.000***
BMI	1.17	1.14,1.9	**0.000***	1.01	0.92,1.11	0.797
WC	1.1	1.09,1.12	**0.000***	1.092	1.04,1.14	**0.000***
TG	4.18	3.4,5.1	**0.000***	5.007	2.8,8.9	**0.000***
FBG	1.45	1.37,1.57	**0.000***	1.44	1.2,1.67	**0.000***
HDL	0.18	0.13,0.27	**0.000***	0.03	0.008,0.121	**0.000***
SEAT	2.9	2.19,3.8	**0.000***	1.17	0.699,1.9	0.558
LAVI	1.06	1.04,1.08	**0.000***	1.08	1.007,1.16	**0.031***
LVMI	1.01	1.009,1.02	**0.000***	1	0.986,1.015	0.972
Diast.Abn	0.928	0.85,1.35	0.526			
Diast. Abn#	1.070		0.582			

Diast.Abn: diastolic abnormalities present; # age adjusted
***indicates statistical significance**

Using linear regression we found that a 1mm in SEAT thickness was associated with a 3 times likelihood of being diagnosed with MS. (Table 3-21). However, subjects with blood pressure ≥130 / ≥85 mmHg were 11 times more likely to have the MS. An increase in triglyceride level of 1mmol/l predicted a 4- fold increase for the diagnosis of MS. Subjects with diastolic abnormalities was not conferred with additional risk for MS, even after adjusting for age (Table 3-21). After multivariate stepwise linear correlation, the greatest

odds of being diagnosed with MS was the IDF blood pressure cut-off (OR= 16), as well an increase in every unit of triglyceride (OR= 5; Table 3-21).

In order to determine whether MS was an independent predictor of diastolic abnormalities, logistic regression was performed to disentangle the effects of several other predictor variables including age, gender, hypertension, diabetes and anthropometry (Table 3-22). After adjustment, gender, age and both anthropometry parameters (BMI and waist circumference) emerged as significant predictors of diastolic abnormalities. Using the crucial Wald statistic, we found that age, which showed the highest Wald statistic, made the most significant contribution to the outcome.

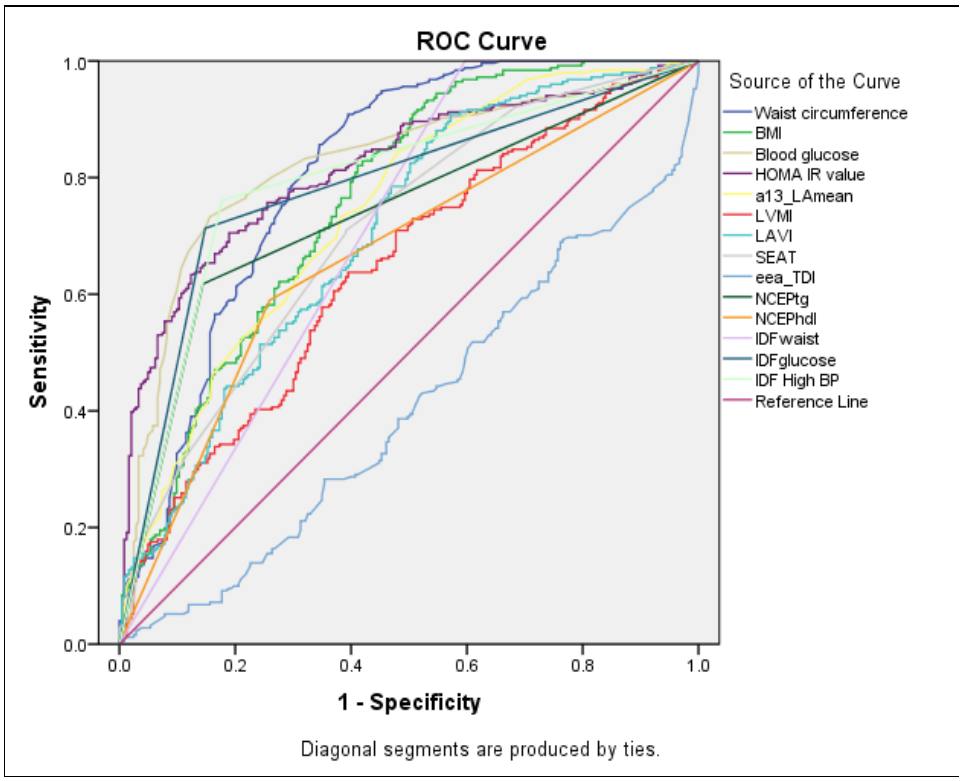
Table 3-22: Logistic regression for independent predictors of diastolic abnormalities

Parameters	Wald	OR	95% C.I.	p-value
Gender	8.603	1.554	1.158; 2.087	**.003***
Hypertension	.230	1.078	.793; 1.467	.631
Diabetes Mellitus	1.380	1.193	.889; 1.601	.240
Metabolic syndrome	1.142	.833	.596; 1.165	.285
Age	23.010	.959	.943; .976	**.000***
BMI	13.785	1.075	1.035; 1.117	**.000***
Waist circumference	19.507	1.025	1.014; 1.037	**.000***

***indicates statistical significance**

We tested the discriminating capacity of all MS components (IDF), as well as echocardiographic and biochemical variables, to detect the presence or absence of the MS, by constructing receiver operator curves (Figure 3.7). Indices relating to glycaemia control yielded the highest area under the curve (AUC): fasting blood glucose levels (AUC = 0.822), followed by HOMA-IR values (AUC = 0.816). The waist circumference had an AUC = 0.807, followed by the triglyceride levels (AUC = 0.806), while the mean LA dimension was the echocardiographic measure with the highest AUC = 0.745. Blood pressure categorized into >or <130/85 yielded an AUC= 0.792.

FIGURE 3.7: Receiver operator curves for detection of Metabolic Syndrome (IDF)



3.4.5 The determination of normal echocardiography values in the Phoenix population

This study sought to propose normal echocardiography ranges in healthy subjects in the Phoenix population, as this has not been done previously (refer to the *Literature review* for rationale). From the 1428 subjects who underwent echocardiography, there were 38 subjects who were found to have structural and functional abnormalities. There were 667 subjects who were diagnosed with the MS (IDF criteria), and these were excluded from the analysis. There were 377 subjects with CV risk factors who did not meet the criteria for the diagnosis of MS: these subjects were also excluded. Therefore, the measurements from 346 subjects (89 males and 257 females) were analysed and are presented in Tables 3-23 and 3-24 below.

The physical and anthropometric characteristics of the normal subjects are shown in Table 3-23, and are stratified for gender. The raw data showed significant differences between male and female height, BSA and mean systolic blood pressure. Males were significantly taller, with a higher BSA, and recorded a higher mean systolic blood pressure. The BMI was significantly higher in females. There were no gender differences for weight and mean diastolic blood pressure.

Inter-gender differences were significant for echocardiography variables, where males had larger chamber dimensions, wall thickness and higher Doppler (Em) measurements (Table 3-24).

When the echocardiographic dimensions were indexed to height, these differences were not apparent, and were replaced by significantly larger LA dimensions in females, higher LVMI and thicker SEAT in males (Table 3-25). These changes persisted when echocardiographic variables were indexed to the BSA, but SEAT thickness was higher in females (Table 3-25).

Table 3-23: Gender stratification of physiologic and anthropometry parameters (median, IQR)

	Males (n=89)	Females (n=257)	p
Age	32 (18,46)	40 (30,49)	**0.01***
Height	169 (165,176)	156 (152,161)	**0.000***
Weight	67 (57,76)	63 (55,75)	0.373
BSA	1.78 (1.62,1.85)	1.6 (1.5,1.8)	**0.000***
BMI	22.7 (19,26)	26 (22,31)	**0.000***
Systolic BP(mean)	125 (115,135)	119 (111,129)	0.036
Diastolic BP(mean)	75 (69,81)	76 (70,83)	0.169

***indicates statistical significance**

Table 3-24: Echocardiographic parameters (raw data) in men and women (mean/95th percentile)

Parameters	Males		Females		p
	Mean	95th percentile	Mean	95th percentile	
LVEDD (mm)	48	56	46	55	**0.000***
LVESD	30	37	28	35	**0.000***
LA dimension	35.65	44	35.36	44	0.53
EF	67	78	69	80	0.094
LVS	8	11	7	10	**0.000***
LVPW	6	8	6	9	**0.003***
LV Mass (g)	134	200	110	180	**0.000***
SEAT	3.3	4.0	3.5	5.0	0.263
E(mitral)	1.55	2.5	1.4	2.3	**0.005***
E/A (transmitral ratio)	1.2	1.9	1.1	1.9	0.319
IVRT	101	144	100	128	0.294
TDI: Em/Ea	7	11	7	11	0.272
LAVI (ml/m)	13.90	22.8	14.42	22.5	0.334

***indicates statistical significance**

Table 3-25: Echocardiography variables in men and women (mean/95th percentile): indexed to height and BSA

Parameters	HEIGHT					BODY SURFACE AREA				
	Males		Females			Males		Females		
	Mean	95th	Mean	95th	p	Mean	95th	Mean	95th	p
LVEDD (mm/m)	29	33	29	34	0.365	28	33	28	33	0.787
LVESD (mm/m)	18	22	17.5	22.5	0.5	17	21	17	22	0.169
LA dimension (mm/m)	21	26	23	28	**0.000***	20	25	21.5	26	**0.000***
LVS (mm/m)	4.6	6.5	4	6	0.173	4	6	4	6	**0**.450
LVPW (mm/m)	3.8	4.9	3.8	5.5	0.932	3.6	5	3.5	5.3	0.478
LV Mass Index (g/m)	80	120	70.5	113	**0.000***	76	112	67	108	**0.000***
SEAT(mm/m)	2.2	4	2.2	3.2	**0.003***	2	2.5	2	3	**0.019***
LVMI ($h^{2.7}$)	36	45	33	56	0.511					

***indicates statistical significance**

3.5 The analysis of gene polymorphisms

3.5.1 The Hardy-Weinberg Equilibrium

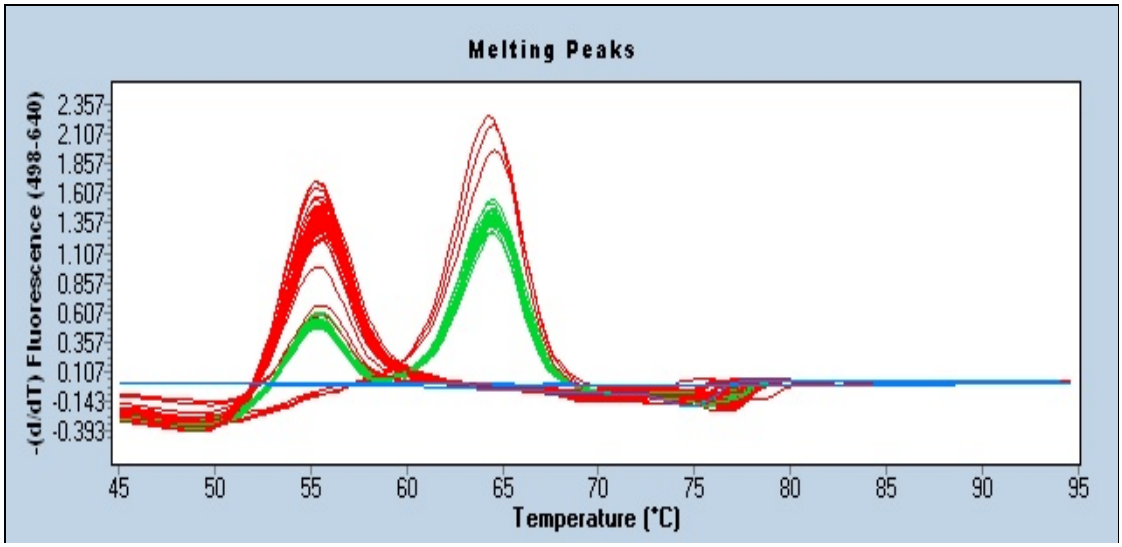
The genotype and allele frequencies for the polymorphisms investigated were computed by gene counting and are presented in Table 3-26. There were no polymorphisms which were in Hardy-Weinberg equilibrium in this sample. All four polymorphisms showed an almost equitable distribution of frequencies of the genotypes and alleles between males and females with no significant differences observed. The frequency of the heterozygous variant genotype of the S447X, Q192R and L55M polymorphisms were higher than the homozygous wild-type genotypes.

There were no heterozygous or homozygous mutant genotypes found in this sample for the N291S polymorphism: this polymorphism was therefore excluded from further analysis.

3.5.2. LPL polymorphisms

The frequency of the homozygous wild-type genotype (SS) of the S447X polymorphism was found in 25% of subjects (Table 3-26). There was a much higher prevalence of the heterozygous variant, which was found in 73% of subjects with a small number (2%) presenting with the homozygous mutant genotype (Figure 3.8). There was a dominance of the c-allele, detected in 62% of the sample, as compared to the g-allele, which was found in 38% of participants.

FIGURE 3.8: Illustration of melting peaks for the S447X polymorphism



Single peak red- homozygous: mutant (55°)/ wild-type (65°)

Double-peak green: heterozygous

Table 3-26: Genotype and allele frequencies in the sample

	genotype	n	%	Allele frequencies
Lipoprotein Lipase	SS	226	25	c : 0.62
S 447 X	SX	662	73	g: 0.38
	XX	16	2	
Total		904	100	1
Lipoprotein Lipase	NN	903	100	a:1
N291S	NS	0	0	g:0
	SS	0	0	
Total		903		1
PON-1	QQ	288/843	34	a: 0.66
L55M	QR	537/843	64	g: 0.34
	RR	18/843	2	
Total		843		1
PON-1	LL	290	35	a: 0.67
Q192R	LM	524	63	t: 0.33
	MM	15	2	
Total		828		1

The distribution of genotypes and alleles in the sample shows that a third of subjects presented with mutations.

3.5.3 PON-1 polymorphisms

3.5.3.1 THE L55M POLYMORPHISM

The frequency of the homozygous wild-type (LL) genotype of the L55M polymorphism was 34% in this sample (Table 3-26), with a high prevalence of the heterozygous (LM) variant (64%). There were only 2% of subjects with the homozygous mutant (MM) genotype (Figure 3.9).

There was a dominance of the a-allele (0.66), as compared to the g-allele (0.34) in the sample.

FIGURE 3.9: Illustration of melting peaks for the L55M polymorphism

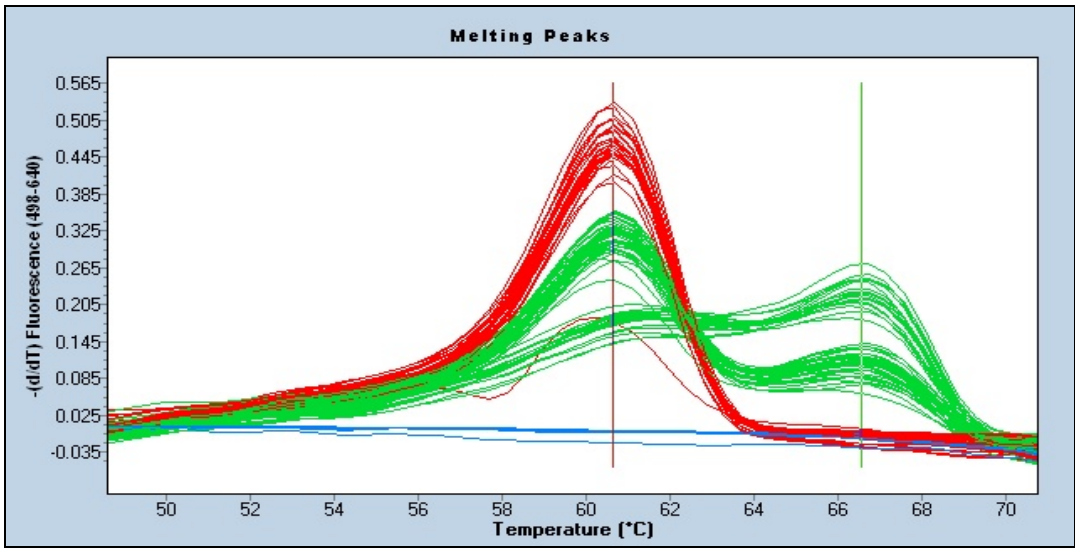


Table 3-27: Genotype frequencies in the subjects with and without MS

Polymorphism	genotype	MS (n/%)	No MS (n/%)	p
Lipoprotein Lipase	SS	112 (25%)	114(25%)	
S 447 X	SX	331 (73%)	331 (73%)	
	XX	10 (2.2%)	6 (1.3%)	
PON-1	N# (%)	407	422	
Q192R	QQ	135(33%)	155 (37%)	0.537
	QR	265 (65%)	259 (61%)	
	RR	7 (2%)	8 (2%)	
L55M	N# (%)	447	440	
	LL	297 (66%)	300 (68%)	0.518
	LM	134 (30%)	127 (29%)	
	MM	16 (3%)	13 (3%)	

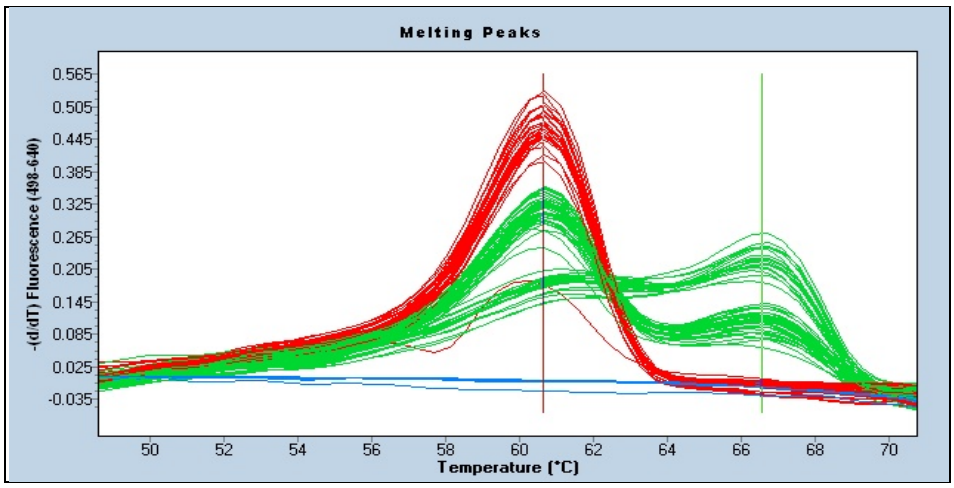
n # varies due to missing values

The distribution of genotypes shows no significant differences in the frequencies of genotypes between subjects with and without the metabolic syndrome

3.5.3.2. The Q192R polymorphism

The frequency of this polymorphism in our sample was 35% for the wild-type, 63% for the heterozygous variant and 2% for the homozygous mutant (Table 3-27). There was a dominance of the a-allele (0.67) as compared to the t-allele (0.33).

FIGURE 3.10: Illustration of melting peaks for the Q192R polymorphism



Single peak red: homozygous wild-type (66.1°)/mutant (61.1°)

Double-peak green: heterozygote

3.5.4 Distribution of polymorphisms in subjects with/without Metabolic Syndrome

There was a very similar distribution of the genotypes in those subjects with and without MS (Table 3-27), with no significant associations detected (p = 0.599) between males and females, even when the heterozygous and homozygous mutant variants were combined. The diagnosis of MS and the distribution of the S447X mutation were analysed, with no significant statistical significance (Table 3-28) detected. Similarly, the L55M and the Q192R genotypes were almost equally distributed between subjects with and without MS, with a consequently non- significant association (Table 3-27) detected. Associations were also sought between the variant polymorphisms and the individual components of the MS.

Although the variants were distributed equally between subjects with and without MS (Table 3-27), non-parametric testing using the Mann-Whitney test showed a statistically significant difference in the Q192R genotype. Subjects with this mutation (Table 3-28), were more inclined to have decreased levels of HDL (p= 0.026) as defined by the IDF criteria.

Table 3-28: Associations between median values of MS components and genotype

MS component	S447X			L55M			Q192R		
	Mean rank		p	Mean rank		p	Mean rank		p
Triglyceride	429	452	0.682	439	453	0.364	406	420	0.322
Increased WC	523	451	0.127	440	452	0.348	426	409	0.197
FBG	500	453	0.397	444	447	0.822	407	429	0.365
HDL	383	451	0.226	443	440	0.827	391	425	**0.026***
Elevated BP	475	440	0.533	434	430	0.841	410	406	0.805

***indicates statistical significance**

3.5.5 The association between polymorphisms and obesity

Considering that obesity was very prevalent in this sample, the frequency of gene polymorphisms in obese subjects using both the WHO and Pan-Asian cut-off, was determined. Statistical significance was found in obese women using the Pan-Asian cut-off with the PON-1 polymorphisms, as a higher number of these obese subjects were more likely to have the Q192R polymorphism (Table 3-28).

Table 3-29: Frequency of polymorphisms in obese subjects

	BMI (kg/m^2)	Wild-type			Mutants		
		SS447	LL55	QQ192	S447X	L55M	Q192R
Sample	>25[#]	12	402	190	603	204	371
	>30	7	221	95	324	103	208
Men	>25[#]	2	71	38	102	33	58
	>30	2	20	10	30	11	20
Women	>25[#]	326	326	152	495	170	**307***
	>30	198	198	85	290	91	184

p < 0.05
#: Asian cut-off

The frequency of the presence of wild-types and mutations in obese subjects using the WHO and Asian cut-offs: there were a significantly higher number of female subjects with the Q192R mutation

CHAPTER FOUR

Discussion, Conclusion and Limitations

This is the first community-based study of South African Asian Indians which documents CV risk factor prevalence and clustering, and relates them to structural and functional changes using echocardiography. This study also determined the relationships and patterns between CV risk factors and single nucleotide polymorphisms (SNPs) of the Lipoprotein Lipase (LPL) and the Human Paraoxonase (PON-1) gene.

The results show that there is a very high prevalence of cardiovascular (CV) risk factors and the metabolic syndrome (MS) in this population, which are associated with alterations in cardiac structure and physiology. Genotyping revealed significant associations between HDL levels and the Q192R polymorphism of the PON-1 gene, which also showed an association with obesity in women.

4.1 Cardiovascular risk factor profiles and Lifestyle patterns

The high prevalence of cardiovascular risk factors in urban Asian Indians has been well documented previously (Balarajen, 1991; Enas *et al*., 1992; Cappucio, 1997; Yusuf *et al*., 2001). Although there is limited data on CV risk factor profiles in South Africa, especially in Asian Indians (Rheeder, 2006), this study shows that the magnitude of change has been of epidemic proportions, from what has been previously reported (Seedat *et al.*, 1990).

Currently, there is a global increase in the prevalence of obesity (Rheeder, 2006; Goedecke *et al.*, 2005), particularly in developing countries. Recent estimates from the South African Demographics and Health Survey published in 2002 (Puoane *et al*., 2002)

reported that 29.2% of men were overweight or obese (≥ 25kg/m^2), and over half of all South African women (56.6%) were obese. Our study showed that the frequency of raised BMI (> 25 kg/m^2), using the World Health Organisation (WHO) Expert Consultation (2004) criteria, was 66% in our sample (Table 3-7a). Of note, there was a steep increase in the prevalence of raised BMI from the 1st (15-24 years old) to the 2nd (25-34 year olds) age groups. We attributed the reasons for this significant increase in weight to socioeconomic changes that occur at this age, namely, financial independence, dietary changes, transition from an active to sedentary working lifestyle, as well as psychological factors related to the individual's social environment (Marmot, 2000).

The frequency of increased BMI in our study is also substantially higher than what has been reported in a study of the coloured community in Mamre (23%), Cape Town (Steyn *et al*., 2004). More worryingly, it is also in excess of what was reported on in a cohort of Asian Indians in the Durban Metropolitan area by Seedat *et al* in 1990, where 48% of subjects were recorded as having a BMI > 25kg/m^2. This translates into a 50% increase in the prevalence of overweight subjects with similar environmental exposure in a span of under 20 years.

It is known that both general and central obesity is associated with a simultaneous increase in CV risk factor development, like hypertension, as well as glucose and lipid abnormalities (Gupta *et al*., 2007). Therefore, it is no surprise that the WHO, amongst other bodies (National Institute of Health 1998; Seidell *et al*., 2001) still recommend the BMI and waist circumference measurements for risk scoring. One of the main limitations

associated with these parameters in Asian Indians is that Asian Indians are known to have higher levels of CV risk factors compared to Caucasians at any given BMI value (Low *et al*., 2009). The WHO subsequently redefined the criteria for obesity to be population-specific: in Asians, the cut-off for obesity has been reduced to 23kg/m^2 from 25kg/m^2 (WHO Expert Consultation, 2004).

In our sample the prevalence of raised BMI (>23 kg/m^2) changed from 66% to 77% with the application of Asian cut-offs for BMI. These results are very similar to what was reported by the Heart and stroke statistics from the American Heart Association (2011), where 67% of subjects tested were overweight; and 37% of these were classified as being obese. The increased prevalence of obesity, higher than what has been previously documented in this sample, may reflect the effects of a community that is undergoing an epidemiological transition. The consumption of a high-calorie diet and the adoption of a more sedentary lifestyle, together with ensuing obesity, is likely the driver for the development of other associated CV risk factors like hypertension, Type 2 Diabetes Mellitus (DM) and dyslipidemia (Tibazarwa *et al*., 2009).

One of the important uses of the waist circumference measurement in CV risk stratification lies in its strong correlation with insulin resistance (Fonseca *et al*., 2004), therefore serving as a surrogate for the measurement of intra-abdominal fat. Since this was a study of subjects of Asian Indian ethnicity, we used the IDF ethnic-specific criteria for the measurement of the waist circumference (male ≥ 90 cm/female ≥ 80cm) as an obligatory component for the diagnosis of MS. There were 1048 (79%) subjects who had an increased waist circumference in this sample, which was most apparent in females

(82%) and in the 45-54 age groups (Table 3-7a). This prevalence is much higher than has been documented in urban Indians in India (Gupta *et al*., 2007), where only 22% of males, and 42.2% of females were documented with increased waist circumference. There was a clear increase in abdominal obesity prevalence within industrialised settings, as shown by Kaur *et al* (2007), where improvement in socio-economic status accompanied the increasing prevalence of abdominal obesity (50%) in men.

4.1.1 Smoking

South Africa is one of the six countries in Africa that has adopted a national smoke-free legislation, where a ban on tobacco advertising, sponsorship and promotion was implemented in 1993 (Tobacco Products Control Act, 1993). In spite of this, there was a prevalence of 26% of subjects in the sample who continued to smoke, with almost half the males in this study being smokers (Table 3-7a). This was comparable with other studies in India, where the prevalence of smoking ranged from 10% in industrialised samples (Mohan *et al*., 2003) to 24.3% in an urbanised sample (Gupta *et al*., 2007). Although there is extensive research (Jonas *et al*., 1992; Ockene & Miller, 1997) that links smoking with CV disease, as well as clear diminution of CV risk with cessation of smoking, these figures suggest that there still remain barriers to intervention in this community.

4.1.2 Hypertension

There was a high prevalence of hypertension in the present study - recorded in almost half (49%) of the sample (Table 3-7a): this was higher than what was reported in the United States, where 44% of subjects were recorded as being hypertensive. The prevalence in our study was also higher than recent estimates in Asian Indians, where a prevalence of 37% was recorded in urban subjects in Kumarokam in 2006 (Thankappen *et al*., 2006). The fact that these significant differences exist in Asian Indians in different parts of the world suggest that environmental and behavioural factors may be important underlying factors for promoting the development of hypertension in our sample.

4.1.3 Diabetes

According to the American Diabetes Association (2012), individuals with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) are regarded as having prediabetes, which in itself, confers a relatively higher risk for the development of future DM. It is now well established that DM, in association with other CV risk factors, increases the risk of macrovascular complications. In fact, the risk of a diagnosis of DM has been likened to having a previous myocardial infarct (Heller, 2005) by the American College of Cardiology and the American Heart Association.

The present study found a prevalence of 20% in male subjects and 25% in female subjects with prediabetes (24% in total), with a third (32%) of the sample being classified as being diabetic. Sixty three percent (63%) of these diabetics self-reported their status.

Using fasting blood glucose testing and the two-hour glucose tolerance test, we identified 37% of subjects with DM who were previously unaware of their status (Table 3-5).

The prevalence of DM has almost quadrupled since an investigation by Omar *et al.* in 1993 (11%), which showed an increase of 2% (from 9%) in 1988 (Omar *et al.*) in Asian Indians in Durban. Using the biochemical cut-off described by Seedat *et al.* (1990), there was an almost 20% increase in DM prevalence in women from 16% in 1990 to 35% in the present study, and a 10% increase in men from 15% in 1990 to 25% in the present study. The overall prevalence has increased markedly from 16% (Seedat *et al*., 1990) in 1990 to 32% in our study, and re-affirms previously documented evidence that the prevalence of DM in South Africa is highest (13%) in Indians (Omar *et al*., 1994). The extent of the excessive prevalence becomes clearly evident when compared to other population groups of similar socio-economic demographics, like the coloured population in Mamre, where only 10% of subjects were reported to be diabetic (Steyn *et al*., 2004).

Research shows that the common strand in most of the studies conducted on Asian Indians living in India as well in the diaspora (for example Africa, United Kingdom, Mauritius and the West Indies) is the high prevalence of DM. However, comparison of our diabetic prevalence shows that it is exceedingly higher than reported by Cappuccio (1997) for Asian Indians in Trinidad (20%) and Mauritius (21%). The same was evident when compared to the prevalence of DM in Asian Indians (17.4%) in the United States as reported by Misra *et al* (2010). Furthermore, analysis of previous studies of CV risk in India show that there were substantially more subjects in our cohort who were diabetic

as compared to urban Asian Indians (12.3%) in Jaipur (Gupta *et al*., 2007). This was further highlighted when compared to recent data from the Heart and Stroke Statistics from the American Heart Association (Roger *et al*., 2011), which indicated that the prevalence of DM in our sample (32%) is significantly higher than that which is reported (8%) in the United States.

4.1.4 Positive family history for cardiovascular risk factors

All subjects reported a positive family history for at least one of the CV risk factors that was recorded, with hypertension being the most frequently reported positive family risk factor, in 76% of subjects. In these subjects with a positive family history for hypertension, 37% of these subjects were found to be prehypertensive (Figure 3.2; Table 3-4). Data from the Framingham cohort (Vasan *et al*., 2001) reported that prehypertension was associated with an increased relative and absolute risk for CVD outcomes across the age spectrum, where prehypertensive subjects were 1.65 times more likely to have 1 or more adverse CV risk factor than those with normal blood pressure (Greenlund *et al*., 2004). Prehypertension, in our sample, was associated with an odds ratio of 1.03 (95% CI= 0.8; 1.3) of these subjects developing hypertension themselves (Table 3-4). Furthermore, a positive family history of hypertension was also associated with a doubling of the odds (Table 3-4) of subjects developing hypertension themselves (OR= 2.08; 95% CI=1.6, 2.7).

Similarly, from the 24% of subjects who were prediabetic (Table 3-5), 90% (292) of these had a positive family history for DM, translating into an odds ratio of 1.062 (95% CI= 0.83; 1.36) for these subjects developing frank DM themselves.

4.1.5 Lipid profiles

Hypercholesterolemia is a common feature in Asian Indians, with recent estimates documenting as many as 41% of Asian Indians in an industrial setting with the condition (Mehan *et al*., 2006). The prevalence is higher in migrant Indians, as reported by Misra *et al.* (2010), with almost 44% of subjects presenting with elevated cholesterol levels. Using the conventional cut-offs of > 6.5mmol/l (Seedat *et al*., 1990), only 18% of subjects in the present study were documented with elevated total cholesterol levels; these numbers being marginally lower (18%) than what was reported by Seedat *et al.* (20%) in 1990 (Table 3-7b). However, when the NCEP criteria (Grundy *et al*., 2001) was used (> 5.17 mmol/l), the prevalence of elevated total cholesterol escalated dramatically to 60% (Table 3-7b). This prevalence was also substantially higher when compared to subjects tested in the United States (15%), according to the Heart Disease and Stroke Statistics from the American Heart Association (2011).

There was a 14% prevalence for decreased HDL concentrations (Table 3-7b) observed in our sample when using conventional cut-offs (<1.0mmol/l). This prevalence increased to 23% when using the NCEP cut-points (Grundy *et al*., 2001), but was still lower when compared to the significantly higher prevalence of 55% reported in India in urban

subjects (Gupta *et al*., 2007) and in 87.2% of subjects in rural areas (Chow *et al*., 2007). Misra et al. (2010) reported a 42.3% prevalence of low HDL levels in Asian Indians in the United States.

4.1.6 Summary of cardiovascular risk factors and clustering

Although Asian Indians share similar cardiovascular risk profiles with other ethnic groups (McKeige *et al*., 1991), these risk factors do not clearly account for the marked excess in risk for CHD that has been consistently demonstrated in Asian Indian subjects (Gupta *et al*., 2004; Misra & Khurana, 2011; Chahal *et al*., 2010). This suggests that the mechanism/s underlying the development of CVD may therefore be different in this ethnic group, portending a possible genetic aetiology. Furthermore, previous population-based studies show inter-correlated metabolic abnormalities (like hypertriglyceridemia, low HDL levels, hyperinsulinaemia and central obesity), related to DM, unique to Asian Indians (Cappaccio, 1997; Misra *et al*., 2003; Misra & Khurana, 2011). Possible explanations revolve around low birth-weight and childhood under-nutrition (Victora *et al*., 2008), which were proposed as risk factors for elevated blood pressure, high glucose concentrations and abnormal lipid profiles once these babies gained weight rapidly during the postnatal period. This stems from the "thrifty phenotype" hypothesis alluded to by Neel in 1962, most evident in those that are undergoing socio-economic changes from poverty, which, in the presence of excessive calories, becomes disadvantageous and leads to obesity and glucose intolerance (Hales

& Barker, 2001, Misra *et al*., 2004). In addition, the theory of a prothrombotic state in Asian Indians has been suggested (Anand *et al*., 2000; Hoogeveen *et al*., 2001), promoted by higher homocysteine, lipoprotein (a), and plasminogen activator inhibitor-1 levels than are observed in Black (McKeige *et al*., 1991; Kain *et al*., 2008) or Caucasian populations.

The high CV risk profile observed in our study may be explained by the mechanisms discussed above, and particularly by the changes in socio-economic status with resultant changes in dietary patterns to more high-fat-and highly processed carbohydrate-based meals and a sedentary lifestyle. Consequently, these were the factors that were suggested to have accelerated the development of over-weight and obesity in the Indian diaspora in Mauritius (Dowse *et al*., 1995) and in Trinidad (Gulliford *et al*., 2001; PAHO/WHO, 1999). Similar conclusions have also been made previously in subjects sharing the same environmental exposure in KwaZulu-Natal (Motala *et al*., 2011; Seedat *et al*., 1990; Omar *et al*., 1988, Ranjith *et al*., 2002). Our study has drawn attention to the very high risk profile in the same community developing only two decades later, which has been signaled by a surge in the prevalence of obesity, dysglycaemia and DM beginning in early adolescence.

4.2 The Metabolic Syndrome

The clustering of biochemical, anthropometric and physiological abnormalities that constitute the metabolic syndrome is known to amplify an individual's risk for CVD. Subjects with MS are considered to have a twofold increased risk for CVD (Scholl, 2012)

even before the development of overt DM. Currently there still remains disagreements about the diagnostic criteria of MS and debate about whether MS is a true syndrome or a mixture of various phenotypes (Alberti *et al*., 2009). The current research, however, suggests that the components may have common genetic mechanisms as they tend to occur concomitantly, or cluster together.

4.2.1 The prevalence of the Metabolic Syndrome

The present study shows that there is a very high prevalence of MS in the Phoenix community, with the varying prevalence attributed to the definitions that were used. The crude prevalence of MS was found to be 38%, 46% and 41% when the NCEP ATP III, IDF and Harmonizing criteria were applied, respectively (Table 3-9). The increase in prevalence using the different criteria was mainly attributable to the ethnic-specific waist circumference cut-offs used in the IDF and Harmonizing criteria, which, in this case, identified more subjects with the syndrome than did the NCEP ATP III criteria.

This prevalence was higher than what has been reported recently in the United States (34%) by the American Heart Association (2011), and much higher than those reported for other population groups in South Africa, namely, 30% in Caucasians (Ker *et al*., 2007) and 23% in the African population (Motala *et al*., 2011). By whatever criteria used, our sample showed a much higher prevalence when compared to the 24.9% which was reported for an urban Asian population in India (Gupta *et al*., 2004) and in also excess of that reported recently in Asian Indians in the United States (Misra *et al*., 2010) as 26.7% and 38.2 % for the NCEP and IDF criteria respectively. This study therefore highlights the

importance of determining ethnic-specific analysis of the prevalence of the syndrome, and not just extrapolating results from other studies (Al-Shaer, 2005).

The present study also shows significant differences in the prevalence of MS amongst men and women, as females were almost twice as likely to have the syndrome as males (Table 3-9), in contrast to the general view that MS is equitably distributed between the sexes (Grundy, 2008). This finding was not altogether unexpected and may be ascribed to the greater frequency of general and abdominal obesity, as well as DM (Table 3-11) in females. This pattern has also been reported in other Asian Indian migrants by Misra & Khurana (2009), and by Ford *et al.* (2002) who described a higher prevalence of MS in the females of their respective cohorts. Recent studies in Asian Indian males (Chow *et al*., 2007; Deepa *et al*., 2007), in Germany (Dekker *et al*., 2005) and in Greece (Skoumas *et al*., 2007) have begun to show increasing prevalence data for MS in males.

The prevalence of MS also increased with ageing (Figure 3-4), and is probably due to the increased exposure of ageing individuals to CVD risk factors to a point where they are clinically evident. The increase in prevalence with advancing age was most discernible in females (Figure 3-4), and has also been reported in females from other ethnicities (Ford *et al*., 2004; Ford 2005). The pattern seen in males was different, with the prevalence of MS peaking in the 4th age groups (45-54 age groups), and decreasing in the 5th age groups (55-64 age groups). This pattern has also been recently described by Misra *et al.* (2010), who describes it as the characteristic Asian Indian male pattern, where dysmetabolism is observed early in life.

The trends with regards to MS and ageing were also different when compared to a recent study conducted in Botswana on health personnel, where the researchers (Garrido *et al*., 2009) found that highest prevalence was in the 35-54 age groups. Interestingly, this was attributed to the changing lifestyle patterns to Westernisation, which the authors believed, afflicted the young more than the old (who remained set in their traditional habits). Their findings therefore underscored the importance of early detection and intervention in the younger subjects of the sample.

4.2.2 The distribution and relationship of individual MS components

McNeill *et al*., (2005) describes a gradient of risk for the development of CVD that is associated with increasing numbers of MS components. The 45-54 age groups appear to be the worst affected in terms of the number of MS components, as this group comprised of the largest number of subjects with more than 1 MS component (Figure 3.5). The greatest number of subjects with all 5 MS components was in the 55-64 age groups. This is probably attributed to the increased exposure of these older individuals to CVD risk factors, developing from the effects of a sedentary lifestyle, a cholesterol-rich diet and the genetic contribution (Grundy, 1997), with ensuing insulin resistance and obesity, which are regarded as the main contributors of MS. What was also emerged from our study was that the MS was not confined to those subjects with DM, but was also present in subjects with prediabetes and even normal glucose tolerance.

The MS in prediabetic subjects is characterised by dyslipoproteinaemia (small-dense LDL, high ApoB, low HDL, low Apo-A1, high triglycerides) and fasting, as well as postprandial hyperinsulinaemia (NCEP ATPIII, 2001), with hypertension being a common finding. Our results showed a worsening of the lipid profile in the transition from the normal glycaemic profile to prediabetes, to DM (Table 3-12). Subjects with prediabetes and DM had markedly high HOMA-IR values, waist circumference measurements and BMI values as compared to those with normal fasting blood glucose.

Our study also showed that there were a high proportion of subjects (63.4%) with MS who had an impaired fasting glucose value (Table 3-11); the underlying mechanism which may be related to the impaired fasting glucose forming the intermediate stage in the pathogenesis of DM (Pietropaolo & Roith, 2001). Further decline in beta-cell function leads to more loss of insulin secretion, and eventual insulin resistance.

Insulin resistance is known to be strongly associated with MS (NCEP ATPIII, 2001; IDF, 2005) and may not be a condition exclusive to the obese, as was reported by Whincup *et al.* (2002) who found insulin resistance in non-obese subjects. There was a high prevalence of insulin resistance in our study, which correlated strongly ($r_s > 0.500$) with the fasting blood glucose, BMI and the waist circumference measurement (Table 3-8).

Our study also showed that there were 20% of subjects with MS who were normoglycaemic (Table 3-12), with 25% of MS subjects manifesting with prediabetes and 55% of subjects classified with MS being diabetic. A previous study on young Indian subjects presenting with acute myocardial infarction (MI) performed at the R K Khan

hospital in 2008 (Ranjith *et al*., 2008) reported a 57% prevalence of MS in his cohort, with 91% of subjects with MS in that cohort having impaired fasting glucose. Similarly, studies on other ethnic groups with DM in South Africa (Joffe & Kalke, 2008), found that MS was present in 46.5% of African and in 74.1% of Caucasian patients. This increasing trend has been observed in the US as well, as shown by the NHANES study (Lin et al., 2007), with 69.9% being reported in Whites, 64.8% for Blacks and 62.4% for Mexican Indians. The lower number of MS subjects with DM in our sample may be explained by the fact that apparently healthy, non-hospitalised subjects were recruited for our study. Other studies on Asian Indians (Misra *et al*., 2010) reported a higher prevalence of impaired fasting glucose and DM in their cohort of subjects with MS. Since these conditions were detected much more frequently in their participants than obesity, insulin resistance and DM were considered as the key drivers for the development of MS.

While obesity is known to contribute towards the development of metabolic risk factors and MS (Wasir *et al*., 2008), its value as a contributor to CVD risk, independent of the accompanying metabolic abnormalities is not well elucidated, and has been recently questioned (Bergman *et al*., 2007). A large, long-term follow-up study of 20 293 subjects showed that MS and DM were more important prognostic indicators of CVD risk, than abdominal obesity (Wildman *et al*., 2011). This was not the case in our study, which showed that although 45% of subjects with MS did not have DM (55% of subjects with MS were diabetic) and 25% of these were prediabetic (Table 3-12), abdominal obesity

was present in all subjects (Table 3-11) irrespective of the criteria that were applied (NCEP, IDF and Harmonizing).

The notion that the increased waist circumference measurement, being an obligatory inclusion for the definition of MS using the IDF criteria could somewhat water-down the effects of the other cardio-metabolic risk factors of the MS (Kalke & Joffe, 2008) did not appear to weaken the definition, since the IDF identified the highest number of subjects with MS in our sample. This may be explained by the propensity of Asian Indians to gain fat in the abdominal area, as opposed to the hip, buttock, and limb areas, and hence develop central, rather than generalised obesity (Misra *et al*., 2004). In addition, Das (2003) suggested that higher levels of TNF-α and IL-6 in Asian Indians may trigger increased expression of 11ß-HSD-1, which promotes the development of abdominal obesity, and may possibly explain why Asian Indians are more prone to develop abdominal, rather than general obesity. This was also shown in our study, by the strong, positive correlations between abdominal obesity and HOMA-IR, and abdominal obesity and MS (Table 3-8), confirming the close relationship between increased waist circumference, MS and insulin resistance. Additionally, the differing trends in obesity prevalence with respect to Asian Indians in the diaspora may once again be attributed to the potential contribution of environmental and lifestyle factors.

In terms of the frequency of the other components of MS observed in our study: there was a high prevalence of elevated triglyceride levels, followed by decreased HDL and then hypertension and DM consecutively, in descending order of prevalence (Table 3-

11). Ntyintyane *et al.* (2007) also found hypertriglyceridemia a common abnormality in their cohort of MS subjects; the difference being that while hypertriglyceridemia was the most common component in Black subjects with MS, the increased waist circumference was the most common in ours. The increased presence of raised triglycerides, particularly when using the IDF criteria, may be explained by the mechanisms proposed by Stannard & Johnson (2004). They reported that intramyocellular triglyceride content was inversely associated with whole-body insulin sensitivity, independent of general obesity.

The combination of abdominal obesity, elevated triglyceride levels and low HDL levels (Table 3-11) differs slightly to what has been reported in other studies, where hypertension (Hsieh *et al*., 2008) and impaired fasting glucose predominated (Dhanaraj *et al*., 2009). A possible explanation for this trend in our study may lie in the pathophysiology of MS, which has been related to systemic inflammation (Das, 2002; Rutter *et al.,* 2004). Healthy Asian Indians have been documented as having raised CRP and hence higher levels of TNF-α and IL-6 (through the feedback mechanism) as compared to Caucasians (Das, 2001). But hypertriglyceridemia, and glucose intolerance have also been associated with elevated TNF-α levels (Jovinge *et al*., 1998), which suggests increased susceptibility to these conditions, and hence, to MS.

4.2.3 The agreement between the IDF, NCEP ATP III and Harmonizing definitions of MS

The Harmonizing criteria, which includes raised ethnic-specific waist circumference measurement as one of the features of MS, was proposed to address the previous limitations of the IDF criteria and MS as a whole. Its merit lies in the removal of the obligatory waist circumference measurement, but includes it as part of the five criteria for the diagnosis of MS, to ensure that the effects of the remaining cardio-metabolic risk factors are not diluted. In keeping with studies done in the Brazilian population (Dutra *et al.,* 2012), our study reflects the effects of using the Harmonized criteria, which yielded a prevalence of 41%; a value that lies between the NCEP criteria (38%), and the IDF (46%) criteria (Table 3-9). We found that there was a strong agreement between the Harmonized and the IDF criteria when diagnosing MS (Kappa = 0.883), The Kappa statistic was slightly lower, when ascertaining the level of agreement between the NCEP ATP III definitions and the Harmonized criteria (0.812), although both levels of agreements are classified in the "almost perfect agreement" category (Landis & Koch, 1997).

4.3 *The Echocardiographic analysis of this sample*

The use of echocardiography for the evaluation of the left ventricular (LV) function and structure is regarded as one of the significant developments of modern medicine, especially in the area of risk stratification. Its value is especially relevant in the face of

the global epidemic of CVD and the concern with CV risk factors and MS appearing in younger individuals. There is evidence that indicates that, prior to the emergence of risk factors; vascular disease would have already developed in 30–50% of patients (Yusuf *et al.,* 2001). This supports the view that it may be possible to detect subtle, preclinical changes before overt structural and functional alterations of the heart occurs. This is the first population-based study that shows alterations in cardiac structure in the presence of CV risk factors, in Asian Indians in South Africa. It is also the first study to derive reference ranges for echocardiography parameters from a sample of apparently normal individuals for this population group in South Africa.

Although the baseline parameters were within the normal ranges (Lang *et al*., 2006), we found that the median value of the left atrium (LA) dimension was much higher as compared to values published by Lang *et al.* (2006). This feature was also described by Cuspidi *et al*. (2005), where the increase in the LA dimension was a reflection of the increased LV mass associated with hypertension. Increases in the LV mass in our subjects (Table 3-13) was also a prominent feature, with increased wall thickness, and altered Doppler parameters also observed in females with CV risk factors (Table 3-14). These findings were similar to those reported by Rutter *et al.* (2003) where these structural changes were attributed to the effects of insulin resistance. This theory may well be applicable to our cohort since we documented a high prevalence of insulin resistance and DM. Non-enzymatic glycation of vascular and membrane proteins, increased cellular fatty acid uptake, and hyperglycemia-induced oxidative stress (Dhalla *et al*., 1998) are

known sub-cellular changes that occur in response to DM, which promote these structural alterations in the heart.

4.3.1 Diastolic abnormalities and dysfunction

Our study confirms the association reported between age and diastolic dysfunction, as the median (IQR) age of subjects with diastolic dysfunction was higher than in those without diastolic abnormalities (Table 3-16). The increases in left ventricular mass index (LVMI) and the attenuation of transmitral indices with age were similar to those found in other community-based studies (Fischer *et al*., 2003; Redfield *et al*., 2003; Abhayrathna *et al*., 2006). Recent studies have focused on the prognosis associated with asymptomatic early LV dysfunction (Kuznetsova *et al*., 2010) and on the relatively high prevalence of heart failure with preserved LV ejection fraction (LVEF) (Redfield *et al*., 2003; Abhayaratna *et al*., 2006) in community-based settings. In keeping with these findings we have shown an elevation in the TDI diastolic filling ratio (Table 3-16), consistent with increased LV filling pressures in those subjects with diastolic abnormalities. This was evident in the almost one third of our cohort (31%) who manifested with diastolic abnormalities (Table 3-16). We also showed a greater prevalence of DM in subjects with diastolic abnormalities (32%) as compared to those subjects with normal diastolic profile (22%) in keeping with a recent study (Kim *et al*., 2011) which reported a significant worsening of diastolic parameters as the duration of DM increased in a cohort of 547 patients. In contrast to the limited number of community-based studies (Fischer *et al*., 2002; Redfield *et al*., 2003; Abhayrathna *et al*.,

2006), we found that there was a very low prevalence (<0.5%) of diastolic dysfunction per se in this community, when using the European Study Group on Diastolic Heart Failure (1998) guidelines.

Univariate associations revealed that an increasing age, BMI, left atrial volume index (LAVI) and the LVMI were independent predictors of diastolic abnormities (Table 3-17). Using multivariate step-wise regression, and after adjusting for age, female gender, hypertension, DM, LAVI and LVMI, we found that subjects were more likely to present with diastolic abnormalities if they were older (OR=0.986), female (OR =0.658) or hypertensive (OR= 0.73) (Table 3-17). The only independent echocardiographic predictor of diastolic abnormalities was an increase in the LAVI (0.882), in contrast to the TDI ratio, which is supposed to reflect LV filling pressure, independent of loading conditions. This may be explained by the fact that the TDI diastolic filling ratio (Em/Ea) represents a one-off evaluation of the LV filling pressure, while an increased LAVI provides physiological and morphological evidence of the chronic elevation in the LVEDP.

This study shows that the prevalence of diastolic dysfunction in this community is low. However, the changes to LV dimensions, as well as the increased LVM and LVMI, in the absence of diastolic dysfunction may well be due to the pathophysiological consequences of hypertension and obesity (Alpert, 2001). Cardiac remodeling in obese subjects is characterised by increased LV mass and end-diastolic dimensions (Powell *et al*., 2006) and is not without risk (Levy *et al*., 1990) since an increased LV mass is an independent predictor for cardiovascular morbidity and mortality in individuals

previously free of clinical CVD.

4.3.2 Echocardiographic changes in the presence of MS

In this study we have demonstrated that significant differences exist between subjects with and without the MS for chamber dimensions, wall thickness, transmitral flow velocities and TDI diastolic filling ratio (Em/Ea) (Table 3-18). There are many causative factors that may result in the alteration of LV structure and function in subjects with MS, namely obesity, hypertension, endothelial dysfunction, CAD, autonomic dysfunction and metabolic abnormalities (Farkouh *et al*., 2008). In fact, the MS was found to be one of the main determinants of increased LA dimension (Cuspidi *et al*., 2005). Considering that almost half our sample was diagnosed with the MS, with significant changes already apparent in the LA dimension in subjects with MS (Table 3-18), further complications related to this adverse remodeling are almost inevitable. Therefore, the major finding in our study is that changes in cardiac structure are present in the preclinical phase, when there is already interplay of the components constituting the syndrome (MS). Our study therefore supports the evidence that abnormal LV geometry and function, related to the MS (Chinali *et al*., 2006; Schillaci *et al.,* 2006) are present in subjects who may not be diabetic or hypertensive.

Our study has also documented changes in diastolic filling parameters, with attenuation of the early mitral (E-wave) filling velocity and the transmitral filling ratio (Em/Am), as well as an increase in the Tissue Doppler (Em/Ea) ratio in subjects with MS (Table 3-18).

These changes could all be secondary to the increase in the LVM and left ventricular mass index (LVMI). The effect of MS on LV diastolic function has previously been reported (Chinali *et al*., 2006) in the Strong Heart study, which showed that MS was associated with a higher prevalence of abnormal early left ventricular relaxation. However, we found a low prevalence of impaired diastolic function in this sample, with the alteration to diastolic parameters more likely being attributed to the very high prevalence of obesity and hypertension in this sample. A possible mechanism may be that the high prevalence of hypertension in this group contributed to ventricular remodeling; hence the resultant increases in LVM and LVMI. This was supported by a good correlation between blood pressure and the LVM and LVMI (r_s =0.337) (Table 3-19). Interestingly, these significant changes in LVM between subjects with and without MS persisted after controlling for hypertension (Table 3-18), suggesting that there may be other pathophysiological mechanisms driving cardiac remodeling, aside from hypertension. Although data regarding the relationships of diabetes and insulin resistance are conflicting, Sundström *et al.* (2006) reported that insulin resistance is a mitogenic stimulus for cardiac hypertrophy, with consequent increases in the LV mass, ventricular wall thickness and LA dimension. This underlying mechanism is plausible considering the high prevalence of insulin resistance (in 58% of subjects) in this sample (Table 3-7a).

The present study was one of the first to measure sub-epicardial fat tissue (SEAT) thickness echocardiographically, and showed a significantly increased thickness of sub-epicardial fat (Table 3-18) in subjects with MS as compared to those without ($p < 0.005$).

SEAT thickness has been recently validated as a measure of visceral adiposity (Iacobellis *et al*., 2005), which is accompanied by an increase in adipocyte size and subsequent adipose tissue dysfunction. The resultant production of inflammatory cytokines by adipose tissue macrophages (Baker *et al*., 2006) has been shown to contribute to the formation of an intimal atherosclerotic lesion (Miyata *et al*., 2000; Verhagen & Visseren, 2011), explaining the increased risk for coronary artery disease in subjects with MS.

The measurement of SEAT thickness has been shown to provide a more accurate assessment of metabolic risk, which could not be accounted for by anthropometric indexes and intra-abdominal visceral fat (Wang *et al.,* 2009). The present study showed that SEAT was moderately correlated with fasting blood glucose, blood pressure and the presence of MS (Table 3-19). SEAT thickness was significantly increased in subjects with DM, hypertension, abdominal and general obesity (Table 3-20). Bivariate correlations (Table 3-20) indicated that correlations were strongest between SEAT and BMI (r_s= 0.416) as well as the waist circumference (r_s = 0.415) measurement. There was a weaker correlation with DM (r_s = 0.249). Linear regression showed that a 1mm in SEAT thickness was associated with a 3 times likelihood of being diagnosed with MS. (Table 3-21). This association may be related to the inflammatory milieu that underpins the pathophysiology of both conditions (Das, 2002; Mazurek *et al*., 2003; Baker *et al*., 2006, Samaras *et al*., 2011; Ahn *et al*., 2007).

The measurement of SEAT thickness routinely in the echocardiography laboratory is feasible, as well as reliable in trained hands. Previous studies indicate that there is good

reproducibility in SEAT measurements, if adherence to standardised guidelines as proposed by Iacobellis *et al.* (2003) is complied with, and would minimise the inter-observer variability in a unit with many echocardiographers. Future studies in this aspect are promising, as the measurement of sub-epicardial fat has been used to evaluate therapeutic targets that modulate adipose tissue. Epicardial fat loss has been documented to be much faster than other indices of body fat (Iacobellis *et al*., 2008), and has been found in subjects undergoing weight loss interventions (Iacobellis *et al*., 2005).

4.4 The receiver operator curve and discriminating capacity to determine the presence of MS

A receiver operator curve (Figure 3.7) was constructed to assess discriminating capacity of individual MS components, anthropometry, and biochemical and echocardiography parameters to identify the MS diagnosis (IDF criteria). The highest AUC was yielded by the fasting blood glucose levels (AUC = 0.822), followed by HOMA-IR values (AUC = 0.816). The waist circumference had an AUC = 0.807, followed by the triglyceride levels (AUC = 0.806), while the mean LA dimension had the highest echocardiographic measure of AUC = 0.745 (Table 3-21). Categorising blood pressure (>or <130/85) using the IDF criteria, yielded the highest AUC was for this parameter. (AUC= 0.792).

In spite of the current controversies surrounding the MS, and its relevance as a syndromic entity, we share the views expressed by Reaven (2007) in that to date, there

is no alternative hypothesis to understanding of the clustering of these components in an individual and how each component correlates strongly with the "same defect-insulin resistance and compensatory hyperinsulinemia". The present study shows that MS is clearly related to an adverse cardiac phenotype, in the presence of even subtle CV risk factors.

4.5 Normal echocardiography reference ranges for this sample

One of the strengths of the present study was the opportunity to derive normal echocardiography ranges from this sample that is specific for the inhabitants of the Phoenix community. Initial studies looking at the effect of ethnicity on cardiac geometry recommended the development of ethnic-specific reference ranges (Chahal *et al*., 2010). Currently, there are very few data available on the echocardiography reference ranges in Asian Indians (Chahal *et al*., 2010) to reliably detect early cardiac structural and functional changes, since most studies have been mainly conducted between African-Caribbean and Caucasian populations. It is possible that these measurements, which form the basis of recent guidelines regarding the echocardiographic quantification and diagnosis of diastolic heart failure, may not be wholly applicable to this population group.

The present study examined the echocardiography variables of 1428 subjects: there were 38 subjects who were found to have structural and functional abnormalities, a

further 667 subjects who were diagnosed with MS (IDF criteria), and an additional 377 subjects with isolated and clustered risk factors who did not meet the criteria for the diagnosis of MS, and were subsequently excluded. Therefore, the measurements from 384 subjects (89 males and 257 females) who were free of cardiac structural and functional abnormalities, as detected by echocardiography, and without documented CV risk factors, were analysed (results are presented in Tables 3-23 and 3-24). The influence of the results by subjects who were replaced by substitutes could not be directly determined in this study. However, since the substitutes were recruited from the same geographical area, and were of similar socio-economic statues, we assume that the bias on selection was minimal.

The chamber dimensions, wall thickness and LV mass were significantly higher in males (Table 3-24). These differences in gender, which have been described previously by other researchers, were not unexpected, and may be attributed to the strong correlation that exists between linear measurements of LV dimensions and body habitus (Salton *et al*., 2002), since males were generally taller, and weighed more than females (Table 3-24).

But considering that the prevalence of over-weight and obesity in this sample was very high, the effects of obesity on LV geometry (Gottdiener *et al*., 1994, Salton *et al*., 2002) may be confounding, especially when assessing these variables to ascertain pathology. But, since height is strongly associated with lean body mass (Devereux *et al*., 1986), it is considered to be the best indication of the metabolic demands on the heart, negating

the effects of weight (or excess weight in our case). Indexing echocardiographic dimensions to height revealed a significantly larger LA dimension in females, but a higher LVMI and thicker SEAT in males (Table 3-25).

The analysis of the raw data (Table 3-24) showed that there was an increase in the LA dimension in our population, as compared to those proposed by Lang *et al*. (2005), as the 95th percentile values were in excess of the upper range for men (44mm cf 40mm) and women (44mm cf 38mm). However, a smaller LAVI was calculated in the present study, when compared to the reference values from Lang *et al.* (Appendix 4). The differences in the ranges may be explained by the fact that these values were calculated from subjects that are of Asian Indian ethnicity, while Lang *et al.* used subjects of Caucasian descent. These differences may therefore reflect the variances that exist in anthropometry (as shown by the lower 95^{th} percentile value in the LVEDD measurement in Table 3-24). So, the smaller LAVI seen in this population may well be due to the relatively smaller heart sizes of the subjects in our cohort, rather than a mere reflection of lower filling LV pressures.

What appears to be apparent are the effects of obesity on cardiac remodeling (in the LA) in this sample, since this group of subjects was not diagnosed with hypertension, glycaemic or lipid abnormalities. The MONICA/KORA study also shown a similar trend (Stritzke *et al*., 2009); where the effect of obesity was reported as being almost twice the effect of hypertension on increased LA size. These authors suggested that a possible mechanism for LA dilation was the hemodynamic alterations due to increased

intravascular volume and increased cardiac output associated with an obese state. Worryingly, an increase in the LA dimension, associated with obesity, has also been reported to confer increased risk for the development of atrial fibrillation (Wang *et al*., 2004), with its accompanying risk of stroke and heart failure in the general population.

With regards to the median LAVI values obtained in this study (13.9): this value was almost identical to that reported by Chahal *et al.* (2010) in a group of normal migrant Asian Indians residing in West London (14.2±4). However, there were differences in the other echocardiography parameters, as the ejection fraction that we recorded was higher, with the LVM and the LVM indexed to BSA, being smaller than the West London cohort. In spite of the similarity in Asian Indians, the LVMI may not be valid for comparison between different ethnic groups (Chahal *et al*., 2010), as was shown in our study, with the mean and 95th percentile values for the LVMI in our sample being higher than published ranges (Lang *et al*., 2006).

The benefit of indexing echocardiographic parameters to the body surface area is that the BSA makes provisions for obesity (as weight is used in conjunction with height to determine BSA). When echocardiographic variables were indexed to BSA, the significant differences between males and females in the LVM and LA dimension, as well as SEAT thickness persisted, but with females having larger LA dimensions and higher SEAT measurements (Table 25).

The echocardiography findings on normal subjects show that there are definite variations in terms of the prescribed normal reference ranges. Other studies which have

found discrepant reference values to published guidelines (Vasan *et al*., 2000) attribute them to the differences in sampling techniques, and different models of equipment used to acquire the images and the software for calculation: this may also be the case in our study. Furthermore, the variations in ethnicity are a very likely cause for the differences in parameters that we have shown. The new reference ranges that we have proposed will be valuable in clinical practice where CV risk stratification is required in this population.

4.6 Genetic patterns in the Metabolic Syndrome

In recent times, there has been an escalation in interest in the field of genetics, with the focus in cardiovascular medicine being the contribution of genetic variation to CVD risk (Gohlke *et al.,* 2009). Although the benefits of association studies have been recognised, its weakness lies in the heterogeneous nature of CVD, which would account for the different sets of genetic factors which predispose to CVD in different populations. Furthermore, atherosclerosis is known to be a multifactorial disease, without a single pathophysiological pathway. In this context, this means that some polymorphisms may be strongly associated with the disease in one population, but weak in others due to the presence of genetic factors. This therefore underscores the importance of population-based studies.

The Asian Indian population is known to demonstrate the triad of high triglyceride levels, with high LDL and low HDL levels, as described by Ravi *et al.* (2004), and hence, an

increased predisposition for CVD. The increasing rate of MS in this population also remains a concern. We therefore determined the genetic patterns associated with conventional CV risk factors and the metabolic syndrome, and the allele frequencies associated with particular CV risk factors, as well as risk factor clustering.

4.6.1 Lipoprotein Lipase polymorphisms

Lipoprotein Lipase has a direct role in pathophysiology of atherogenesis (Malloy, 2001). Lipid abnormalities are modulated by multiple gene-gene and gene-environmental interactions (Corella *et al*., 2002; Lee *et al*., 2004), and are hence subject to environmental and genetic variations. Since plasma fatty acid composition has been reported as a strong determinant in the development of MS (Garcia-Rios *et al*., 2011), polymorphisms which are of major importance to lipid metabolism were studied to determine their possible role in relation to MS and specific phenotypes.

4.6.1.1 FREQUENCY OF THE S447X POLYMORPHISM

The distribution of this genotype was almost equally distributed amongst males and females, with a much higher prevalence of the heterozygous variants of the S447X polymorphism than the homozygous wild-type (Table 3-26). There were no polymorphisms detected for the S291N variant, although Spence *et al.* (2003) also reported a 0.9% frequency for the S291N mutation in a sample of 452 subjects, while the EARS study (Gerdes *et al*., 1997) reported an allelic frequency of 3.1%.

There have been inconsistent observations made with regards to the allelic frequencies of the S447X polymorphism. Corella *et al.* (2002) reported a frequency of 0.14 in the Mediterranean population, while Spence *et al.* (2003) reported an allelic frequency of 4.4% for the S447X. The HuGe Association review and meta-analysis (Sagoo *et al*., 2008) reported a frequency of 9.9% in the X allele and overall, with this mutation being more common in East Asians (12.2%) than Caucasians (10.3%). Our study reports a comparatively high allelic frequency of the 447X in our sample (0.38), as compared to the frequency of (0.08) as reported by Bhanushali & Das (2010) in Asian Indians.

4.6.1.2 GENOTYPIC-PHENOTYPIC ASSOCIATIONS OF THE LPL POLYMORPHISMS

The lipoprotein lipase (LPL) gene is considered as a strong candidate gene for atherogenic lipid profiles and CAD. In particular, the S447X polymorphism has been reported as a putative gain-of-function mutation, with substantial debate as to whether it demonstrates increased lipolytic activity (Ross *et al*., 2005). Previous studies, including a meta-analysis on Caucasians by Whittrup *et al.* (1999) reported that the X447 allele was significantly associated with decreased triglyceride and increased HDL levels. This allele has therefore been associated with a reduced risk of CV disease, including myocardial infarction (Samani *et al*., 2007). This polymorphism is consequently regarded as conferring a cardio-protective effect, although this has been an inconsistent finding in some studies of Asian Indians (Lee *et al*., 2004; McGladdery *et al*., 2000). Our results did not suggest this protective effect, and indeed, were in contrast to what was found in a local study, which examined this polymorphism in subjects with MI (Ranjith *et al*., 2009).

These authors found a significant relationship between the X allele of S447X polymorphism and the presence of MS (NCEP criteria) with this particular polymorphism being associated with a favourable lipid profile. Since we found no significant relationships between this SNP and favourable or adverse lipid levels, it may be that the S447X SNP may not be the major causal SNP within LPL that influences lipid levels in this community-based population. In the same vein, we did not find any associations between this polymorphism and the presence of MS (Table 3-27) or with its components (Table 3-28).

Kern *et al.* (1990) reported that the relationship between LPL and BMI may alter clinical phenotypes, as LPL activity is up-regulated in response to caloric restriction. In spite of the high prevalence of this mutation in this sample, our study did not find any significant relationships between the S447X polymorphism and BMI.

The N291S variant has been reported to be associated with dyslipidemia, DM and CAD, and more especially with ageing and obesity (Yaomin *et al*., 2006). The underlying mechanisms relate to the increased rate of dissociation of LPL dimerisation, and hence, alteration of LPL enzymatic activity (Razzaghi *et al*., 2001). This mutation was not observed in our study, similar to the findings reported by Bhanushali & Das (2010), who, in their study of South Indian Asians, suggested that this mutation may be extremely rare or absent in the Indian population. This highlights the importance of population-based studies as disparities with other studies may be explained by the fact that particular associations found in one population may not necessarily be extrapolated to

others. In addition, inter-sample variations are likely to occur because of the linkage disequilibrium between a measured marker and an unmeasured functional allele at or near the LPL locus (Spence *et al*., 2003).

4.6.2 Polymorphisms of the Human Paraoxonase gene

The contribution of oxidation on the pathogenesis of atherosclerosis has been well-established, as oxidized low-density lipoproteins (LDL) are thought to promote endothelial injury and foam cell formation (Lusis, 2001). There has therefore been great clinical interest in polymorphisms conferring risk or protection from the effects of systemic oxidation. Previous studies (Getz *et al*., 2004) found a strong association between PON-1 polymorphisms and systemic measures of oxidative stress, which, in turn has been linked to the development of CVD (Stocker & Keany, 2004). PON-1 has also been reported to possess significant anti-atherogenic properties (Shih *et al*., 1998) and hence, has been suggested to lower susceptibility to CAD, although the effects of PON-1 polymorphisms to systemic atherogenesis in Asian Indians have been inconsistent (Sanghera *et al*., 1998). PON-1 has been reported to be associated with the oxidative modification of HDL and LDL, as well as in preventing the induction of monocyte-endothelial interactions on the arterial wall (Watson *et al*., 1995). PON-1 is also predictive of CVD risk (Jarvik *et al*., 2003; Mackness *et al*., 2003), and since MS has been reported to alter oxidative stress and contribute to atherosclerosis-related conditions (Tabur *et al*., 2010; Lavi *et al.,* 2008), we examined the association of these common

polymorphisms with MS and its components.

4.6.2.1 FREQUENCY OF THE PON-1 POLYMORPHISMS

The frequency of both polymorphisms (Q192R and L55M) were similarly distributed in males and females with no significance detected (Table 3-26).

The frequency of the PON-1 polymorphisms has been comprehensively described by Ginsberg *et al.* (2009). The 192R allele has been documented in 20–30% of Caucasian subjects, with similar percentages observed in Turkish (28%) and Asian Indian (31%) populations. This mutation appeared to be the dominant allele in certain populations, where the frequency was significantly increased: in African Americans (64%), Japanese (66%) and the Chinese (57%).

The frequency of the 192R-alelle in our sample was similar to those reported by Ginsberg *et al.* (2009), and was detected in 33% of subjects.

The frequency of the 55M allele is documented as being lower than the 192R allele, with estimates ranging from 26-38% in Caucasians, 18% in Afro-Americans and 21% in Asian Indians (Ginsberg *et al*., 2009). We found a frequency of 34% for the distribution of the 55M allele in the present study.

4.6.2.2 GENOTYPIC-PHENOTYPIC ASSOCIATIONS OF THE PON 1 POLYMORPHISMS

PON-1 polymorphisms have been somewhat inconsistently linked to the risk of coronary artery disease in population studies. For example, a meta-analysis by Wheeler *et al*.

(2004) reported that there was a weak association with CAD with the Q192R mutation, whereas, in contrast, Bhattacharya *et al.* (2008) reported an increased risk of all-cause mortality in subjects with the QQ192 genotype, the increased risk being ascribed to low PON-1 activity.

Insulin resistance and adiposity, which are considered to be the key components of MS, have also been associated with altered oxidative stress, which increases with increasing BMI. There are few available association studies with MS, with the focus being on PON-1 activity. Senti *et al.* (2003) reported lower PON-1 activity in subjects with MS compared to controls, due to the influence of oxidative stress, whereas Tabur *et al.* (2010) reported low or unchanged PON-1 activity, in the presence of oxidative stress. In terms of the polymorphisms, Martinelli *et al.* (2005) found that carriers of the 55Leu and 192Arg allele were at an increased risk of CAD in subjects with MS, although the genotypes were equally distributed between subjects with and without MS.

Since we did not measure PON-1 activity in our study, we were not able to make these comparisons. Furthermore, we did not find significant associations between MS and PON-1 polymorphisms (Table 3-27), as there were similar distributions in subjects with and without MS, the same trend that was noted with Senti *et al.* (2003).

In terms of the association with MS components: there was only one significant association detected in terms of the association between MS components, that being with HDL levels. Carriers of the 192R allele, which is known to reduce protection against lipid peroxidation, were inclined to have lower HDL levels (Table 3-28). This is plausible since 192R-allele has a lower activity for hydrolyzing lipid peroxide (Aviram *et al.,* 1999,

2000), hence resulting in lower anti-atherogenic properties, with subjects manifesting with lower HDL levels as an adverse phenotype.

Similarly, we found a significant interaction between the Q192R variant and obese females, where this polymorphism appeared to confer a predisposition for the development of obesity (Table 3-29), using the Asian cut-off for obesity (>25 kg/m^2). Although these findings were similar to those found in Portuguese women (Veiga *et al*., 2011), where the R-allele was found to be a risk factor for obesity, significant associations between the homozygous L (LL) variant and obesity has also been found in recent studies in the Mexican (Martínez-Salazar *et al*., 2011) population.

Vincent & Taylor (2006) reported that obesity was linked to increased oxidative stress and decreased concentrations of plasma antioxidants, hence, a higher susceptibility to lipid peroxidation. Therefore the relationship between obesity and the R-allele is not difficult to understand, as the R-allele has been reported as being less effective in protection from the effects of oxidative stress (Aubo *et al*., 2000; Levy, 2003). Another mechanism that has recently been suggested is that oxidized LDLs may encourage the development of obesity when endocytosed by adipocytes (Marsella *et al*., 2006). Adipocyte proliferation and differentiation is then stimulated, which consequently contributes to the increase in adipose tissue mass (Martínez-Salazar *et al*., 2011).

However, recent research by Galinier *et al.* (2006) reported that the adipose tissue of obese rats showed lesser indications of lipid peroxidation than that of lean rats, suggesting that obesity may not be associated with an inflammatory state since it is

related to a reduced redox state. Therefore, larger studies are necessary to confirm whether PON-1 polymorphisms indeed confer greater risk for obesity.

4.6.3 The Hardy-Weinberg equilibrium

We did not document Hardy Weinberg equilibrium (HWE) for any of the polymorphisms that we studied. The lack of HWE in population-based studies may be due to many reasons: existence of migration, selection, mutation and absence of random mating (Wittke-Thompson *et al*., 2005). The fact that these Asian Indian community members display significant heterogeneity in terms of their place of origin in India, their marriage and customs, their dietary and cultural habits (Seedat *et al*., 2005) may have contributed to this finding. Subjects with differences between groups of ethnic origin or differences between groups of similar ethnic origin but with limited admixture are known to contribute to lack of HWE (Radha *et al*., 2010) may have been included in this sample.

HWE violation may also be due to an excess of homozygotes or heterozygotes detected in the sample (Sen & Burmeister, 2008): our population reflects the latter. This pattern is probably indicative of over dominant selection or of outbreeding. Interestingly, several large studies have failed to show Hardy-Weinberg equilibrium (Zhong *et al*., 2013; Tan *et al*., 2010). Finally, it may also be possible that our study was underpowered to detect HWE deviation, as has been the case in many published studies (Salanti *et al*., 2005).

4.7 *Conclusion and recommendations*

The interdependent relationship between lifestyle "risk factors" and CVD appear to be strongly influenced by lifestyle patterns (dietary habits, physical inactivity, adiposity and harmful behavioural traits) via the effects on endothelial function, inflammation/oxidative stress, thrombosis/coagulation, arrhythmia, and other pathways (Mozaffarian *et al.,* 2008), which are all modifiable to some degree. However, certain ethnic groups have a higher propensity to develop clustering of risk factors, leading to a heightened predisposition to CV disease (Chaturvedi, 2003). In particular, Asian Indians are at a particularly higher risk of developing non-communicable diseases (Misra & Khurana, 2011; Yusuf *et al.,* 2001) related to obesity, and have a higher incidence and mortality rate from CVD than other population groups, with a plethora of research conducted to explain possible mechanisms for this excess in risk. Notwithstanding the accelerative effects of urbanisation and westernisation on CVD, Asian Indians have an additional increased susceptibility arising from altered biochemistry (dyslipidaemia, hyperinsulinaemia, and hyperglycaemia) (McKeige *et al.,* 1991), anthropometry ("thin-fat" phenotype) (Misra, 2003), perinatal conditioning (Yajnik *et al.,* 2002), a pro-coagulant state (Anand *et al.,* 2000), as well as genetic factors (Yusuf *et al.,* 2001). Furthermore, the prevalence of CV risk factors and risk factor clustering has been documented to be higher in Indians than the native populations of the diaspora. Epidemiologists have been sounding warning bells on the rising burden of cardiovascular disease (CVD) which has now exploded into epidemic proportions, including subjects from the Phoenix community.

The data from this study adds to the evidence that cardiovascular diseases now contribute, and continue contributing towards an ever-increasing proportion of non-communicable diseases in developing countries (WHO, 2002), promoted by the increase in life expectancy, together with the increasing global prevalence of CV risk factors like hypertension, obesity and DM. More importantly, for us, this study has drawn attention to the evolving epidemic of CV risk in the young in this population, particularly in the form of a high prevalence (~ 50%) of insulin resistance, hypercholesterolemia, increased BMI and waist circumference (Table 3-7a; 3-7b). These subjects are now manifesting with changes to their cardiac structure, fuelled by the high prevalence of CV risk factors, particularly, insulin resistance and obesity. These observations suggest a greater burden of CV disease in the near future, as these individuals reach adulthood, with these estimates projected to increase substantially in keeping with data extrapolated from other developing countries (Kruger *et al.,* 2002).

The clustering of risk factors into the metabolic syndrome (MS) is believed to account for approximately half of CVD risk for the development of atherosclerosis (Grundy, 2008), with the risk conferred deemed to be much higher than currently estimated. The main role of MS (Kahn *et al.,* 2005), therefore, seems to be its potential for identifying risk factors that are likely to occur concurrently. It is believed that the key to delaying the onset of a higher CV risk profile is early detection and intervention, particularly from childhood, considering the premature, malignant nature of CAD amongst Asian Indians

(Thankappen *et al.,* 2006). This will permit early detection and treatment of multiple risk factors, and the implementation of lifestyle modification, if necessary.

Our study has shown that there is a 46% prevalence of MS in a sample of the Phoenix community, driven largely by the presence of obesity and insulin resistance and is associated with significant alterations in cardiac structure. In our sample, the extremely high prevalence of MS in this community is a call to alert Health authorities to realise the long-term effects, and to expect a greater lifetime burden of CV disease, if interventions, specific to this population, are not implemented.

Since the high prevalence of MS in this community appears to be driven by central and abdominal obesity and insulin resistance, an emphasis on the developed risk factors like hypertension, dyslipidemia, and DM does not address the root causes of adiposity or its full cardiovascular consequences, since adiposity develops as a result of inadequate physical activity and poor dietary (high-caloric) habits, One of the key suggestions from the INTERHEART study was that common strategies be adopted for MI prevention for individuals from all backgrounds, age-groups and ethnicities, as well as both genders with these risk factors (Yusuf *et al.,* 2004). Changes in lifestyle (Ratner *et al.,* 2005) may be the most important factor for primary prevention, where subjects increase their physical activity and change their diet to one richer in fresh vegetables and fruit. This approach has been shown to produce great benefits with regards to other established CV risk factors related to inactivity, adiposity, and poor dietary habits at a community level.

These interventions must start as part of the public health strategy, particularly targeted at population and individuals who are prone to develop chronic diseases of lifestyle. Changes in legislation to incorporate physical education programmes at schools must be made, to reduce the burden of childhood obesity, which is already rife in South Africa. The population at large should be actively engaged and educated on the prevention and treatment of chronic diseases of lifestyle, and on how to take responsibility for their own health. This should be driven primarily by local, provincial and central government, together with the food industry, non-governmental agencies, and the media. These strategies, in addition to increasing awareness and possibly curtailing the burden of CVD on many levels, are also critical in order to prevent premature cardiovascular remodeling, considering that cardiac remodeling is already manifesting as a consequence of the obesity epidemic in this population, even in subjects free of CV risk factors. This study therefore strongly advocates echocardiographic evaluations on high-risk subjects. In so doing, structural changes may be identified early in the course of the "obesity cardiomyopathy", considering the high prevalence of CV and metabolic risk factors in this community, along with the absence of guidelines and detection strategies for control of CVD in our population.

Recent population-based investigations (Mollentze *et al*., 1995; Kruger *et al*., 2003; Tibazarwa *et al*., 2009) consistently recommend strategic and systematic surveillance of CV risk factors, particularly in communities undergoing epidemiological transition, like the Phoenix community. Therefore, longitudinal studies in this particular population should

be supported and implemented in order to determine the epidemiology patterns of CV risk factor development. This is critical since it is now established that clinical DM and coronary artery disease, which are the more common non-communicable diseases in developing countries, are preceded by risk factor clustering. If not, the current situation augurs a pandemic of CVD in this population in the near future, which is further intensified by the high CV risk profile of its younger members. Screening and intervention should therefore be focused in school-going individuals, when CV risk factors are already manifesting in this population.

A clearer understanding of many modern health conditions will emerge as one considers that most of human evolution took place when our ancestors were hunter-gatherers (Trevathan *et al*., 1999). This study therefore recommends that immediate strategies be developed and implemented to curtail the epidemic of cardiovascular disease, in keeping with the recent call from the World Heart Federation, Geneva, Switzerland (Ralston, 2012). In doing so modern man may regain the 'normal' physiology of our hunter–gatherer ancestors and thereby express the phenotype as it has evolved to be.

4.8 Limitations of the study

We have identified several limitations in our study, and they are discussed briefly below:

1. The design of this study was cross-sectional in nature: we could not observe and were therefore not able to report on trends that could have developed over time. We were therefore unable to ascertain risk associated with CV events, which could have been better addressed with a longitudinal study.
2. There was a predominance of females in our sample. This was attributed to the nature of randomly sampling subjects for this project. In hindsight, a stratified sample would have yielded a higher number of male participants. However, in terms of the comparability of our demographics with other population studies, it appears that the predominance of females appears to be a common trend in South African population studies (Erasmus et al., 2012). This high female to male participation ratios (2:1) have been observed in other community-based studies, looking at cardiovascular risk profiles and/or the metabolic syndrome (Omar et al, 1988; Tibazarwa et al.,2008, Motala et al. 2011) in South Africa, with the male component at times being as low as 19% (Erasmus et al. 2012).

3. It is established that the presence of MS does not predict absolute risk for CVD, as a significant portion of the pathogenesis of CV risk factors may be related to other factors such as gender, age and smoking, which are not included in the MS criteria, but included in other risk factor scores (like the Framingham criteria).

However, substantial data indicate that there is an increased CV risk associated with the presence of MS (Grundy, 2008). The Framingham criteria does not include features like abdominal obesity, increased fasting glucose and hypertriglyceridemia in its risk score, but uses age, gender and smoking status as well as cholesterol to predict CV risk (D'Augustino *et al*, 2001). The diagnosis of MS could therefore be used to refine CV risk in subjects with risk factors, and thereby extend the concept of cardiovascular risk (Opie, 2007).

4. In the present study, measurement of inflammatory markers (like CRP) was not done. CRP levels correlate strongly with the number of metabolic disorders (dyslipidemia, upper body adiposity, insulin resistance, and hypertension) that together constitute the MS. Since the metabolic syndrome has been associated with an inflammatory state (Fester *et al*., 2000), CRP might have served as a composite marker of risk and inflammation. In its absence, it would not be possible to assess the effect of these parameters on the presence of MS.

5. An important and recent enhancement of the assessment of regional LV function has been the calculation of the myocardial velocity gradient or strain rate imaging (SRI). Therefore future studies in this field will pursue an evaluation of systolic and diastolic ventricular function using global strain rate, strain, and regional systolic velocity and diastolic velocity, as was undertaken by Wong *et al.* (2005), to further evaluate the role of echocardiography as a screening tool for the early detection

of CV risk factors and MS.

6. The current study did not measure PON-1 serum concentrations or enzymatic activity, which would have been useful to correlate with such CV risk factors as prediabetes, hypertriglyceridemia and smoking. However, in retrospect, measurement of PON-1 activity and concentration would have been made from blood drawn possibly after the development or CV risk factors or a cardiovascular event. It would then not be possible to know whether the reflected PON-1 activity/concentration was due to the consequence of the event or the cause of the event, apart from the fact that wide variability exists, in terms of the enzymatic activity of PON-1 in humans (Balcerzyk *et al*., 2007). The challenges of deeming causality based on genetic association were also highlighted in this study, as shown by the lack of significant relationships between LPL SNPs and biochemical and anthropometric parameters. These associations have been reported elsewhere in other population groups, but were not found in this population.

7. The present project looked at four SNPs from two genes. Given the complexity of lipid and insulin regulation, as well as the multifactorial pathophysiology that is characteristic of the metabolic syndrome, it is reasonable that many genes may be responsible for these functions. Future studies in this population should look at genome-wide association scans (Zabaneh & Balding, 2010), which may yield more

information about the genetic pathways contributing to the common pathogenesis of the metabolic syndrome.

REFERENCES

1. Adkins, S.S., Gan, K.N. Mody, M. La Du, B.N. (1993) 'Molecular basis for the polymorphic forms of human serum paraoxonase/arylesteras: glutamine or argentine at position 191 for respective A or B allozymes.' *Am J Hum Genet*, 52:598-608
2. Abhayaratna, W.P. Marwick, T.H. Smith, W.T. Becker, N.G. (2006) 'Characteristics of left ventricular diastolic dysfunction in the community: an echocardiographic survey.' *Heart*, 92(9):1259-1264
3. Aguilera, C.M. Gil-Campos, M. Canete, R. Gil, A. (2008) 'Alterations in plasma and tissue lipids associated with obesity and the metabolic syndrome.' *Clin Sci*, 114:183-193
4. Ahn, S.G. and Lim, S. (2008) 'Relationship of epicardial adipose tissue by echocardiography to coronary artery disease.' *Heart,* 94(3): e7
5. Aijaz, B.K. Ammar, A. Lopez-Jimenez, F. Redfield, M.M, Jacobsen, S.J. Rodeheffer, R.J. (2008) 'Abnormal cardiac structure and function in the metabolic syndrome: a population-based study.' *Mayo Clin Proc*, 83(12);1350-7
6. Al-Shaer, M.H. (2005) 'The impact of ethnicity on the lifetime risk of metabolic syndrome and diabetes mellitus.' *The American Journal of Cardiolog,* 95(6):819-820
7. Alam, M. Wardell, J. Andersson, E. Samad, B.A. Nordlander, R. (1999) 'Characteristics of mitral and tricuspid annular velocities determined by pulsed wave Doppler tissue imaging in healthy subjects.' *J Am Soc Echocardiogr*, 12:618–628
8. Alberti, K.G. Eckel, R.H. Grundy, S.M., *et al* (2009) 'Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task

Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.' *Circulation,*120:1640–1645

9. Alberti, K.G. Zimmet, P. and Shaw, J. (2005) 'The metabolic syndrome—a new worldwide definition.' *Lancet*, 366:1059–1062
10. Alberti, G. Zimmet, P. Shaw, J. Grundy, S.M. The IDF consensus worldwide definition of the metabolic syndrome. available at: http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf (accessed on 13/10/2010)
11. Alexander, C.M. Landsman, P.B. Teutsch, S.M. Haffner, S.M. (2003) 'NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older.' *Diabetes*, 52:1210-14
12. Alpert, M.A. (2001) 'Obesity cardiomyopathy pathophysiology and evolution of the clinical syndrome.' *Am J Med Sci,*321:225–236
13. American Diabetes Association. (2012) 'Standards of medical care in diabetes—2012.' *Diabetes Care,* 35 (Suppl 1):S11-63
14. American Diabetes Association. (2006) 'Diagnosis and classification of diabetes mellitus.' *Diabetes Care,* 29 (Suppl 1):S43-8
15. Anand, S.A. Enas, A.E. Pogue, J. Haffner, S. Pearson, T. Yusuf, S. (1998)'Elevated Lipoprotein (a) levels in South Asians in North America.' *Metabolism*, 47(2): 182-184

16. Ascaso, J.F. Pardo, S. Real, .JT. Lorente, R.I. Priego, A., *et al*. (2003) 'Diagnosing Insulin Resistance by Simple Quantitative Methods in Subjects With Normal Glucose Metabolism.' *Diabetes Care,* 26: 3320–3325
17. Aubo, C. Senti M. Marrugat, J. Tomas, M. Vila, J. Sala, J. Masia, R. (2000) 'Risk of myocardial infarction associated with Gln/Arg 192 polymorphism in the human paraoxonase gene and diabetes mellitus. The REGICOR investigators.' *Eur Heart J*,21:33-38
18. Aviram, M. Hardak, E. Vaya, J. Mahmood, S. Milo, S., *et al*. (2000) 'Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities.' *Circulation,* 101: 2510-2517
19. Aviram, M. Rosenblat, M. Billecke, S. *et al*. (1999) 'Human serum paraoxonase (PON1) is inactivated by oxidised low density lipoprotein and preserved by antioxidants.' *Free Radic Biol Med*, 26:892-904
20. Baik, S.H. Ahn, S.G. Choi, J.H. Koh, B.R. Yoo, J.H. Kang, S.J. Choi, B.J. Choi, S.Y. Yoon, M.H. Tahk, S.J. Shin, J.H. (2007) 'The relationship of epicardial adipose tissue to metabolic syndrome and cardiovascular risk factors.' *Korean J Med*, 72:290-297
21. Baker, A.R. Silva, N.F. Quinn, D.W. Harte, A.L. Pagano, D. Bonser, R.S., *et al*. (2006) 'Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease.' *Cardiovasc Diabetol,* 5:1
22. Balarajan R. (1991). 'Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England and Wales.' *BMJ,* 302:560–564

23. Balcerzyk, A. Zak, I. and Krauze, J. (2007) 'Synergistic effects between Q192R polymorphism of paraoxonase 1 gene and some conventional risk factors in premature coronary artery disease.' *Arch Med Res*, 38:545–550
24. Balkau, B. and Charles, M.A. (1999) 'Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR).' *Diabet Med*, 16:442–443
25. Banerjee, I. (2010) 'Relationship between Paraoxonase 1 (PON1) gene polymorphisms and susceptibility of stroke: a meta-analysis.' *Eur J Epidemiol*.,25(7):449-589
26. Barbieri, M. Bonafe, M. Marfella, R. Ragno, E. Guigliano, D. Franceschi, C., *et al*. (2002) 'LL_paraoxonase genotype is associated with a more severe degree of homeostasis model assessment of IR in healthy subjects.' *J Clin Endocrinol Metab*, 87:2225
27. Bayturan, O. Tuzcu, E.M. Lavoie, A., *et al*. (2010) 'The metabolic syndrome, its component risk factors and progression of coronary atherosclerosis.' *Arch Intern Med*, 170:478
28. Beaglehole R & Bonita R. (2008) 'Global public health: a scorecard.' *Lancet* 372: 1988–1996
29. Bergman, R.N. Kim, S.P. Hsu, I.R. Catalano, K.J. Chiu, J.D. Kabir, M. Richey, J.M. Ader, M. (2007) 'Abdominal obesity: role in the patho- physiology of metabolic disease and cardiovascular risk.' *Am J Med*, 120:S3–S8
30. Bhattacharyya, T. Nicholls, S.J. Topol, E.J. Zhang, R. Yang, X. Schmidtt, D., *et al*. (2008) 'Relationship of Paraoxonase 1 (PON1) Gene Polymorphisms and Functional Activity With Systemic Oxidative Stress and Cardiovascular Risk.' *JAMA*, 299 (11): 1265-1276

31. Bhanushali, A.A. Das, B.R. (2010) 'Genetic variants at the APOE, lipoprotein lipase (LpL), cholesteryl ester transfer protein (CETP), and endothelial nitric oxide (eNOS) genes and coronary artery disease (CAD): CETP Taq1 B2B2 associates with lower risk of CAD in Asian Indians.' *J Community Genet,* 1:55–62
32. Blatter-Garin, M.C. James, R.W. Messmer, S. Barja, F. Pometta, D. (1994) 'Quantification of human serum paraoxonase by enzyme linked immunoassay: population differences in protein concentrations.' *Biochem,* 304:549-554
33. Borch-Johnsen, K. and Wareham, N. (2010) 'The rise and fall of the Metabolic Syndrome.' *Diabetologia*. Available from: http://dx.doi.org/10.1007/s00125-009-1620-4 [accessed 15 February 2012]
34. Bosello, O. and Zamboni, M. (2000) 'Visceral obesity and metabolic syndrome.' *Obes Rev*, 1:47–56.
35. Bradshaw, D. Nannan, N. Laubscher, R., *et al*. (2007) 'South African national burden of disease study 2000. Estimates of provincial mortality.' Cape Town: Medical Research Council. Available at http://www.mrc.ac.za/bod/estimate.pdf (Accessed on 21 April 2010)
36. Buysschaert, M. and Bergman, M. (2011) 'Definition of Prediabetes.' *Med Clin N Am,* 95: 289–297
37. Cabré, J.J. Martín, F. Costa, B. Piñol, J.L. Llor, J.L. Ortega, Y. Basora, J. Baldrich, M. Solà, R. Daniel, J. Hernández, J.M. Saumell, J., *et al*. (2008) 'Metabolic syndrome as a cardiovascular disease risk factor: patients evaluated in primary care.' *BMC Public Health,* 8:251

38. Cappuccio, C.P. (1997) 'Ethnicity and cardiovascular risk: variations in people of African ancestry and South Asian origin.' *Journal of Human Hypertension,* 11: 571–576
39. Ceriello, A. and Motz, E. (2004) 'Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes and cardiovascular disease? The common soil hypothesis revisited.' *Aterioscler Thromb Vasc Biol*, 24:816-823
40. Chahal, N.S. Lim, T.K. Jain, P. Chambers, J.C. Kooner, J.S. Senior, R. (2010) 'Ethnicity-related differences in left ventricular function, structure and geometry: a population study of UK Indian Asian and European white subjects.' *Heart*, 96:466-471
41. Chaowalit, N. Somers, V.K. Pellikka, P.A. Rihal, C.S. Lopez-Jimenez, F. (2006) 'Subepicardial adipose tissue and the presence and severity of coronary artery disease.' *Atherosclerosis*, 18:6354–6359
42. Chaturvedi, N. (2003) 'Ethnic differences in cardiovascular disease.' *Heart*, 89(6): 681–686
43. Cheal, K.L. Abbasi, F. Lamendola, C. McLaughlin, T. Reaven, G.M. Ford, E.S. (2004) 'Relationship to insulin resistance of the Adult Treatment Panel III diagnostic criteria for identification of the metabolic syndrome.' *Diabetes*, 53:1195-1200
44. Cheatham, B. Vlahos, C.J. Cheatham, L. Wang, L. Blenis, J. Kahn, C.R. (1994) 'Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis and glucose transporter translocation.' *Mol Cell Biol*, 14:4902–4911

45. Chinali, M. de Simone, G. Roman, M.J., *et al*. (2006) 'Impact of obesity on cardiac geometry and function in a population of adolescents: the Strong Heart study.' *J Am Coll Cardiol,* 47:2267–2273

46. Chobanian, A.V. Bakris, G.L. Black, H.R. Cushman, W.C. Green, L.A. Izzo, J.L Jr. Jones, D.W. Materson, B.J. Oparil, S. Wright, J.T Jr. Roccella, E.J. (2003) 'Seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure.' *Hypertension,* 42: 1206–1252

47. Chow, C.K. Naidu, S. Raju, K., *et al*. (2008) 'Significant lipid, adiposity and metabolic abnormalities amongst 4535 Indians from a developing region of rural Andhra Pradesh.' *Atherosclerosis,* 196(2): 943–952

48. Christiansen, L. Bathum, L. Frederiksen, H. Chrisitiansen, K. (2004) 'Paraoxonase 1 polymorphisms and survival.' *Eur J Hum Genet*, 12:843-847

49. Clendenning, J.B. Humbert, R. Green, E.D. Wood, C. Traver, D. Furlong, C.E. (1996) 'Structural organization of the human PON1 gene.' *Genomics*, 35: 586–589

50. Conus, F. Allison, D.B. Rabasa-Lhoret, R., *et al*. (2004) 'Metabolic and behavioral characteristics of metabolically obese but normal-weight women.' *J Clin Endocrinol Metab,* 89:5013

51. Corella, D. Guillen, M. Saiz, C., *et al*. (2002) 'Associations of LPL and APOC3 gene polymorphisms on plasma lipids in a Mediterranean population: interaction with tobacco smoking and the APOE locus.' *J Lipid Res,* 43:416-427

52. Cornier, M. Dabelea, D. Hernandez, T.L. Lindstrom, R.C. Steig, A.J. (2008) 'The Metabolic syndrome.' *Endocrine Reviews*, 29(7):777–822

53. Cosson, S. and Kevorkian, J.P. (2003) 'Left ventricular diastolic dysfunction: an early sign of diabetic cardiomyopathy?' *Diabetes Metab*, 29:455-466

54. Cusi, K. Maezono, K. Osman, A. Pendergrass, M. Patti, M.E. Pratipanawatr, T. DeFronzo, R.A. Kahn, C.R. Mandarino, L.J. (2000) 'Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle.' *J Clin Invest*, 105:311-320

55. Cuspidi, C. Meani, S. Valerio, C. Fusi, V. Sala, C. Zanchetti, A. (2005) 'Prevalence and correlates of left atrial enlargement in essential hypertension: role of ventricular geometry and the metabolic syndrome: The Evaluation of Target Organ Damage in Hypertension study.' *J Hypertens,* 23(4):875-822

56. D'Agostino, R.B Sr. Grundy, S. Sullivan, L.M. Wilson, P. (2001) 'Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation.' *JAMA,* 286(2):180-187

57. Dahabreh, I.J. Kitsios, G.D. Kent, D.M. Trikalinos, T.A. (2010) 'Paraoxonase 1 polymorphisms and ischemic stroke risk: A systematic review and meta-analysis.' *Genet Med.*, 12(10):606-15

58. Damani, S.B. (2011) 'Emerging Genomic Applications in Coronary Artery Disease.' *J Am Coll Cardiol Intv., 4(5):473-482*

59. Das, U.N. (2002) 'Is metabolic syndrome X an inflammatory condition?' *Exp Biol Med*, 227:989

60. De las Fuentes, L. Brown, A. and Mathews, S. (2007) 'Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass.' *European Heart Journal*, 28; 553–559

61. De Marco, M. De Simone, D. Roman, M.J. Chinali, M. Lee, E.T. Calhoun, D. Howard, B.V. Devereux, R.B. (2012) 'Cardiac Geometry and Function in Diabetic or Prediabetic Adolescents and Young Adults. The Strong Heart Study.' *Diabetes Care*, 34(10): 2300-2305

62. Deepa, M. Farooq, S. Datta, M. Deepa, R, Mohan, V. (2007) 'Prevalence of metabolic syndrome using WHO, ATPIII and IDF definitions in Asian Indians: the Chennai Urban Rural Epidemiology Study (CURES-34).' *Diabetes Metab Res Rev*, 23:127–134

63. Dekker, J.M. Girman, C. Rhodes, T. Nijpels, G. Stehouwer, C.D. Bouter, L.M. (2005) 'Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn study.' *Circulation,* 112:666-673

64. Devereux, R.B. Lutas, E.M. Casale, P.N., *et al*. (1984) 'Standardization of M-mode echocardiographic left ventricular anatomic measurements.' *Am Coll Cardiol,* 4:1222–1230

65. Devereux, R.B. Alonso, D.R. Lutas, E.M. Gottlieb, G.J. Campo, E. Sachs, I. Reichek, N. (1986) 'Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings.' *Am J Cardiol,* 57:450–458

66. Devereux, R.B. Roman, M.J. Paranicas, M. O'Grady, M.J. Lee, E.T. Welty, T.K. Fabsitz, R.R. Robbins, D. Rhoades, E.R. Howard, B.V. (2000) 'Impact of Diabetes on Cardiac Structure and Function. The Strong Heart Study.' *Circulation*, 101:2271-2276

67. Dhalla, N.S. Liu, X. Panagia, V., *et al*. (1998) 'Subcellular remodeling and heart dysfunction in chronic diabetes.' *Cardiovasc Res,* 40: 239–247

68. Dhanaraj, E. Bhanshali, A Jaggi, S. Dutta, P. Jain, S. et al. (2009)' Predictors of metabolic syndrome in Asian north Indians with newly detected type 2 diabetes.' *Indian J Med Res,* 129:506-514

69. Dhawan, S. (1995) 'Birth weights of infants of first generation Asian women in Britain compared with second generation Asian women.' *BMJ*, 311:86

70. Didenko, V.V. (2001) 'DNA probes using fluorescence resonance energy transfer (FRET): designs and applications.' *Biotechniques,* 31:1106-1116

71. Ding, J. Hsu, F.C. Harris, T.B. Liu, Y. Kritchevsky, S.B. Szklo, M. Ouyang, P. Espeland, M.A. Lohman, K.K. Criqui, M.H. Allison, M. Bluemke, D.A. Carr, J.J. (2009) 'The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA).' *Am J Clin Nutr,* 90(3):499-504

72. Dowse, G.K. Gareeboo, H. Alberti, K.G. Zimmet, P. Tuomilehto, J. Purran, A. Fareed, D. Chitson, P. Collins, V.R. (1995) 'Changes in population cholesterol concentrations and other cardiovascular risk factor levels after five years of the non-communicable disease intervention programme in Mauritius. Mauritius Non-communicable Disease Study Group.' *BMJ*, 311(7015): 1255–1259

73. Drukteinis, J.S. Roman, M.J. Fabsitz, R.R., *et al*. (2007) 'Cardiac and systemic hemodynamic characteristics of hypertension and prehypertension in adolescents and young adults. The Strong Heart Study.' *Circulation*; DOI: 10.1161/CIRCULATIONAHA.106.668921

74. Dumesnil, J.G. Paulin, C. Pibarot, P. Coulombe, D. Arsenault, M. (2002) 'Mitral annulus velocities by Doppler tissue imaging: Practical implications with regard to preload alterations, sample position, and normal values.' *J Am Soc Echocardiogr,* 15:1226–1231.

75. Durrington, P.N. Mackness, B. and Mackness, M.I. (2001) 'Paraoxonase and atherosclerosis.' *Arterioscler Thromb Vasc Biol*, 4(Apr 21):473–480

76. Dutra, E.S. Baiocchi de Carvalho, K.M. Miyazaki, E., *et al*. (2012) 'Metabolic syndrome in central Brazil: prevalence and correlates in the adult population.' *Diabetology & Metabolic Syndrome,* 4:20

77. Eapen, D. Kalra, G.L. Merchant, N. Arora, A. Khan, B.V. (2009) 'Metabolic syndrome and cardiovascular disease in South Asians.' *Vascular Health and Risk Management*, 5: 731–743

78. Eckel, R.H. Grundy, S.M. Zimmet, P.Z. (2005) 'The metabolic syndrome.' *Lancet,* 365:1415

79. Enas, E.A. Chacko, V. Pazhoor, S.G. Chennikkara, H. Devarapalli, H.P. (2007) 'Dyslipidemia in South Asian patients. *Current Atherosclerosis Report,* 9 (5):367–374

80. Enas, E.A. Singh, V. Gupta, R. Patel, R., *et al*. (2009) 'Recommendations of the Second Indo-US Health Summit for the prevention and control of cardiovascular disease among Asian Indians.' *Indian heart journal*, 61:265-274

81. Eroglu, S. Sade, L.E. Yildirir, A. Bal, U. Ozgul, A.S. et al. (2009) 'Epicardial adipose tissue thickness by echocardiography is a marker for the presence and severity of

coronary artery disease.' *Nutrition, Metabolism and Cardiovascular diseases,*19(3):211-217

82. European Study Group on Diastolic Heart Failure. (1998) 'How to diagnose diastolic heart failure.' *Eur Heart J*, 19:990–1003
83. Executive Summary of The Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. (2002) *Circulation*, 106:3143–421.
84. Farkouh, M.E. Rayfield, E. and Fuster, W. (2008) 'Diabetes and cardiovascular disease.' In: Fuster, W.. O'Rourke, R.A Poole-Wilson, P. (Eds.), *Hurst's The Heart* (12th ed.), McGraw-Hill Company: pp. 2077–2081
85. Festa, A. D'Agostino, R.J. Howard G., *et al*. (2000) 'Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS).' *Circulation*, 102: 42–47
86. Fischer, M. Baessler, A. Hense, H.W. Hengstenberg, C., *et al*. (2003) 'Prevalence of left ventricular diastolic dysfunction in the community. Results from a Doppler echocardiographic-based survey of a population sample.' *European Heart Journal*, 24: 320–328
87. Flekac, M. Skrha, J. Zı´dkova´, K. Lacinova´, Z. Hilgertova´, J. (2008) 'Paraoxonase 1 gene polymorphisms and enzyme activities in diabetes mellitus.' *Physiological Research*, 57: 717–726

88. Florentin, M, Liberopoulos, E.N. Wierzbicki, A.S. Mikhailidis, D.P. (2008) 'Multiple actions of high-density lipoprotein.' *Curr. Opin. Cardiol*, 23: 370–378

89. Ford, E.S. Giles, W.H. and Dietz, W.H. (2002) 'Prevalence of metabolic syndrome among US adults: findings from the Third National Health and Nutrition Examination Survey.' *JAMA,* 287:356– 359

90. Ford, E.S. and Giles, W.H. (2003) 'A comparison of the prevalence of the metabolic syndrome using two proposed definitions.' *Diabetes Care*, 26:575–581

91. Ford, E.S. (2005) 'Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence.' *Diabetes Care*, 28:1769

92. Fox, E.R. Alnabhan, N. Penman, A.D. Butler, K.R. Taylor, H.A Jr. Skelton, T.N., *et al*. (2007) 'Echocardiographic left ventricular mass index predicts incident stroke in African Americans: Atherosclerosis Risk in Communities (ARIC) Study.' *Stroke*, 38: 2686-2691

93. Fraser, A.G. (2009) 'Echocardiographic epidemiology—an emerging tool for early diagnosis, studying pathophysiology, predicting prognosis, and testing treatments.' *European Heart Journal*, 30: 642–644

94. Fujihara, F. Yasuda, T. Kawai, Y. Morikawa, N. Arakawa, K. Koda, Y. Soejima, M. Kimura-Kataoka, K. Takeshita, H. (2011) 'First survey of the three gene polymorphisms (PON1 Q192R, eNOS E298D and eNOS C-786T) potentially associated with coronary artery spasm in African populations and comparison with worldwide data.' *Cell Biochemistry and Function*, 29 (2):156–163

95. Fuster, V. Kelly, B.B., editors. (2010) 'Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health. Institute of Medicine (US) Committee on Preventing the Global Epidemic of Cardiovascular Disease: Meeting the Challenges in Developing Countries.' National Academies Press (US); Washington DC

96. Gaidukov, L. Rosenblat, M. Aviram, M. Tawfik, D.S. (2006) 'The 192R/Q polymorphs of serum paraoxonase PON1 differ in HDL binding, lipolactonase stimulation, and cholesterol efflux.' *J Lipid Res,* 47(11):2492–2502

97. Galassi, A. Reynolds, K. and He, J. (2006) 'Metabolic syndrome and risk of cardiovascular disease: a meta-analysis.' *Am J Med*, 119:812

98. Galinier, A. Carrière, A. Fernandez, Y. Carpéné, C. André, M. Caspar-Bauguil, S., *et al*. (2006) 'Adipose tissue proadipogenic redox changes in obesity.' *Journal of Biological Chemistry*, 281:12682–12687

99. Gami, A.S. Witt, B.J. Howard, D.E. Erwin, P.J., *et al*. (2007) 'Metabolic Syndrome and Risk of Incident Cardiovascular Events and Death A Systematic Review and Meta-Analysis of Longitudinal Studies.' *JACC*, 49(4): 403-414

100. Garcia-Rios, A. Delgado-Lista, J. Perez-Martinez, P. Phillips, C.M. Ferguson, J.F. Gjelstad, I.M. (2011) 'Genetic variations at the lipoprotein lipase gene influence plasma lipid concentrations and interact with plasma n-6 polyunsaturated fatty acids to modulate lipid metabolism.' *Atherosclerosis,* 218(2):416-422

101. Garrido, R.A. Semeraro, M.B. Temesgen, S.M. Simi, M.R. (2009) 'Metabolic syndrome and obesity among workers at Kanye Seventh-day Adventist Hospital, Botswana.' *SAMJ,* 99(5): 331-334

102. Gazino, J.M. (2005) 'Global burden of cardio vascular disease.' In: Braunwald Eugene, ed. *Heart disease a text book of cardiovascular medicine.* USA. 7th edition. WB Saunders Company. Philadelphia, Pensylvania

103. Genuth, S. Alberti, K.G. Bennett, P. Buse, J. Defronzo, R. Kahn, R. Kitzmiller, J. Knowler, W.C. Lebovitz, H. Lernmark, A. Nathan, D. Palmer, J., *et al* (2003) 'Follow-up report on the diagnosis of diabetes mellitus.' *Diabetes Care*, 26: 3160–3167

104. Getz, G.S. and Reardon, C.A. (2004) 'Paraoxonase: a cardioprotective enzyme: continuing issues.' *Curr Opin Lipidiol*, 15:261-267

105. Ghanim, H. Aljada, A. Hofmeyer, D, Syed, T. Mohanty, P. Dandona, P. (2004) 'Circulating mononuclear cells in the obese are in a proinflammatory state.' *Circulation,* 110:1564-1571

106. Ginsberg, H.N. Zhang, Y.L. and Hernandez-Ono, A. (2005) 'Regulation of plasma triglycerides in insulin resistance and diabetes.' *Arch Med Res,* 36:232–240

107. Goedecke, J.H. Jennings, C.L. and Lambert, E.V. (2005) 'Obesity in South Africa. Chronic Diseases of Lifestyle in South Africa since 1995 – 2005.' Available on: http://www.mrc.ac.za/chronic/cdl1995-2005.htm. Accessed on 19 August 2010

108. Gohlke, J.M. Thomas, R. Zhang, Y. Rosenstein, M.C. Davis, A.P. Murphy, C. (2009) 'Genetic and environmental pathways to complex diseases.' *BMC Syst Biol* , 3: 46

109. Goodarzi, M.O. Guo, X. Taylor, K.D. Quinones, M.J. Saad, M.F. Yang, H. Hsueh, W.A. Rotter, J.I. (2004) 'Lipoprotein lipase is a gene for insulin resistance in Mexican Americans.' *Diabetes*, 53:214–220

110. Goodarzi, M.O. Guo, X. Taylor, K.D. Quiñones, M.J. Samayoa, C. Yang, H. Saad, M.F. Palotie, A. Krauss, R.M. Hsueh, W.A. Rotter, J.I. (2004) 'Determination and use of haplotypes: ethnic comparison and association of the lipoprotein lipase gene and coronary artery disease in Mexican-Americans.' *Genet Med* , 5:322–327

111. Goodarzi, M.O. Taylor, K.D. Guo, X. Hokanson, J.E. Haffner, S.M. Cui, J. Chen, Y.D. Wagenknecht, L.E. Bergman, R.N. Rotter, J.I. (2007) 'Haplotypes in the lipoprotein lipase gene influence fasting insulin and discovery of a new risk haplotype.' *J Clin Endocrinol Metab*, 92:293–296

112. Gordon, G.W. Berry, G. Liang, X.H. Levine, B. Herman, B. (1998) 'Quantitative fluorescence resonance energy transfer measurements using fluorescence microscopy.' *Biophys J.*, 74(5):2702-2713

113. Gottdiener, J.S. Reda, D.J. Materson, B.J., *et al*. (1994) 'Importance of obesity, race and age to the cardiac structural and functional effects of hypertension.' *J Am Coll Cardiol*, 24:1492–1498

114. Greenlund, K.J. Croft, J.B. and Mensah, G.A. (2004) 'Prevalence of heart disease and stroke risk factors in persons with prehypertension in the United States, 1999–2000.' *Arch Intern Med,* 164:2113–2118

115. Groop, L. and Orho-Melander, M. (2001). 'The dyssmetabolic syndrome.' *J Intern Med*, 250:105-120

116. Grundy, S.M. (1997) 'Small LDL, atherogenic dyslipidemia, and the metabolic syndrome.' *Circulation*, 95:1–4

117. Grundy, S.M. Brewer, H.B Jr. Cleeman, J.I. Smith, S.C Jr. Lenfant, C. American Heart Association; National Heart, Lung, and Blood Institute. (2001) 'Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).' *JAMA,* 285(19): 2486–2497

118. Grundy, S.M. Cleeman, J.I. Daniels, S.R. Donato, K.A. Eckel, R.H. Franklin, B.A. Gordon, D.J. Krauss, R.M. Savage, P.J. Smith Jr, S.C. Spertus, J.A. Costa, F. (2005) 'Diagnosis and management of the metabolic syndrome. An American Heart Association/ National Heart, Lung, and Blood Institute Scientific Statement.' *Circulation,* 112:2735–2752

119. Grundy, S.M. (2007) 'Metabolic syndrome: a multiplex cardiovascular factor.' *Journal of Clinical Endocrinology and Metabolism*, 92(2):399–404

120. Grundy, S.M. (2008) 'Metabolic syndrome pandemic.' *Arterioscler Thromb Vasc Biol*, 28:629–636

121. Gulliford, M.C. Mahabir, D. Rocke, B. Chinn, S. Rona, R. (2001) 'Overweight, obesity and skinfold thicknesses of children of African or Indian descent in Trinidad and Tobago.' *Int J Epidemiol*, 30(5):989-998

122. Gupta, R. Deedwania, P.C. Gupta, A. Rastogi, S. Panwar, R.B. Kothari, K. (2004) 'Prevalence of the metabolic syndrome in an Indian urban population.' *International Journal of Cardiology,* 97: 257– 261

123. Gupta, R. Rastogi, P. Sarna, M. Gupta, V.P. Sharma, S.K. Kothari, K. (2007) 'Body-mass index, waist-size, waist-hip ratio and cardiovascular risk factors in urban subjects.' J Assoc Physicians India, 55: 621-627

124. Gupta, N. Singh, S. Maturu, V.N. Sharma, Y.P. Gill, K.D. (2011) 'Paraoxonase 1 (PON1) polymorphisms, haplotypes and activity in predicting cad risk in North-West Indian Punjabis.' *PLos One* 6(5):e17805. Epub 2011 May 24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21629682. (Accessed on 24 June 2012)

125. Hales, N.P. and Barker, D.J.P. (2001) 'The Thrifty phenotype hypothesis: Type 2 Diabetes.' *Br Med Bull*, 60 (1): 5-20

126. Hansel, B. Giral, P. Nobecourt, E. Chantepie, S. Bruckert, E. Chapman, M.J. Kontush, A. (2004) 'Metabolic Syndrome Is Associated with Elevated Oxidative Stress and Dysfunctional Dense High-Density Lipoprotein Particles Displaying Impaired Antioxidative Activity.' *Journal of Clinical Endocrinology & Metabolism,* 89(10): 4963-4971

127. Haring, H.U. (1991) 'The insulin receptor: signally mechanism and contribution to the pathogenesis of insulin resistance.' *Diabetologica,* 34:848-861

128. Harding, S. (2003) 'Mortality of migrants from the Indian subcontinent to England and Wales: effect of duration of residence.' *Epidemiology*, 14:287–292

129. Hayden, M.R. Ma, Y. Brunzell, J. Henderson, H.E. (1991) 'Genetic variants affecting human lipoprotein and hepatic lipases.' *Curr Opin Lipidol*, 2:104–109

130. Heller, G.V. (2005) 'Evaluation of the patient with diabetes mellitus and suspected coronary artery disease.' *Am J Med,* 118(Suppl 2):9S-14S

131. Hsieh, C.H. Pei, D. Hung, Y.J. Kuo, S.W. He, C.T. Lee, C.H., *et al*. (2008) 'Identifying subjects with insulin resistance by using the modified criteria of metabolic syndrome.' *J Korean Med Sci*, 23: 465-9. Published online 2008 June 20. doi: 10.3346/jkms.2008.23.3.465 (accessed on 10 September 2010)

132. Himsworth, H. (1936) 'Diabetes mellitus: a differentiation into insulin-sensitive and insulin-insensitive types.' *Lancet* i:127–130

133. Hokanson, J.E. and Austin, M.A. (1996) 'Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol levels: a meta-analysis of population-based prospective studies.' *J Cardiovasc Risk*, 3 (2):213-219

134. Hokanson, J.E. (1999) 'Functional variants in the lipoprotein gene and cardiovascular disease.' *Curr Opin Lipidol*, 10(5):393-399

135. Hoogeveen, R.C. Gambhir, J.K. Gambhir, D.S. Kimball, K.T. Ghazzaly, K. Gaubatz, J.W. Vaduganathan, M. Rao, R.S. Koschinsky, M. Morrisett, J.D. (2001) 'Evaluation of Lp(a) and other independent risk factors for CHD in Asian Indians and their USA counterparts.' *J Lipid Res.*, 42:631– 638

136. Hu, G. Qiao, Q. Tuomilehto, J. Balkau, B. Borch-Johnsen, K. Pyorala, K. (2004) 'Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women.' *Arch Intern Med*, 164:1066-1076

137. Huang, P.L. (2009) 'A comprehensive definition for metabolic syndrome.' *Dis Model Mech*; 2(5-6): 231-237

138. Huang AQ, Hu YH, Zhan SY, Xu B, Pang ZC, Cao WH, Lu J,Qin Y, Lee LM (2006) Lipoprotein lipase gene S447X polymorphism modulates the relation between central obesity and serum lipids, a twin study. Int J Obes (Lond) 30:1693–1701

139. Huddle, K.R.L. and Kalk, W.J. (1994) 'Practical Diabetes Management', 3rd edition Johannesburg: Wits Diabetes Group

140. Humphries, S.E. Nicaud, V. Margalef, J. Tiret, L. Talmud, P.J. (1998) 'Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and and postprandial plasma trigylcerides: the European Atherosclerosis Research Study (EARS).' *Atheroscler Thromb Vasc Biol*, 18:526-534

141. Iacobellis, G. Ribaudo, M.C. Assael, F. Vecci, E. Tiberti, C. Zappaterreno, A. Di Mario, U. Leonetti, F. (2003) 'Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk.' *J Clin Endocrinol Metab,* 88: 5163–5168

142. Iacobellis, G. Assael, F. Ribaudo, M.C. Zappaterreno, A. Alessi, G. Di Mario, U. Leonetti, F. (2003) 'Epicardial fat from echocardiography: a new method for visceral adipose tissue prediction.' *Obes Res*., 11(2):304-310

143. Iacobellis, G. and Leonetti, F. (2005) 'Epicardial adipose tissue and insulin resistance in obese subjects.' *J Clin Endocrinol Metab,* 90:6300–6302

144. Imai, Y. Morita, H. Kurhara, H. Sugiyama, T. Kato, N. Ebihara, A. Hamada, C., *et al*. (2000) 'Evidence for association between paraoxonase gene polymorphisms and atherosclerotic diseases.' *Atherosclerosis*, 149:435-442

145. International Diabetes Federation. (2005) 'The IDF consensus worldwide definition of the metabolic syndrome.' Available from http://www.idf.org/webdata/docs/Metabolic_syndrome_definition. (Accessed 24 February 2009)

146. Jarvik, G.P. Hatsukami, T.S. Carlson, C., *et al*. (2003) 'Paraoxonase activity, but not haplotype utilizing the linkage disequilibrium structure, predicts vascular disease.' *Arterioscler Thromb Vasc Biol*, 231:1465-1471

147. Jarvik, G.P. Rozek, L.S. Brophy, V.H., *et al*. (2000) 'Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1192 or PON155 genotype.' *Arterioscler Thromb Vasc Bio,* 20:2441-2447

148. Jennings, C.L. Lambert, E.V. Collins, M. Levitt, N.S. Goedecke, J.H. (2009) 'The atypical presentation of the metabolic syndrome components in black African women: the relationship with insulin resistance and the influence of regional adipose tissue distribution.' *Metabolism*, 58(2):149-157

149. Jensen, M.K. Rimm, E.B. Rader, D. Schmidt, E.B. *et al*. (2009) 'S447X variant of the lipoprotein lipase gene, lipids, and risk of coronary heart disease in 3 prospective cohort studies.' *Am Heart J*, 157:384-390

150. Jiang, Z.Y. Lin, Y.W. Clemont, A. Feener, E.P. Hein, K.D. Igarashi, M. Yamauchi, T. White, M.F. King, G.L. (1999) 'Characterization of selective resistance to insulin signaling in the vasculature of obese Zuker (fa / fa) rats.' *J Clin Invest,* 104:447-457

151. JiangPing, L. Xiaobin, W. Yong, H. TianHua, N. ChangZhong, C. GuoYing, Z., *et al*. (2005) 'PON-1 polymorphism, diabetes, obesity and risk of myocardial infarction:

modifying effects of diabetes mellitus and myocardial infarction.' *Genet Med*, 7(1):58-63

152. Jonas, M.A. Oates, J.A. Ockene, J.K. Hennekens, C.H. (1992) 'Statement on smoking and cardiovascular disease for healthcare professionals: American Heart Association.' *Circulation*, 86:1664-1669

153. Joy, T. Lahiry, P. Pollex, R.L. Hegele, R.A. (2008) 'Genetics of the metabolic syndrome.' *Current Diabetes Reports*, 8: 141–148

154. Jovinge, S. Hamsten, A. Tomvall, P., *et al*. (1998) 'Evidence for the role of tumor necrosis factor alpha in disturbances of triglycerides and glucose metabolism predisposing to coronary heart disease). *Metabolism*, 47:113

155. Juhan-Vague, I. Alessi, M.C. Mavri, A. Morange, P.E. (2003) 'Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk.' *J Thromb Haemost,* 1:1575–1579

156. Kaan, O. Balcioglu, A.S. Tavil, Y., *et al*. (2008) 'A relationship between echocardiographic subepicardial adipose tissue and metabolic syndrome.' *Int J Cardiovasc Imaging,* 24:577–583

157. Kahn, R. Buse, J. Ferrannini, E. Stern, M. (2005). 'The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes.' *Diabetes Care,* 28:2289 –2304

158. Kain, K. Catto, A.J. and Grant, P.J. (2003) 'Associations between insulin resistance and thrombotic risk factors in high-risk south Asian subjects.' *Diabet Med*, 20:651-655

159. Kalk, W. and Joffe, B. (2007) 'Differences in coronary heart disease prevalence and risk factors in African and White patients with type 2 diabetes.' *Diabetes Res Clin Pract*, 77(1):107-112

160. Kalk, W. and Joffe, B. (2008) 'The metabolic syndrome, insulin resistance and its surrogates in African and White subjects with type 2 diabetes in South Africa.' *Metab Syndr Relat Disord*, 6:247– 255

161. Kassi, E. Pervanidou, P. Kaltsas, G. Chrousos, G. (2011) 'Metabolic Syndrome: definitions and controversies.' *BMC Medicine*, 9:48

162. Kastelein, J.J. Ordovas, J.M. Wittekoek, M.E. Pimstone, S.N. Wilson, W.F. Gagne, S.E. Larson, M.G. Schaefer, E.F. Boer, J.M. Gerdes, C. Hayden, M.R. (1999) 'Two common mutations (D9N, N291S) in lipoprotein lipase: a cumulative analysis of their influence on plasma lipids and lipoproteins in men and women.' *Clin Genet*, 56:297–305

163. Kastelein, J.J. Jukema, J.W. Zwinderman, A.H. Clee, S.M. van Boven, A.J. Jansen, H. Rabelink, T.J. Peters, R.J. Lie, K.I. Liu, G. Bruschke, A.V. Hayden, M.R. for the REGRESS Study Group. (2000) 'Lipoprotein lipase activity is associated with severity of angina pectoris.' *Circulation,* 102:1629–1633

164. Kathiresan, S. Melander, O. Guiducci, C., *et al*. (2008) 'Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans.' *Nat Genet.*, 40:189–197

165. Kedia, G. Habibzadeh, M.R. Kudithipudi, V., *et al*. (2008) 'Using traditional measurements of the left atrial diameter to predict the left atrial volume index.' *Echocardiography*, 25(1):36-39

166. Ker, J.A. Rheeder, P. and Van Tonder, R. (2007) 'Frequency of the metabolic syndrome in screened South African corporate executives.' *CVJSA*, 8(1): 30-31

167. Kern, P.A. Ong, J.M. Saffari, B. Carty, J. (1990) 'The effects of weight loss on the activity and expression of adipose-tissue lipoprotein lipase in very obese humans.' *N Engl J Med.*, 322:1053–1059

168. Kerkeni, M. Addad, F. Chauffert, M. Chuniaud, L. Miled, A. Trivin, F. Maaroufi, K. (2006) 'Hyperhomocysteinemia, paraoxonase activity and risk of coronary artery disease.' *Clinical Biochemistry*, 39 821–825

169. Kershaw, E.E. Flier, J.S. (2004) 'Adipose tissue as an endocrine organ.' *J Clin Endocrinol Metab.,* 89(6):2548-2556

170. Kim, C.H. and Younossi, Z.M. (2008) 'Non-alcoholic fatty liver disease: a manifestation of the metabolic syndrome.' *Cleveland Clinic Journal of Medicine,* 75: 721–728

171. Kim, N.S. Kang, K. Cha, M.H. Kang, B. Moon, J. Kang, B. Yu, B. Kim, Y. Choi, S.M. Bang, O. (2007) 'Decreased paraoxonase-1 activity is a risk factor for ischemic stroke in Koreans.' *Biochemical and Biophysical Research Communications*, 364:157–162

172. Kim, Y. Shin, M. Kim, Y. Kang, W.C. Kim, B.R. Moon, J. Chung, W. Ahn, T.H. Shin, E.K. (2011) 'The impact of diabetes duration on left ventricular diastolic function and cardiovascular disease.' *Postgrad Med J,* 10:1136

173. Koh, K.K. Han, S.H. and Quon, M.J. (2005) 'Inflammatory markers and the metabolic syndrome: insights from therapeutic interventions.' *J Am Coll Cardiol,* 46:1978

174. Komurcu-Bayrak, E. Onat, E. Poda, M. Humphries, S.E., *et al*. (2007) 'The S447X variant of lipoprotein lipase gene is associated with metabolic syndrome and lipid levels among Turks.' *Clinica Chimica Acta*, 383 :110–115

175. Krishnaveni, G.V. Hill, J.C. Veena, S.R. Leary, S.D. Saperia, J. Chachyamma, K.J. Karat, S.C. Fall, C.H. (2005) 'Truncal adiposity is present at birth and in early childhood in South Indian children.' *Indian Pediatr,* 42:527–538

176. Kruger, H.S. Venter, C.S. Vorster, H.H. (2003) 'Physical inactivity as a risk factor for cardiovascular disease in communities undergoing rural to urban transition: the THUSA study.' *Cardiovasc J S Afr*, 14:16–23

177. Kusminski, C.M., Shetty, S., Orci, L.,Unger,R.H., Scherer,P.E. (2009)'Diabetes and apoptosis:Lipotoxicity.'*Apoptosis,* 14:1484-1495

178. Kuznetsova, T. Herbots, L. Jin, Y. Stolarz-Skrzypek, K. Staessen, J.A. (2010) 'Systolic and diastolic left ventricular dysfunction: from risk factors to overt heart failure.' *Expert Review of Cardiovascular Therapy,* 8 (2):251-258

179. Kwok, P.Y. and Chen, X. (2003) 'Detection of single nucleotide polymorphisms.' *Curr Issues Mol Biol,* 5(2):43-60

180. Kylin, E. (1923) 'Studien ueber das Hypertonie-hyperglykamie-Hyperurikamiesyndrom.' *Zentralblatt fuer Innere Medizin*, 44:105-127 Available on: http://www.medscape.org/viewarticle/458372. (Accessed on 17 September 2009)

181. Lakka, H.M. Laaksonen, D.E. Lakka, T.A. Niskanen, L.K. Kumpusalo, E. Tuomilehto, J. Salonen, J.T. (2002) 'The metabolic syndrome and total cardiovascular disease mortality in middle-aged men.' *JAMA*, 288:2709–2716

182. Landis, J.R. and Koch, G.G. (1977) 'The measurement of observer agreement for categorical data.' *Biometrics*, 33:159-174

183. Lang, R.M. Bierig, M. Devereux, R.B., *et al*. (2006) 'Recommendations for chamber quantification.' *Eur J Echocardiogr*, 7(2):79-108

184. Larsson, B. (1991) 'Obesity, fat distribution and cardiovascular disease.' *Int J Obesity*, 15:53–57

185. Lavi, S. McConnell, J.P. Lavi, R. Barsness, G.W. Rihal, C.S. Novak, G.D., *et al*. (2008) 'Association between the Paraoxonase-1 192Q>R Alleleic Variant and Coronary Endothelial Dysfunction in Patients with Early Coronary Artery Disease.' *Mayo Clin Proc*., 83(2):158-164

186. Lee, J. Tan, C.S. Chia, K.S., *et al*. (2004) 'The lipoprotein lipase S447X polymorphism and plasma lipids: interactions with APOE polymorphisms, smoking and alcohol consumption.' *J Lipid Res*, 45:1132-1139

187. Leeder, S. Raymond, S. Greenberg, H. (2004) 'Race against time: The challenge of cardiovascular disease in developing economies.' New York: Columbia University

188. Leviev, I. Kalix, B. Brulhart, M. James, R.W. (2001) 'The paraoxonase PON 1 promoter polymorphism is associated with increased serum glucose concentrations in non-diabetic patients.' *Diabetologia,* 44:1177-1183

189. Levy, A.P. (2003) 'Genetics of cardiovascular disease: identification of a major susceptibility gene.' *Acta Diabetol*, 40:S330–S333

190. Levy, D, Garrison, R.J. Savage, D.D. Kannel, W.B. Castelli, W.P. (1990) 'Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study.' *N Engl J Med*, 322: 1561-1566

191. Libby, P. (2002) 'Inflammation in atherosclerosis.' *Nature*, 420(6917):868-874

192. Li, H. Liu, D. and Liang, C. (2003) 'Paraoxonase gene polymorphisms, oxidative stress, and diseases.' *J Mol Med*, 81:766-779

193. Lim, W.Y.A. Chia, Y.Y. Liong, S.Y. Ton, S.H. Kadir, K.A. Husain, S.N. (2009) 'Lipoprotein lipase expression, serum lipid and tissue lipid deposition in orally-administered glycyrrhizic acid-treated rats.' *Lipids in Health and Disease,* 8:31

194. Lin, S.X. Pi, L. and Sunyer, E.X. (2007) 'Prevalence of the metabolic syndrome among US middle-aged and older adults with and without diabetes-a preliminary analysis of the NHANES 1999-2002 data.' *Ethn Dis*, 17:35-39

195. Lindsay, R.S. and Howard, B.V. (2004) 'Cardiovascular risk associated with the metabolic syndrome.' *Curr Diab Rep*, 4:63

196. Liu, J.E. Palmieri, V. Roman, M.J. Bella, J.N. Fabsitz, R. Howard, B.V. Welty, T.K. Lee, E.T. Devereux, R.B. (2001) 'The impact of diabetes on left ventricular filling pattern in normotensive and hypertensive adults: The Strong Heart Study.' *J Am Coll Cardiol,* 37:1943–1949

197. López-Ruiz, A. Jarabo, M.M. Martínez-Triguero, M.L. Morales-Suárez-Varela, M. Solá, E. Bañuls, C. (2009) 'Small and dense LDL in familial combined hyperlipidemia and N291S polymorphism of the lipoprotein lipase gene.' *Lipids in Health and Disease,* 8:12

198. Low, S. Chin, M.C. Ma, S. Heng, D. Deurenberg-Yap, M. (2009) 'Rationale for Redefining Obesity in Asians.' *Ann Acad Med Singapore*, 38:66-74

199. Lusis, A.J. Fogelman, A.M. and Fonarow, G.C. (2001) 'Genetic basis of atherosclerosis: part I: new genes and pathways.' *Circulation*, 110:1868–1873

200. Lusis, A.J. Mar, R. and Pajukanta, P. (2004) 'Genetics of atherosclerosis.' *Annu Rev Genomics Hum Genet*, 5:189–218

201. Mackness, M.I. Arrol, S. Abbot, C. Durrinton, P.N. (1993) 'Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase.' *Atherosclerosis* ,104:129-135

202. Mackness, B. Davies, G.K. Turkie, W., *et al*. (2001) 'Paraoxonase status in coronary heart disease: are activity and concentration more important than genotype?' *Arterioscler Thromb Vasc Biol*, 21:1451-1457

203. Mackness, B. Durrington, P. McElduff, P., *et al*. (2003) 'Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study.' *Circulation,* 107:2775-2779

204. Maddux, B.A. See, W. Lawrence, J.C. Goldfine, A.L. *et al*. (2001) 'Protection Against Oxidative Stress–Induced Insulin Resistance in Rat L6 Muscle Cells by Micromolar Concentrations of α-Lipoic Acid.' *Diabetes*, 50:404–410

205. Mahabadi, A.A. Massaro, J.M. Rositi, G.A. Levy, D. et al. (2009) 'Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study.' *European Heart Journal*, 30: 850–856

206. Malloy, M.J. and Kane, J.P. (2001) 'A risk factor for atherosclerosis: triglyceride-rich lipoproteins.' *Adv Intern Med*, 47:111-136

207. Marchington, J.M. Mattacks, C.A. and Pond, C.M. (1989) 'Adipose tissue in the mammalian heart and pericardium: structure, foetal development and biochemical properties.' *Comp Biochem Physiol B*, 94: 225–232

208. Märin, P. Andersson, B. Ottosson, M. Olbe, L. Chowdhury, B. Kvist, H. Holm, G. Sjöström, L. Björntorp, P. (1992) 'The morphology and metabolism of intra-abdominal adipose tissue in men.' *Metabolism*, 41:1242–1248

209. Maritim, A.C. Sanders, R.A. and Watkins, J.B. (2003) 'Diabetes, oxidative stress and antioxidants: a review.' *J Biochem Mol Toxicol*, 17:24-38

210. Marmot, M. (2000) 'Multilevel approaches to understanding social determinants.' In: Berkman LF, Kawachi I, editor. *Social epidemiology.* Oxford. Oxford University Press; pp. 349–367

211. Marsella, R. Vari, R. D`Archivio, M., *et al*. (2006) 'Oxidised LDL modulate adipogenesis in 3T-L1 preadipocytes by affecting the balance between cell proliferation and differentiation.' *FEBS Lett.*, 580:2421–2429

212. Martinelli, N. Girelli, D. Olivieri, O.,*et al*. (2005) 'Interaction between metabolic syndrome and PON1 polymorphisms as a determinant of the risk of coronary artery disease.' *Clin Exp Med*, 5:20–30

213. Martínez-Salazar, M.F. Almenares-López, D. García-Jiménez, S. Sánchez-Alemán, M.A. Juantorena-Ugás, A. Ríos, C. Monroy-Noyola, A. (2011) 'Relationship between

the paraoxonase (PON1) L55M and Q192R polymorphisms and obesity in a Mexican population: a pilot study.' *Genes Nutr.*, 6(4):361-368

214. Matthews, D.R. Hosker, J.P. Rudenski, A.S. Naylor, B.A. Treacher, D.F. Turner, R.C. (1985) 'Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.' *Diabetologia,* 28 (7): 412–419

215. Mayosi, B.M. Flisher, A.J. Lalloo, U.G. Sitas, F. Tollman, S.M. Bradshaw, D. (2009) 'The burden of non-communicable diseases in South Africa.' *Lancet*, 374:934–947

216. Mazurek, T. Zhang, L. Zalewski, A. Mannion, J.D. Diehl, J.T. Arafat, H., *et al*. (2003) 'Human epicardial adipose tissue is a source of inflammatory mediators.' *Circulation*, 108: 2460–2466

217. Mbanya, J.C. Motala, A.A. Sobngwi, E. Assah, F.K. Enoru, S.T. (2010) 'Diabetes in sub-Saharan Africa.' *Lancet* , 375:2254–2266

218. McGladdery, S.H. Pimstone, S.N. Clee, S.M. Bowden, J.F. Hayden, M.R. Frohlich, J.J. (2001) 'Common mutations in the lipoprotein lipase gene (LPL): effects on HDL-cholesterol levels in a Chinese Canadian population.' *Atherosclerosis,* 156(2):401-407

219. McKeigue, P.M. Shah, B. and Marmot, M.G. (1991) 'Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in south Asians.' *Lancet*, 337:382-386

220. McNeill, A.M. Rosamond, W.D. Girman, C.J., *et al*. (2005) 'The metabolic syndrome and 11-year risk of incident cardiovascular disease in the Atherosclerosis Risk in Communities study.' *Diabetes Care*, 28(2):385-390

221. McPhee, S.J. and Hammer, G.D. (2010) 'Cardiovascular diseases". In: *Pathophysiology of Disease: An Introduction to Clinical Medicine. 6th Edition.* McGraw-Hill Medical

222. Mead, J.R. and Ramji, D.P. (2005) 'The pivotal role of lipoprotein lipase in atherosclerosis.' *Cardiovasc Res*, 55:261–269

223. Mehan, M.B. Srivastava, N. and Pandya, H. (2006) 'Profile of NCD risk factors in an Industrial setting.' *J Postgrad Med*, 52 : 167-173

224. Meigs, J.B. Wilson, P.W. Nathan, D.M., *et al*. (2003) 'Prevalence and characteristics of the metabolic syndrome in the San Antonio Heart and Framingham Offspring studies.' *Diabetes*, 52:2160-2167

225. Mente, A. Yusuf, S. Islam, S. McQueen, M.J. Tanomsup, S. Onen, C.L. Rangarajan, S. Gerstein, H.C. Anand, S.S. INTERHEART Investigators. (2010) 'Metabolic syndrome and risk of acute myocardial infarction a case-control study of 26,903 subjects from 52 countries.' *J Am Coll Cardiol*, 55:2390-2398.

226. Misra, A. (2003) 'Body composition and the metabolic syndrome in Asian Indians: a saga of multiple adversities.' *Natl Med J India*, 16 : 3-7

227. Misra, A. Vikram, N.K. Arya, S. Pandey, R.M. Dhingra, V. Chatterjee, A., *et al*. (2004) 'High prevalence of insulin resistance in postpubertal Asian Indian children is associated with adverse truncal body fat patterning, abdominal adiposity and excess body fat.' *Int J Obes Relat Metab Disord*, 28 : 1217-1226

228. Misra, A. and Khurana, L. (2011) 'Obesity-related non-communicable diseases: South Asians vs White Caucasians.' *Int J Obes (London)*, 35(2):167-87.

229. Misra, K.B. Endemann, S.W. and Ayer, M. (2005) 'Leisure time physical activity and metabolic syndrome in Asian Indian immigrants residing in northern California.' *Ethn Dis*, 15 : 627-634

230. Misra, R. Patel, T. Kotha, P. Raji, A. Ganda, O. Banerji, M., *et al*. (2010) 'Prevalence of diabetes, metabolic syndrome and cardiovascular risk factors in US Asian Indians: results from a national study.' *Journal of Diabetes and its complications*, 24:145-153

231. Miyata, K. Shimokawa, H. Kandabashi, T. Higo, T. Morishige, K. Eto, Y., *et al*. (2000) 'Rho-kinase is involved in macrophage-mediated formation of coronary vascular lesions in pigs in vivo.' *Arterioscler Thromb Vasc Biol*, 20: 2351–2358

232. Modan, M. Halkin, H. Almog, S., *et al*. (1985) 'Hyperinsulinemia: a link between hypertension obesity and glucose intolerance.' *J Clin Invest.,* 75: 809–811

233. Mogelvang, R. Sogaard, P. Pedersen, S.A. Olsen, N.T. Schnohr, P. Jensen, J.S. (2009) 'Tissue Doppler echocardiography in persons with hypertension, diabetes, or ischaemic heart disease: the Copenhagen City Heart Study.' *Eur Heart J*, 30:731–739.

234. Mohamed, R.H. Karam, R.A. and Abd El-Aziz, T.A. (2010) 'The relationship between paraoxonase1-192 polymorphism and activity with coronary artery disease.' *Clin Biochem*, 43(6):553-558

235. Mollentze, W.F. Moore, A.J. Weigh, D.J., *et al*. (1995) 'Coronary heart disease risk factors in a rural and urban Orange Free State black population.' *S Afr Med J* 85(2):90-96

236. Mouhamed, D.H. Ezzaher, A. Mechri, A. Neffati, F. Omezzine, A. Bouslama, A. Gaha, L. Douki, W. Najjar, M.F. (2011) 'Effect of cigarette smoking on paraoxonase 1

activity according to PON1 L55M and PON1 Q192R gene polymorphisms.' *Environ Health Prev Med*, Dec 29. [Epub ahead of print]

237. Mohan, V. Shanthirani, C.S. and Deepa, R. (2003) 'Glucose intolerance (diabetes and IGT) in a selected south Indian population with special reference to family history, obesity and lifestyle factors: the Chennai Urban Population Study (CUPS 14).' *J Assoc Physicians India*, 51 : 771-777

238. Morcos, M. Humpert, P. Bierhaus, A. Nawroth, P. (2009) 'The unsolved problem of diabetes mellitus type 2 and associated complications.' *Chirurg*, 80 (5): 400-404

239. Motala, A.A. Omar, M.A. and Gouws, E. (1993) 'High Risk of Progression to NIDDM in South - African Indians with Impaired Glucose Tolerance.' *Diabetes*, 42, 556-563

240. Motala, A. Esterhuizen, T. Piries, F.J. Omar, M.A.K. (2011) 'The Prevalence of Metabolic Syndrome and Determination of the Optimal Waist Circumference Cutoff Points in a Rural South African Community.' *Diabetes Care*, February 17

241. Motulsky, A.G. and Brunzell, J.D. (2002) 'Genetics of coronary atherosclerosis.' In: *The genetic basis of Common Diseases.* 2nd ed. King RA, Rotter JI, Motulsky AG, Eds. New York, Oxford University Press

242. Mozaffarian, D. Wilson, P.W.F. Kannel, W.B. (2008) 'Beyond Established and Novel Risk Factors. Lifestyle Risk Factors for Cardiovascular Disease.' *Circulation*, 117: 3031-3038

243. Mukhopadhyay, B. Sattar, N. and Fisher, M. (2005) 'Diabetes and Cardiac Disease in South Asians.' *British Journal of Diabetes and Vascular Disease*, 5(5):253-259

244. Murray, C.J.L. and Lopez, A.D. (1994) 'Global comparative assessments in the health sector.' Geneva, Switzerland: World Health Organization

245. Murthy, V. Julien, P. and Gagne, C. (1996) 'Molecular pathobiology of the human lipoprotein lipase gene.' *Pharmacol Ther*, 70**:** 101–135

246. Nagueh, S.F. Appleton, C.P. Gilleber, T.C., *et al*. (2009) 'Recommendations for the evaluation of left ventricular diastolic function by echocardiography.' *J Am Soc Echocardiogr*, 22:107

247. Nagueh, S.F. Sun, H. Kopelen, H.A. Middleton, K.J. Khoury, D.S. (2001) 'Hemodynamic determinants of the mitral annulus diastolic velocities by tissue Doppler.' *J Am Coll Cardiol*, 37:278-285

248. Nagueh, S.F. Middleton, K.J. Kopelen, H.A. Zoghbi, W.A. Quinones, M.A. (1997) 'Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures.' *J Am Coll Cardiol.,* 30: 1527–1533

249. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). (2001) 'Third report of the National Cholesterol Education Program (NCEP).' *JAMA*, 285:2486-2497

250. National Institutes of Health. (1998) 'Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults.' *Am J Clin Nutr* ,68(4):899–917

251. Nauck, M. Wieland, H. and Ma¨rz, W. (1999) 'Rapid, Homogeneous Genotyping of the 4G/5G Polymorphism in the Promoter Region of the PAI1 Gene by Fluorescence Resonance Energy Transfer and Probe Melting Curves.' *Clinical Chemistry,* 45(8): 1141–1147

252. Neel, J.V. (1962) 'Diabetes Mellitus: a 'thrifty' genotype rendered detrimental by 'progress'?' *Am J Hum Genet*, 14: 353-362

253. Ntyintyane, L.M. Panz, V.R. Raal, V.R. Gill, G.V. (2007) 'Postprandial lipaemia, metabolic syndrome and LDL particle size in urbanised South African blacks with and without coronary artery disease.' *International Journal of Medicine*, 101 (2): 111-119

254. Nugent, A.P. (2004) 'The metabolic syndrome.' *Nutrition Bulletin*, 29 (1): 36-43

255. Ockene, I.S. and Miller, N.H. (1997) 'Cigarette Smoking, Cardiovascular Disease, and Stroke. A Statement for Healthcare Professionals from the American Heart Association.' *Circulation,* 96: 3243-3247

256. Ogah, O.S. Adebanjo, A>T. Adedeji, S.O. Tarwanger, J.J. (2006) 'Echocardiography in Nigeria: use, problems, reproducibility and potentials.' *Cardiovascular Ultrasound*, 4:13

257. Oh, J.K Hatle, L. Tajik, J. Little, W.C. (2006) 'Diastolic Heart Failure Can Be Diagnosed by Comprehensive Two-Dimensional and Doppler Echocardiography.' *J Am Coll Cardiol*., 47(3):500-506

258. Oka, K. Tkalcevic, G.T. Nakano, T. Tucker, H. Ishimura-Oka, K. Brown, W.V. (1990) 'Structure and polymorphic map of human lipoprotein lipase gene.' *Biochim. Biophys. Acta,* 1049:21-26

259. Olivera, S.A. Mansur, A.P. Ribiero, C.C. Ramires, J.A. Annichino-Bizzacchi, J.M. (2004) 'PON1 M/L 55 mutation protects high-risk patients against coronary heart disease.' *Int J Cardiol.*, 94:73-77

260. Omar, M.A. Seedat, M.A. Dyer, R.B. Motala, A.A. (1988) 'Diabetes and hypertension in South African Indians. A community study.' *S. Afr. Med. J.*, 73:635-637

261. Omar, M.A. Seedat, M.A. Motala, A.A. Dyer, R.B. Becker, P. (1993) 'The prevalence of diabetes mellitus and impaired glucose tolerance in a group of urban South African blacks.' *S. Afr. Med. J.,* 83:641-643

262. Omar, M.A. Seedat, M.A Dyer, R.B. Motala, A.A. Knight, L.T. Becker, P.J. (1994) 'South African Indians show a high prevalence of NIDDM and bimodality in plasma glucose distribution patterns.' *Diabetes Care,* 17:70-73

263. Ommen, S.R. Nishimura, R.A. Appleton, C.P. Miller, F.A. Oh, J.K. Redfield, M.M. Tajik, A.J. (2000) 'Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: a comparative simultaneous Doppler-catheterization study.' *Circulation*, 102:1788 –1794

264. Onat, A. Ozhan, H. Erbilen, E. Albayrak, S. Küçükdurmaz, Z. Can, G. Keleş, I. Hergenç, G. (2009) 'Independent prediction of metabolic syndrome by plasma fibrinogen in men, and predictors of elevated levels.' *Int J Cardiol*, 135:211-217

265. Ong, K.K and Loos, R.J.F. (2007) 'Rapid infancy weight gain and subsequent obesity: Systematic reviews and hopeful suggestions.' 29 MAR 2007 DOI: 10.1080/08035250600719754. (Accessed on 06/02/2012)

266. Opie, L.H. (2007) 'Metabolic syndrome.' *Circulation,* 115: e32-e35

267. Opie, L.H and Mayosi, B. (2005) 'Cardiovascular disease in sub-Saharan Africa.' *Circulation*, 112:3536-3540

268. Owan, T.E. Hodge, D.O. Herges, R.M. Jacobsen, S.J. Roger, V.L. Redfield, M.M. (2006) 'Trends in prevalance and outcome of heart failure with preserved ejection fraction.' *N Engl J Med*, 355:251–259.

269. PAHO/WHO. (1999).a. Trinidad and Tobago: Basic Country Health Profiles, Summaries. PAHO/WHO Web page. Country Health Profiles Available on (http://www.paho.org) (Accessed 31 March 2012)

270. Park, J.H. Park, Y.S. Kim, Y.J. Lee, I.S. Kim, J.H. Lee, J.H. Choi, S.W. Jeong, J.O. Seong, I.W. (2010) 'Effects of statins on the epicardial fat thickness in patients with coronary artery stenosis underwent percutaneous coronary intervention: comparison of atorvastatin with simvastatin/ezetimibe.' *J Cardiovasc Ultrasound*, 18(4):121-126

271. Pan, C.Y. So, W.Y. Khalid, B.A.K., *et al*., on behalf of the ASDIAB Study Group. (2004) 'Metabolic, immunological and clinical characteristics in newly diagnosed Asian diabetes patients aged 12-40 years.' *Diabet Med*, 21:1007- 1013

272. Patel, J.V. Vyas, A. Cruickshank, J.K. Prabhakaran, D. Hughes, E. Reddy, K.S. Mackness, M.I. Bhatnagar, D. Durrington, P.N. (2006) 'Impact of migration on

coronary heart disease risk factors: comparison of Gujaratis in Britain and their contemporaries in villages of origin in India.' *Atherosclerosis*, 185:297–306

273. Pati, N. and Pati, U. (1998) 'Paraoxonase gene polymorphism and coronary artery disease in Indian subjects.' *International Journal of Cardiology*, 66:165-168

274. Paulus, W.J. Tsch¨ope,C. Sanderson, J.E., *et al*. (2007) 'How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology.' Eur Heart J, 28:2539-2550

275. Pearson, T.A. Bazzarre, T.L. Daniels, S.R. Fair, J.M. Fortmann, S.P. Franklin, B.A., *et al*. (2003) 'American Heart Association guide for improving cardiovascular health at the community level: a statement for public health practitioners, healthcare providers, and health policy makers from the American Heart Association Expert Panel on Population and Prevention Science.' *Circulation*, 107; 645-651

276. Peterson, L.R. Waggoner, A.D. Schechtman, K.B. Meyer, T. Gropler, R.J. Barzilai, B. Davila-Roman, V.G. (2004) 'Alterations in left ventricular structure and function in young healthy obese women: Assessment by echocardiographyand tissue Doppler imaging.' *J Am Coll Cardiol*, 43:1399–1404

277. Phillips, C.M. Tierney, A.C. and Roche, H.M. (2008) 'Gene-Nutrients in the Metabolic Syndrome.' *J Nutrignet Nutrigenomics*, 1: 135-151

278. Pietropaolo, M. and Le Roith, D. (2001) 'Pathogenesis of diabetes: our current understanding.' *Clin Cornerstone,* 4:1–16

279. Pillarisetti, S, and Saxena, U. (2003) 'Lipoprotein lipase as a therapeutic target for dyslipidemia.' *Front Biosci*, 8:d238-241

280. Poirer, P. Bogaty, P. Garneau, C Marois, L. Dumesnil, L.G. (2001) 'Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy.' *Diabetes Care*, 24 (1): 5–10

281. Pollare, T. Lithell, H. and Vessby, B. (1991) 'Lipoprotein lipase activity in the skeletal muscle is related to insulin sensitivity.' *Atheroscler Throm Vasc Biol*, 11:1192-1203

282. Pont-Kingdon, G, and Lyon, E. (2005) 'Direct molecular haplotying by melting curve analysis of hybridization probes: beta 2-adrenergic receptor haplotypes as an example.' *Nucleic Acids Research,* 10 (33):e89

283. Poole-Wilson, P. (2005) 'The prevention of cardiovascular disease worldwide: Whose task and WHO's task?' *Clinical Medicine,* 5: 379–384

284. Powell, B.D. Redfield, M.M. Bybee, K.A. Freeman, W.K. Rihal, C.S. (2006) 'Association of Obesity with Left Ventricular Remodeling and Diastolic Dysfunction in Patients without Coronary Artery Disease.' *Am J Cardiol,* 98(1):116-120

285. Preiss-Landi, K. Zimmermann, R. Hammerle, G. Zechner, R. (2002) 'Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism.' *Curr Opin Lipidol*, 13(5):471-481

286. Primo-Parmo, S.L. Sorenson, R.C Teiber, J. LaDu, B.N. (1996) 'The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family.' *Genomics*, 1;33(3):498-507

287. Pritchett, A.M. Jacobsen, S.J. Mahoney, D.W. Rodeheffer, R.J. Bailey, K.R. Redfield, M.M. (2003) 'Left atrial volume as an index of left atrial size: a population-based study.' *J Am Coll Cardiol,* 41:1036–1043

288. Puoane, T. Steyn, K. Bradshaw, D., *et al*. (2002) 'Obesity in South Africa: the South African demographic and health survey.' *Obes Res,*;10:1038–1048

289. Quinones, M.A. Otto, C.M. Stoddard, M. Waggoner, A. Zoghbi, W.A. (2002) 'Recommendations for quantification of Doppler echocardiography: A report from the Doppler quantification task force of the nomenclature and standards committee of the American Society of Echocardiography.' *J Am Soc Echocardiogr*, 15:167–184

290. Radha, V. Vimaleswaran, K.S. Ayyappa, K.A. Mohan, V. (2007) 'Association of lipoprotein lipase gene polymorphisms with obesity and type 2 diabetes in an Asian Indian population.' *Int J Obes (Lond),* 31:913–918

291. Ralston, J. (2012) 'New global target on non-communicable diseases: a call to action for the global cardiovascular disease community.' *CVJA,* 23(5):241-242

292. Ramachandran, A. Ching Wan, Ma. Snehalatha, R. (2010) 'Diabetes in Asia.' *Lancet,* 375(9712):408 – 418

293. Ranjith, N. Pegeraro, R. Naidoo, D.P. Shanmugam, R. Rom, L. (2008) 'Genetic variants associated with Insulin Resistance and Metabolic syndrome in Young Asian Indians with Myocardial Infarction.' *Metabolic Syndrome and Related disorders,* 6(3): 209-214

294. Ranjith, N. Verho, N.K. Verho, M. Winkelmann, B.R. (2002) 'Acute Myocardial Infarction in a Young South African Indian-Based Population: Patient Characteristics

on Admission and Gender-Specific Risk Factor Prevalence.' *Curr Med Res Opin*, 18, 242-248

295. Ratner, R. Goldberg, R. Haffner, S. Marcovina, S. Orchard, T. Fowler, S. Temprosa, M. (2005) 'Impact of intensive lifestyle and metformin therapy on cardiovascular disease risk factors in the diabetes prevention program.' *Diabetes Care,* 28: 888–894

296. Ravi, G.R. Pradeepa, R. and Mohan, V. (2004) 'Hypertriglyceridemia and coronary artery disease-an update.' *Indian Heart J,* 56:21–26

297. Razzaghi, H.B. Day, W. McClure, R.J. Kamboh, M.I. (2001) 'Structure-function analysis of D9N and N291S mutations in human lipoprotein lipase using molecular modeling.' *J. Mol. Graph. Model.,* 19: 487–490

298. Reaven, G.M. (1988) 'Role of insulin resistance in human disease.' *Diabetes,* 37:1595-1607

299. Reaven, G.M. (2007) 'The Individual Components of the Metabolic Syndrome: Is There a Raison d'Etre?' *J Am Coll Nutr,* 26(3): 191-195

300. Redfield, M.M. Jacobsen, S.J. Burnett, J.C Jr. Mahoney, D.W. Bailey, K.R. Rodeheffer, R.J. (2003) 'Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic.' *JAMA,* 289(2):194-202

301. Rheeder, P. (2006) 'Type 2 diabetes: the emerging epidemic.' *SA Fam Pract*, 48(10): 20

302. Rip, M. Nierman, C. Ross, C.J., *et al*. (2006) 'Lipoprotein lipase S447X: a naturally occurring gain-of-function mutation.' *Arterioscler Thromb Vasc Biol.*, 26:1236–1245

303. Ririe, K.M. Rasmussen, R.P. and Wittwer, C.T. (1997) 'Product differentiation by analysis of DNA melting curves during the polymerase chain reaction.' *Anal Biochem,* 245: 154-160

304. Ristow, B. Ali, S. Whooley, M.A. Schiller, N.B. (2008) 'Usefulness of Left Atrial Volume Index to Predict Heart Failure Hospitalization and Mortality in Ambulatory Patients With Coronary Heart Disease and Comparison to Left Ventricular Ejection Fraction (from the Heart and Soul Study).' *Am J Cardiol,* 102:70 –76

305. Roche, H.M. (2005) 'Fatty acids and the metabolic syndrome.' *Proc Nutr Soc,* 64:23-29

306. Roger V.L., Go A.S., Lloyd-Jones D.M., *et al.* (2011) 'Heart Disease and Stroke Statisitics - 2011 Update: A Report From the American Heart Association. *Circulation*, 123:e18-e209

307. Rosenzweig, J.L. Ferrannini, E. Grundy, S.M., *et al*. (2008) 'Primary prevention of cardiovascular disease and type 2 diabetes in patients at metabolic risk: an endocrine society clinical practice guideline.' *J Clin Endocrinol Metab,* 93:3671

308. Ross, C.J. Liu, G. Kuivenhoven, J.A., *et al*. (2005) 'Complete rescue of lipoprotein lipase–deficient mice by somatic gene transfer of the naturally occurring LPLS447X beneficial mutation.' *Arterioscler Thromb Vasc Biol.,* 25: 2143–2150

309. Rudich, A. Kozlovsky, N. Potashnik, R. Bashan, N. (1997) 'Oxidant stress reduces insulin responsiveness in 3T3-L1 adipocytes.' *Am J Physiol*, 272:E935-940

310. Ruderman, N. Chisholm, D. Pi-Sunyer, X.,*et al*. (1998) 'The metabolically obese, normal-weight individual revisited.' *Diabetes,* 47: 699–713

311. Rutter, M.K. Parise, H. Benjamin, E.J., *et al*. (2003) 'Impact of Glucose Intolerance and Insulin Resistance on Cardiac Structure and Function: Sex-Related Differences in the Framingham Heart Study.' *Circulation*, 107: 448-454

312. Rutter, M.K. Meigs, J.B. Sullivan, L.M., *et al*. (2004) 'C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study.' *Circulation,* 110:380

313. Sagoo, G.S. Tatt, I. Salanti, G. Butterworth, A.S. Sarwar, N. Van Maarle, M., *et al*. (2008) 'LPL polymorphisms, lipid fractions and coronary disease: a HUGE association, review and meta-analysis.' *Am J Epidemiol,* 168:1233-1246

314. Salanti, G. Amountza, G. Ntanzi, E.E. Ionnidid, J.P. (2005) 'Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power.' *Eur J Hum Genet.*,13(7):840-848

315. Salton, C.J. Chuang, M.L. o'Donnell, C.J. Kupka, M.J. *et al*(2002) 'Gender Differences and Normal Left Ventricular Anatomy in an Adult Population Free of Hypertension.' J *Am Coll Cardiol,* 39:1055–1060

316. Samani, N.J. Erdmann, J. Hall, A.S. Hengstenberg, C. Mangino, M.*, et al*. (2007) 'Genomewide association analysis of coronary artery disease.' *N Engl J Med.* 357:443–453.

317. Samaras K. Botelho, N.K. Chisholm, D.J. Lord, R.V. (2010) 'Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes.' *Obesity*, 18(5):884-889

318. Sandeep, S. Gokulakrishnan, K. Deepa, M. Mohun, V. (2011) 'Insulin resistance is associated with increased cardiovascular risk in Asian Indians with normal glucose tolerance--the Chennai Urban Rural Epidemiology Study (CURES-66).' *J Assoc Physicians India*, 59:480-484

319. Sanghera, D.K. Saha, N. and Kamboh, M.I. (1998) 'The codon 55 polymorphism in the paraoxonase 1 gene is not associated with the risk of coronary heart disease in Asian Indians and Chinese.' *Atherosclerosis*, 136:217–223

320. Sattar, N. McConnachie, A. Shaper, A.G., *et al*. (2008) 'Can metabolic syndrome usefully predict cardiovascular disease and diabetes? Outcome data from two prospective studies.' *Lancet*, 371:1927–1935

321. Schejba, l.V. (1989) 'Epicardial fatty tissue of the right ventricle morphology, morphometry and functional significance.' *Pneumologie*, 43: 490–499

322. Schillaci, G. Pirro, M. Pucci, G., *et al*. (2006) 'Different impact of the metabolic syndrome on left ventricular structure and function in hypertensive men and women.' *Hypertension,* 47 : 881–886

323. Schiller, N.B. Shah, P.M. Crawford, M. De Maria, A. Devereux, R. Feigenbaum, H. Gutgesell, H. Reichek, N. Sahn, D. Schnittger, I. (1989) 'Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography committee on standards, subcommittee on quantitation of two-dimensional echocardiograms.' *J Am Soc Echocardiogr,* 2:358–367

324. Schinner, S. Scherbaum, W.A. Bornstein, W.A. Barthel, A. (2005) 'Molecular mechanisms of insulin resistance.' *Diabet Med*, 22:674-682

325. Scholl, J. (2012) 'Traditional Dietary Recommendations for the Prevention of Cardiovascular Disease: Do They Meet the Needs of Our Patients?' *Cholesterol*, 2012, Article ID 367898, 9 pages. Available on http://www.hindawi.com/journals/chol/2012/367898/ (accessed on 5 August 2012)

326. Seedat, Y. K. Seedat, M.A. and Reddy, K. (1978) 'The prevalence of hypertension in the Indian population of Durban.' *S. Afr. Med. J.,* 54:10-15

327. Seedat, Y.K. Mayet, F.G. Khan, S. Somers, S.R. Joubert, G. (1990) 'Risk factors for coronary heart disease in the Indians of Durban.' *S. Afr. Med. J.*, 78: 447-454

328. Seedat, Y. Mayet, F.G. and Gouws, E. (1994) 'Risk factors for coronary heart disease in the white community of Durban.' *S. Afr. Med. J.*, 84: 257-262

329. Seedat, Y.K. (2005) 'Review: Diabetes mellitus in South African Indians' *British Journal of Diabetes & Vascular Disease*, 5 (5): 249-251

330. Seidell, J.C. Kahn, H.S. Williamson, D.F. Lissner, L. Valdez, R. (2001) 'Report from a Centers for Disease Control and Prevention Workshop on use of adult anthropometry for public health and primary health care.' *Am J Clin Nutr*,73(1):123–126

331. Sen, S. and Burmeister, M. (2008) 'Hardy-Weinberg analysis of a large set of published association studies reveals genotyping error and a deficit of heterozygotes across multiple loci.' *Hum Genomics,* 3(1):36-52

332. Senti, M. Tomas, M. Fito, M. Weinbrenner, T. *et al*. (2003) 'Antioxidant Paraoxonase 1 Activity in the Metabolic Syndrome.' J Clin Endocrinol Metab 88: 5422–5426

333. Sewdarsen, M. Vythilingum, S. Jialal, I., *et al*. (1987) 'Risk factors in young Indian males with myocardial infarction.' *S Afr Med J*.,71: 261–262

334. Shapiro, L.M. Howat, A.P. and Calter, M.M. (1981) 'Left ventricular function in diabetes mellitus. I. Methodology, and prevalence and spectrum of abnormalities.' *Br Heart J,* 45: 122-128

335. Shih, D.M. Gu, L. Xia, Y.R. Navab, M. Li, W.F. Hama, S. Castellani, L.W. Furlong, C.E. Costa, L.G. Fogelman, A.M. Lusis, A.J. (1998) 'Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis.' *Nature,* 394:284–287

336. Simmons, R.K. Alberti, K.G.M.M., Gale, E.A.M., *et al*. (2010) 'The metabolic syndrome: useful concept or clinical tool? Report of a WHO Expert Consultation.' *Diabetologia*, 53 (4):600-605

337. Skoumas, J. Papadimitriou, L. Pitsavos, C. Masoura, C., *et al*. (2007) 'Metabolic syndrome: prevalence and characteristics in Greek adults with familial combined hypertension.' *Metabolis,* 56:135-141

338. Snehalatha, C. Viswanathan, V. Ramachandran, A. (2003) 'Cutoff Values for Normal Anthropometric Variables in Asian Indian Adults.' *Diabetes Care*, 26(5): 1380-1384

339. Sniderman, A.D. and Faraj, M. (2007) 'Apolipoprotein B, apolipoprotein A-I, insulin resistance and the metabolic syndrome.' *Curr Opin Lipidol,* 18:633–637

340. Souverein, O.W. Jukema, J.W. Boekholdt, S.M. Zwinderman, A.H. Tanck, M.W. (2005) 'Polymorphisms in APOA1 and LPL genes are statistically independently associated with fasting TG in men with CAD.' *Eur. J. Hum. Genet.*, 13: 445–451

341. Spence, J.D. Ban, M.R. and Hegele, R.A. (2003) 'Lipoprotein Lipase (LPL) Gene Variation and Progression of Carotid Artery Plaque.' *Stroke,* 34:1176-1180

342. Stannard, S.R. and Johnson, N.A. (2004) 'Insulin resistance and elevated triglyceride in muscle: more important for survival than "thrifty" genes?' *Physiol,* 1; (554):595-607

343. Statistics South Africa. (2011), 27 July p. 3. (Accessed on 13/08/2011) http://www.statssa.gov.za/publications/P0302/P03022011.pdf

344. Stein, Y. and Stein, O. (2003) 'Lipoprotein lipase and atherosclerosis.' *Atherosclerosis*, 170:181-189

345. Stern, M.P. Williams, K. González-Villalpando, C. Hunt, K.J. Haffner, S.M. (2004) 'Does the metabolic syndrome improve identification of individuals at risk of type 2 diabetes and/or cardiovascular disease?' *Diabetes Care*, 27:2676–2681

346. Steyn, K. Levitt, N.S. Hoffman, M. Marais, A.D. Fourie, J.M., *et al*. (2004) 'The global cardiovascular diseases risk pattern in a peri-urban working class community in South Africa.' *Ethn Dis.*, 14(2):233-242

347. Stewart, S. Carrington, M. Pretoriuous, S. Methusi, P. Sliwa, K. (2011) 'Standing at the crossroads between new and historically prevalent heart disease: effects of migration and socio-economic factors in the Heart of Soweto cohort study.' *Eur Heart J*, 32 (4): 492-499

348. St-Onge, M.P. Janssen, I. Heymsfield, S.B. (2004) 'Metabolic syndrome in normal-weight Americans: new definition of the metabolically obese, normal-weight individual.' *Diabetes Care,* 27:2222

349. Stocker, R. and Keaney, J.F. (2004) 'Role of oxidative modifications in atherosclerosis.' *Physiol Rev,* 84:1381–1388

350. Stritzke, J. Markus, R.P. Duderstadt, S. Lieb, W. Luchner, A. Döring, A., *et al*. (2009) 'The Aging Process of the Heart: Obesity Is the Main Risk Factor for Left Atrial Enlargement During Aging. The MONICA/KORA (Monitoring of Trends and Determinations in Cardiovascular Disease/Cooperative Research in the Region of Augsburg) Study.' *J Am Coll Cardiol.,* 54(21):1982-1989

351. Sundström, J. Vallhagen, E. Risérus, U., *et al*. (2006) 'Risk associated with the metabolic syndrome versus the sum of its individual components.' *Diabetes Care,* 29:1673

352. Syvanen, A. (2001) 'Accessing genetic variation: genotyping single nucleotide polymorphisms.' *Nature*, 2: 930 – 942

353. Tabur, S. Torun, A.N. Sabuncu, T. Turan, M.N. Celik, H. Ocak, A.R. Aksoy, N. (2010) 'Non-diabetic metabolic syndrome and obesity do not affect serum paraoxonase and arylesterase activities but do affect oxidative stress and inflammation.' *Eur J Endocrinol*., 162: 535-541

354. Taegtmeyer, H. McNulty, P. and Young, M.E. (2002) 'Adaptation and maladaptation of the heart in diabetes: Part I: general concepts.' *Circulation,* 105:1727–1733

355. Talmund, P.J. Flavell, D.M. Alfakih, K. Cooper, J.A. Balmford, A.J. Sivananthan, M., *et al*. (2007) 'The lipoprotein lipase gene serine 447 stop variant influences hypertension-induced left ventricular hypertrophy and risk of coronary heart disease.' *Clin Sci (London),* 112(12): 617-624

356. Tan, J.T. Ng, D.P.K. Nurbaya, S. Ye, S. Lim, X.L. Leong, H., et al. 'Polymorphisms Identified through Genome-Wide Association Studies and Their Associations with Type 2 Diabetes in Chinese, Malays, and Asian-Indians in Singapore. 'The Journal of Clinical Endocrinology & Metabolism 2010; 95(1): 390-397

357. Tarek, A. Abd, E. Rasha, H.M. Reem, M.H. (2011) 'Association of lipoprotein lipase and apolipoprotein C-III genes polymorphism with acute myocardial infarction in diabetic patients.' *Mol Cell Biochem,* 354:141–150

358. Taşkiran, P. Cam, S.F. Sekuri, C. Tüzün, N. Alioğlu, E. Altintaş, N. Berdeli, A. (2009) 'The relationship between paraoxanase gene Leu-Met (55) and Gln-Arg (192) polymorphisms and coronary artery disease.' *Turk Kardiyol Dern Ars.*, 37(7):473-478

359. Thankappan, K.R. Sivasankaran, S. Khader, S.A. Padmanabhan, P.G. Sarma, P.S. Mini, G.K., *et al*. (2006) 'Prevalence, correlates, awareness, treatment, and control of hypertension in Kumarakom, Kerala: Baseline results of a community-based intervention program.' *Indian Heart J,* 58 : 28-33

360. The International Expert Committee. (2009) 'International Expert Committee Report on the role of the A1C assay in the diagnosis of diabetes.' *Diabetes Care,* 32:1327–1334

361. Tibazarwa, K. Ntyintyane, L. Sliwa, K. Gerntholtz, T. Carrington, M. Wilkinson, D. Stewart, S. (2009) 'A time bomb of cardiovascular risk factors in South Africa: Results from the Heart of Soweto Study "Heart Awareness Days".' *International Journal of Cardiology,* 132: 233–239

362. Tobacco Products Control Act. (1993) Downloaded from: www.acts.co.za/tobacco_products_control_act_1993.htm. Accessed 10 August 2012

363. Took, J.E. (2000) 'Possible pathophysiological mechanisms for diabetic angiopathy in Type-2 diabetes.' *J Diabetes Complications,* 14:197-200

364. Tremblay, M. Gaudet, D. and Brisson, D. (2011) 'Metabolic Syndrome and Oral Markers of Cardio metabolic Risk.' *J Can Dent Assoc,*;77:b125

365. Trevathan, W.R. Smith, E.O. and McKenna, J.J. (1999) 'Introduction.' *In: Evolutionary Medicine,* Trevathan WR, Smith EO, and McKenna JJ (eds). New York: Oxford Univ. Press pp. 3-6

366. Tsang, T.S. Barnes, M.E. Gersh, B.J. Bailey, K.R. Seward, J.B. (2002) 'Left atrial volume as a morphophysiologic expression of left ventricular diastolic dysfunction and relation to cardiovascular risk burden.' *Am J Cardiol,* 90:1284 –1289

367. Tward, A. Xia, Y.R. Wang, X.P. Shi, Y.S. Pak, C. Castellani, L.W. Lusis, A.J. Shih, D.M. (2002) 'Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice.' *Circulation*, 106:484-940

368. UK Prospective Diabetes Study Group. (1998) 'The incidence of myocardial infarction in White, south Asian and Afro-Caribbean patients with type diabetes (UKPDS).' *Diabetes Care,* 21:1271-1277

369. Vague, J. (1947) 'La differénciation sexuelle, facteur determinant des formes de l'obésité.' *Presse méd,* 55:339–340

370. Van Bockxmeer, F.M. Liub, Q. Mamotteb, C. Burked, V. Taylor, R. (2001) 'Lipoprotein lipase D9N, N291S and S447X polymorphisms: their influence on premature coronary heart disease and plasma lipids.' *Atherosclerosis*, 157 (1): 123-129

371. Van de Weijer, T. Schrauwen-Hinderling, V.B. and Schrauwen P. (2011) 'Lipotoxicity in type 2 diabetic cardiomyopathy.' *Cardiovascular Research,* 92: 10–18

372. Vasan, R.S. Levy, D. Larson, M. Benjamin, E. (2000) 'Interpretation of Echocardiographic measurements: a call for standardization.' *Am Heart J*, 139(3):412-422

373. Vasan, R.S. Larson, M.G. Leip, E.P. Kannel, W.B. Levy, D. (2001) 'Assessment of frequency of progression to hypertension in non-hypertensive participants in the Framingham Heart Study: a cohort study.' *Lancet*, 358: 1682–1686

374. Victora, C. Adair, G. Hallal, L. Martorell, P.C. Richter, R. Singh, L. Sachdev, H., for the Maternal and Child Undernutrition Study Group. (2008) 'Maternal and child under nutrition: consequences for adult health and human capital.' *Lancet,* 371(9609): 340–357

375. Veiga, L. Silva-Nunes, J. Mela, A. Oliveira, A. Duarte, L. Brito, M. (2011) 'Q192R polymorphism of the paraoxonase-1 gene as a risk factor for obesity in Portuguese women.' *European journal of Cardiology*, 164(2):213-218

376. Verhagen, S.N. and Visseren F.L.J. (2011) 'Perivascular adipose tissue as a cause of atherosclerosis.' *Atherosclerosis,* 214:3–10

377. Vincent, H.K. and Taylor, A.G. (2006) 'Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans.' *International Journal of Obesity,* 30: 400–418

378. Virmani, R. Burke, A.P. Farb, A. Kolodgie, F.D. (2006) 'Pathology of the vulnerable plaque.' *J Am Coll Cardiol,* 47:C13-18

379. Wallace, C. Newhouse, S.J. Braund, P. Zhang, F. Tobin, M. Falchi, M. Ahmadi, K. Dobson, R.J. Marcano, A.C. Hajat, C., et al. (2008) 'Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia.' Am. J. Hum. Genet., 82:139-149.

380. Wang, T.D Lee, W. Shih, F. Huang, C.Chang, Y. *et al*. (2009). 'Relations of Epicardial Adipose Tissue Measured by Multidetector Computed Tomography to Components of the Metabolic Syndrome Are Region-Specific and Independent of Anthropometric Indexes and Intra-abdominal Visceral Fat'. *The Journal of Clinical Endocrinology & Metabolism,* 94 (2): 662-669

381. Wang, T.J. Parise, H. Levy, D., *et al*. (2004) 'Obesity and the risk of new-onset atrial fibrillation.' *JAMA,* 292: 2471-2477

382. Wang, C.C.L. Goalstone, M.L. and Draznin, B. (2004) 'Molecular Mechanisms of Insulin Resistance That Impact Cardiovascular Biology.' *Diabetes,* 53(11): 2735-2740

383. Wang, H. and Eckel, R.H. (2009) 'Lipoprotein lipase: from gene to obesity.' *Am J Physiol Endocrinol Metab,* 297:E271-288

384. Wajchenberg, B.L. (2000) 'Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome.' *Endocrine Reviews,* 21 (6): 697-738

385. Wasir, J.S. Misra, A. Vikram, N.K. Pandey, R.M. Gupta, R. (2008) 'Comparison of definitions of the metabolic syndrome in adult Asian Indians.' *J Assoc Physicians India*, 56 :158-164

386. Watson, A.D. Berliner, J.A. Hama, S.Y. La Du, B.N. Faull, K.F. Fogelman, A.M. Navab, M. (1995) 'Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein.' *J. Clin. Invest.*, 96: 2882–2891

387. Watzinger, N. Schmidt, H. Schumacher, M. Schmidt, R. Eber, B. Feuhwald, F.M., *et al*. (2002) 'Human Paraoxonase 1 gene polymorphisms and the risk of coronary heart disease: a community based study.' *Cardiology*, 98:116-122

388. Tang, W. Apostol, G. Schreiner P.J. Jacobs Jr, D.R, *et al.* (2010) 'Associations of Lipoprotein Lipase Gene Polymorphisms with Longitudinal Plasma Lipid Trends in Young Adults: the CARDIA Study.' *Circ Cardiovasc Genet.,* 3(2): 179–186.

389. Weissglas-Volkov, D. and Pajukanta, P. (2010) 'Genetic causes of high and low serum HDL-cholesterol.' *Journal of Lipid Research*, 51:2032-2057

390. Wellen, K.E. and Hotamisligil, G.S. (2003) 'Obesity-induced inflammatory changes in adipose tissue.' *J Clin Invest*, 112:1785–1788

391. Whaley-Connell, A, McCullough, P.A. and Sowers, J.R. (2011) 'The role of oxidative stress in the metabolic syndrome.' *Reviews in Cardiovascular Medicine*, 12 :21–29

392. Wheeler, J.G. Keavney, B.D. Watkins, H., *et al*. (2004) 'Four paraoxonase gene polymorphisms in 11 212 cases of coronary heart disease and 12 786 controls: meta-analysis of 43 studies.' *Lancet*, 363(9410): 689–695

393. Whincup, P.H. Gilg, J.A. Papacosta, O. Seymour, C. Miller, G.J. Alberti, K.G. Cook, D.G. (2002) 'Early evidence of ethnic differences in cardiovascular risk: cross sectional comparison of British South Asian and white children.' *BMJ,* 324(7338): 635

394. WHO Expert Consultation. (2004) 'Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies.' *Lancet,* 363:157–163

395. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation 1999.

396. Wildman, R.P. McGinn, A.P. Lin, J. Wang, D. Muntner, P., *et al*. (2011) 'Cardiovascular Disease Risk of Abdominal Obesity vs. Metabolic Abnormalities.' *Obesity*, 19: 853-860

397. Wittke-Thompson, Pluzhnikov, A. Cox, N.J. (2005) 'Rational inferences about departures from Hardy-Weinberg equilibrium.' Am J Hum Genet.,76(6):967-986

398. Wittrup, H.H. Tybjaerg-Hansen, A. and Nordestgaard, B.G. (1999) 'Lipoprotein lipase mutations, plasma lipids and lipoproteins, and the risk of ischemic heart disease: a metal-analysis.' *Circulation*, 99:2901-2907

399. Wong, C.Y. O'Moore-Sullivan, T. Fang, Z.Y. Haluska, B. Leano, R. Marwick, T.H. (2005) 'Myocardial and vascular dysfunction and exercise capacity in the metabolic syndrome.' *Am J Cardiol,* 96:1686–1691

400. Wu, Z. and Schimmele, M.A. (2011) 'Racial/Ethnic Variation in Functional and Self-Reported Health. *Am J Public Health*, 95(4): 710–716

401. Wung, S. Kulkarni, M.V. Pullinger, C.R. Malloy, M.J. Kane, J.P. Aouizerat, B.E. (2006) 'The lipoprotein lipase gene in combined hyperlipidemia: evidence of protective allele depletion.' *Lipids in Health and Disease,* 5:19

402. Yajnik, C.S. Lubree, H.G. Rege, S.S., *et al*. (2002) 'Adiposity and hyperinsulinaemia in Indians are present at birth.' *J Clin Endocrinol Metab,* 87:5575-5580

403. Yaomin, H. Wei, L. Rong, H. Xiaoying, Z. (2006) 'A systematic review and meta-analysis of the relationship between lipoprotein lipase Asn291Ser variant and diseases.' *J. Lipid Res.*, 47: 1908–1914

404. Young, M.E. McNulty, P. and Taegtmeyer, H. (2002) 'Adaptation and maladaptation of the heart in diabetes: Part II: potential mechanisms.' *Circulation,* 105:1861–1870

405. Yusuf, S. Reddy, S. Oˆ unpuu, S. Anand, S. (2001) 'Global Burden of Cardiovascular diseases Part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies.' *Circulation*, 104: 2855–2864

406. Yusuf, S. Hawken, S. Ounpuu, S. Dans, T. Avezum, A. Lanas, F. McQueen, M. Budaj, A. Pais, P. Varigos, J. Lisheng, L. INTERHEART Study Investigators. (2004) 'Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study.' *Lancet,* 364: 937–952

407. Zabaneh, D. and Balding, D.J. (2010) 'A Genome-Wide Association Study of the Metabolic Syndrome in Indian Asian Men.' *PLoS ONE,* 5(8): e11961

408. Zabaneh, D. Chambers, J.C. Elliott, P. Scott, J. Balding, D.J., *et al*. (2009) 'Heritability and genetic correlations of insulin resistance and component phenotypes in Asian Indian families using a multivariate analysis.' *Diabetologia,* 52 (12): 2582–2589

409. Zechner, R. (1997) 'Tissue-specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism.' *Curr Opin lipidol*, 8:77-88

410. Zhang, H. Henderson, H. Gagne, S.E. Clee, S.M. Miao, L. Liu, G. Hayden, M.R. (1996) 'Common sequence variants of lipoprotein lipase: standardized studies of in viro expression and catalytic expression.' *Biochimica et Acta.*, 1302: 159-166

411. Zhong ,J.H. Rodríguez, A.C. Yang, N.N. Li, L.Q. 'Methylenetetrahydrofolate Reductase Gene Polymorphism and Risk of Type 2 Diabetes Mellitus'. PLoS ONE 2013; 8(9): e74521. doi:10.1371/journal.pone.007452

412. Zile, M.R. Baicu, C.F. and Gaasch, W.H. (2004) 'Diastolic heart failure—abnormalities in active relaxation and passive stiffness of the left ventricle.' *N Engl J Med,* 350:1953–1959

413. Zimmet, P. Alberti, K.G. and Shaw, J. (2001) 'Global and societal implications of the diabetes epidemic.' *Nature,* 414: 782–787

APPENDIX 1: INSTRUCTIONS FOR RECRUITER

1. RECRUITING INSTRUCTIONS

The sample for the study has already been selected. There are 1200 people in the sample.

The sample has been selected to ensure that participants are spread out evenly over the township.

The sample has also been chosen to ensure that a specific number of men and women in different age categories are included in the study.

The sampling has been designed in theory, but the YOU THE RECRUITER must make sure that it is carried out in practice. The methods and rules have been worked out to help you in the selection of the participants. Following these methods and rules may at times seem difficult and unnecessary, but the success of the study depends on it.

Methods for recruiting

Sampling will include the following:

(1) Finding a selected household in Phoenix.

(2) Selecting participants out of the chosen household.

1 Finding a selected household in Phoenix

For the aim of this study, Phoenix has been divided into different areas. In each of these areas, households that have been selected for the study have been marked. We will provide each field-worker with a list that contains the addresses of household listed by household study number (i.e. 1 to nth) household. If nobody is home, try to visit the household on different times of the day, for instance, once in the morning and once in the evening or during the weekends. If you are unable to find anybody at a particular household on several attempts the write down the number of visits you have made to the household as well as the reasons why you were not successful in the relevant section in the STEPS questionnaire.

Each recruiter will commence with house number 1 on their list

2. Selecting participants from the household

2.1 Introduce yourself to the household and hand them the information page for the Phoenix study and explain the reason for your visit.

.At house #1:

2.2 write down the names, ages and relation of all persons 15 years and older who stay at this address in Form A (appendix 1) and record date of birth, relation to householder.

i) To select a person for the study ask who has had a birthday most recently, and select that person. If birth dates are unknown then select the oldest person in the household.

ii) Next, you will record this household study number in the table below in a cell that best describes the person who was selected. For example, if the person selected is a 38-year old male enter household 1 in grid marked 5 address in the table (see record in yellow highlight).

	Male	Female
Age (yrs) 15-24	1 Address	2 Address
25-34	3 Address	4 Address
35-44	5 Address	6 Address
45-54	7 Address	8 Address
55-64	9 Address	10 Address
≥65	11 Address	12 Address

iii) Next you will go to household number 2 on your list. Try to find someone in this household who will fit the next cell (6 Address) moving from right to left and top to bottom on your list. So, the next cell will be for a female aged 35-44 years (6 Address). Select that person from this household and record household study number in this cell. Now you will have:

	Male	Female
Age (yrs) 15-24	1 Address	2 Address
25-34	3 Address	4 Address
35-44	Household 1	Household 2
45-54	7 Address	8 Address
55-64	9 Address	10 Address
≥65	11 Address	12 Address

iv) Next you will go to household number 3 on your list. Try to find someone in this household who will fit the next cell moving from right to left and top to bottom on your table. So, the next cell will be for a male aged 45-54 years.

v) Continue this process until you get to the bottom of the list and then move to the first cell which should be a male aged 15-24 years. Continue downward until all cells are complete.

vi) If you visit a household where there is no person that can fill the description of your cell then move on to the next cell. For example, if at household number 2 there was no female aged 35-44 years then try to select someone for the next cell which requires a male aged 45-54 years, and so on, until you have selected someone from the household. At your next household, household number 3, you will once again try to select someone to fill the cell that was not successful at the previous home.

This is done so that we do not have more than one subject from each home and so that the selection is random and represents all ages. Remember to obtain written consent for participation into the programme and also remember that in certain age categories people are more likely to be at school, college or work when you visit; so always enquire about all members and include them in your list for selection. This may mean re-visiting the home to contact them and obtain consent.

We will give you lists with additional homes in the event that there are refusals to participate or no-one at home on at least 3 occasions. To ensure an unbiased sample we need to keep careful records of reasons for not including a person in the study. You will be required to keep a log of each home approached, date and time, outcome and reason. The possible outcomes are consented to participation/refused/not present at 1st visit/ not present at 2nd visit/ not present at 3rd visit. For everything except consented to participation, you need to ascertain and record a reason and the gender and age of the person who refused or was not present on all occasions in the relevant section in the STEPS Questionnaire.

Remember: Pregnant women and handicapped people (bedridden and in a wheelchair) are not selected for the study.

(3) Hand the consent form to participant who has been selected for the study and who has accepted to participate. Participant must sign this form and hand it back to you.

(4) Make an appointment with the participant to meet with the research assistant to administer the STEPS QUESTIONNAIRE Write the date and time of the appointment on the appointment card. Also note the names and appointment times in your book.

APPENDIX 2: INFORMATION AND CONSENT DOCUMENT FOR PARTICIPANTS

Dear Member of the Phoenix community,

Greetings to You,

I, Professor DP Naidoo, together with other members of my team from the University of KwaZulu-Natal, am carrying out a research study on residents in the Phoenix. You may have read of this research in pamphlets circulated to residents and in advertisements in the local newspapers. In this study we want to learn about the health, lifestyle (physical activity, dietary patterns, smoking and drinking of alcohol habits), psychological well-being, diseases of lifestyle and sleeping disorders of the residents.

You, perhaps, may know that heart attack, stroke, kidney failure and complications of diabetes (high blood sugar levels) – gangrene, problems with the eyes and kidneys are becoming a big problem amongst the South African Indian population. The main reason for this study is to try to reduce the factors which cause these cardiovascular problems in our community.

We are inviting you to participate in this research study.

During this study, a brief detail about yourself and your family will be taken by one of our researchers and a questionnaire regarding your lifestyle, diet, physical activity, smoking habits, psychological well-being, cardiovascular problems, sleep and family history of cardiovascular problems and diabetes will be filled by the researcher. This should take about 30-45 minutes to complete and this interview will be held at your residence at a time convenient to you. In addition your blood pressure will also be measured by the trained researcher.

Following the above an appointment will be then made for you to meet, at a day and time convenient to you, at a local doctor's rooms (at the Sunford Medical Centre) the address will be given to you by our researcher. The night before the scheduled appointment you will be required to fast for about 10 hours prior to this date. You will be transported from the Medical Centre to the Albert Luthuli Central Hospital in Cato Manor. At this venue a qualified person (either a nurse or a medical doctor) will measure your blood pressure and take an ultrasound picture and ECG recording of your heart. Fasting blood and urine samples (to measure blood glucose, insulin and cholesterol and urine protein levels) will be done. If you agree, you will then be given 75 grams of glucose to drink over a two-minute period and after 2 hours another sample of blood will be taken (to measure blood sugar and insulin levels). In addition, weight and height will be measured and measurements of the circumference of the waist, arm, and neck and skinfold thickness will be made by the researcher before taking blood samples. In total the time required for these procedures will be over two and half hours.

Should you be employed, or should you be attending a school/college a leave form for being absent will be given which has to be handed to the employer or school teacher before the scheduled visit.

You will also be required to participate in a programme designed to reduce the risk of developing cardiovascular disease. Our research assistant will give you the details of the programme. This programme will go on for one year. During this one year you will be visited by our researcher at the end of six months and again at the end of the one year during which the questionnaire will again be filled and all the measurements will again be made and your blood and urine samples will also be taken in the same way as the initial time. We will then compare the results to see whether this intervention programme has benefited you. You will be regularly informed of your progress

Individuals in the age range 15-64 years, both males and females will be selected. In all 2500 people will be randomly selected from the Phoenix community. The person selected must not be pregnant or confined to a wheel chair or bed or have any neurological problems e.g., stroke.

There will be no risks to you if you participate in this research programme. Only slight discomfort will be experienced during withdrawal of blood. You are free to withdraw from the programme at any time should you so desire. However, if you are identified as having disorders such as hypertension and diabetes for the first time you will be referred to your family doctor or a clinic or a hospital for appropriate treatment:

In order to benefit optimally from the programme it will be advisable for you to adhere to the prescribed physical activity, the dietary and psychological prescriptions given by one of our researchers for the one year period. You will also be expected to reliably answer questions posed to you by our interviewer and agree to taking of blood samples and spot urine samples

You will be informed of all the results regarding yourself, including the effects of the intervention programme. Besides yourself nobody else will have access to your information and results other than your family doctor.

You are assured of confidentially of your details and the data of the results. Your name will not appear on the questionnaire, forms and containers for blood & urine samples. You will be assigned an identity number by the leader of the research team. This identity number will appear on the questionnaire, forms, and blood and urine sample containers. Your information will be securely kept. Should any research publications or reports be compiled your identity will not be disclosed. Your anonymity and right to privacy will be retained at all times. However, absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law.

If you agree to participate in this research, please sign the informed consent form, however, if you are under 18 years of age we will require your parent/guardian to also sign a similar form giving you permission to participate. Remember you can decline to participate or withdraw at any time.

If you have any doubts or questions, please ask for further explanation from the researcher/s. Please feel free to contact me. I am the head of the research team and

Head of Cardiology Department at the Inkosi Albert Luthuli Central Hospital should you require any further clarification or explanation please feel free to contact me. My contact details are: Tel. (031) 240 2207 (W) 2617588 (H); Fax (031) 240 2225; email: datshananai@ialch.co.za

This study has received ethics approval from the University of KwaZulu-Natal, Nelson R Mandela School of Medicine, Research Ethics Committee. Should you have any queries about the ethics of this study, you may contact: The Medical Research Administration – tel (031) 260 4495; fax: (031) 260 4410; email: borresen@ukzn.ac.za.

INFORMED CONSENT FORM

Consent to Participate in Research

You have been invited to participate in this research study and an information leaflet about this study has been given to you and the details of the study and your involvement has also been explained to you by our researchers.

You may contact Professor DP Naidoo at Tel No. 031 240 2207 (w) or 031 2617588 (h) at any time if you have questions about the research or if you are injured as a result of the research.

You may contact the Medical Research Office at the Nelson R Mandela School of Medicine at

031-260 4604 if you have questions about your rights as a research subject.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to stop at any point in this study.

If you agree to participate, please sign this document and you may keep the information sheet which is a written summary of the research.

Freedom of Consent

I agree to voluntarily participate in this research programme. I understand that I can stop participating in this programme at any time I may wish without giving any explanation or being prejudiced in any way.

I acknowledge that I have read this form in its entirety or it has been read to me or its entire contents have been explained to me, and I understand my responsibility in the research programme in which I will be participating. I accept the risks, rules and regulations set forth. Knowing these, and having had the opportunity to ask questions which have been answered to my satisfaction, I consent to participate in this research programme and consent to my blood sample being stored and used for any additional investigation.

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Signature of Participant Date

(Please print your name)

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Signature of Witness Date

INFORMATION DOCUMENT FOR PARENTS

Dear Member of the Phoenix community,

Greetings to You,

I, Professor DP Naidoo, together with other members of my team from the University of KwaZulu-Natal, am carrying out a research study on residents in the Phoenix. You may have read of this research in pamphlets circulated to residents and in advertisements in the local newspapers. In this study we want to learn about the health, lifestyle (physical activity, dietary patterns, smoking and drinking of alcohol habits), psychological well-being, diseases of lifestyle and sleeping disorders of the residents.

You, perhaps, may know that heart attack, stroke, kidney failure and complications of diabetes (high blood sugar levels) – gangrene, problems with the eyes and kidneys are becoming a big problem amongst the South African Indian population. The main reason for this study is to try to reduce the factors which cause these cardiovascular problems in our community.

Your son/daughter who is under 18 years has agreed to participate in this research study.

During this study, a brief detail about yourself and your family will be taken by one of our researchers and a questionnaire regarding your son's/daughter's lifestyle, diet, physical activity, smoking habits, psychological well-being, cardiovascular problems, sleep and family history of cardiovascular problems and diabetes will be filled by the researcher. This should take about 30-45 minutes to complete and this interview will be held at your residence at a time convenient to your son/daughter. In addition, his/her blood pressure will also be recorded.

Following the above an appointment will be then made for your son/daughter to meet, at a day and time convenient to him/her at a local doctor's rooms (at the Sunford Medical Centre) the address will be given to you by our researcher. The night before the scheduled appointment he/she will be required to fast for about 10 hours. He/she will be transported from the Medical Centre to the Albert Luthuli Central Hospital in Cato Manor. At this venue a qualified person (either a nurse or a medical doctor) will measure your blood pressure and take an ultrasound picture and ECG recording of your heart. Fasting blood and urine samples (to measure blood glucose, insulin and cholesterol and urine protein levels) will be done. If your son/daughter agrees, he/she will be given 75 grams of glucose to drink over a two-minute period and after 2 hours another sample of blood will be taken (to measure blood sugar and insulin levels). In addition, weight and height will be measured and measurements of the circumference of the waist, arm, and neck and skinfold thickness will be made by the researcher before taking any blood samples. For these procedures the total time time required will be over two and half hours. .

Should your son/daughter be employed or should he she be attending a school/college a leave form for being absent will be given which has to be handed to the employer or school teacher before the scheduled visit.

Your son/daughter will also be required to participate in a programme designed to reduce the risk of developing cardiovascular disease. Our research assistant will give your son/daughter the details of the programme. This programme will go on for one year.

During this one year your son/daughter will be visited by our researcher at the end of six months and again at the end of the one year during which the same questionnaire will again be filled and all the measurements will again be made and blood and urine samples will also be taken in the same way as the initial time. We will then compare the results to see whether this intervention programme has benefited your son/daughter. You and your son/daughter will be regularly informed of any progress

Individuals in the age range 15-64 years, both males and females, will be selected to participate in this research project. In all 2500 people will be randomly selected from the Phoenix community. The person selected must not be pregnant or confined to a wheel chair or bed or have any neurological problems e.g., stroke.

There will be no risks to your son/daughter if he/she participates in this research programme. Only slight discomfort will be experienced during withdrawal of blood. Your son/daughter will be free to withdraw from the programme at any time should he/she so desire. However, if your son/daughter is identified as having disorders such as hypertension and diabetes for the first time you will be referred to your family doctor or a clinic or a hospital for appropriate treatment:

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In order to benefit optimally from the intervention programme it will be advisable for your son/daughter to adhere to the prescribed physical activity, the dietary and psychological prescriptions given by one of our researchers for at least one year. Your son/daughter will also be expected to reliably answer questions posed to him/her by our interviewer and agree to taking of blood samples and spot urine samples

You and your son/daughter will be informed of all the results, including the effects of the intervention programme. Besides yourself and your son/daughter nobody else will have access to any information and results other than your family doctor.

Your son/daughter is assured of confidentially of his/her details and the data of the results. Your son's/daughter's name will not appear on the questionnaire, forms and containers for blood and urine samples. He/she will be assigned an identity number by the leader of the research team. This identity number will appear on the questionnaire, forms, and blood and urine sample containers. All information will be securely kept. Should any research publications or reports be compiled your son's/daughter's identity will not be disclosed. Your son's/daughter's anonymity and right to privacy will be retained at all times. However, absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law.

If you agree to your son/daughter to participate in this research, please sign the informed consent form, however. Remember your son/daughter can decline to participate or withdraw at any time.

If you have any doubts or questions, please ask for further explanation from the researcher/s. Please feel free to contact me. I am the head of the search team and Head of Cardiology at the Inkosi Albert Luthuli Central Hospital. Should you require any further clarification or explanation please feel free to contact me. My contact details are: Telephone No. (031) 240 2207 (w) or (031) 2617588 (H) ; Fax (031) 2225; email: datshananai@ialch.co.za

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INFORMED CONSENT FORM

Consent to Participate in Research

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You may contact Professor DP Naidoo, the head of the research team. His contact details are: Telephone (031) 240 2207 (W) or (031) 2617588 (H); Fax (031) 2225; email: datshananai@ialch.co.za, at any time if you have questions about the research or if your son/daughter is injured as a result of the research.

You may contact the Medical Research Office at the Nelson R Mandela School of Medicine at

031-260 4604 if you have questions about your rights as a research subject.

Your son's/daughter's participation in this research is voluntary, and he/she will not be penalized or lose benefits if you refuse to allow him/her to participate or if you decide he/she should stop at any point in this study.

If you agree to your son/daughter to participate, please sign this document and you may keep the information sheet which is a written summary of the research.

Freedom of Consent

I agree to voluntarily allow my son/daughter to participate in this research programme. I understand that I my son/daughter can stop participating in this programme at any time

he/she may wish without giving any explanation or being prejudiced in any way.

I acknowledge that I have read this form in its entirety or it has been read to me or its entire contents have been explained to me, and I understand my son's/daughter's responsibility in the research programme in which he/she will be participating. I accept the risks, rules and regulations set forth. Knowing these, and having had the opportunity to ask questions which have been answered to my satisfaction, I consent to my son/daughter to participate in this research programme and consent to my son's/daughter;s blood sample being stored and used for any additional investigation/s.

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Signature of parent/guardian) Date

(Please print your name)

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Signature of Witness Date