Cape Peninsula University of Technology

Validation of a recently proposed equation for the estimation of small, dense LDL particles from routine lipid measures in a population of mixed ancestry South Africans

by

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DECLARATION

I, Mohamed Abdulsalam Masoud, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

Background: Cardiovascular diseases (CVD) are the leading cause of global mortality, of which over 75% occurred in low- and middle-income countries such as South Africa. The lipid profile, specifically decreased levels of high density lipoprotein cholesterol (HDL-C), elevated triglyceride levels and the presence of small-dense low density lipoprotein (sdLDL) has been reported associated with CVD. An increased number of sdLDL is also common in metabolic syndrome (MetS), visceral obesity and diabetes mellitus, the last a known risk factor for CVD. The modification of low density lipoprotein (LDL) size, or number of sdLDL particles, has been reported to significantly reduce CVD risk, but not conclusively so and needs further investigation. In this regard, sdLDL particles are seldom estimated routinely for clinical use because of financial and other limitations. Currently, an alternative approach for estimating sdLDL is to use equations derived from routine lipid measures, as has been proposed by several groups. However, there is a need for extensive evaluation of this equation across different ethnic and disease groups, especially since reports showed an inadequate performance of the equation in a Korean population.

Aim of the study: The aim of this study was to assess the performance of a recently proposed equation for the estimation of sdLDL in healthy and diabetic mixed ancestry South Africans. Furthermore, we also investigated the role of sdLDL as a cardiometabolic risk factor, as measured against known risk factors such as the glycemic and lipid profiles.

Methods: The study was part of a larger project which investigated diabetes and vascular diseases in Bellville South, a predominantly mixed ancestry community. Blood samples were drawn from 239 consenting participants for routine biochemical analysis, including blood glucose (mmol/L), insulin (IU/l), HDL-C (mmol/L), LDL-C (mmol/L) and triglycerides (mmol/L). The sdLDL-C (mmol/L) was measured directly (Randox sLDL-EX“SEIKEN”) and calculated according to the following formula: $sdLDL\text{-}C_{mmol/L}$ = 0.580 (non–HDL-C) + 0.407 (dLDL-C) – 0.719 (cLDL-C) – 0.312.

Results: Although the medians of the two methods were comparatively similar (0.87 vs 0.84), the interquartile ranges were quite divergent (min to max: -1.85 to 2.52 vs 0.17 to 3.39). This could possibly be explained by the skewed distribution shown in both calculated and measured sdLDL-C as tested by the Shapiro-Wilk’s W test (both $P < 0.0001$). Known risk factors for CVD, including the glycemic and lipid profiles were evaluated across percentiles of measured sdLDL. The hyperglycemic subgroups did not show a significant difference in numbers across the sdLDL percentiles, but the fasting glucose ($P = 0.0008$), 2-hour glucose ($P = 0.0020$) and 2-hour insulin ($P = 0.0007$) levels were significantly increased across the sdLDL percentiles.

Similarly, the MetS positive cases ($P < 0.0001$), waist circumference ($P = 0.0042$), systolic blood pressure ($P = 0.0002$), all lipids tested ($P < 0.0001$) and age ($P = 0.0109$) showed significant increases across the sdLDL percentiles.

Discussion: Our validation study of the proposed equation showed a good agreement between the formula and measured sdLDL-C as tested in the mixed ancestry population. However our data did point to a possibility that the two methods sometimes identified different individuals based on the varying interquartile ranges. Furthermore, our data also confirmed the link between sdLDL and CVD risk factors, such as the glycemic and lipid profiles, including LDL-C, triglycerides and cholesterol as previously reported for CVD risk in the mixed ancestry population. Results showed a marked increase of both glucose and insulin mean values in the higher sdLDL percentiles and suggested a role for sdLDL in the evaluation of CVD risk assessment, as both DM and the presence of sdLDL have been associated with CVD and MetS. In the current study we have shown a high percentage of both screen-detected (7.2%) and known diabetes (14.8%). Similarly, results across the sdLDL percentiles showed highly significant increases in all lipids measured except HDL-C, which was non-significantly decreased over the sdLDL percentiles. These findings showed that sdLDL was closely associated with the lipid profile and importantly with the triglycerides which were significantly increased over the sdLDL percentiles. We have also shown an increasing trend in MetS cases and its components with increasing sdLDL quartiles, which further buttress the value of sdLDL in determining CVD risk. Therefore, in conclusion, we report that the use of equations to estimate sdLDL levels is a viable alternative cost-effective method to preselect patients at risk for CVD. However, the need for extensive evaluation across different ethnic and specific groups remains, before it can be widely recommended.

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DEDICATION

To my mother, father and my wife, Reem

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ATP	Adenosine tri-phosphate
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CVD	Cardiovascular diseases
DALYs	Disability-adjusted life years
DM	Diabetes mellitus
HDL-C	High density lipoprotein cholesterol
lbLDL	Large, buoyant low density lipoprotein
IDF	International Diabetes Federation
IDL-C	Intermediate density lipoprotein cholesterol
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
JIS	Joint Interim Statement
LDL-C	Low density lipoprotein cholesterol
MetS	Metabolic syndrome
mmol/L	Millimoles per liter
NACB LMPG	National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines
NCEP	National Cholesterol Education Program
OGTT	Oral glucose tolerance test

ox-LDL	Oxidized low density lipoprotein
ROC	Receiver operating characteristic
sdLDL	Small dense low density lipoprotein
SD	Standard deviation
SR-BI	Scavenger Receptor class B type I
T2DM	Type 2 diabetes mellitus
VLDL	Very low density lipoprotein
WHO	World Health Organization

CHAPTER 1
LITERATURE REVIEW

1.1 Introduction

Cardiovascular diseases (CVD) are still the leading cause of mortality globally, accounting for 31% (17.5 million) of all deaths in 2012 (World Health Organization {WHO}, 2015). A vast majority of these deaths were due to coronary heart disease (CHD) (7.4 million) and stroke (6.7 million), the two main forms of CVD, and mostly (over 75%) occurred in low- and middle-income countries such as South Africa (WHO, 2015). Beyond mortality, the burden of CHD alone is globally expected to increase from about 47 million disability-adjusted life years (DALYs) in 1990 to 82 million DALYs by 2020 (Mackay & Mensah, 2004). The lipid profile is one of the tools used more widely to predict CHD (Mora et al., 2008). Decreased levels of high density lipoprotein cholesterol (HDL-C), elevated triglyceride levels and the presence of small-dense low density lipoprotein (sdLDL) have been termed the “lipid triad” which usually occurs in people with CVD (Rizzo & Berneis, 2005). Several studies have found that the increased low density lipoprotein cholesterol (LDL-C) level is strongly associated with CHD (Alsheikh-Ali et al., 2007; Keevil et al., 2007).

1.2 Risk factors for CVD

Risk factors for CVD include family history, ethnicity and age (non- modifiable) as well as tobacco exposure, hypertension, high cholesterol, obesity, physical inactivity, diabetes, metabolic syndrome (MetS), unhealthy diets and harmful use of alcohol (modifiable). The modifiable risk factors are responsible for about 80% of CVD (WHO, 2011). The effects of an unhealthy diet and physical inactivity manifest as raised blood pressure, raised blood glucose, raised blood lipids, and overweight/obesity. These “intermediate risk factors” are assessed as risk indicators for developing a heart attack, stroke, heart failure and other complications. High LDL-C is one of the most important lipid disorders reported among smokers, leading to an increased susceptibility to atherosclerosis which is an early indicator of CVD (Daniels et al., 2011; Sharma & Garg, 2012).

1.2.1 Impaired glucose tolerance, impaired fasting glucose, diabetes mellitus and the risk of CVD

Both impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are identified as pre-diabetic states of hyperglycemia (Lin et al., 2007) and IGT has been observed as a risk factor for CVD as well (Alcolado et al., 1994; Barr et al., 2007) and coronary heart disease (Califf et al., 2008). In this regard, while IFG has been shown to be an independent risk factor for T2DM, it is not clear yet whether it is also independently associated with an increased incidence of CVD. Yeboah et al., (2011) reported the absence of this association, while Ford et al. (2010)

reported IFG associated with a modest increased risk for CVD. Levitzky et al. (2008) identified IFG as an independent risk factor for CVD in women, but not in men, while Kanaya et al. (2005) showed that IFG was not associated with an increased risk of cardiovascular events in post-menopausal women with coronary artery disease.

1.2.2 Metabolic Syndrome and the risk of CVD

Metabolic syndrome (MetS) is a cluster of risk factors of metabolic origin, which include insulin resistance, dyslipidemia, hypertension, hyperglycemia and obesity (Marchetti et al., 2012; Reaven, 2012). The prevalence of MetS is high in countries with higher incomes and is also increasing in countries with fast-growing economies (Arora et al., 2013). CVD death has been reported to be due to a large extent to hypertension and dyslipidemia associated with MetS (Karalis, 2014), with MetS associated with a 5-fold increase in the risk of T2DM and a 2-fold increased risk for CVD within 5 to 10 years of onset (Alberti et al., 2009).. Dyslipidemia, often associated with MetS correlates with elevated levels of sdLDL particles and has been recognized as a major risk factor for atherosclerosis and CVD. Arora et al. (2013) suggested that sdLDL should be included as an evaluation criteria for MetS risk, which would improve management and treatment of complications that are associated with MetS.

1.3 Atherosclerosis

Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in large arteries, forming plaques. The plaques constrict the lumen of the blood vessel thereby increasing the shear force of blood to a point that it may result in its rupture, potentially leading to the formation of thrombus, ischemic stroke or heart attack (Frink, 2002; Libby et al., 2002; Moreno, 2010). The process of plaque development begins with a lesion in the arterial endothelium, allowing LDL to move from the blood into the intima, the innermost layer of the artery (Figure 1) and where it is oxidized (ox-LDL) by free radicals which are continuously released from biochemical reactions within the body, including the intima (Ryu, 2000; Cohen et al., 2014). In the presence of ox-LDL, endothelial cells secrete the monocyte chemoattractant protein, which triggers the recruitment of monocytes into the intima and the differentiation of monocytes into macrophages (Reape & Groot, 1999; Harrington, 2000). Macrophages have a high affinity for the ox-LDL and when they ingest large amounts of ox-LDL, they transform into foam cells which secrete chemokines that attract even more macrophages (Osterud & Bjorklid, 2003; Little et al., 2009; Gui et al., 2012). The appearance of lipid-laden foam cells represents a clear manifestation of an atherosclerotic lesion (Chrysohoou et al., 2007). A fibrous tissue (cap) forms over the lesion which continues to grow and invade the lumen of the vessel as the foam cells accumulate. Thinning of the fibrous cap can lead to rupture of the plaque, leading to thrombus and subsequent stroke or infarction (Vita, 2005).

As the major carrier of cholesterol in human plasma and its intimate involvement in the process of atherosclerosis, LDL-C is a key diagnostic and primary therapeutic target for CVD (Upadhyay, 2015), however, analysing other lipoproteins may reveal useful information.

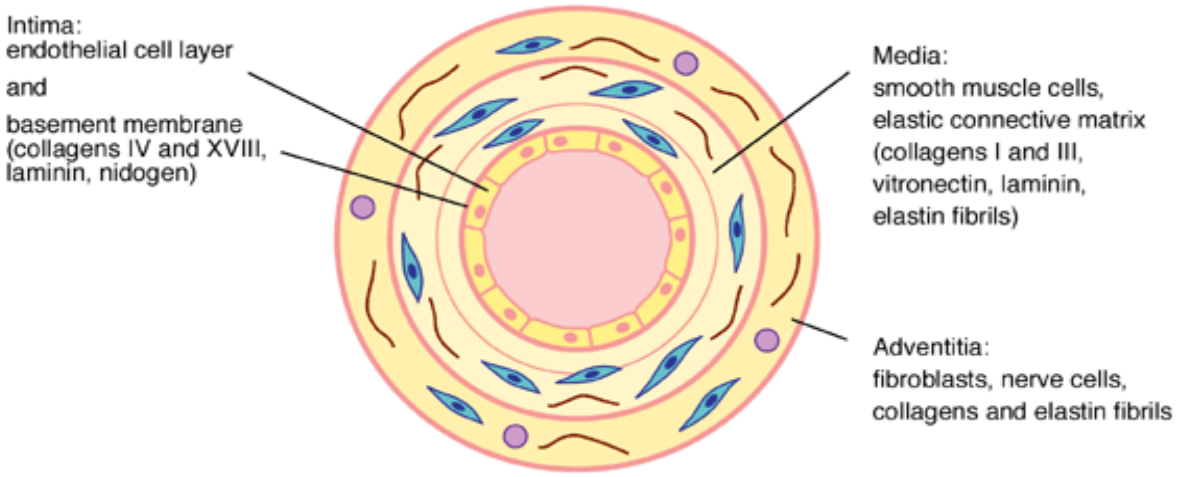

Figure 1: Schematic view of an arterial wall in cross-section (Expert Review in Molecular Medicine © 2002 Cambridge University Press http://www-ermm.cbcu.cam.ac.uk)

1.4 Classification of lipoproteins

Lipoproteins can be classified into five groups, based on their density (ultracentrifugation characteristics), electrophoretic mobilities and LDL particle size. (Table 1).

Table 1: Classification of lipoproteins

Class	Density (g/mL)	Electrophoretic mobility	Diameter (nm)	Molecular weight (Da)
Chylomicrons	0.93	Remain at origin	75 - 1200	(50 – 1000) x 10^6
VLDL	0.93 – 1.006	pre-β	30 - 80	(10 – 80) x 10^6
IDL	1.006 – 1.019	Slow pre-β	25 - 35	(5 – 10) x 10^6
LDL	1.019 – 1.063	β	18 - 25	(2 – 3) x 10^6
HDL	1.063 – 1.21	α	5 - 12	(65 – 386) x 10^6

Reference: Davidson, M., Toth, P.P. & Maki, K.C. 2007. Therapeutic Lipidology. Springer Science & Business Media.

1.4.1 Lipoproteins based on ultracentrifugation characteristics

It is well known that pure fat is less dense than water and therefore lipoproteins that contain a high proportion of lipids are less dense than those that contain a high proportion of protein. Therefore, using this characteristic of lipoproteins, ultracentrifugation classification is used to classify lipoproteins into four major groups: chylomicrons, very low density lipoproteins (VLDL), LDL and HDL. HDL can be subdivided into HDL-1, HDL-2 and HDL-3, whereas LDL can be further divided into LDL-1, LDL-2 and intermediate density lipoproteins cholesterol (IDL) (Chatterjea & Shinde, 2012). According to size, two distinct phenotypes of LDL particles have been identified, as large, buoyant LDL (pattern A: large buoyant LDL; diameter ≥ 25.5 nm) and small, dense LDL (pattern B: sdLDL; diameter < 25.5 nm) (Miyashita et al., 2006).

1.4.2 Based on electrophoretic mobilities

Lipoproteins can also be divided depending on their electrophoresis properties, where those containing higher protein content show a faster movement towards the anode. On this basis, HDL is called alpha, LDL beta, VLDL pre-beta, and IDL broad beta lipoproteins, while chylomicrons remain stable at the origin since they have more fat content (Chatterjea & Shinde, 2012).

1.4.3 Based on the nature of the Apo- protein content

Lipoproteins can also be divided depending on the number of apolipoproteins (proteins or polypeptides) they contain. The main apolipoproteins of HDL are designated A (apo AI and apo AII) in addition to apo E and apo C (apo CI, II, III), which are found also in VLDL, IDL and LDL. The major apolipoprotein B (apo B100 is synthesized in the liver and is found in VLDL and LDL. Chylomicrons contain apolipoprotein B (apo B48 which is synthesized in the intestine), apo A (AI and AII), apo C (CII and CIII) and apo E (Vasudevan et al., 2011).

1.5 Lipoprotein metabolism

The metabolism of lipoprotein particles can be divided into external (exogenous) and internal (endogenous) pathways (Figure 2). The different pathways depend largely on whether the lipoprotein particles are derived mainly from the diet (exogenous) or whether they originated in the liver (endogenous).

1.5.1 Exogenous pathway

Cholesterol and fatty acids from the diet are absorbed by the epithelial cells (enterocytes) lining the small intestine. They are then re-esterified to cholesterol esters and triglycerides. These lipids together with phospholipids and apolipoprotein B48 form nascent chylomicrons. These nascent chylomicrons can be released into the lymphatic circulation and reach the bloodstream through the thoracic duct (Ginsburg & Willard, 2012). In the bloodstream HDL particles donate apolipoprotein C-II, apolipoprotein C-III and apolipoprotein E to the nascent chylomicrons, which lead to their maturity (Covic et al., 2014). Chylomicrons then activate the enzyme

lipoprotein lipase which is found in the endothelial cell lining of the blood vessels of muscle and adipose tissues. This process stimulates the hydrolysis of triacylglycerides to glycerol and fatty acids which are absorbed by muscle and adipose cells. The chylomicron remnants deliver triglycerides and cholesterol to the liver (Crook, 2012). The chylomicron remnants are eventually destroyed by hepatocytes in the liver.

1.5.2 Endogenous pathway

The liver is one of the most important sources for the production of lipoproteins, especially VLDL. VLDL particles consist of triglycerides, phospholipids, cholesterol and apolipoprotein B-100. They mature when they gain apolipoproteins (CII and E) from HDL2 particles in the bloodstream. They can bind with lipoprotein lipase on the endothelial cells of the peripheral tissue via apolipoprotein C-II, which activates lipoprotein lipase, resulting in the decomposition of VLDL particles and release of glycerol and fatty acids to be absorbed by adipose and muscle cells. The hydrolyzed VLDL particles are now considered a VLDL remnant or IDLs. VLDL remnants transfer to the liver where they can interact with the LDL- receptors-related protein via apolipoprotein E, ending up absorbed by the liver, or they can be further hydrolyzed by hepatic lipase. Thus, the VLDL remnants lose more glycerol and fatty acids, leading to shrinking in size, and are now called IDL remnants or LDL, which contain a high level of cholesterol (Covic et al., 2014). In normal individuals, approximately 60 to 80% of LDL-C is absorbed by the liver and peripheral tissues through a process of interaction between the LDL receptors and apolipoprotein B-100 on LDL particles (Kwan et al., 2007). The cholesterol ester transfer protein assists LDL and VLDL particles to inter exchange their cholesterol and triglyceride molecules, resulting in triglycerides-rich LDL and VLDL remnants respectively. The triglycerides-rich LDL is followed by lipolysis of the triglyceride in hepatocytes by hepatic lipase, resulting in generating sdLDL which are more atherogenic than general LDL particles (Carlson, 2011).

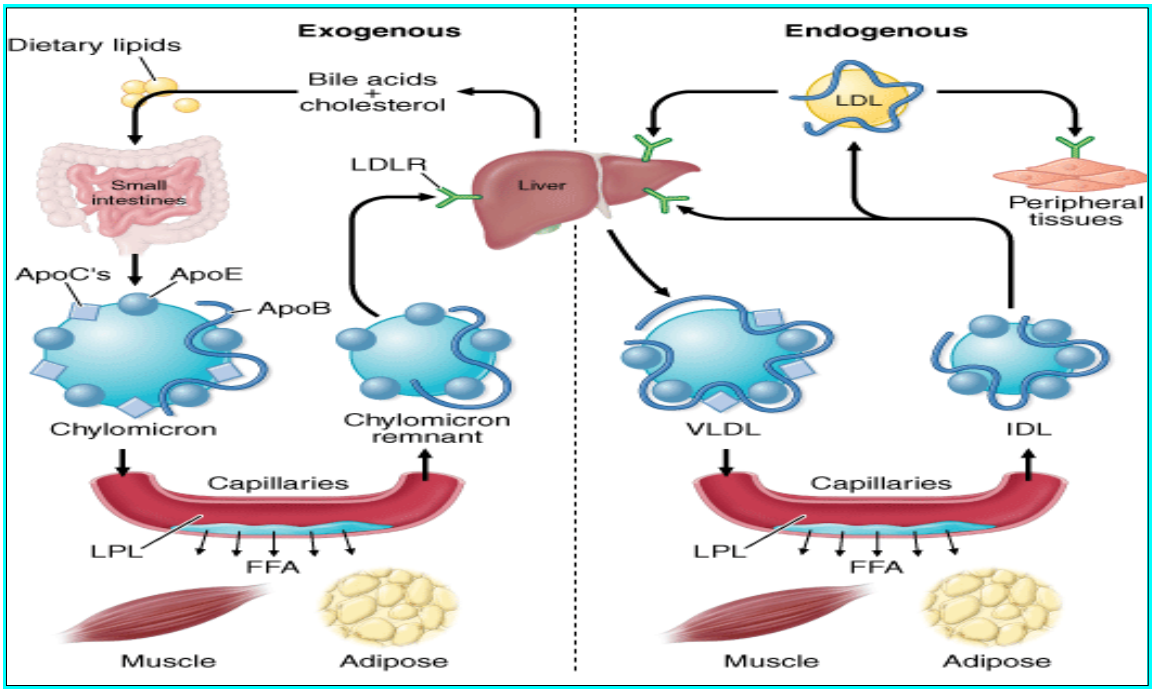

Figure 2: Exogenous and endogenous pathways of lipoprotein metabolism. Fauci, A.S., Kasper, D.L., Braunwald, E., Hauser, S.L., Longo, D.L., Jameson, J.L., Loscalzo, J. Harrison's Principles of Internal Medicine. 17th Edition: http://www.accessmedicine.com. Copyright (c) The McGraw-Hill Companies, Inc.

1.5.3 Reverse cholesterol transport

Reverse cholesterol transport is the process followed to transport cholesterol from the cells to the liver, including cholesterol metabolism through cholesterol efflux from peripheral tissues/macrophage to metabolic diversion into bile acids or final secretion of cholesterol into the faeces. The cholesterol is supplied to peripheral cells through a pathway after a dietary uptake or from synthesis within the liver or intestine in the form of apoB-containing lipoproteins (Cuchel & Rader, 2006). These lipoproteins are taken up by macrophages, which then become foam cells (Tabas et al., 2007). The cholesterol can be an efflux from these cells as free cholesterol via the adenosine tri-phosphate (ATP) binding cassette transporter A1 (ABCA1) with a few of lipidated apoA-1 as acceptors or via ATP-binding cassette sub-family G member 1 (ABCG1) with further HDL particles as acceptors. An additional efflux capacity might be supplied by scavenger receptor class B type 1 (SR-B1) (Cuchel & Rader, 2006). The cholesterol inside HDL is esterified by lecithin-cholesterol acyltransferase, enabling the uptake of additional free cholesterol. This is then taken via the plasma in the reverse pathway to the liver. Hepatocytes take up HDL esterified cholesterol via SR-BI receptors, after which the esterified cholesterol is de-esterified and excreted into the bile or as bile acids (Annema & Tietge, 2012).

1.6 High density lipoprotein cholesterol

HDL particles are also called “good cholesterol”, because they play a role in protecting against the development of atherosclerosis and thus reduce the risk of CVD. This process is called “reverse cholesterol transport”. In general, a high proportion of HDL particles leads to an increase in the ability to get rid of cholesterol and thereby preventing the development of serious blockages in the arteries (Toth, 2005). HDL can also suppress the endothelium from expressing adhesion molecules and the migration of monocytes due to a response to ox-LDL (Rosenson, 2006). Although increased levels of HDL particles have been reported to participate positively to reduce the risk of CHD, conversely, low level of HDL particles have also been reported to lead to an increased risk of Coronary artery disease (CAD) (Toth, 2005). The HDLs are anti-atherogenic lipoproteins and can be separated by ultracentrifugation into two major subclasses, HDL2 and the smaller, denser HDL3 (Roheim & Asztalos, 1995). Some studies have reported that a decline in the level of one of the HDL subgroups, HDL3, is one of the strongest indicators of CHD (Roheim & Asztalos, 1995) and that a decrease in the level of HDL3 in relation to that of HDL2 has been found associated with myocardial infarction and atherosclerosis.

1.7 Low density lipoprotein cholesterol

LDL is one of the five major classes of lipoprotein (Table 1) which serve to transport lipids around the body in the extracellular fluid. Like the other lipoproteins, the LDL structure is composed of a neutral lipid core surrounded by a surface monolayer of phospholipids, free cholesterol and apolipoproteins (Figure 3) that allow it to ferry lipids around the body.

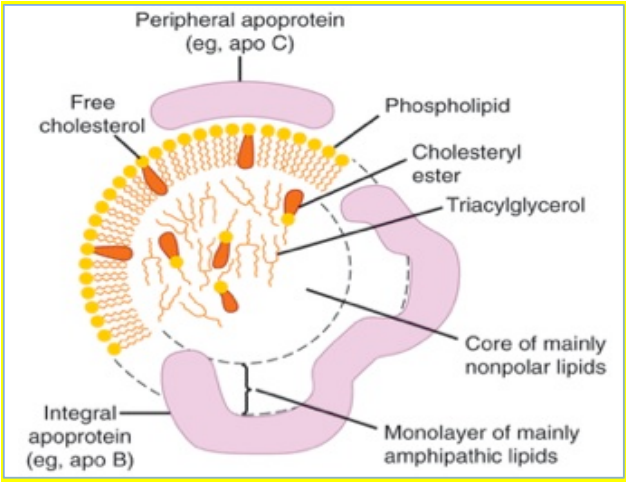

Figure 3: The structure of a lipoprotein. http://www.guwsmedical.info/amino-acids/free-fatty-acids-are-rapidly-metabolized.html Accessed date: 28 March 2016

LDL plasma particles are composed of heterogeneous sub-fractions that range in size from 18 to 25 nm in diameter (Davidson et al., 2007). The size of LDL particles depends on the amount of lipids in its core, and the lipid content determines its density. Based on size and density, two distinct LDL phenotypes have been described: pattern A, > 25.5 nm with a predominance of large, buoyant LDL particles and pattern B, ≤ 25.5 nm, with a predominance of sdLDL particles (Palazhy et al., 2014). Approximately 30% of circulating LDL in normolipidemic individuals is comprised of sdLDL and its proportion increases substantially in individuals with CAD, depending on the severity of the disease (Khan, 2012).

Ensign et al. (2006) have identified differences in the interpretation of 4 different methods for assessing LDL particle characteristics. The Gradient Gel Electrophoresis (GGE) method separates LDL into 7 subfractions based on size and shape, which are characterized into three patterns A, B and AB. Particles between 26.35 –28.5 nm are classified as large LDL Pattern A; sizes 25.75 –26.34 nm as Intermediate LDL Pattern AB and 22.0 –25.74 nm as small LDL Pattern B. The Density Gradient Ultracentrifugation (DGU) method produces 6 LDL subclasses which are identified from LDL1, being the most buoyant to LDL6, being the most dense. The Nuclear Magnetic Resonance (NMR) method generates three LDL subclasses LDL-1, LDL-2 and LDL-3. The Tube Gel Electrophoresis (TGE) method identifies 7 possible LDL subfractions, where lipoproteins are separated into different LDL patterns; LDLSF score for normal < 5.5 (pattern A); for Intermediate 5.5 – 8.5 (pattern AB) and for atherogenic > 8.5 (pattern B).

The role of LDL as a large holder of cholesterol and levels of LDL-C in the plasma identifies a real risk factor for the development of CHD (Vance & Vance, 2008). LDL particles are considered as “bad cholesterol”. Although their function is to carry cholesterol through the blood stream to peripheral tissues for cell building, they leave the excess cholesterol to build up in the walls of the coronary arteries. This may lead to the development of atherosclerosis, where the plaques narrow the arteries and thus resulting in CHD and heart attack (Cohen & Hasselbring, 2007). Although LDL-C has been considered a critical risk factor for CHD (Murray & Lopez, 1997), it has also been reported that up to 46% of the initial events of cardiovascular injuries occur in people with normal levels of LDL-C (Rifai & Ridker, 2001) and that ox-LDL may be the major factor in atherosclerosis injuries in humans (Gleissner et al., 2007) because they are more toxic to endothelial cells.

1.8 Small, dense low density lipoprotein

sdLDL, a fraction of LDL is a potent risk factor for CHD, even at levels within the normal range of LDL-C (Koba et al., 2008; Packard & Libby, 2008). Genetic and environmental factors are involved in the expression of LDL subclass phenotype B, with Its heritability estimated at 35 to 45% (Rizzo & Berneis, 2006). People could be at risk of increased sdLDL in the blood as a

result of high carbohydrate intake, trans fat intake, uncontrolled diabetes, high triglycerides, low HDL and MetS (Galeano et al., 1998). The presence of sdLDL is broadly related to lipid abnormalities. The formation of sdLDL particles from VLDL is related to insulin resistance since the hepatic production of VLDL is stimulated by and is an early complication of hepatic IR (Berneis & Krauss, 2002).

sdLDL has several characteristics that are linked to atherogenesis and an increased risk for CVD (Austin et al., 1988; Campos et al., 1992; Coresh et al., 1993) Compared to its larger counterparts, sdLDL binds less strongly to the LDL receptor (Chen et al., 1994) which prolongs its lifetime in circulation. In addition, the particle interacts more strongly with arterial wall proteoglycans which further increases the time it spends trapped in the subendothelial space of the artery wall, and hence increases the opportunity to be modified by oxidation (Tan et al., 1999). Lastly, a decreased LDL-C size is associated with an increased susceptibility to oxidation and low concentrations of antioxidants (Tribble et al., 1992). Collectively, these characteristics of sdLDL have been hypothesized to be used as a risk prediction and assessment for the response to lipid treatment rather than LDL-C as such (Austin, 2000). The therapeutic modification of LDL size, or number of sdLDL particles, significantly reduces CVD risk (Rizzo & Berneis, 2006). As such, the measurement of sdLDL is increasingly viewed as a powerful tool for evaluating atherogenic risk and has subsequently been recognised as an emerging cardiovascular risk factor by the National Cholesterol Education Program (NCEP) (Srisawasdi et al., 2011).

1.9 Small, dense low density lipoprotein as a marker for Cardiovascular Risk Assessment

The level of sdLDL cholesterol in a normolipidemic condition is approximately 30% of the total LDL-C in the blood. An increase of sdLDL-C has been reported to be associated with an increased risk of CVD and has also been found closely correlated with glucose tolerance and insulin resistance (Maeda et al., 2012). The ratio of sdLDL-C to LDL-C increases several-fold in hyperlipidemia depending on the degree and severity of the disease (Packard, 2003; Hirano et al., 2004; Khan et al., 2011). Results from the Quebec Cardiovascular Study which had included 2072 males aged between 46 - 75 years showed that over a 13 year follow-up period a predominance of sdLDL rather than LDL was found to be a strong and independent predictor for CHD in males, especially in the first years of follow-up (St-Pierre et al., 2005; Gentile et al., 2013) reported sdLDL particles as a sign of early carotid atherosclerosis and useful in the risk evaluation for atherosclerotic disease in females after menopause. Several studies have confirmed the reported independent association of increased sdLDL with CHD risk (St-Pierre et al., 2005; Kathiresan et al., 2006; Rizzo et al., 2009; Zeljkovic et al., 2010; Hoogeveen et al., 2014) and has subsequently been acknowledged as an emerging CVD risk factor by both the NCEP (2002) and the National Academy of Clinical Biochemistry Laboratory Medicine

Practice Guidelines (NACB LMPG) (2009). In contrast, results from an EPIC-Norfolk study showed that after adjustment for elevated triglyceride levels and reduced HDL-C particles, the estimation of sdLDL did not offer a significant advantage in evaluating the risk of CVD (Arsenault et al., 2007).

1.10 Diabetes and sdLDL

Both impaired insulin secretion and insulin resistance precede hyperglycemia, which leads to a pre-diabetic event, and subsequent T2DM phenotype (Codario, 2010). Diabetes and pre-diabetes are included as some of the main risk factors for CVD. According to the International Diabetes Federation (IDF), there will be a huge increase of people with diabetes by 2030 (Whiting et al., 2011). Females with diabetes have been reported to have an increased risk for CVD compared to males, due to an increased concentration of Apo B in diabetic females, Apo B has been reported as 113.7 ± 1.7 mg/dl in diabetic females and 100.6 ± 2.1 in diabetic males (Williams et al., 2008).

Similarly, a significant increase in the LDL subclass phenotype B has been reported among diabetic children as compared to a healthy control group (Alabakovska et al., 2008). The authors demonstrated that this link contributes to the increased vulnerability of children to the risk of diabetes and atherosclerosis (Alabakovska et al., 2008) and insulin resistance (Stan et al., 2005). sdLDL particles consist of less cholesterol than normal-sized LDL particles, but they are more atherogenic compared to large, buoyant low density lipoprotein (lbLDL) (Marcovina & Packard, 2006). An increased number of sdLDL is very common in diabetes mellitus, visceral obesity and MetS (Sniderman et al., 2002). MetS and diabetic dyslipidemia are defined by the lipid triad of increased triglyceride levels, decreased HDL-C and the presence of sdLDL particles and which together have been reported to be strong risk factors for diabetes (Wierzbicki, 2006).

Patients with diabetes have a 2 to 3 fold increased risk of CVD death (Betteridge, 2004). Moreover, a study conducted in the United States found that in people with both diabetes and obesity, there was an 18% increase in CHD mortality. Therefore, it was proposed by the authors that the ability to control obesity and diabetes could achieve a possible reduction in CHD deaths (Ford et al., 2007). Additionally, statin therapy for patients who suffer from T2DM, has shown a significant reduction in LDL-C, decrease elevated triglycerides and a slight increase of HDL-C (Knopp et al., 2006).

1.11 Assessment of plasma sdLDL levels

In spite of its potential clinical value, sdLDL particles are seldom routinely measured although there are various methods available for this purpose. These include density gradient ultracentrifugation (Griffin et al., 1990), gradient gel electrophoresis (Alabakovska et al., 2002), tube gel electrophoresis (Hoefner et al., 2001) and nuclear magnetic resonance (Kuller et al.,

2002). These methods suffer from one or more limitations relating to exorbitant running costs, technical complexity and a requirement for elaborate instrumentation making them unfeasible for routine clinical use or population screening. A simpler enzymatic method that has potential for routine clinical application was recently developed (Ito et al., 2011) and although it is presently the only available direct automated sdLDL method, the prohibitive cost of the reagents has also restricted its wider use.

In the absence of a suitable, routinely applicable method, the alternative approach for determining sdLDL is to use equations derived from routine lipid measures as has been proposed by several groups. Ratios of triglycerides/HDL-C ≥ 3.0 (Mohan et al., 2005) and LDL-C /LDL-apolipoprotein B < 1.2 (Hattori et al., 1998) have previously been suggested as useful surrogate markers for sdLDL. The most promising equation however, with the potential for a wider application, is the one derived from a Thai population by Srisawasdi et al. (2011). They did correlation studies between classic lipid indices and sdLDL-C measured directly via the homogeneous enzymatic assay. They used the equation [(sdLDL-$C_{mg/dl}$ = 0.580 (non–HDL-C) + 0.407 (dLDL-C) – 0.719 (cLDL-C) – 12.05) or (sdLDL-$C_{mmol/L}$ = 0.580 (non–HDL-C) + 0.407 (dLDL-C) – 0.719 (cLDL-C) – 0.312)] to evaluate sdLDL-C levels from routinely measured lipid parameters in healthy individuals. The authors reported reliable results across a wide spectrum of sdLDL-C levels. They did not, however, evaluate the performance of the equation in key patient groups with abnormal lipoprotein metabolism, including diabetes mellitus and MetS. There is therefore a need for extensive evaluation of the equation across different ethnic and disease groups, especially after the recent demonstration of its inadequate performance in healthy Koreans and those with MetS (Cho et al., 2012). In this regard, Erasmus et al. (2012) reported the prevalence of diabetes, a key risk factor for CVD, almost quadrupled in the current study population from 7.1% in 1999 to 28.2% in 2012, making this study population a good target population for the evaluation of the Srisawasdi formula (2011).

1.12 Aim

The aim of this study was:

1. To assess the performance of the recently proposed equation for the estimation of sdLDL-C in healthy mixed ancestry South Africans and those with diabetes to determine whether it is applicable to this population in general and to a specific group with abnormal lipoprotein metabolism.

1.13 Objectives

To evaluate the following two methods, measured versus calculated sdLDL-C in a mixed ancestry study population:

1. Measured: A novel homogenous enzymatic assay (Randox sLDL-EX“SEIKEN”) to measure sdLDL-C.

2. Calculated: A recently equation proposed by Srisawasdi et al. (2011) to estimate sdLDL-C from classic lipid measures.

CHAPTER 2
RESEARCH METHODOLOGY

2.1 Background and ethical considerations

The main prospective study was approved by the Cape Peninsula University of Technology Health and Wellness Sciences Research Ethics Committee (Ref #: NHREC: REC - 230 408 – 014) and was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave consent in writing after all relevant procedures were explained to them. For this sub-study, ethical approval was also requested and granted by the Cape Peninsula University of Technology Health and Wellness Sciences Research Ethics Committee (CPUT/HW-REC 2014/H08). The study was part of a larger project whose overarching aim was to develop well informed, easy to use and high quality tools that offer screening, prevention, management and diagnosis for diabetes and vascular diseases in the urban communities of Bellville South, Western Province. Bellville South is a predominantly "Coloured" township formed in the late 1950s and, according to the 2011 population census, its population stands at approximately 29 301 with an average household size of 4.84 individuals. The population is predominantly of coloured or mixed ancestry (76%) followed by black Africans (18.5%) and Caucasian and Asians making only (1.5%) and other minority groups (4%) (Stats, S.A. 2012). The sampling for the present study was commenced when the assays were available for testing. Samples for all participants who were eligible and who consented to the study were used.

2.2 Eligibility for the study population

Inclusion criteria

Participants were recruited if they were:

- of mixed ancestry descent
- residents of Bellville South, Ward 9 area
- consenting adults aged 20 years or older

Exclusion criteria

Participants were not recruited if they were:

- pregnant females

2.3 Materials and methods

2.3.1 Sample and data collection

Questionnaires and measurements were used to obtain demographics, lifestyle, clinical and anthropometric data. Overnight fasting blood samples were drawn from all participants. To

evaluate glucose tolerance status, all participants except those with a known diabetic status were asked to consume a standard glucose solution (75 g glucose powder + 250 mL water) and additional blood samples were drawn two hours later as prescribed by the WHO (2016). The blood samples were sent to an ISO 15189 accredited Pathology practice (PathCare, Reference Laboratory, Cape Town, South Africa) for routine biochemical analysis or frozen at -80°C until needed for further analysis.

2.3.2 Physical examination

Body weight (kg) was measured using an Omron body fat meter HBF-511digital bathroom scale and height (cm) with a stadiometer. Body Mass Index (BMI) was calculated as weight per square meter (kg/m^2). Waist (cm) and hip (cm) circumference was measured with a tape. The blood pressure (mmHg) was measured using the Omron M6 Comfort-preformed Cuff Blood Pressure Monitor.

2.3.3 Biochemical Assays

Blood samples were obtained from each participant after 10 to 12 hours of overnight fasting and processed for further biochemical analysis at an accredited laboratory (PathCare, Reference Laboratory, Cape Town, South Africa). Blood glucose (mmol/L) was measured using the enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa). Insulin (IU/l) was determined by a paramagnetic particle chemiluminescence assay (Beckman DXI, Beckman Coulter, South Africa). HDL-C (mmol/L) was by enzymatic immunoinhibition – End Point (Beckman AU, Beckman Coulter, South Africa). LDL-C (mmol/L) was measured by enzymatic selective protection – End Point (Beckman AU, Beckman Coulter, South Africa). Triglycerides (mmol/L) were measured by glycerol phosphate oxidase-peroxidase, End Point (Beckman AU, Beckman Coulter, South Africa). Non-HDL-C (mmol/L) values were calculated by subtracting the measured HDL-C (mmol/L) values from the total cholesterol (TC) (mmol/L) values (Srisawasdi et al. 2011). LDL-C (mmol/L) was calculated according to Friedewald's formula, cLDL-C = TC – HDL-C – (TG/5) (Friedewald et al., 1972). sdLDL (mmol/L) was measured directly, using a homogenous enzymatic assay (Randox sLDL-EX"SEIKEN") on an ABX Pentra 400 analyser (HORIBA ABX, Montpellier, France) as well as calculated sdLDL, using the formula proposed by Srisawasdi et al (2011): $sdLDL\text{-}C_{mmol/L}$ = 0.580 (non–HDL-C) + 0.407 (dLDL-C) – 0.719 (cLDL-C) – 0.312, where dLDL is direct LDL and cLDL is calculated LDL. The assay and quality control on the ABX Pentra 400 analyser was carried out according to the manufacturer's instructions (Randox Laboratory Limited, South Africa). The following principles were adapted from the assay insert. The assay consists of two steps and is based on the technique to use well-characterized surfactants and enzymes that selectively react with certain groups of lipoproteins. In the first step, non-sdLDL lipoproteins, that is, chylomicrons, VLDL, IDL, LDL and HDL are decomposed by a surfactant and sphingomyelinase (SPC) in Reagent-I that is reactive to those non-sdLDL lipoproteins. The

cholesterol released from such non-sdLDL lipoproteins is then degraded to water and oxygen by the action of enzymes. Cholesterol ester is hydrolyzed by the cholesterol esterase (CHE) and then oxidized by the cholesterol oxidase (CO). Produced hydrogen peroxides are finally decomposed to water and oxygen by the catalase. In the second step, another surfactant in Reagent-2 releases cholesterol only from sdLDL particles and cholesterol released from sdLDL is then subject to the enzymatic reactions. As catalase in the reaction mixture is inhibited by sodium azide in Reagent-2, hydrogen peroxides, produced from the reaction with the cholesterol esterase and cholesterol oxidase, then develop a purple-red color with the coupler in the presence of peroxidase (POD). sdLDL controls at levels 1, 2 and 3 were done at least once a day. These levels were within the specified range, with a coefficient of variance (CV) of less than 3%.

2.4 Definition of diabetes mellitus

The diabetes status of the study population was based on a history of doctor-diagnosis, a fasting plasma glucose ≥ 7.0 mmol/L or a 2-hour post-oral glucose tolerance test (OGTT), with plasma glucose ≥ 11.1 mmol/L (Table 2) (WHO, 2016).

Table 2: The WHO criteria for the diagnosis of diabetes

	Fasting plasma glucose (mmol/L)		**2-hour plasma glucose (mmol/L)**
Diabetes	≥ 7.0 mmol/L	OR	≥ 11.1 mmol/L
IGT	< 7.0 mmol/L	AND	≥ 7.8 and < 11.1 mmol/L
IFG	6.1 to 6.9 mmol/L	AND	< 7.8 mmol/L

2.5 Definition of Metabolic syndrome

Metabolic syndrome (MetS) was defined based on the 2009 Joint Interim Statement (JIS) criteria (Alberti et al., 2009), with modification of a waist circumference cut-off of 90 cm for both men and women in this population as reported by Matsha et al. (2013). This includes the presence of any three of the following conditions: increased waist circumference (men: ≥ 94 cm and women: ≥ 80 cm), low HDL (men < 40 mg/dl (1 mmol/L) and women < 50 mg/dl (1.3 mmol/L), hypertriglyceridemia, triglycerides ≥ 150 mg/dl (1.7 mmol/L), elevated blood pressure (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg or drug treatment for hypertension) and elevated blood sugar (fasting blood sugar ≥ 100 mg/dl (5.6 mmol/L) or diagnosed diabetes mellitus.

2.6 Statistical Analysis

A software program, Statistica 12 (StatSoft, Southern Africa) was used to analyse the collected data. Variables are summarized as mean and standard deviation (SD) or median and 25th-75th percentiles (ANOVA). The Bonferroni test was used to compare differences, with significance level $P < 0.05$. The Shapiro-Wilk's W test was employed to determine whether the data were normally distributed based on probability thresholds of $P > 0.1$. The association between measured and estimated sdLDL measurements was assessed using Pearson's correlation test, with significance level $P < 0.05$.

CHAPTER 3
RESULTS

3.1 General description

Of a total of 239 participants, 2 were excluded from the study as their triglyceride levels were greater than 4.5 mmol/L (de Cordova & de Cordova, 2013). Therefore, the final total number of participants in the study was 237, of whom 55 (23.2%) were males and 182 (76.8%) were females. The mean and SD for age in years of the participants was 54.2 (14.7) years. The biochemical and anthropometric measurements of the participants according to gender and glucose tolerance status are summarized in Tables 3 and 4 respectively.

3.2 Biochemical and anthropometric measurements according to gender

The characteristics of participants according to gender are summarized in Table 3. The results showed a significant difference in the 2-hour glucose levels (mmol/L) between males, mean (SD) 5.8 (3.3) and females, mean (SD) 7.2 (3.1) (P = 0.0076). Although the females were significantly shorter than males (P < 0.0001), their weight was non-significantly higher than their male counterparts but with a significant corresponding higher BMI (kg/m^2), (P < 0.0001). Similarly, the other obesity indices were significantly higher in females, that is, waist circumference (P = 0.0201) and hip circumference (P < 0.0001). The lipids and lipoproteins concentrations showed no significant differences between males and females, except HDL-C (mmol/L) which was significantly lower in males (P = 0.0002).

Table 3: Biochemical and anthropometric measurements according to gender

	Overall Mean (SD) N237	**Male Mean (SD) N55**	**Female Mean (SD) N182**	**P-value**
Age (years)	54.2 (14.6)	53.1 (16.3)	54.6 (14.2)	0.4916
Fasting glucose (mmol/l)	6.0 (3.4)	5.6 (2.6)	6.1 (3.6)	0.2697
2-hour glucose (mmol/l)	6.8 (3.2)	5.8 (3.3)	7.2 (3.1)	0.0076
Fasting insulin (IU/l)	7.5 (4.7-12.2)	5.6 (3.6-9.5)	8.0 (5.0-12.7)	0.1119
2-hour insulin (IU/l)	46.1 (27.9-82.4)	30.1 (12.9-63.2)	50.7 (31.2-89.0)	0.0590
Weight (kg)	79.0 (21.7)	75.7 (23.0)	79.7 (21.2)	0.2069
Height (cm)	159.3 (8.8)	169.5 (6.8)	156.2 (6.8)	< 0.0001
Body mass index (kg/m^2)	31.2 (8.6)	26.3 (7.7)	32.8 (8.3)	< 0.0001
Waist circumference (cm)	97.0 (17.5)	92.2 (19.4)	98.4 (16.7)	0.0201
Hip circumference (cm)	110.2 (16.3)	100.9 (14.1)	113.0 (15.9)	< 0.0001
Systolic blood pressure (mmHg)	129.4 (23.9)	129.9 (25.9)	129.2 (23.4)	0.8519
Diastolic blood pressure (mmHg)	81.9 (12.2)	80.7 (12.5)	82.3 (12.1)	0.3992
Total cholesterol (mmol/l)	5.1 (1.2)	4.9 (1.3)	5.1 (1.1)	0.2728
HDL-C (mmol/l)	1.3 (0.3)	1.1 (0.3)	1.3 (0.3)	0.0002
Measured LDL-C (mmol/l)	3.2 (1.0)	3.1 (1.2)	3.2 (1.0)	0.8436
Calculated LDL-C (mmol/l)	3.2 (1.0)	3.2 (1.1)	3.2 (1.0)	0.7413
Calculated sdLDL-C (mmol/l)	0.93 (0.42)	0.91 (0.47)	0.93 (0.41)	0.7031
Measured sdLDL-C (mmol/l)	0.95 (0.51)	0.96 (0.53)	0.94 (0.51)	0.8511
Non-HDL-C (mmol/l)	3.8 (1.2)	3.8 (1.2)	3.8 (1.1)	0.8845
Triglycerides (mmol/l)	1.4 (0.7)	1.4 (0.9)	1.5 (0.7)	0.6994

3.3 The biochemical and anthropometric measurements according to glucose tolerance status.

Table 4 shows the general characteristics of participants according to the glucose tolerance status. The participants were categorized into the following glucose tolerance status, normotolerant, prediabetes (IFG or impaired glucose tolerance (IGT) or both), screen-detected diabetes and known diabetes. One hundred and fifty six (65.8%) of the participants were normotolerant, 29 (12.2%) were prediabetic, 17 (7.2%) were screen-detected diabetes and 35 (14.8%) were known diabetes. A significant difference in mean age (years) between normotolerant and both prediabetes and known diabetes, $P = 0.0001$ and $P < 0.0001$ was respectively determined. As expected, there were significant differences in fasting glucose and post 2-hour glucose between the normotolerant, prediabetic and/or diabetes groups ($P \leq 0.0002$). Similarly, a significant difference in fasting insulin levels (IU/l) was found between the normotolerant and known diabetes groups ($P = 0.0002$), while the 2-hour insulin levels (IU/l) were significantly different between normotolerant and prediabetes ($P = 0.0262$). The BMI (kg/m^2) showed a significant difference between normotolerant and known diabetes subjects ($P = 0.0012$). The only significant difference in waist circumference was observed between normotolerant and known diabetes ($P < 0.0001$). There was a significant difference between normotolerant and known diabetes in systolic blood pressure (mmHg) ($P = 0.0079$), but none between any of the groups for diastolic blood pressure (mmHg). There was also no significant differences between all the groups for total cholesterol (mmol/L), HDL-C (mmol/L), measured LDL-C (mmol/L), calculated LDL-C (mmol/L) and non-HDL-C (mmol/L), There were however significant differences in triglycerides (mmol/L) between normotolerant, and diabetic subjects (both screen-detected diabetes, and known ($P = 0.0043$ and 0.0083 respectively).

Table 4: Baseline characteristics overall and by glucose tolerance status

	Overall	Normo tolerant[a]	Pre diabetes[b]	Screen-detected diabetes[c]	Known diabetes[d]	P - value
Age, years	54.2 (14.6)	50.3 (14.7)	62.4 (12.3)	59.5 (10.0)	62.4 (11.2)	P^{ab} = 0.0001 P^{ad} < 0.0001
Male, n (%)	55 (23.2)	40 (25.6)	5 (17.2)	2 (11.8)	8 (22.9)	
Female, n (%)	182 (76.8)	116 (74.4)	24 (82.8)	15 (88.2)	27 (77.1)	
Glucose tolerance, n (%)	237	156 (65.8)	29 (12.2)	17 (7.2)	35 (14.8)	
Fasting glucose (mmol/L)	6.0 (3.4)	4.7 (0.54)	5.4 (0.55)	8.8 (5.43	10.7 (5.63)	P^{ac} < 0.0001 P^{ad} < 0.0001 P^{bc} = 0.0002 P^{bd} < 0.0001
2-hour glucose (mmol/L)	6.8 (3.2)	5.6 (1.38)	8.9 (1.05)	15.2 (3.66)	Not applicable	P^{ab} < 0.0001 P^{ac} < 0.0001 P^{bc} < 0.0001
Fasting insulin (IU/l)	7.5 (4.7-12.2)	6.4 (4.2-9.6)	9.0 (5.7-13.9)	13.1 (10.6-19.1)	11.7 (6.1-16.5)	P^{ad} = 0.0002
2-hour insulin (IU/l)	46.1 (27.9-82.4)	39.1 (22.6-70.3)	64.5 (52.1-118.4)	90.7 (33.7-114.9)		P^{ab} = 0.0262
Body mass index (kg/m^2)	31.2 (8.6)	29.7 (8.3)	33.4 (7.9)	33.6 (5.6)	35.6 (10.0)	P^{ad} = 0.0012
Waist circumference (cm)	97.0 (17.5)	92.9 (17.6)	101.5 (13.3)	103.6 (10.7)	107.9 (16.4)	P^{ad} < 0.0001
Hip circumference (cm)	110.2 (16.3)	108.3 (15.9)	112.6 (14.5)	111.2 (11.9)	116.1 (19.8)	Not significant
Systolic blood pressure (mmHg)	129 (24)	126 (25)	133 (20)	133 (19)	140 (21)	P^{ad} = 0.0079
Diastolic blood pressure (mmHg)	82 (12)	81 (13)	82 (12)	83 (10)	84 (11)	Not significant
Total cholesterol (mmol/L)	5.1 (1.2)	5.0 (1.1)	5.2 (0.9)	5. 7 (1.7)	5.0 (1.2)	Not significant
HDL cholesterol (mmol/L)	1.3 (0.3)	1.3 (0.3)	1.2 (0.3)	1.3 (0.2)	1.2 (0.3)	Not significant
Measured LDL-C (mmol/L)	3.2 (1.0)	3.1 (1.0)	3.3 (0.9)	3.6 (1.5)	3.1 (1.0)	Not significant
Calculated LDL-C (mmol/L)	3.2 (1.0)	3.1 (1.0)	3.3 (1.1)	3.5 (1.5)	3.0 (1.1)	Not significant
Non-HDL-C (mmol/L)	3.8 (1.2)	3.7 (1.1)	4.0 (1.0)	4.4 (1.7)	3.8 (1.2)	Not significant
Triglycerides (mmol/L)	1.4 (0.7)	1.3 (0.7)	1.6 (1.1)	2.0 (0.8)	1.8 (0.8)	P^{ac} = 0.0043 P^{ad} = 0.0083

3.4 Measured sdLDL versus calculated sdLDL

The mean (SD) of calculated and measured sdLDL-C were 0.91 (0.45) (mmol/L) and 0.95 (0.51) (mmol/L) respectively and their difference was not significant (P = 0.6066) (Table 5). Although the medians of the two methods were comparatively similar (0.87 vs 0.84 mmol/L), the interquartile ranges were quite divergent (min to max: -1.85 to 2.52 vs 0.17 to 3.39) indicating that individuals identified by the two methods are likely to be sometimes different. This could possibly be explained by the skewed distribution shown in both calculated and measured sdLDL as tested by the Shapiro-Wilk's W test (P < 0.0001 for both) as well as the Agostino test (P = 0.0009 and P < 0.0001 respectively). In addition, the Anscombe-Glynn test for kurtosis showed that variance was the result of infrequent extreme deviations. The coefficient of variation for calculated and measured sdLDL-C showed similar dispersions of data points around the mean (49.5% and 53.9% respectively), showing both methods to have irregular distributions of data to a similar extent. Linear regression analysis showed a moderate continuous (43%) association between calculated and measured sdLDL-C, adjusted R^2 = 0.432, beta coefficient 0.580 with a significance level of P < 0.0001 (Fig 4). The Bland-Altman plots showed good agreement between the methods used to determine sdLDL-C with limits of agreement shown in Fig 5. The total error for the current study was 4.83% and which falls within the total allowable error for sdLDL which is 13% (Ricos, 2014).

Table 5: Calculated vs measured sdLDL-C (mmol/l)

	Calculated sdLDL-C (mmol/l)	**Measured sdLDL-C (mmol/l)**
Mean (SD)	0.91 (0.45)	0.95 (0.51)
Median (min-max)	0.87 [-1.85 to 2.52]	0.84 [0.17 to 3.39]
Shapiro-Wilk's W (P-value)	< 0.0001	< 0.0001
Skewness (Agostino test, P-value)	0.0009	< 0.0001
Kurtosis (Anscombe-Glynn test, P-value)	< 0.0001	< 0.0001
Coefficient of variation (%)	49.5%	53.9%

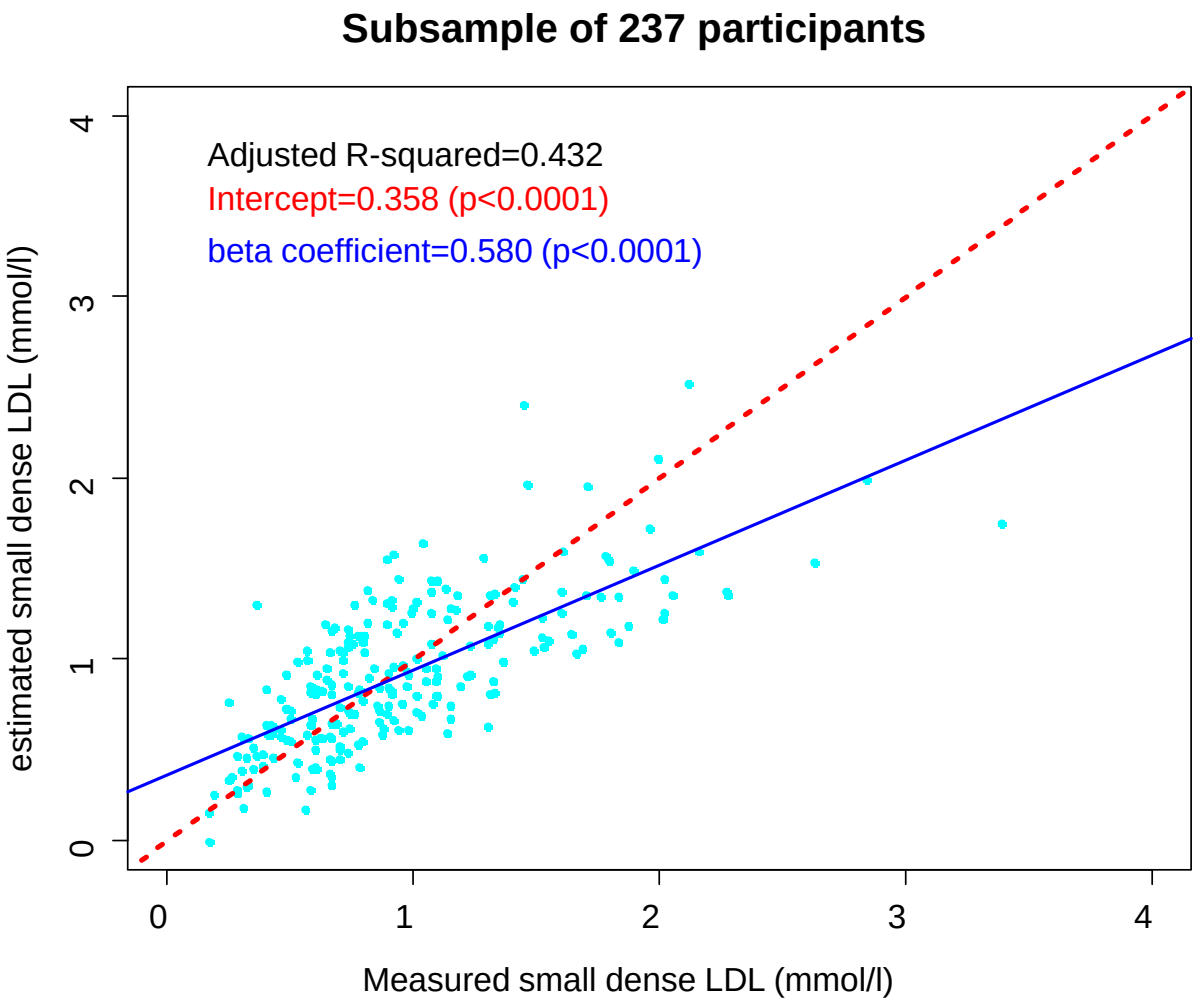

Figure 4: Linear regression curves showing the continuous association of measured with estimated sdLDL-C in participants with data available on both measures. The dotted diagonal line is the line of perfect agreement

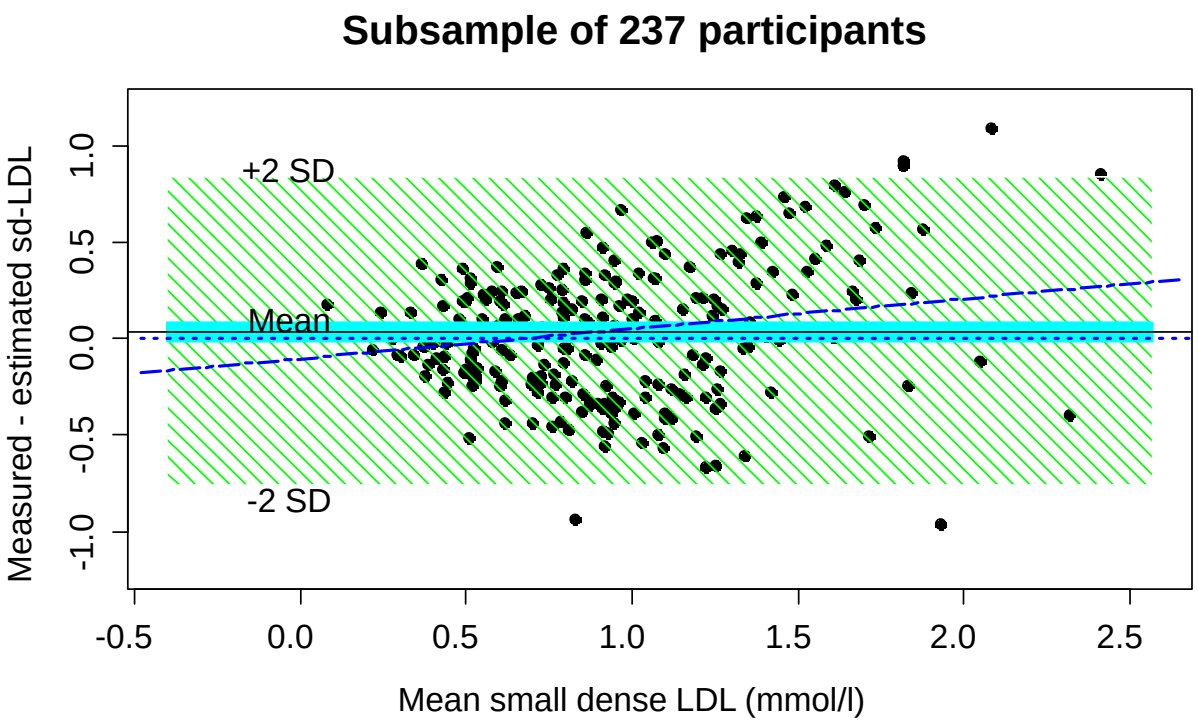

Figure 5: Bland and Altman plots comparing measured with estimated sdLDL-C distribution curves for measured sdLDL-C. The difference of Measured minus Estimated value for each participant is plotted against the mean of the two measurements. The horizontal dashed line through 0 (zero) is the line of perfect agreement between the two measurements. The parallel solid black line is the bias line (mean difference), and the colored band about it for the 95% confidence interval, while the shaded area delineates the zone of agreement between the two measurements. The lines of best fit from linear regressions are also shown (dotted superimposed curves).

3.5 Biochemical and anthropometric measurements evaluated against measured sdLDL percentiles

The baseline characteristics of participants across percentiles of measured sdLDL are summarized in Table 6. The hyperglycemic subgroups did not show a significant difference between the sdLDL percentiles (P = 0.2423, data not shown), but there is a trend towards an increased number of both screen detected diabetes mellitus (DM) and known DM in the 4th quartile, with a simultaneous decrease in the normotolerant group. The JIS MetS positive cases showed a significant increase over the sdLDL percentiles (P < 0.0001). Across percentiles of sdLDL levels, significant differences were also observed in the distribution of age (P = 0.0109), fasting (P = 0.0008), 2-hour glucose levels (P = 0.0020), 2-hour insulin levels (P = 0.0007), waist circumference (P = 0.0042), systolic blood pressure (P = 0.0002) and all lipid profile measurement, (all P < 0.0001); with increasing trends across percentiles of sdLDL levels.

Table 6: Biochemical and anthropometric measurements evaluated against measured sdLDL percentiles

	Q1	Q2	Q3	Q4	
	Mean (SD) [and minimum, maximum range]				P-value
Measured sdLDL-C (mmol/L)	0.43 (0.12) [0.17, 0.60]	0.72 (0.06) [0.61, 0.84]	0.99 (0.09) [0.85, 1.15]	1.66 (0.43) [1.17, 3.39]	**< 0.0001**
Total numbers	60	59	59	59	
Normotolerant N (%)	43 (27.6)	44 (28.2)	37 (23.7)	32 (20.5)	
Prediabetes N (%)	8 (27.6)	6 (20.7)	9 (31.0)	6 (20.7)	
Known DM N (%)	7 (20.0)	6 (17.1)	9 (25.7)	13 (37.1)	
Screen-detected DM N (%)	2 (11.8)	3 (17.6)	4 (23.5)	8 (47.1)	
MetS (Yes) N (%)	19 (15.6)	24 (19.7)	35 (28.7)	44 (36.1)	**< 0.0001**
Male N (%)	14 (25.5%)	12 (21.7%)	14 (25.5%)	15 (27.3%)	
Age (years)	49 (17) [21,88]	55 (16) [25,85]	57 (13) [22,79]	57 (12) [25,85]	**0.0109**
Fasting glucose (mmol/L)	5.41 (2.29) [3.20, 16.40]	5.44 (3.09) [3.30, 27.60]	5.62 (2.37) [3.50, 19.90]	7.56 (4.90) [4.00, 27.70]	**0.0008**
2-hour glucose (mmol/L)	6.25 (2.57) [2.40, 15.60]	6.32 (2.34) [2.70, 17.30]	6.55 (2.82) [2.60, 17.10]	8.38 (4.34) [3.40, 22.70]	**0.0020**
Fasting insulin (IU/l)	9.85 (13.06) [0.80, 96.70]	9.46 (10.54) [1.90, 73.10]	9.60 (8.32) [1.40, 52.80]	11.66 (6.74) [3.60,30.80]	0.6035
2-hour insulin (IU/l)	51.2 (52.0) [3.6, 236.8]	57.1 (46.1) [4.9, 265.2]	53.1 (38.0) [2.3, 166.9]	89.9 (63.9) [12.0, 300.0]	**0.0007**
Body mass index (kg/m^2)	29.6 (10.4) [16.2, 62.7]	31.1 (8.3) [17.4, 51.9]	30.9 (7.5) [17.7, 54.2]	33.6 (7.9) [19.5, 62.9]	0.0904
Waist circumference (cm)	91.5 (19.4) [63.5, 149.8]	96.7 (17.9) [64.5, 143.0]	96.7 (16.3) [62.5, 135.5]	103.0 (14.4) [77.5, 162.5]	**0.0042**
Hip circumference (cm)	106.9 (20.0) [80.5, 169.8]	110.2 (15.4) [83.8, 156.8]	109.9 (13.4) [89.5, 146.0]	113.8 (15.1) [89.5, 175.8]	0.1456
Systolic blood pressure (mmHg)	119.9 (21.4) [67.0, 176.0]	126.9 (22.8) [77.0, 172.0]	133.2 (24.9) [92.0, 186.0]	137.7 (23.1) [94.0, 199.0]	**0.0002**
Diastolic blood pressure (mmHg)	79.7 (12.3) [50.0, 109.0]	80.9 (12.2) [59.0, 111.0]	82.0 (12.9) [54.0, 118.0]	85.0 (10.9) [65.0, 122.0]	0.1074
Total cholesterol (mmol/L)	4.09 (0.86) [2.40, 6.50]	4.84 (0.88) [2.50, 6.60]	5.33 (0.84) [3.70, 7.40]	6.12 (1.01) [4.80, 9.10]	**< 0.0001**
HDL cholesterol (mmol/L)	1.34 (0.34) [0.40, 2.10]	1.27 (0.31) [0.70, 2.30]	1.28 (0.30) [0.80, 1.90]	1.21 (0.27) [0.70, 2.00]	0.1340
Measured LDL-C (mmol/L)	2.24 (0.75) [0.80, 4.30]	3.00 (0.78) [1.20, 4.60]	3.37 (0.81) [1.70, 5.50]	4.08 (0.86) [2.80, 6.60]	**< 0.0001**
Non-HDL-C (mmol/L)	2.76 (0.80) [1.20, 4.90]	3.58 (0.81) [1.80, 5.20]	4.06 (0.81) [2.90, 6.10]	4.91 (0.93) [3.50, 7.70]	**< 0.0001**
Triglycerides (mmol/L)	1.00 (0.40) [0.36, 1.98]	1.23 (0.53) [0.50, 3.31]	1.55 (0.89) [0.41,6.51]	2.02 (0.81) [0.90, 4.34]	**< 0.0001**

CHAPTER 4
DISCUSSION

4.1 Background

Elevated levels of sdLDL are very common in diabetes mellitus, visceral obesity and MetS (Sniderman et al., 2002), with patients with diabetes reported to have a 2 to 3 fold higher risk of CVD death compared to a control group (Betteridge, 2004). Although reports showed that patients with CVD had the same range of LDL-C compared to normal subjects, the distribution of LDL particle size has been shown to show a shift towards the smaller sized subgroups, including sdLDL (Akosah et al., 2003; St-Pierre et al., 2005). Currently available tests for sdLDL are expensive and laborious for use in a general clinical practice or for population screening, making alternative, reliable methods desirable. Therefore, we conducted a validation study of a recently proposed equation (Srisawasdi et al., 2011) for the estimation of sdLDL-C from classic lipid measures in a sample of mixed ancestry South Africans with varying degrees of glucose tolerance. Overall, we observed a moderate to good agreement between the two methods in a total of 237 subjects as shown by the Bland-Altman plot and linear regression. Moreover, the two methods showed similar dispersion of data points around the mean. Although the means and medians were comparatively similar between the two points, the interquartile ranges were divergent indicating that the two methods sometimes identified different individuals. Furthermore, our data also confirmed the link between sdLDL and certain cardiometabolic risk factors as previously also reported by Hattori et al. (1998).

4.2 Measured versus calculated sdLDL

Previous studies have used different equations to establish possible reliable methods to replace measured sdLDL-C with reliable calculated sdLDL-C values. For example, Hattori et al. (1998) used the LDL-C /LDL-apolipoprotein B ratio as a predictive tool for the level of sdLDL in subjects with CVD. They reported this formula to be useful in lipoprotein disorders. Wagner et al. (2002) used the formula suggested by Hattori et al. (1998) and reported that the LDL-C /LDL-apolipoprotein B ratio is a good predictor of LDL particle size, but that this formula is only an estimate and could therefore at best be used in risk assessment in diabetes. In contrast to the positive findings by Hattori et al. (1998) and Wagner et al. (2002), Furuya et al. (2000), using gradient-gel electrophoresis did not observe a good agreement between the two methods. The equation used in this study to evaluate measured versus calculated sdLDL-C, was originally proposed by Srisawasdi et al. (2011) for the calculation of sdLDL-C. The formula, ($sdLDL\text{-}C_{mmol/L}$ = 0.580 (non–HDL-C) + 0.407 (dLDL-C) – 0.719 (cLDL-C) – 0.312) was optimized in a Thai population and showed that the formula gave reliable values over a wide range of sdLDL values (7 - 187 mg/dL or 0.18 - 4.84 mmol/L) and furthermore was not affected by either gender or age. However, the need for extensive evaluation of the equation across

different ethnic and specific disease groups was underlined in a recent study that demonstrated the inadequate performance of this formula in Korean subjects with MetS and healthy controls (Cho et al., 2012). In our study, we observed a good agreement between Srisawasdi et al formula and measured sdLDL. However our data did point to a possibility that the two methods sometimes identified different individuals based on the varying interquartile ranges.

sdLDL has been shown to increase with a decrease in HDL-C and an increase in triglycerides (Dobiasova et al., 2004), and these markers have been used in combination to evaluate increases in sdLDL. Maruyama et al. (2003) used the triglycerides/HDL-C ratio to assess sdLDL presence in healthy Japanese subjects not on any medication. The authors suggested the use of this formula to evaluate therapy outcomes for the prevention of sdLDL formation. Mohan et al. (2005) used receiver operating characteristic (ROC) curve analysis (for highest sensitivity and specificity) to show that a triglycerides/HDL-C ratio of 3 was optimal for the detection of elevated sdLDL and that this ratio could be used as a possible surrogate marker for sdLDL in CAD and diabetes in an Asian Indian population. In this study, although triglycerides showed a significant increase across sdLDL quartiles, HDL-C was similar in all quartiles. On the other hand, total cholesterol increased with increasing sdLDL-C and this was driven mainly by the LDL-C and non HDL-C confirming the combined CVD risk associated with both LDL and sdLDL.

4.3 CVD risk in this population

Overweight and obesity have been strongly linked to a high prevalence of T2DM, leading to cardiometabolic complications which are the main cause of death in patients with T2DM (Wilding, 2014). Results from this study showed that the mean (SD) for BMI in males was higher than the cutoff for overweight and for females this was increased to a higher value than the cutoff for obesity, categorized according to the WHO Global Database on Body Mass Index (2004). These results were also shown for both waist and hip circumference, which were significantly increased in females as compared to males. Waist circumference has been reported as a useful surrogate marker for abdominal fat and has been associated with cardiometabolic diseases (Klein et al., 2007). Furthermore, results showed that the 2-hour glucose levels were increased in females as compared to males, confirming reports on gender differences in glucose intolerance reported by others (Janghorbani & Amini, 2008; Sicree et al., 2008) and possibly an overweight/obesity association with T2DM (Wilding, 2014). In this regard, our findings showed an increase in both BMI and waist circumference in subjects with known diabetes as compared to normotolerant subjects, supporting the findings of Wilding (2014) on the relationship between overweight and obesity with a high prevalence of T2DM.

There was no difference in blood pressure between males and females, but the systolic blood pressure (mmHg) was significantly increased in known DM as compared to normotolerant subjects, but not in screen detected DM as compared to normotolerent subjects, suggesting

that an increase in blood pressure in DM could be time related, and possibly a contributor to high blood pressure. In this regard, Laakso (2010) reported that chronic hyperglycemia contributes to heart and blood vessel diseased states and that subjects with long-lasting T2DM are at high risk of CHD and stroke. Raised levels of free fatty acids in obese people could be a major contributor to hyperinsulinemia (Shanik et al., 2008), which is well associated with glucose intolerance, causing obese hypertension and dyslipidemia which include increased sdLDL levels (Modan et al., 1985).

Of the lipids tested in this study, only HDL-C showed a significant difference between males and females, but the means for these values were similar to the reference range for normal HDL-C values in males and females, mean (SD) 1.1 (0.3) and 1.3 (0.3) respectively. However, HDL-C is known to be increased in females (Davis et al., 1996; Habib et al., 2005) and has been reported to show antioxidative activity (Kontush et al., 2003) which reduces the risk of CHD (Toth, 2005). We also did not find any differences in the lipid profile among the glycemic sub groups, except the triglycerides (mmol/L) were significantly increased in both screen detected and known DM as compared to the normotolerant group. Banu et al. (2014), also showed an association between triglycerides and DM, but also between total cholesterol and DM, but not between HDL-C and LDL-C and DM status. Although results from this study only showed an increase in triglycerides in subjects with DM, elevated triglyceride levels are known to be central to an abnormal lipid profile and have been associated with insulin resistance and T2DM (Ginsberg et al., 2005; Wierzbicki, 2006). Rising plasma triglyceride levels lead to the formation of hepatic triglyceride -enriched large circulating VLDL, resulting in an increased generation of sdLDL (Avramoglu et al., 2006; Meshkani & Adeli, 2009).

4.4 Association of sdLDL and CVD risk in this population

Results from a previous study (Matsha et al., 2012) showed a high risk for CVD in the mixed ancestry population of South Africa, as tested for in the Bellville South community. The 30-year CVD interactive risk calculator was used to predict CVD risk in subjects without a history of CVD. The CVD risk was reported to be highest in subjects with DM, but a high CVD risk factor (> 20%) was also reported in normoglycemic subjects younger than 35 years. Significant risk factors for CVD in this population included LDL-C, triglycerides and cholesterol. Other studies have shown that sdLDL-C should be evaluated together with LDL-C as a risk factor for the assessment for CAD in T2DM (Hirayama & Miida, 2012; Huang et al., 2014). Indeed, we showed an increasing trend in MetS and its components with increasing sdLDL quartiles, which further buttress the value of sdLDL in determining CVD risk. Similarly, Hoogeveen et al. (2014) reported that they found sdLDL to be a better predictor of risk assessment for incident CHD even in subjects at low cardiovascular risk as measured by LDL-C. These results suggested that sdLDL should be included in the CVD risk factor profile investigated in the current study population.

In the present study, we therefore evaluated the association of sdLDL with known cardiometabolic risk factors, such as the glycemic and lipid profiles, as both hyperglycemia and dyslipidemia are known cardiometabolic risk factors (Klein et al., 2007), as well as LDL-C, triglycerides and cholesterol specifically as reported by Matsha et al. (2012) for CVD risk in the mixed ancestry population.

4.4.1 Hyperglycemia

We found that both fasting and 2 hour glucose (mmol/L) as well as the 2-hour insulin (IU/l) values were highly significantly increased across the sdLDL percentiles, although the hyperglycemic subgroups as such only showed a trend towards an increased number of both screen detected subjects with DM and known DM in the 4th quartile, with a simultaneous decrease in the number of subjects in the normotolerant group, possibly not significantly so, because of rather low numbers in each sub-group. However, these results showed a marked increase of both glucose and insulin mean values in the higher sdLDL percentiles and suggested a role for sdLDL in the evaluation of CVD risk assessment, as both DM (Betteridge, 2004) and the presence of sdLDL have been reported associated with CVD and MetS (Rizzo & Berneis, 2005). Currently, the measurement of sdLDL has been advanced as an evaluator of atherogenic risk and has been recognised as a cardiovascular risk factor by the (NCEP) (Srisawasdi et al., 2011). These findings may however need further testing as results from the EPIC-Norfolk study showed that adjustment for elevated triglyceride levels and reduced HDL-C levels, reduced the significance of sdLDL in evaluating the risk of CVD (Arsenault et al., 2007). It is of importance to evaluate the role for DM as a risk factor in the development of other diseased states such as CVD as the incidence of diabetes has been reported to be very high in the mixed ancestry population of South Africa (Erasmus et al., 2012).

4.4.2 Dyslipidemia

Similarly, results across the sdLDL percentiles showed highly significant increases in all lipids measured except HDL-C, which as can be expected, decreased over the sdLDL percentiles, but not significantly so. These findings showed that sdLDL was closely associated with the lipid profile and importantly also with the triglycerides (mmol/L) which were shown to be highly significantly increased over the sdLDL percentiles. sdLDL has been shown to increase with an increase in triglycerides (Dobiasova et al., 2004) and elevated triglyceride levels and the presence of sdLDL have been reported in subjects with CVD (Rizzo & Berneis, 2005). These findings further confirm previous reports which have suggested that both sdLDL-C and LDL-C should be evaluated together in CVD risk assessment (Hoogeveen et al., 2014).

Dyslipidaemia, including elevated sdLDL levels have been reported to be associated with obesity (Nikolic et al., 2013) and these findings were confirmed in the current study. We found a significant increase in waist circumference and a near significant increase in BMI (kg/m^2) over the sdLDL percentiles. Waist circumference as a measure of body fat distribution has

been associated with DM and CVD (Klein et al., 2007; Dudina et al., 2011) as well as cardiometabolic complications which are among the main cause of death in patients with T2DM (Wilding, 2014).

4.4.3 Metabolic Syndrome

Metabolic syndrome (MetS), a metabolic disorder, is similarly obesity-related and has also been reported to be associated with dyslipidaemia (Nikolic et al., 2013) and CVD (Kang et al., 2012). sdLDL, similarly reported as a CVD risk factor (Nikolic et al.. 2013), and more so, possibly independently of other risk factors such as plasma lipids (Nikolic et al.. 2013) has also been reported to be increased in MetS (Gazi et al., 2006). In this study, we have found a highly significant increase in the percentage of subjects with MetS as measured over the sdLDL percentiles, with 31.7% in the 1st quartile against 74.6% in the 4th quartile. The components that were used to evaluate MetS presence are blood pressure, waist circumference, HDL-C, triglycerides and fasting blood glucose. As discussed above, waist circumference, fasting blood glucose and triglycerides were all increased over the sdLDL percentiles and HDL non-significantly decreased. Similarly, the systolic blood pressure (mmHg) was significantly increased and diastolic blood pressure (mmHg) non-significantly over the sdLDL percentiles. In this regard, Kang et al. (2012) reported systolic blood pressure to be more closely associated with CVD than the other measures of MetS, with hypertension reported as a known risk factor for MetS (Kirkman et al., 2012)**.** Our observations therefore confirm previous reports on the association between sdLDL and MetS, also with regards to the increase in systolic blood pressure with an increase in sdLDL as a measure of both hypertension and MetS. In this regard, an increase in sdLDL has been reported with CVD risk in association with MetS (Kirkman et al., 2012). Furthermore, age, a traditional risk factor for CVD (Shen et al., 2015) also showed a significant increase across the sdLDL percentiles in this study, although this increase was not highly significant, with the mean age in the 3rd and 4th quartile basically the same (57 years). In this regard, sdLDL has been reported to increase with age (Shen et al., 2015) and our results may indicate an older study population in general

4.5 Limitations of this study

Although the study sample number was sufficient for reliable statistical analysis, the number of males in the study population was rather low. The age of the study population was also high, mean (SD) 49 (17) years, which could have had an effect on the study results, as several of the parameters tested are known to increase with age. Similarly, the majority of our study population had high BMIs which may have influenced the results of sdLDL levels since sdLDL-C correlated positively with the BMI in our study population. A further limitation to the study could possibly be the storage of serum samples before use, as sample recovery rate may have been less than optimal. However, our samples were stored at -80°C and good stability of samples stored at -70°C has been reported by Lee et al. (2014).

4.6 Recommendation

The use of equations to estimate sdLDL levels is a viable alternative that could provide a cost-effective method to preselect patients at risk for CVD. The Srisawasdi et al. (2011) equation showed a moderate to good agreement between the two methods tested, however, the need for extensive evaluation across different ethnic and specific groups with abnormal lipoprotein metabolism remains, before it can be widely recommended.

4.7 Conclusion

The study showed a moderate to good performance for the recent equation proposed by Srisawasdi et al. (2011) for the estimation of sdLDL particles from routine lipid measures as tested for in mixed ancestry South Africans. Furthermore, our data confirmed the association between sdLDL particles and other established markers for CVD risk, which highlighted the importance of the use of an easily available method for the determination of sdLDL in the current population. In this regard, sdLDL has been reported as a CVD risk factor, independently of risk factors such as other plasma lipids (Nikolic et al., 2013).

REFERENCES

Akosah, K.O., Schaper, A., Cogbill, C. & Schoenfeld, P. 2003. Preventing myocardial infarction in the young adult in the first place: How do the national cholesterol education panel III guidelines perform? *Journal of the American College of Cardiology*, 41(9): 1475–1479.

Alabakovska, S.B., Todorova, B.B., Labudovic, D.D. & Tosheska, K.N. 2002. Gradient gel electrophoretic separation of LDL and HDL subclasses on BioRad Mini Protean II and size phenotyping in healthy Macedonians. *Clinica Chimica Acta*, 317(1): 119–123.

Alabakovska, S.B., Labudovic, D.D., Tosheska, K.N., Spiroski, M.Z. & Todorova, B.B. 2008. Low density lipoprotein subclass distribution in children with diabetes mellitus. *Bratislavské lekárske listy*, 109(4): 155.

Alberti, K., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., Fruchart, J.-C., James, W.P.T., Loria, C.M. & Smith, S.C. 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(16): 1640–1645.

Alcolado, J.C., Clark, P.M., Rees, A., Hales, C.N., Yudkin, J. & Coppack, S. 1994.Insulin resistance and impaired glucose tolerance. *The Lancet*, 344(8932): 1293–1295.

Alsheikh-Ali, A.A., Lin, J., Abourjaily, P., Ahearn, D., Kuvin, J.T. & Karas, R.H. 2007. Prevalence of Low High-Density Lipoprotein Cholesterol in Patients with Documented Coronary Heart Disease or Risk Equivalent and Controlled Low-Density Lipoprotein Cholesterol. *American Journal of Cardiology*, 100(10): 1499–1501.

Annema, W. & Tietge, U.J. 2012. Regulation of reverse cholesterol transport - a comprehensive appraisal of available animal studies. *Nutrition & Metabolism*, 9(1): 1–18.

Arora, A., Khan, Q.A., Arora, V., Setia, N. & Khan, B.V. 2013. Growing Cardiovascular and Metabolic Diseases in the Developing Countries: Is there a Role for Small Dense LDL Particles as an Inclusion Criteria for Individuals at Risk for the Metabolic Syndrome. Journal of Community Medicine & Health Education, 3(243): 2161–711.

Arsenault, B.J., Lemieux, I., Després, J.P., Wareham, N.J., Luben, R., Kastelein, J.J.P., Khaw, K.T. & Boekholdt, S.M. 2007. Cholesterol levels in small LDL particles predict the risk of coronary heart disease in the EPIC-Norfolk prospective population study. *European Heart Journal*, 28(22): 2770–2777.

Austin, M.A., Breslow, J.L., Hennekens, C.H., Buring, J.E., Willett, W.C. & Krauss, R.M. 1988. Low-density lipoprotein subclass patterns and risk of myocardial infarction. Journal of Community Medicine & Health Education, 260(13): 1917–1921.

Austin, M.A. 2000. Triglyceride, small, dense low-density lipoprotein, and the atherogenic lipoprotein phenotype. *Current Atherosclerosis Reports*, 2(3): 200–207.

Avramoglu, R.K., Basciano, H. & Adeli, K. 2006. Lipid and lipoprotein dysregulation in insulin resistant states. *Clinica Chimica Acta*, 368(1): 1–19.

Banu, S., Jabir, N.R., Manjunath, N.C., Firoz, C.K., Kamal, M.A., Khan, M.S. & Tabrez, S. 2014. Comparative study of non-high density lipoproteins cholesterol level and lipid profile in pre-diabetic and diabetic patients. *CNS & Neurological Disorders Drug Targets*, 13(3): 402–407.

Barr, E.L., Zimmet, P.Z., Welborn, T.A., Jolley, D., Magliano, D.J., Dunstan, D.W., Cameron, A.J., Dwyer, T., Taylor, H.R., Tonkin, A.M. & others. 2007. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance The Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation*, 116(2): 151–157.

Berneis, K.K. & Krauss, R.M. 2002. Metabolic origins and clinical significance of LDL heterogeneity. *Journal of Lipid Research*, 43(9): 1363–1379.

Betteridge, D.J. 2004. The interplay of cardiovascular risk factors in the metabolic syndrome and type 2 diabetes. *European Heart Journal Supplements*, 6(suppl G): G3–G7.

Califf, R.M., Boolell, M., Haffner, S.M., Bethel, M.A., McMurray, J., Duggal, A., Holman, R.R., Group, N.S. & others. 2008. Prevention of diabetes and cardiovascular disease in patients with impaired glucose tolerance: rationale and design of the Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research Trial. *American heart journal,* 156(4): 623–632.

Campos, H., Genest, J.J., Blijlevens, E., McNamara, J.R., Jenner, J.L., Ordovas, J.M., Wilson, P.W. & Schaefer, E.J. 1992. Low density lipoprotein particle size and coronary artery disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 12(2): 187–195.

Carlson, L. 2011. *Comprehensive lipid testing and management*. Springer Science & Business Media.

Chatterjea, M.N. & Shinde, R. 2012. *Textbook of Medical Biochemistry*. JP Medical Ltd.

Chen, G.C., Liu, W., Duchateau, P., Allaart, J., Hamilton, R.L., Mendel, C.M., Lau, K., Hardman, D.A., Frost, P.H. & Malloy, M.J. 1994. Conformational differences in human apolipoprotein B-100 among subspecies of low density lipoproteins (LDL). Association of altered proteolytic accessibility with decreased receptor binding of LDL subspecies from hypertriglyceridemic subjects. *Journal of Biological Chemistry*, 269(46): 29121–29128.

Cho, Y., Kim, Y., Kim, J.H., Jee, S.H. & Han, K. 2012. The Plasma Small Dense LDL-Cholesterol Calculation Formula Proposed by Srisawasdi et al Is Not Applicable to Koreans Who Are Healthy or Have Metabolic Syndrome. *American Journal of Clinical Pathology*, 138(5): 754–756.

Chrysohoou, C., Panagiotakos, D.B., Pitsavos, C., Skoumas, I., Papademetriou, L., Economou, M. & Stefanadis, C. 2007. The implication of obesity on total antioxidant capacity in apparently healthy men and women: the ATTICA study. *Nutrition, Metabolism, and Cardiovascular Diseases: NMCD*, 17(8): 590–597.

Codario, R.A. 2010. *Type 2 Diabetes, Pre-Diabetes, and the Metabolic Syndrome*. Springer Science & Business Media.

Cohen, A., Myerscough, M.R. & Thompson, R.S. 2014. Athero-protective Effects of High Density Lipoproteins (HDL): An ODE Model of the Early Stages of Atherosclerosis. *Bulletin of Mathematical Biology*, 76(5): 1117–1142.

Cohen, B. & Hasselbring, B. 2007. *Coronary Heart Disease: A Guide to Diagnosis and Treatment*. Addicus Books.

Coresh, J., Kwiterovich, P.O., Smith, H.H. & Bachorik, P.S. 1993. Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. *Journal of Lipid Research*, 34(10): 1687–1697.

Coutinho, M., Gerstein, H.C., Wang, Y. & Yusuf, S. 1999. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care*, 22(2): 233–240.

Covic, A., Kanbay, M. & Lerma, E. V. 2014. *Dyslipidemias in Kidney Disease*. Springer Science & Business Media.

Crook, M.A. 2012. *Clinical Biochemistry and Metabolic Medicine Eighth Edition*. CRC Press.

Cuchel, M. & Rader, D.J. 2006. Macrophage Reverse Cholesterol Transport Key to the Regression of Atherosclerosis? *Circulation*, 113(21): 2548–2555.

Daniels, S.R., Pratt, C.A. & Hayman, L.L. 2011. Reduction of risk for cardiovascular disease in children and adolescents. *Circulation*, 124(15): 1673–1686.

Davidson, M.B., Landsman, P.B. & Alexander, C.M. 2003. Lowering the criterion for impaired fasting glucose will not provide clinical benefit. *Diabetes Care*, 26(12): 3329–3330.

Davidson, M., Toth, P.P. & Maki, K.C. 2007. *Therapeutic Lipidology*. Springer Science & Business Media.

Davis, C.E., Williams, D.H., Oganov, R.G., Tao, S.C., Rywik, S.L., Stein, Y. & Little, J.A. 1996. Sex Difference in High Density Lipoprotein Cholesterol in Six Countries. *American Journal of Epidemiology*, 143(11): 1100–1106.

De Cordova, C.M.M. & de Cordova, M.M. 2013. A new accurate, simple formula for LDL-cholesterol estimation based on directly measured blood lipids from a large cohort. *Annals of Clinical Biochemistry*, 50(1): 13–19.

Dobiášová, M. 2004. Atherogenic index of plasma [log (triglycerides/HDL-cholesterol)]: theoretical and practical implications. *Clinical Chemistry*, 50(7): 1113–1115.

Dudina, A., Cooney, M.T., Bacquer, D.D., Backer, G.D., Ducimetière, P., Jousilahti, P., Keil, U., Menotti, A., Njølstad, I., Oganov, R., Sans, S., Thomsen, T., Tverdal, A., Wedel, H., Whincup, P., Wilhelmsen, L., Conroy, R., Fitzgerald, A., Graham, I. & SCORE investigators. 2011. Relationships between body mass index, cardiovascular mortality, and risk factors: a report from the SCORE investigators. *European Journal of Cardiovascular Prevention and Rehabilitation: Official Journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology*, 18(5): 731–742.

Ensign, W., Hill, N. & Heward, C.B. 2006. Disparate LDL phenotypic classification among 4 different methods assessing LDL particle characteristics. *Clinical chemistry*, 52(9): 1722–1727.

Erasmus, R.T., Soita, D.J., Hassan, M.S., Blanco-Blanco, E., Vergotine, Z., Kengne, A.P. & Matsha, T.E. 2012. High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: Baseline data of a study in Bellville, Cape Town. *SAMJ: South African Medical Journal*, 102(11): 841–844.Ford, E.S., Ajani, U.A., Croft, J.B., Critchley, J.A., Labarthe, D.R., Kottke, T.E., Giles, W.H. & Capewell, S. 2007. Explaining the Decrease in U.S. Deaths from Coronary Disease, 1980–2000. *New England Journal of Medicine*, 356(23): 2388–2398.

Ford, E.S., Zhao, G. & Li, C. 2010. Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. *Journal of the American College of Cardiology*, 55(13): 1310–1317.

Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. 1972. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clinical Chemistry*, 18(6): 499–502.

Frink, R.J. 2002. *Inflammatory Atherosclerosis*. Heart Research Foundation.

Furuya, D., Yagihashi, A., Nasu, S., Endoh, T., Nakamura, T., Kaneko, R., Kamagata, C., Kobayashi, D. & Watanabe, N. 2000. LDL particle size by gradient-gel electrophoresis cannot be estimated by LDL-cholesterol/apolipoprotein B ratios. *Clinical Chemistry*, 46(8): 1202–1203.

Galeano, N.F., Al-Haideri, M., Keyserman, F., Rumsey, S.C. & Deckelbaum, R.J. 1998. Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. *Journal of Lipid Research*, 39(6): 1263–1273.

Gazi, I., Tsimihodimos, V., Filippatos, T., Bairaktari, E., Tselepis, A.D. & Elisaf, M. 2006. Concentration and relative distribution of low-density lipoprotein subfractions in patients with metabolic syndrome defined according to the National Cholesterol Education Program criteria. *Metabolism*, 55(7): 885–891.

Gentile, M., Panico, S., Mattiello, A., Ubaldi, S., Iannuzzo, G., De Michele, M., Iannuzzi, A. & Rubba, P. 2013. Association between small dense LDL and early atherosclerosis in a sample of menopausal women. *Clinica Chimica Acta*, 426: 1–5.

Ginsberg, H.N., Zhang, Y.L. & Hernandez-Ono, A. 2005. Regulation of plasma triglycerides in insulin resistance and diabetes. *Archives of Medical Research*, 36(3): 232–240.

Ginsburg, G.S. & Willard, H.F. 2012. *Genomic and Personalized Medicine*. 2nd ed. Academic Press.

Gleissner, C.A., Leitinger, N. & Ley, K. 2007. Effects of Native and Modified Low-Density Lipoproteins on Monocyte Recruitment in Atherosclerosis. *Hypertension*, 50(2): 276–283.

Griffin, B.A., Caslake, M.J., Yip, B., Tait, G.W., Packard, C.J. & Shepherd, J. 1990. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. *Atherosclerosis*, 83(1): 59–67.

Gui, T., Shimokado, A., Sun, Y., Akasaka, T. & Muragaki, Y. 2012. Diverse roles of macrophages in atherosclerosis: From inflammatory biology to biomarker discovery. *Mediators of Inflammation*, 2012: 693083.

Habib, S.S., Aslam, M. & Hameed, W. 2005. Gender differences in Lipids and Lipoprotein (A) profiles in healthy individuals and patients with type 2 Diabetes Mellitus. *Pak J Physiol*, 1: 1–2.

Harrington, J.R. 2000. The Role of MCP-1 in Atherosclerosis. *Stem Cells*, 18(1): 65–66.

Hattori, Y., Suzuki, M., Tsushima, M., Yoshida, M., Tokunaga, Y., Wang, Y., Zhao, D., Takeuchi, M., Hara, Y., Ryomoto, K.I., Ikebuchi, M., Kishioka, H., Mannami, T., Baba, S. & Harano, Y. 1998. Development of approximate formula for LDL-chol, LDL-apo B and LDL-chol/LDL-apo B as indices of hyperapobetalipoproteinemia and small dense LDL. *Atherosclerosis*, 138(2): 289–299.

Hirano, T., Ito, Y., Koba, S., Toyoda, M., Ikejiri, A., Saegusa, H., Yamazaki, J. & Yoshino, G. 2004. Clinical significance of small dense low-density lipoprotein cholesterol levels determined by the simple precipitation method. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 24(3): 558–563.

Hirayama, S. & Miida, T. 2012. Small dense LDL: an emerging risk factor for cardiovascular disease. *Clinica Chimica Acta*, 414: 215–224.

Hoefner, D.M., Hodel, S.D., O'Brien, J.F., Branum, E.L., Sun, D., Meissner, I. & McConnell, J.P. 2001. Development of a rapid, quantitative method for LDL subfractionation with use of the Quantimetrix Lipoprint LDL System. *Clinical Chemistry*, 47(2): 266–274.

Hoogeveen, R.C., Gaubatz, J.W., Sun, W., Dodge, R.C., Crosby, J.R., Jiang, J., Couper, D., Virani, S.S., Kathiresan, S., Boerwinkle, E. & others. 2014. Small Dense Low-Density Lipoprotein-Cholesterol Concentrations Predict Risk for Coronary Heart Disease The Atherosclerosis Risk in Communities (ARIC) Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 34(5): 1069–1077.

Huang, Y.C., Chang, P.Y., Hwang, J.S. & Ning, H.C. 2014. Association of small dense lowdensity lipoprotein cholesterol in type 2 diabetics with coronary artery disease. *Biomedical Journal*, 37(6): 375–379.

Ito, Y., Fujimura, M., Ohta, M. & Hirano, T. 2011. Development of a homogeneous assay for measurement of small dense LDL cholesterol. *Clinical Chemistry*, 57(1): 57–65.

Janghorbani, M. & Amini, M. 2008. Effects of Gender and Height on the Oral Glucose Tolerance Test: The Isfahan Diabetes Prevention Study. *The Review of Diabetic Studies : RDS*, 5(3): 163–170.

Kanaya, A.M., Herrington, D., Vittinghoff, E., Lin, F., Bittner, V., Cauley, J.A., Hulley, S. & Barrett-Connor, E. 2005. Impaired fasting glucose and cardiovascular outcomes in postmenopausal women with coronary artery disease. *Annals of Internal Medicine*, 142(10): 813–820.

Kang, G.D., Guo, L., Guo, Z.R., Hu, X.S., Wu, M. & Yang, H.T. 2012. Continuous metabolic syndrome risk score for predicting cardiovascular disease in the Chinese population. *Asia Pacific Journal of Clinical Nutrition*, 21(1): 88–96.

Karalis, D.G. 2014. Achieving optimal lipid goals in the metabolic syndrome: A global health problem. *Atherosclerosis*, 237(1): 191–193.

Kathiresan, S., Otvos, J.D., Sullivan, L.M., Keyes, M.J., Schaefer, E.J., Wilson, P.W., D'Agostino, R.B., Vasan, R.S. & Robins, S.J. 2006. Increased small low-density lipoprotein particle number a prominent feature of the metabolic syndrome in the Framingham Heart Study. *Circulation*, 113(1): 20–29.

Keevil, J.G., Cullen, M.W., Gangnon, R., McBride, P.E. & Stein, J.H. 2007. Implications of Cardiac Risk and Low-Density Lipoprotein Cholesterol Distributions in the United States for the Diagnosis and Treatment of Dyslipidemia Data From National Health and Nutrition Examination Survey 1999 to 2002. *Circulation*, 115(11): 1363–1370.

Khan, M.S., Khan, M.K.A., Siddiqui, M.H. & Arif, J.M. 2011. An in vivo and in silico approach to elucidate the tocotrienol-mediated fortification against infection and inflammation induced alterations in antioxidant defense system. European Review for Medical and Pharmacological Sciences, 15(8): 916–930.

Khan, M.S. 2012. Analytical Biochemistry Small Dense LDL : New Marker for Cardiovascular Risk Assessment and its Therapeutic Inflection. *Biochemistry & Analytical Biochemistry*, 1(6): 1–4.

Kirkman, M.S., Briscoe, V.J., Clark, N., Florez, H., Haas, L.B., Halter, J.B., Huang, E.S., Korytkowski, M.T., Munshi, M.N., Odegard, P.S., Pratley, R.E. & Swift, C.S. 2012. Diabetes in Older Adults. *Diabetes Care*, 35(12): 2650–2664.

Klein, S., Allison, D.B., Heymsfield, S.B., Kelley, D.E., Leibel, R.L., Nonas, C. & Kahn, R. 2007. Waist circumference and cardiometabolic risk: A consensus statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, the

Obesity Society; the American Society for Nutrition; and the American Diabetes Associat. *Diabetes Care*, 30(6): 1647–1652.

Knopp, R.H., d'Emden, M., Smilde, J.G. & Pocock, S.J. 2006. Efficacy and Safety of Atorvastatin in the Prevention of Cardiovascular End Points in Subjects With Type 2 Diabetes the Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in Non-Insulin-Dependent Diabetes Mellitus (ASPEN). *Diabetes Care*, 29(7): 1478–1485.

Koba, S., Yokota, Y., Hirano, T., Ito, Y., Ban, Y., Tsunoda, F., Sato, T., Shoji, M., Suzuki, H., Geshi, E., Kobayashi, Y. & Katagiri, T. 2008. Small LDL-cholesterol is superior to LDL-cholesterol for determining severe coronary atherosclerosis. *Journal of Atherosclerosis and Thrombosis*, 15(5): 250–260.

Kontush, A., Chantepie, S. & Chapman, M.J. 2003. Small, Dense HDL Particles Exert Potent Protection of Atherogenic LDL Against Oxidative Stress. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 23(10): 1881–1888.

Kuller, L., Arnold, A., Tracy, R., Otvos, J., Burke, G., Psaty, B., Siscovick, D., Freedman, D.S. & Kronmal, R. 2002. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the cardiovascular health study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 22(7): 1175–1180.

Kwan, B.C.H., Kronenberg, F., Beddhu, S. & Cheung, A.K. 2007. Lipoprotein Metabolism and Lipid Management in Chronic Kidney Disease. *Journal of the American Society of Nephrology*, 18(4): 1246–1261.

Laakso, M. 2010. Cardiovascular Disease in Type 2 Diabetes From Population to Man to Mechanisms The Kelly West Award Lecture 2008. *Diabetes Care*, 33(2): 442–449.

Lee, M.N., Kim, J.R., Huh, H.J., Lee, S.Y., Kang, E.S. & Park, H..D. 2014. Evaluation of the Analytical Performance of a Direct Quantitative Assay of Small Dense LDL. *The Journal of Laboratory Medicine and Quality Assurance,* 36(2): 84–91.

Levitzky, Y.S., Pencina, M.J., D'Agostino, R.B., Meigs, J.B., Murabito, J.M., Vasan, R.S. & Fox, C.S. 2008. Impact of impaired fasting glucose on cardiovascular disease: the Framingham Heart Study. *Journal of the American College of Cardiology*, 51(3): 264–270.

Libby, P., Ridker, P.M. & Maseri, A. 2002. Inflammation and atherosclerosis. *Circulation*, 105(9): 1135–1143.

Lin, J.D., Wan, H.-L., Li, J.-C., Wu, C.-Z., Kuo, S.-W., Hsieh, C.-H., Lian, W.-C., Lee, C.-H., Kao, M.-T. & Pei, D. 2007. Impaired glucose tolerance and impaired fasting glucose share similar underlying pathophysiologies. *The Tohoku journal of experimental medicine*, 212(4): 349–357.

Little, M.P., Gola, A. & Tzoulaki, I. 2009. A model of cardiovascular disease giving a plausible mechanism for the effect of fractionated low-dose ionizing radiation exposure. *PLoS Computational Biology*, 5(10): 1000539.

Mackay, J. & Mensah, G.A. 2004. Global burden of coronary heart disease. *The Atlas of Heart Disease and Stroke*: 1–12.

Maeda, S., Nakanishi, S., Yoneda, M., Awaya, T., Yamane, K., Hirano, T. & Kohno, N. 2012. Associations between small dense LDL, HDL subfractions (HDL2, HDL3) and risk of atherosclerosis in Japanese-Americans. *Journal of Atherosclerosis and Thrombosis*, 19(5): 444–452.

Marchetti, E., Monaco, A., Procaccini, L., Mummolo, S., Gatto, R., Tetè, S., Baldini, A., Tecco, S. & Marzo, G. 2012. Periodontal disease: the influence of metabolic syndrome. *Nutrition & Metabolism*, 9(1): 1.

Marcovina, S. & Packard, C.J. 2006. Measurement and meaning of apolipoprotein AI and apolipoprotein B plasma levels. *Journal of Internal Medicine*, 259(5): 437–446.

Maruyama, C., Imamura, K. & Teramoto, T. 2003. Assessment of LDL particle size by triglyceride/HDL-cholesterol ratio in non-diabetic, healthy subjects without prominent hyperlipidemia. *Journal of Atherosclerosis and Thrombosis*, 10(3): 186–191.

Matsha, T.E., Hassan, M.S., Kidd, M. & Erasmus, R.T. 2012. The 30-year cardiovascular risk profile of South Africans with diagnosed diabetes, undiagnosed diabetes, pre-diabetes or normoglycaemia: the Bellville, South Africa pilot study. *Cardiovascular Journal of Africa*, 23(1): 5–11.

Matsha, T.E., Hassan, M.S., Hon, G.M., Soita, D.J. Kengne, A.P.,. Erasmus, R.T. 2013. Derivation and validation of a waist circumference optimal cutoff for diagnosing metabolic syndrome in a South African mixed ancestry population. *International journal of cardiology*, *168*(3), 2954-5.

Meshkani, R. & Adeli, K. 2009. Hepatic insulin resistance, metabolic syndrome and cardiovascular disease. *Clinical Biochemistry*, 42(13): 1331–1346.

Miyashita, M., Okada, T., Kuromori, Y. & Harada, K. 2006. LDL particle size, fat distribution and insulin resistance in obese children. *European Journal of Clinical Nutrition*, 60(3): 416–420.

Modan, M., Halkin, H., Almog, S., Lusky, A., Eshkol, A., Shefi, M., Shitrit, A. & Fuchs, Z. 1985. Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. *Journal of Clinical Investigation*, 75(3): 809–817.

Mohan, V., Deepa, R., Velmurugan, K. & Gokulakrishnan, K. 2005. Association of small dense LDL with coronary artery disease and diabetes in urban Asian Indians - the Chennai Urban Rural Epidemiology Study (CURES-8). *The Journal of the Association of Physicians of India*, 53(2): 95–100.

Mora, S., Rifai, N., Buring, J.E. & Ridker, P.M. 2008. Fasting Compared With Nonfasting Lipids and Apolipoproteins for Predicting Incident Cardiovascular Events. *Circulation*, 118(10): 993–1001.

Moreno, P.R. 2010. Vulnerable Plaque: Definition, Diagnosis, and Treatment. *Cardiology Clinics*, 28(1): 1–30.

Murray, C.J. & Lopez, A.D. 1997. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *The Lancet*, 349(9063): 1436–1442.

National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines (NACB LMPG). 2009. Emerging Biomarkers for Primary Prevention of Cardiovascular Disease. Committee Members: Myers, G.L., Christenson, R.H., Cushman, M., Ballantyne, C.M., Cooper, G.R., Pfeiffer, C.M., Grundy, S.M., Labarthe, D.R., Levy, D., Rifai, N. & Wilson, P.W.F. *Clinical Chemistry*, 55(2): 378–384.

National Cholesterol Education Program (NCEP). 2002. 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. *Circulation*, 106(25): 3143–3421.

Nikolic, D., Katsiki, N., Montalto, G., Isenovic, E.R., Mikhailidis, D.P. & Rizzo, M. 2013. Lipoprotein subfractions in metabolic syndrome and obesity: clinical significance and therapeutic approaches. *Nutrients*, 5(3): 928–948.

Osterud, B. & Bjorklid, E. 2003. Role of monocytes in atherogenesis. *Physiological Reviews*, 83(4): 1069–1112.

Packard, C.J. 2003. Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein. *Biochemical Society Transactions*, 31(5): 1066–1069.

Packard, R.R.S. & Libby, P. 2008. Inflammation in Atherosclerosis: From Vascular Biology to Biomarker Discovery and Risk Prediction. *Clinical Chemistry*, 54(1): 24–38.

Palazhy, S., Kamath, P. & Vasudevan, D.M. 2014. Estimation of Small, Dense LDL Particles Using Equations Derived From Routine Lipid Parameters as Surrogate Markers. *Biochemistry & Analytical Biochemistry*, 3(1): 1–5.

Reape, T.J. & Groot, P.H.E. 1999. Chemokines and atherosclerosis. *Atherosclerosis*, 147(2): 213–225.

Reaven, G. 2012. Insulin resistance and coronary heart disease in nondiabetic individuals. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 32(8): 1754–1759.

Ricos, C., Alvarez, V., Cava, F., Garcia-Lario, J.V., Hernandez, A., Jimenez, C.V., Minchinela, J., Perich, C., Simon, M. 1999, updated 2014. Current databases on biologic variation: pros, cons and progress. *Scandinavian Journal* of *Clinical* and *Laboratory Investigation*, 59:491-500.

Rifai, N. & Ridker, P.M. 2001. High-Sensitivity C-Reactive Protein: A Novel and Promising Marker of Coronary Heart Disease. *Clinical Chemistry*, 47(3): 403–411.

Rizzo, M. & Berneis, K. 2005. Lipid triad or atherogenic lipoprotein phenotype: a role in cardiovascular prevention? *Journal of Atherosclerosis and Thrombosis*, 12(5): 237–239.

Rizzo, M. & Berneis, K. 2006. Low-density lipoprotein size and cardiovascular risk assessment. *QJM*, 99(1): 1–14.

Rizzo, M., Pernice, V., Frasheri, A., Di Lorenzo, G., Rini, G.B., Spinas, G.A. & Berneis, K. 2009. Small, dense low-density lipoproteins (LDL) are predictors of cardio-and cerebro-vascular events in subjects with the metabolic syndrome. *Clinical Endocrinology*, 70(6): 870–875.

Roheim, P.S. & Asztalos, B.F. 1995. Clinical significance of lipoprotein size and risk for coronary atherosclerosis. *Clinical Chemistry*, 41(1): 147–152.

Rosenson, R.S. 2006. Low high-density lipoprotein cholesterol and cardiovascular disease: risk reduction with statin therapy. *American Heart Journal*, 151(3): 556–563.

Ryu, B. 2000. Low density lipoprotein (LDL), atherosclerosis and antioxidants. *Biotechnology and Bioprocess Engineering*, 5(5): 313–319.

Shanik, M.H., Xu, Y., Škrha, J., Dankner, R., Zick, Y. & Roth, J. 2008. Insulin resistance and hyperinsulinemia is hyperinsulinemia the cart or the horse? *Diabetes Care*, 31(Supplement 2): S262–S268.

Sharma, S.B. & Garg, S. 2012. Small dense LDL: risk factor for coronary artery disease (CAD) and its therapeutic modulation. *Indian Journal of Biochemistry & Biophysics*, 49(2): 77–85.

Shen, H., Xu, L., Lu, J., Hao, T., Ma, C., Yang, H., Lu, Z., Gu, Y., Zhu, T. & Shen, G. 2015. Correlation between small dense low-density lipoprotein cholesterol and carotid artery intima-media thickness in a healthy Chinese population. *Lipids in Health and Disease*, 1(14): 1–6.

Sicree, R.A., Zimmet, P.Z., Dunstan, D.W., Cameron, A.J., Welborn, T.A. & Shaw, J.E. 2008. Differences in height explain gender differences in the response to the oral glucose tolerance test— the AusDiab study. *Diabetic Medicine*, 25(3): 296–302.

Sniderman, A.D., Lamarche, B., Tilley, J., Seccombe, D. & Frohlich, J. 2002. Hypertriglyceridemic hyperapoB in type 2 diabetes. *Diabetes Care*, 25(3): 579–582.

Srisawasdi, P., Chaloeysup, S., Teerajetgul, Y., Pocathikorn, A., Sukasem, C., Vanavanan, S. & Kroll, M.H. 2011. Estimation of plasma small dense LDL cholesterol from classic lipid measures. *American Journal of Clinical Pathology*, 136(1): 20–29.

Stan, S., Levy, E., Delvin, E.E., Hanley, J.A., Lamarche, B., O'Loughlin, J., Paradis, G. & Lambert, M. 2005. Distribution of LDL Particle Size in a Population-Based Sample of Children and Adolescents and Relationship with Other Cardiovascular Risk Factors. *Clinical Chemistry*, 51(7): 1192–1200.

Stats, S.A. 2012. Census 2011, Statistical release (Revised) P0301. 4. *Statistics South Africa, Pretoria*, 3014. http://www. statssa. gov. za/Publications P [15 April 2016].

St-Pierre, A.C., Cantin, B., Dagenais, G.R., Mauriège, P., Bernard, P.M., Després, J.P. & Lamarche, B. 2005. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men 13-year follow-up data from the Québec Cardiovascular Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 25(3): 553–559.

Tabas, I., Williams, K.J. & Borén, J. 2007. Subendothelial Lipoprotein Retention as the Initiating Process in Atherosclerosis Update and Therapeutic Implications. *Circulation*, 116(16): 1832–1844.

Tan, K.C.B., Ai, V.H.G., Chow, W.S., Chau, M.T., Leong, L. & Lam, K.S.L. 1999. Influence of Low Density Lipoprotein (LDL) Subfraction Profile and LDL Oxidation on Endothelium-Dependent and Independent Vasodilation in Patients with Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism*, 84(9): 3212–3216.

Toth, P.P. 2005. The 'Good Cholesterol' High-Density Lipoprotein. *Circulation*, 111(5): e89–e91.

Tribble, D.L., Holl, L.G., Wood, P.D. & Krauss, R.M. 1992. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis*, 93(3): 189–199.

Upadhyay, R.K. 2015. Emerging risk biomarkers in cardiovascular diseases and disorders. *Journal of Lipids*, 2015: 971453.

Vance, J.E. & Vance, D.E. 2008. *Biochemistry of Lipids, Lipoproteins and Membranes*. 5th ed. Elsevier.

Vasudevan, D., Sreekumari, S., Vaidyanathan, K. & Damodaran, K.G. 2011. *Clinical Chemistry Made Easy*®. 1/e. Jaypee Brothers Medical Publishers (P) Ltd.

Vita, J. a. 2005. Polyphenols and cardiovascular disease : effects on endothelial and platelet function. *The American Journal of Clinical Nutrition*, 81(1): 292–297.

Wägner, A.M., Jorba, O., Rigla, M., Alonso, E., Ordonez-Llanos, J. & Pérez, A. 2002. LDL-cholesterol/apolipoprotein B ratio is a good predictor of LDL phenotype B in type 2 diabetes. *Acta Diabetologica*, 39(4): 215–220.

Whiting, D.R., Guariguata, L., Weil, C. & Shaw, J. 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Research and Clinical Practice*, 94(3): 311–321.

Wierzbicki, A.S. 2006. Diabetic dyslipidaemia: the triad. *European Heart Journal Supplements*, 8(Suppl F): F30–F33.

Wilding, J.P.H. 2014. The importance of weight management in type 2 diabetes mellitus. *International Journal of Clinical Practice*, 68(6): 682–691.

Williams, K., Tchernof, A., Hunt, K.J., Wagenknecht, L.E., Haffner, S.M. & Sniderman, A.D. 2008. Diabetes, abdominal adiposity, and atherogenic dyslipoproteinemia in women compared with men. *Diabetes*, 57(12): 3289–3296.

World Health Organization (WHO). 2004. Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet (London, England)*, 363(9403): 157–163.

World Health Organization & International Diabetes Federation (IDF). 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia : report of a WHO/IDF consultation. *Geneva : World Health Organization*. http://www.who.int/iris/handle/10665/43588 [7 May 2016].

World Health Organization (WHO). 2011. *Global status report on noncommunicable diseases 2010*. Geneva: World Health Organization.

World Health Organization (WHO). 2015. Cardiovascular diseases (CVDs) Fact Sheet N°317. http://www.who.int/mediacentre/factsheets/fs317/en/ [9 May 2016].

World Health Organization (WHO). 2016. Global Report on Diabetes. Recommendations for the Diagnostic Criteria for Diabetes and Intermediate Hyperglycaemia. http://www.who.int , WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland

Yeboah, J., Bertoni, A.G., Herrington, D.M., Post, W.S. & Burke, G.L. 2011. Impaired fasting glucose and the risk of incident diabetes mellitus and cardiovascular events in an adult population: MESA (Multi-Ethnic Study of Atherosclerosis). *Journal of the American College of Cardiology*, 58(2): 140–146.

Zeljkovic, A., Vekic, J., Spasojevic-Kalimanovska, V., Jelic-Ivanovic, Z., Bogavac-Stanojevic, N., Gulan, B. & Spasic, S. 2010. LDL and HDL subclasses in acute ischemic stroke: prediction of risk and short-term mortality. *Atherosclerosis*, 210(2): 548–554.

APPENDICES

Appendix 1: Ethics approval document

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

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06 October 2014
CPUT/HW-REC 2014/H08

Faculty of Health and Wellness Sciences – Biomedical Sciences Department

Dear Mr Masoud

YOUR APPLICATION TO THE HW-REC FOR ETHICAL CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC on 02 October 2014 to Mr. Mohamed Abdulsalam Masoud for ethical clearance. This approval is for research activities related to your MTech Biomedical Technology at CPUT.

TITLE: Small dense low density lipoprotein cholesterol in mixed ancestry South Africans: Calculated versus Measured

SUPERVISOR: Prof. T. Matsha
CO-SUPERVISER: Dr. Macharia and Prof. Erasmus

Comment:
Approval will not extend beyond 07 October 2015. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the conditions under which they are authorized to carry out this study and they should be compliant to these conditions. **It is required that the investigator(s) complete an annual progress report that should be submitted to the HW-REC in December of that particular year, for the HW-REC to be kept informed of the progress and of any problems you may encounter.**

Kind Regards

MR. NAVINDHRA NAIDOO
CHAIRPERSON – ETHICS RESEARCH COMMITTEE
FACULTY OF HEALTH AND WELLNESS SCIENCES

Appendix 2: Questionnaire

Title: The Cape Town Diabetes and Cardiovascular Disease Study (VMH)

Principal Investigator: **Prof Tandi Matsha**

Name of Interviewer: **……**

Date of Interview: **……. /……./…….** **Ref No …….**

To the respondent:

Thank you very much for your willingness to participate in the completion of this questionnaire. The information obtained on this questionnaire will provide us with information on all the possible health, family, life style and dietary risk factors within your house hold that might influence the development of diabetes. This is because many health conditions develop slowly over time yet could be prevented if diagnosed early or if pre-determined. This questionnaire therefore aims at getting information which may be used to determine the extent of diabetes and those likely to develop diabetes in the future. The questionnaire should not take long and we hope you find it interesting and enjoyable. All answers provided will be treated as confidential and anonymous.

Note

No special knowledge is needed to fill this questionnaire. Please feel free to ask for clarification if needed.

Section A: General Questions

1. Language

1. What is your home language?

- □ English
- □ Afrikaans
- □ Xhosa
- □ Other: ……..

2. Which language would you prefer to be communicated in?

- □ English
- □ Afrikaans
- □ Xhosa
- □ Other: ……..

2. Personal Questions

1. Gender ___Male ___Female
2. Date of birth? ____/_____/_____
3. What is your relationship status?

- ☐ Married/registered partnership
- ☐ Cohabiting (living together)
- ☐ Unmarried (never married)
- ☐ Divorced or separated
- ☐ Widow/widower

4. Including yourself, how many people are there in your household? |__|__| person(s) .
   This includes children who live with you only some of the time.

Section B: General Health

3. General Health

3.1 In general, would you say your health is:

- ☐ Excellent
- ☐ Very good
- ☐ Good
- ☐ Fair
- ☐ Poor

3.2 In general, would you say you are physically active (that is, gardening, jogging etc)?

- ☐ Yes
- ☐ No

3.3 In general, would you say you have

emotional problems (such as feeling depressed or anxious)

- ☐ Yes
- ☐ No

3.4 During the past 4 weeks, how much did pain keep you from doing your normal activities?

- ☐ Not at all
- ☐ A little bit
- ☐ Quite a lot
  A lot
- ☐ A very great deal

3.5 The following questions are about how you felt and how you were doing during the past 4

weeks. For each question, please choose the answer that best describes how often you felt this way.

a. During the past 4 weeks, how often did you feel calm and contented?

- ☐ Always
- ☐ Usually
- ☐ Often
- ☐ Sometimes
- ☐ Hardly ever
- ☐ Never

b. During the past 4 weeks, how often did you feel very energetic?

- ☐ Always
- ☐ Usually
- ☐ Often
- ☐ Sometimes
- ☐ Hardly ever
- ☐ Never

c. During the past 4 weeks, how often did you feel down and depressed?

- ☐ Always
- ☐ Usually
- ☐ Often
- ☐ Sometimes
- ☐ Hardly ever
- ☐ Never

3.6 During the past 4 weeks, how often did your physical health or emotional problems limit your social activities (such as visiting friends or family)?

- ☐ Always
- ☐ Usually
- ☐ Often
- ☐ Sometimes
- ☐ Hardly ever
- ☐ Never

3.7 Please indicate the status of your eyesight at the present time, using both eyes (with glasses or contact lenses, if you wear them)

- ☐ Excellent
- ☐ Good
- ☐ Fair
- ☐ Poor
- ☐ Very Poor
- ☐ Completely blind

4. Question for women

If you are a woman, please answer the questions below. If you are a man, please go directly to Question 5.

4.1 How old were you when you had your first period (menstruation)?

If you aren't sure, please try to estimate this.

|___|___|

4.2 How old were you the first time your period stopped for a whole year? |___|___|

Do not include times when your period stopped because of pregnancy, breastfeeding, or using birth control.

4.3 Do you currently use contraceptive medication? (Birth control pill)

☐ Yes

☐ No

4.4 Have you given birth to one or more children?

☐ Yes

☐ No- **Go to question 5**

4.5 How many children have you had? |____|____| children

(This includes still born babies)

Section C: Specific Illness and disorders

5. Illness and disorders

Please indicate which of the following illnesses and disorders you have now or that you have had in the past 12 months, and whether or not this was diagnosed by a doctor.

If you don't know or if you have had a certain illness or disorder, please fill in 'No'. Please give an answer for every illness/disorder. [Interviewer: please explain medical terms if necessary]

	No	**Yes, not diagnosed by a doctor**	**Yes, diagnosed by a doctor**
a. stroke, brain hemorrhage, cerebral infarction, or TIA ('transient ischaemic attack': temporary loss of bodily function)	☐	☐	☐
b. heart attack (myocardial infarction)	☐	☐	☐

c. another serious heart condition (for example, heart failure or angina pectoris (severe chest pain))	☐	☐	☐
d. a form of cancer (malignant disorder)	☐	☐	☐
e. migraine or frequent severe headaches	☐	☐	☐
f. severe or chronic fatigue	☐	☐	☐
g. narrowing of the arteries in the belly or legs (artery stenosis)	☐	☐	☐
h. asthma, chronic bronchitis, lung emphysema, or CNSLD (chronic non-specific lung disease) or COPD (chronic obstructive pulmonary disease)	☐	☐	☐
i. serious or persistent intestinal disorders lasting more than 3months	☐	☐	☐
j. chronic inflammation of the joints (inflammatory rheumatism, chronic rheumatism, rheumatoid arthritis)	☐	☐	☐

5.2 Have you had cancer

☐ Yes

☐ No

5.3 What type of cancer _____

_____

_____

6. Medicine and prescription

6.1 Are you currently on medication?

(Do not include birth control and vitamins)

☐ Yes

☐ No

6.2 How many days in the past have you been able to take your prescribed medicine(s)?

- ☐ Not a single day
- ☐ 1 to 2 days
- ☐ 3 to 4 days
- ☐ 5 to 6 days
- ☐ All 7 days

6.3 Now I would like tell me the **names of all medication(s)** you currently taking on a regular basis. I need you to include vitamins and over-the-counter medicine, as well as herbal remedies that you have taken at least once a day over the past two weeks.

1._____	7._____	13._____
2._____	8._____	14._____
3._____	9._____	15._____
4._____	10._____	16._____
5._____	11._____	17._____
6._____	12._____	18._____

6.4 Are you using or have used any prohibited drugs?

- ☐ Tik
- ☐ Marijuana (Dagga)
- ☐ Cocaine
- ☐ Other:_____

☐ Prefer not to answer

7. Blood pressure

7.1 Have you ever been diagnosed with high blood pressure at a hospital or clinic (by a doctor or nurse etc)?

☐ No- **got to Question 8**

☐ Yes

7.2 How old where you when you were diagnosed with high blood pressure?

___ Years

7.3 Do you use medication for your high blood pressure?

☐ No

☐ Yes

7.4 Are you on a special diet (for example low salt) for your blood pressure

☐ No

☐ Yes

8. Cholesterol

8.1 Have you ever been diagnosed with a high blood cholesterol at a hospital or clinic (by a doctor or nurse etc)?

☐ No- **Go to Question 9**

☐ Yes

☐ I don't know- **go to Question 9**

8.2 When was the last time a doctor checked your cholesterol level?

☐ Never

☐ I don't know

☐ More than 2 years ago

☐ Between 1 and 2 years ago

☐ Between 6 months and 1 year ago

☐ Less than 6 months ago

8.3 How old where you when you were diagnosed with high blood cholesterol?

___ Years

8.4 Do you use cholesterol-lowering medication?

☐ No

☐ Yes

8.5 Are you on a cholesterol-lowering diet right now?

☐ No

☐ Yes

9. Blood sugar and diabetes mellitus

9.1 When was the last time a doctor checked your blood sugar (glucose) level?

☐ Never**- go to Question 9.12**

☐ I don't know

☐ More than 2 years ago

☐ Between 1 and 2 years ago

☐ Between 6 months and 1 year ago

☐ Less than 6 months ago

9.2 Have you ever been diagnosed with diabetes by a doctor or health care worker?

☐ No- **go to Question 9.12**

☐ Yes

☐ I don't know

9.3 Were you diagnosed with diabetes only when you were pregnant?

☐ No, I'm a man

☐ Yes- **go to Question 9.12**

☐ I don't know

9.4 How old were you when you were first diagnosed with diabetes? *If you aren't sure, please try to estimate this.* |__|__| years old

9.5 Has a doctor or specialist treated you for diabetes in the past 12 months?
☐ No
☐ Yes

9.6 Do you use tablets for your diabetes?
☐ No
☐ Yes

9.7 Are you on a diabetic diet right now?
☐ No
☐ Yes

9.8 Do you use insulin injections for your diabetes?
☐ No
☐ Yes

9.9 Did you start insulin injections immediately after being diagnosed with diabetes?
☐ No
☐ Yes

9.10 Did you start insulin injections within 6 months of being diagnosed with diabetes?
☐ No
☐ Yes

9.11 Have you ever been told by a doctor or health care worker that you have eye disease or eye damage as a result of your diabetes (diabetic retinopathy)?
☐ No
☐ Yes

9.12 Has someone in your immediate family (your parents, brothers, sisters, or children) been diagnosed with diabetes?
☐ No
☐ Yes

10. Chest pain

10.1 Have you **ever** had any pain or discomfort in your chest? *This does not include problems caused by a cold, asthma, or a stomach ulcer.*

- ☐ No- **Go to Question 11**
- ☐ Yes

10.2 Do you get this pain when you're exerting yourself (for example, when you're climbing stairs, walking fast, or cycling)?

- ☐ No- **Go to Question 10.8**
- ☐ Yes

10.3 Do you get this pain when you're just walking along the street at a normal pace?

- ☐ No
- ☐ Yes

10.4 If you get pain or discomfort in your chest when walking or cycling, what do you usually do?

- ☐ Continue at the same pace – **go to Question 10.7**
- ☐ Slow down or stop
- ☐ Use tablet or spray under the tongue and continue at the same pace
- ☐ Use a tablet or spray under the tongue and slow down

10.5 If you stop or slow down, or use a tablet or spray under the tongue, does the pain disappear?

- ☐ No- go to **Question 10.7**
- ☐ Yes

10.6 How soon does it disappear?

- ☐ Within 10 minutes
- ☐ After more than 10 minutes

10.7 Where do you get this pain or discomfort? *You can give more than one answer.*

- ☐ In the upper part of my chest
- ☐ In the lower part of my chest
- ☐ On the left side of my chest
- ☐ In my left arm
- ☐ Somewhere else namely:

……

10.8 Have you **ever** had severe pain across the front of your chest that lasted for half an hour or more?

☐ Yes

☐ No

11. Leg pain

11.1 Do you get pain in either leg when walking?

☐ No- **go to Question 12**

☐ Yes

11.2 Does this pain ever start when you're standing still or sitting?

☐ No

☐ Yes

11.3 In which part of your legs do you get this pain? *You can give more than one answer.*

☐ In the calf

☐ Other location, namely:

……..

11.4 Do you get this pain when walking fast or climbing stairs?

☐ No

☐ Yes

☐ I never do this

11.5 Do you ever get this pain when you're just walking along the street at a normal pace?

☐ No

☐ Yes

11.6 Does the pain ever disappear while you're still walking?

☐ No

☐ Yes

11.7 What do you do if this happens while you're walking?

☐ Continue on the same pace

☐ Stop or slow down

11.8 What happens if you stop?

☐ The pain usually disappears within 10 minutes

☐ The pain usually disappears after more than 10 minutes

☐ The pain continues

12. Neurologic dysfunction

Neurologic dysfunction is a temporary loss of bodily function: sudden numbness or weakness in the face or other parts of the body (for example, having difficulty finding the right words, a partial or complete paralysis or 'drop' of your hand, arm, foot, leg, or face).

12.1 Have you **ever** had a loss of bodily function that lasted for **less than one day**?

- ☐ No- go to **Question 12.3**
- ☐ Yes

12.2 On this day, was this on just one side of your body or on both sides?

- ☐ On just one side
- ☐ On both sides(at the same time, or changing from left to right)

12.3 Have you **ever** had a stroke?

- ☐ No
- ☐ Yes

12.4 Have you **ever** fainted?

- ☐ No
- ☐ Yes

13. Family

By cardiovascular disease, we mean a heart attack, a dotter procedure (angioplasty) or bypass operation on the heart or legs, a TIA, or a stroke.

13.1 Has anyone in your immediate family (that is your parents, brothers, sisters, daughters or sons) ever been diagnosed with cardiovascular disease.

- ☐ No – **go to Question 13.3**
- ☐ Yes
- ☐ I don't know

13.2. Please indicate these family member(s), and how old they were when they were **first** diagnosed with cardiovascular disease *you can give more than one answer. If you aren't sure of their age, please try to estimate this.*

- ☐ Father, at the age of **|__|__|**
- ☐ Mother, at the age of **|__|__|**
- ☐ Brother, at the age of **|__|__|**
  (if more than one brother, please put down the youngest age at occurrence)
- ☐ Sister, at the age of **|__|__|**
  (if more than one sister, please put down the youngest age at occurrence)
- ☐ Son, at the age of **|__|__|**
  (if more than one son, please put down the youngest age at occurrence)

☐ Daughter, at the age of **|__|__|**
(if more than one daughter, please put down the youngest age at occurrence)

13.3 Has anyone in your immediate family (that is, your parents, brothers, sisters, daughters or sons) **ever** suddenly died <u>when they were 60</u> <u>years old or younger</u> with no clear cause of death?

☐ No- **go to Question 14**

☐ Yes

☐ I don't know –**go to Question 14**

13.4 Could you please indicate these family member(s), and how old they were when they died suddenly? You can give more than one answer. If you aren't sure of their age, please try to estimate this

☐ Father, at the age of **|__|__|**

☐ Mother, at the age of **|__|__|**

☐ Brother, at the age of **|__|__|**
(if more than one brother, please put down the youngest age at occurrence)

☐ Sister, at the age of **|__|__|**
(if more than one sister, please put down the youngest age at occurrence)

☐ Son, at the age of **|__|__|**
(if more than one son, please put down the youngest age at occurrence)

☐ Daughter, at the age of **|__|__|**
(if more than one daughter, please put down the youngest age at occurrence)

Section D: Country of birth and lifestyle

14. Country of birth

14.1 What is your country of origin?

☐ South Africa

☐ Other: ……..

14.2 What is your ethnicity?

☐ Black

☐ White

☐ Mixed ancestry (Coloured)

☐ Asian

☐ Indian

14.3 How long have you been living in your area of residence?

Less than 6 Months	☐	Less than 1 Year	☐
1-5 Years	☐	5 years and above	☐

14.4 What is your mother's country of birth?

☐ South African

☐ Other: ……

14.5 What is your mother's ethnicity?

☐ Black

☐ White

☐ Mixed ancestry (Coloured)

☐ Asian

☐ Indian

14.6 What is your father's country of birth?

☐ South African

☐ Other: ……

14.7 What is your father's ethnicity?

☐ Black

☐ White

☐ Mixed ancestry (Coloured)

☐ Asian

☐ Indian

15. Smoking

15.1 Do you smoke at all?

☐ Yes – **Go to question 15.4**

☐ No, I've never smoked- **go to question 15.6**

☐ No but I used to smoke **Go to question 15.4**

15.2 How long did you smoke? |__|__| years & |__|__| months

15.3 How long has it been since you quit? |__|__| years & |__|__| months

15.4 How many years have you smoked? If you aren't sure, please try to estimate this. |__|__| years

15.5 What did you smoke and how much? You can give more than one answer.
(*After answering *go to question 16)

☐ About |__| cigarettes from a pack of |___| or hand-rolled a day

☐ About |___| cigars a week

☐ About |__|__| package(s) of pipe tobacco (50 grams) a week

15.6 How many people in your household smoke |__|__|

15.7 For how many hours, on average each day, are you closely subjected to other people's tobacco smoke? |__|__|

16. Alcohol

16.1 Have you ever consumed any alcoholic drinks (Wine, Beer, and Spirits)?

☐ Yes

☐ No

16.2 Do you still consume alcoholic drinks?

☐ Yes

☐ No

16.3 If you consume or consumed alcohol, how old were you when you first started drinking?

|__|___| years

16.4 If you stopped, how old were you when you stopped drinking?

|__|___| years old

16.5 Which type of alcohol do you or did you drink?

☐ Beer

☐ Spirits

☐ Wine

☐ Other:_____

16.6 When you drink or drank alcoholic drinks, how many drinks or glasses do you or did you consume daily? Indicate the number

|__|___|

16.7 How many days a week do you or did you consume alcohol?

|__|___| day(s)

16.8 Have you or did you ever feel you should cut down your drinking?

☐ Yes

☐ No

16.9 Have people ever annoyed you by criticizing your drinking?

☐ Yes

☐ No

16.10 Have you ever felt bad about your drinking?

☐ Yes

☐ No

16.11 Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover (Eye Opener)

☐ Yes

☐ No

17. Physical activity

Next I am going to ask you about the time you spend doing different types of physical activity **in a typical week**. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed].*

In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

17.1 Work

Please describe your physical activity at work

a. Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like (carrying or lifting heavy loads, digging or construction work) for **at least 10 minutes** continuously?

☐ Yes

☐ No- **go to Question 17.1d**

b. In a **typical week**, on how many days do you do vigorous-intensity activities as part of your work?

|__| days

c. How much time do you spend doing vigorous-intensity activities at work on a **typical day**?

Hours |__| Minutes|__|

d. Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking (or carrying light loads) for **at least 10 minutes** continuously?

☐ Yes

☐ No- **go to Question 17.2**

e. In a **typical week**, on how many days do you do moderate-intensity activities as part of your work?

Number of days|__|

f. How much time do you spend doing moderate-intensity activities at work on a **typical day**? Hours |__| Minutes|__|

17.2 Travel to and from places

The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. [*Insert other examples if needed*].

a. Do you walk or use a bicycle (pedal cycle) for **at least 10 minutes continuously to get to and from places?**
☐ Yes
☐ **No- go to Question 17.3**

b. In a **typical week**, on how many days do you walk or cycle for **at least 10 minutes** to get to and from places? |__| days

c. How much time do you spend walking or cycling for travel on a typical day? Hours |__| Minutes|__|

17.3 Recreation al activities

The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure), [*Insert relevant terms*].

a. Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate (like running or football) for **at least 10 minutes** continuously?
☐ Yes
☐ No- **go to Question 17.3d**

b. In a **typical week**, on how many days do you do vigorous-intensity sports, fitness or recreational (leisure) activities? |__| days

c. How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a **typical day**? Hours |__| Minutes|__|

d. Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that cause a small increase in breathing or heart rate such as brisk walking, (cycling, swimming, volleyball) for **at least 10 minutes** continuously?
☐ Yes
☐ No- **go to question 17.4**

e. In a **typical week**, on how many days do you do moderate-intensity sports, fitness or recreational (leisure) activities? Number of days|__|

f. How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a **typical day**? Hours |__| Minutes|__|

17.4 Sedentary behavior

The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, traveling in car, bus, train, reading, playing cards or watching television, but **do not include time spent sleeping**. [*INSERT EXAMPLES*]

How much time do you usually spend sitting or reclining on a **typical day**? Hours |__| Minutes|__|

Section E: Education and Employment

18. Education

18.1. What is the highest level of education you have completed?

This is the highest level of education you completed and for which you received a diploma or a certificate of proficiency.

- ☐ Primary School or less
- ☐ High School (Not Completed)
- ☐ High School graduate
- ☐ College Or Technical College (Not Completed)
- ☐ College or Technical College Graduate
- ☐ University or Technikon (Not Completed)
- ☐ University or Technikon graduate

18.2. Are you going to school at the moment?

- ☐ No - **go to Question 19**
- ☐ Yes, day classes
- ☐ Yes, evening classes
- ☐ Both day and evening classes
- ☐ Secondary education

18.3. Which course of study are you following right now?

- ☐ Secondary education

☐ College

☐ University/Technikon

☐ Other, namely:……

19. Employment

19.1. Which situation <u>most</u> applies to you?

☐ I have a paid job, and work 32 or more hours a week. - **Go to Question 19.4**

☐ I have a paid job, and work between 20 and 32 hours a week. -7 **Go to Question 19.4**

☐ I have a paid job, and work between 12 and 20 hours a week. -7 **Go to Question 19.4**

☐ I have a paid job, and work less than 12 hours a week. - **Go to Question 19.4**

☐ I'm retired.

☐ I'm unemployed and looking for work

☐ I'm unable to work I get social benefits

☐ I'm a full-time homemaker (male or female).

☐ I'm a student without part time work

☐ I'm a student with part time work

19.2. If you're **not** working **right now**, have you had a paid job in the past?

☐ Yes

☐ No- **Go to Question 20**

19.3. When (in what year) did you stop Year |__|__|__|__|

working?

19.4 . What is your job or profession now? Or if you're not working right now, what was your last job or profession?

Please describe this with as much detail as you can (for example, primary school teacher, manager of a software company, or worker in a cheese factory rather than teacher, manager, or factory worker).

……

……

……

……

……

19.5. Do you (or did you) have to work irregular hours, such as shift work or nights?

☐ Yes, namely |__|__| hours a week

☐ No

19.6 Which situation best describes (or described) you?

☐ Salaried job

☐ Self-employed

☐ Working in a family business

20. Household income

20.1. Which of these options add to the net income of your household?

☐ Wages or salary

☐ Income from own company or activities

This relates to the income of the whole

household, not just your own (so you can

give more than one answer

☐ Income from investments

☐ Pension

☐ State pension benefits

☐ Incapacity (sickness) benefits

☐ Unemployment benefits

☐ Social benefits

☐ Student grants and loans

☐ Others, namely:……

20.2 How many people in your household

need to live from this income

(including yourself)?

|__|__| people

20.3 Are there people outside your

household who live wholly or partially

from this income?

☐ Yes, namely: |__|__| person(s)

☐ No

Think of children away at university,

alimony for an ex-partner, etc

20.4 During the past year, did you have

problems managing your household

income?

☐ No, no problems at all

☐ No problems, but I have to watch what I spend

☐ Yes, some problems

☐ Yes, lots of problems

Section F: Personality, Experiences and Well-being

21. Dealing with everyday problems

The following statements are about how you deal with everyday problems.
For each statement please indicate to what extent it applies to you.

	Totally disagree	Disagree	Neutral	Agree	Totally agree
a. I have little control about the things that happen to me.	☐	☐	☐	☐	☐
b. I can't seem to solve some of my problems at all.	☐	☐	☐	☐	☐
c. There isn't much I can do to change important things in my life.	☐	☐	☐	☐	☐
d. I often feel helpless in dealing with the problems of life.	☐	☐	☐	☐	☐
e. Sometimes I feel like a play ball of life.	☐	☐	☐	☐	☐

22. Diet

22.1. What day was it yesterday

Sunday | Monday | Tuesday | Wednesday | Thursday | Friday |

22.2. Would you describe the food that you ate yesterday as typical of your usual food intake?

Yes No

I want to find out everything you ate or drank yesterday, including water or food you picked up from the veld. Please tell me everything you ate from the time you woke up to the time you went to sleep. I will also ask you where you ate the food and how much you ate.

Morning (up to 9:00am)

Time	Place	Description of food ate	Amount

Mid-Morning(9am to 11.59am)

Time	Place	Description of food ate	Amount

Afternoon (12:00pm to 3:00pm)

Time	Place	Description of food ate	Amount

Mid-Afternoon (3pm to 5pm)

Time	Place	Description of food ate	Amount

Evening (5pm to 9pm)

Time	Place	Description of food ate	Amount

Before bed(9pm till late)

Time	Place	Description of food ate	Amount

23. Recent experiences

23.1. We will now mention some events. Please indicate whether you've experienced these events in the **past 12 months**

a. You suffered from a serious illness or injury ☐ No ☐ Yes

b. A close relative had a serious illness or injury ☐ No ☐ Yes

c. Your parent, child, brother, sister, or spouse died ☐ No ☐ Yes

d. Another relative (such as an aunt, cousin, or grandparent) or close friend died. ☐ No ☐ Yes

e. You broke off a steady relationship ☐ No ☐ Yes

f. A long-term friendship with a good friend or family member was broken off ☐ No ☐ Yes

g. You had a serious problem with a good friend, family member, or neighbor ☐ No ☐ Yes

h. You were sacked from your job or became unemployed ☐ No ☐ Yes

i. You had a major financial crisis ☐ No ☐ Yes

23.2. **In the past 12 months**, have you felt stressed (feeling irritable or anxious or having trouble sleeping) because of the situation at work or place of study

☐ Never
☐ Some periods
☐ Several periods
☐ Constantly
☐ Doesn't apply

24. Recent well being

In the **past 2 weeks**, how often have you had the following problems?

	Never	On several days	On more than half of the days	Nearly every day
a. Little interest or pleasure in doing things	☐	☐	☐	☐ .
b. Feeling down, depressed, or hopeless	☐	☐	☐	☐ .
c. Trouble falling or staying asleep, or sleeping too much	☐	☐	☐	☐ .
d. Feeling tired or having little energy	☐	☐	☐	☐ .
e. Poor appetite or overeating	☐	☐	☐	☐ .
f. Feeling bad about yourself or feeling like a failure or like you've let yourself or your family down	☐	☐	☐	☐ .
g. Trouble concentrating on things, like reading the newspaper or watching television	☐	☐	☐	☐ .
h. Moving or speaking so slowly that other people might notice	☐	☐	☐	☐ .
i. Being so fidgety or restless that you move around more than usual	☐	☐	☐	☐ .
j. Thinking that you'd be better off dead, or thinking about hurting yourself in some way	☐	☐	☐	☐ .
k. Feeling stressed due to the financial or material demands of your family/friends/relatives	☐	☐	☐	☐ .
l. Feeling stressed due to the demands of the society (e.g. rules, fast way of living, bureaucratic system)	☐	☐	☐	☐

Section G: Body shape

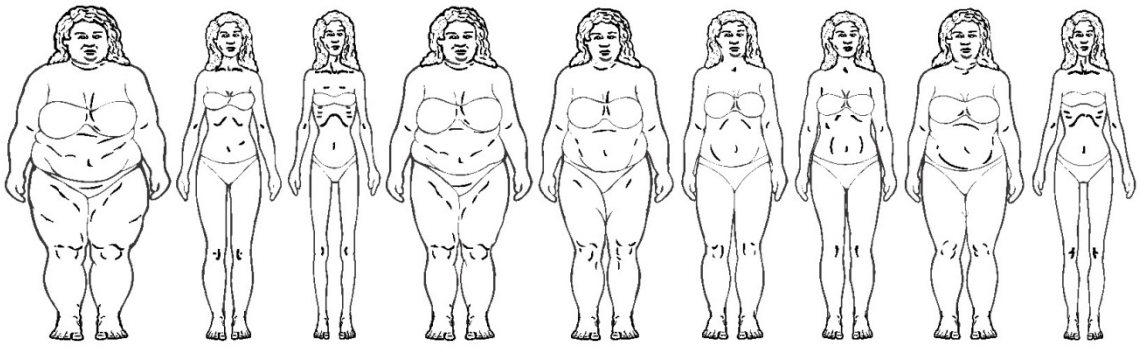
25. Body shape (Females only)

*If you are a **woman**, please answer the questions below. If you are a man, you can go directly to **Question 26**.*

Finally, we want to ask some questions about body shape. For the following questions, you can choose one of the pictures below. Under each picture is a number. Please use this number for your answer

Please put an **X** under **one** of the numbers below

	1	2	3	4	5	6	7	8	9
a. Which picture do you most look like right now?	☐	☐	☐	☐	☐	☐	☐	☐	☐
b. Which picture would you most prefer to look like?	☐	☐	☐	☐	☐	☐	☐	☐	☐
c. Which picture is most like other women your age?	☐	☐	☐	☐	☐	☐	☐	☐	☐
d. Which picture do you think most of the men around you would prefer women to look like?	☐	☐	☐	☐	☐	☐	☐	☐	☐



26. Body shape (Males only)

*If you are a **man**, please answer the questions below.*

Finally, we want to ask some questions about body shape. For the following questions, you can choose one of the pictures below. Under each picture is a number. Please use this number for your answer

Please put an **X** under **one** of the numbers below

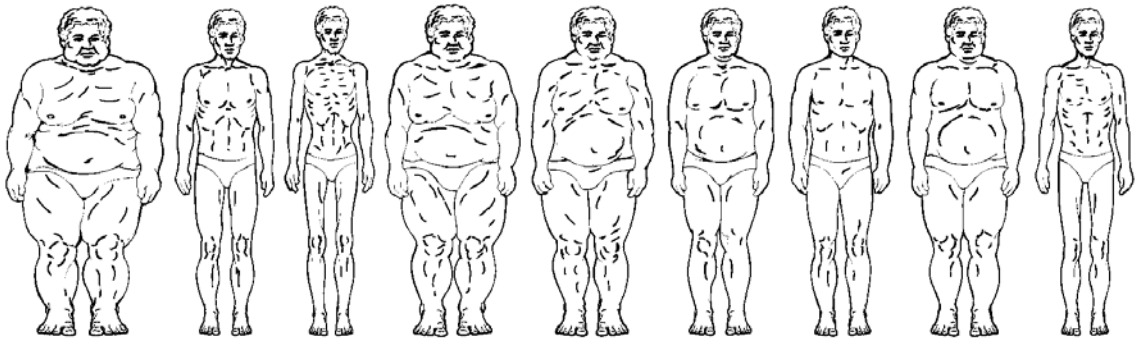
1	2	3	4	5	6	7	8	9

e. Which picture do you most look like right now? ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

f. Which picture would you most prefer to look like? ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

g. Which picture is most like other women your age? ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

h. Which picture do you think most of the men around you would prefer women to look like? ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐



27. Feelings

Now I'm going to ask you questions about how you've been feeling over the past week.

Please tell me the best answer for ***how you have felt over the past week***:

Yes	No	Geriatric Depression Scale
[ ]	[ ]	Are you basically satisfied with your life?
[ ]	[ ]	Have you dropped many of your activities and interests?
[ ]	[ ]	Do you feel that your life is empty?
[ ]	[ ]	Do you often get bored?
[ ]	[ ]	Are you in good spirits most of the time?
[ ]	[ ]	Are you afraid that something bad is going to happen to you?
[ ]	[ ]	Do you feel happy most of the time?

8. [ ] [ ] Do you often feel helpless?

9. [ ] [ ] Do you prefer to stay at home, rather than going out and doing new things?

10. [ ] [ ] Do you feel that you have more problems with memory than most people?

11. [ ] [ ] Do you think it is wonderful to be alive now?

12. [ ] [ ] Do you feel pretty worthless the way you are now?

13. [ ] [ ] Do you feel full of energy?

14. [ ] [ ] Do you feel that your situation is hopeless?

15. [ ] [ ] Do you think that most people are better off than you are?

28. Anxiety (Hopkins Symptom Checklist):

28.1 During the past week, have you felt nervous or shaky inside?

0=No
1=a little
2=sometimes
3=extremely
4=do not know

28.2 During the past week, did you have to avoid certain things, places or activities because they frighten you?

0=No
1=a little
2=sometimes
3=extremely
4=do not know

28.3 During the past week, have you felt tense?

0=No
1=a little
2=sometimes
3=extremely
4=do not know

28.4 During the past week, have you felt fearful?

0=No
1=a little
2=sometimes
3=extremely
4=do not know

29. Mastery:

Please tell me whether you agree or disagree with this statement: I can do just about anything I really set my mind to.

1=strongly agree
2=somewhat agree
3=somewhat disagree
4=strongly disagree

Section I: Clinical Measurements

30. Body Weight

30.1 What do you think of your body weight?

- ☐ I'm much too heavy
- ☐ I'm a little too heavy
- ☐ I'm just about right
- ☐ I'm a little too thin
- ☐ I'm much too thin

30.2 Are you trying to do something about your weight right now?

- ☐ No, nothing
- ☐ Yes, I'm trying to lose weight
- ☐ Yes, I'm trying to stay the same weight
- ☐ Yes, I'm trying to gain weight

31. Weight and height

Body Weight (kg)	☐☐☐	**Comment:** ……
Body height (cm)	☐☐☐	**Comment:** ……
Visceral fat	☐☐☐	**Comment:** ……
Body fat rate	☐☐☐	**Comment:** ……
Muscle percentage	☐☐☐	**Comment:**

		……..
RM		**Comment:……..**
BMI		**Comment:**
		……

32. Circumference measurements

Waist Circumference 1 (cm)				Comment:
Waist Circumference 2 (cm)				
Waist Circumference 3 (cm)				

Hip Circumference (cm)				Comment:
Hip Circumference (cm)				
Hip Circumference (cm)				

33. Blood pressure measurements

Systolic Pressure 1 (mmHg)				Comment:
Systolic Pressure 2 (mmHg)				
Systolic Pressure 3 (mmHg)				

Diastolic Pressure 1 (mmHg)				Comment:
Diastolic Pressure 2 (mmHg)				
Diastolic Pressure 3 (mmHg)				

Pulse 1 (Beat per Minute)				Comment:
Pulse 2 (Beat per Minute)				
Pulse 3 (Beat per Minute)				

Section J: Blood collection

34. Fasting bloods

34.1 Are you a diabetic

☐ Yes, **Complete question 34.1 and skip question 35**

☐ No, **must complete question 34 &35**

34.2 Have you collected the following fasting bloods?

34.2.1 One(1) 5ml Green Top Tubes

☐ Yes

☐ No, why:_____

34.2.2 Two (2) 10ml or Three (3) 5ml Gold Top Tubes

☐ Yes,

☐ No, why:_____

34.2.3 One(1) 5ml Grey Top Tubes

☐ Yes

☐ No, why:_____

34.2.4 Two (2) 5ml Purple Top Tubes

☐ Yes

☐ No ,why:_____

34.2.4 One (1) RNA tube

☐ Yes

☐ No ,why:_____

35. Glucose bloods

Have you collected the following fasting bloods?

35.2.1 One (1) 10ml or Two(2) 5ml Gold Top Tubes

☐ Yes

☐ No, why:_____

35.2.2 One (1) 5ml Grey Top Tubes

☐ Yes

☐ No, why:_____

35.2.3 One(1) 5ml Purple Top Tubes

☐ Yes

☐ No, why:_____

35.2.4 One(1) 4ml Light Blue Top Tubes

☐ Yes

☐ No, why:_____

Appendix 3: Information and Consent form

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM FOR RESEARCH INVOLVING GENETIC STUDIES

TITLE OF RESEARCH PROJECT: PROGRESSIVE RESEARCH ON RISK FACTORS OF TYPE 2 DIABETES AND CARDIOVASCULAR DISEASES IN SOUTH AFRICA

REFERENCE NUMBER:

PRINCIPAL INVESTIGATORS: Professor Tandi Matsha (Cape Peninsula University of Technology)

Professor Rajiv Erasmus (Stellenbosch University) Professor Andre Kengne (SA Medical Research Council)

Project manager: Dr Gloudina Maria Hon (Cape Peninsula University of Technology)

ADDRESS: Obesity and chronic diseases of lifestyle Department of Biomedical Sciences Faculty of Health & Wellness Sciences

Cape Peninsula University of Technology, Bellville

CONTACT NUMBER: Prof T Matsha 021 959 6366 or email: matshat@cput.ac.za

Ethics approval: Cape Peninsula University of Technology Ethics Reference number: CPUT/SW-REC 2015/H01

University of Stellenbosch Ethics Reference number: N14/01/003

We would like to invite you to participate in a research study that involves genetic analysis and possible long-term storage of blood or tissue specimens. Please take some time to read the information presented here which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is *entirely voluntary* and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. *You are also free to withdraw from the study at any point, even if you do agree to take part initially.*

This research study has been approved by the ethics Faculty of Health & Wellness Sciences of the Cape Peninsula University of Technology and it will be conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of

Helsinki, and the SA Department of Health's 2004 Guidelines: *Ethics in Health Research: Principles, Structures and Processes.*

1. What is Genetic research?

Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Occasionally genetic material is obtained from other sources such as saliva or biopsy specimens. (A biopsy is a tiny piece of tissue that is cut out e.g. from the skin or from a lump, to help your doctor make a diagnosis.) Genes are found in every cell in the human body. Our genes determine what we look like and sometimes what kind of diseases we may be susceptible to. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions.

2. What does this particular research study involve?

This research study seeks to address the increasing problem of diabetes and cardiovascular diseases such as heart attack and stroke amongst the mixed ancestry or coloured population of South Africa. In this study we shall identify people with diabetes and those at high risk of diabetes as well as investigate the environmental and genetic risk factors that predispose some individuals to the development of diabetes and cardiovascular diseases. Examples of environmental factors include body weight, diet, and physical activity. Additionally, this project aims to investigate whether oral health is a risk factor for diabetes and cardiovascular diseases. In this study we shall investigate whether some individuals have early cardiovascular diseases by using an ultrasound machine. This project also aims to collect genetic material (blood) to analyze for certain variants and to store excess material for future research. When a large group of patients with similar diseases has been collected, meaningful research into the disease processes may become possible.

3. Why have you been invited to participate?

Our research team has previously conducted a similar research study involving the coloured community and found out that more that 18 out of 100 individuals had diabetes but did not know. We also found that some of the risk factors associated with diabetes in other populations were not necessary the same as those affecting the coloured population of South Africa. You have therefore been invited to take part in this research study to assist in establishing the risk factors for diabetes and cardiovascular diseases affecting the coloured people of South Africa.

4. What procedures will be involved in this research?

You will be requested to provide information about your medical history, family history and information on eating, drinking and smoking habits. Completion of the questionnaire will take no longer than 30 minutes.

You shall be requested to provide a record of the medication you are currently taking, therefore if you are taking chronic medication, you shall be requested to provide this to the research team to record the medication.

Measurement such as weight, height, waist and hip will be done.

Fasting Venous Blood (20ml) will be collected thereafter you will be asked to drink a glucose solution (glucose content 75g). After two hours another venous blood (10ml) will be collected. The blood will be used to determine whether you have diabetes or you are at high risk for developing diabetes.

The other tests that will be determined from your blood sample are: Cholesterol, triglycerides, creatine levels to assess your kidney function, liver enzymes to assess your liver, and biochemical markers for inflammation.

A finger prick blood sample (a drop of blood), to be taken at the same time of the first venous blood sample, may also be required from you. The finger prick blood sample will be used to test for diabetes or the risk of developing diabetes on a point-of-care test instrument. Researchers will compare the finger prick point-of-care diabetes test with that of the send away venous blood laboratory test and would be able to establish whether the point-of-care test provides the same accurate results as that of the laboratory. Point-of-care testing may in the future be used to provide fast and accurate results without the need to send blood away to a laboratory for processing. This may be of benefit to people undergoing testing for diabetes as results would be available within a few minutes.

The remainder of the blood sample will be used for genetic and future research studies. The serum and DNA may be stored for several years until the technology for meaningful analysis becomes available. No pharmaceutical agent (medication) will be tested in the study.

For oral health, research study personnel will extract wooden toothpick, flocked brush, and mouthwash saliva samples from you to test for the presence of Porphyromonas gingivalis as an indicator for periodontal disease. Flocked brush and wood toothpick sampling will involve inserting devices in the subgingival crevice between the last upper premolar and the first upper molar. The device will sweep down the anterior surface of the first upper molar with the direction of motion away from the gum to minimize any potential discomfort. Mouthwash sampling will involve rinsing with 10 ml sterile saline solution for 20 seconds.

Early cardiovascular diseases will be performed by means of an ultrasound machine.

The research team will follow up on you on a yearly basis and some of these test may be repeated. The investigators wish to follow you up for your entire life. In the unfortunate event that you are deceased during the study period. The study team will review stats SA data and/or medical records to ascertain whether the cause of death was due to diabetes or cardiovascular diseases. . *If you do not wish to be followed up on a yearly basis and your Statistics SA and/or medical records not to be accessed in the unfortunate event that you are deceased whilst being a participant in the study, you will have an opportunity to request that it be not accessed when you sign the consent form.*

Radio imaging techniques will be done on consenting subjects. These include (i) ultra sound to assess whether you have signs of early cardiovascular diseases, (ii) computed tomography scan (CT-scan) to accurately assess the fat content that is dangerous for cardiovascular diseases (iii) Dual-energy X-ray absorptiometry (DXA) devices will be used to study the morphology of the liver. These radio imaging techniques involve radiation which can be harmful if one is exposed excessively. For this study a low dose radiation will be used for acquisition of the images thereby minimizing radiation exposure to the participant. . *If you do not wish to undergo any of these radio imaging techniques, you will have an opportunity to decline when you sign the consent form.*

An eye examination will be done to test your eye vision and any other abnormalities inthe eye. For this examination, drops placed in your eyes widen (dilate) your pupils to allow the doctor to better view inside your eyes. The drops may cause your close vision to blur for a short while.

5. Are there any risks involved in genetic research?

A slight bruising might occur after blood has been drawn from the arm but this will heal quickly. After the administration of the glucose solution, you may feel nauseous and dizzy in which case you must notify the medical personnel. A medical nurse or doctor will be present on all occasions. You may also learn that you have diabetes, in which case you will be referred to your health care giver with the results for further treatment and management. If during the study it is discovered that you have changes in your genes that may lead to a serious disease, a genetic counsellor at the expense of the principal investigators will counsel you. Radio imaging techniques such as the CT-scan involves radiation which can be harmful if one is exposed excessively. For this study a low dose radiation will be used for acquisition of the images thereby minimizing radiation exposure to the participant.

6. Are there any benefits to your taking part in this study and will you get told your results?

Your personal results will be made known to you *only if they indicate* that you may:

Have diabetes, thereafter, you will be referred to your local health centre or general practitioner for further investigations and treatment.

Have a condition or predisposition to developing diabetes that is treatable or avoidable

e.g. by a lifestyle modification.

Need genetic counselling.

However, participants with normal results who wish to know their results are free to contact the research team and their results will be given upon written request.

7. How long will your blood be stored and where will it be stored?

The blood samples may be stored *indefinitely* to accommodate new technologies that may develop. In the event that a technology is not available in South Africa to analyse your blood sample, your blood specimen may be sent to another country with the technology either now or at a later date. However, if your specimen is to be sent to another country, permission to do so will be sought from relevant bodies. Your blood specimen will be stored at the Cape Peninsula University of Technology.

8. If your blood is to be stored is there a chance that it will be used for other research?

Your blood will only be used for genetic research that is directly related to Diabetes and cardiovascular diseases. Also if the researchers wish to use your stored blood for *additional research in this field* they will be required to apply for permission to do so from the ethics *Faculty of Health & Wellness Sciences of the Cape Peninsula University of Technology. If you do not wish your blood specimen to be stored after this research study is completed you will have an opportunity to request that it be discarded when you sign the consent form.*

9. **How will your confidentiality be protected?**

Your identity will be recorded once and kept confidential throughout. This is to allow the principal investigators to convey information that may be beneficial to you. Access will be limited to the principal investigators by assigning a special study code to all your data and blood samples. This means that your sample will be identified with a special study code that will remain linked to your name and contact details. However, during the entire research study, your blood specimens will be anonymised and the research staff won't be able to associate it with your name and contact details. You shall also be supplied this code so that if at any time the investigators need to contact you, you may only identify yourself using your special code. Any scientific publications, lectures or reports resulting from the study will not identify you.

Some insurance companies may mistakenly assume that taking part in research indicates a higher risk for disease. Thus no information about you or your family will be shared with such companies.

10. Will you or the researchers benefit financially from this research?

You will not be paid to take part in this study *although your out-of-pocket expenses may be reimbursed.* The expenses that will be covered by the research team are those that include transportation to a hospital radiography department should you consent to radio imaging.

Important information: In the unlikely event that this research leads to the development of a commercial application or patent, you or your family will not receive any profits or royalties, but profits will be reinvested into supporting the cause of further research which may bring benefits to you or your family and to the community, such as health screening, medical treatment, educational promotions, etc.

11. Is there anything else you should know or do?

You should inform your family practitioner or usual doctor that you are taking part in a research study. You can contact Prof T Matsha at 021 959 6366 or matshat@cput.ac.za,

If you have any further queries or encounter any problems, you can also contact the Cape Peninsula University of Technology Health and Wellness Sciences Research Ethics Committee,

Chairperson: Prof Engel-hills at 0219596570 or EngelhillsP@cput.ac.za or

You will receive a copy of this information and consent form for your own records if it is requested.

12. Declaration by participant

By signing below, I ……..……. agree to take part in a research project that includes genetic research study entitled *(*PROGRESSIVE RESEARCH ON RISK FACTORS OF TYPE 2 DIABETES AND CARDIOVASCULAR DISEASES IN SOUTH AFRICA*)*.

I declare that:

I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.

I have had a chance to ask questions and all my questions have been adequately answered.

I understand that taking part in this study is voluntary and I have not been pressurised to take part.

I have received a signed duplicate copy of this consent form for my records.

13. Tick the option you choose:

☐ I agree that my blood or tissue sample can be stored *indefinitely* after the project is completed but that it is anonymised with all possible links to my identity removed, and that the researchers may then use it for additional research in this or a related field. Once my sample is anonymised, my rights to that sample are waivered. My sample may be shipped to another laboratory in SA or abroad to be used in other research projects in this or a related field

OR

I agree that my blood or tissue sample can be stored *indefinitely,* but I can choose to request at any time that my stored sample be destroyed. My sample will be identified with a special study code that will remain linked to my name and contact details. I have the right to receive confirmation that my request has been carried out.

OR

Please destroy my blood sample as soon as the current research project has been completed.

14. Tick the option you choose:

I *consent* that the research team may follow me up for yearly check-up *AND* in the unfortunate event that I am deceased whilst still part of the study, I *consent* that the team may access Statistics SA and/or my medical records to ascertain whether the cause of my death was due to diabetes or cardiovascular diseases.

OR

I *do not consent* to follow me up for yearly check-up *BUT* in the unfortunate event that I am deceased whilst still part of the study, I *consent* that the team may access Statistics SA and/or my medical records to ascertain whether the cause of my death was due to diabetes or cardiovascular diseases.

OR

I do not consent to follow me up for yearly check-up *AND* in the unfortunate event that I am deceased whilst still part of the study, I *do not consent* that the team accessing Statistics SA and/or my medical records to ascertain whether the cause of my death was due to diabetes or cardiovascular diseases.

15. Tick the option you choose: Radio Imaging

I *consent* to ultra sound techniques to assess if I have early cardiovascular diseases

I *do not consent* to ultra sound techniques that assess if I have early cardiovascular diseases

AND

I *consent* computed tomography scan (CT-scan) to accurately assess the fat content that is dangerous for cardiovascular diseases

I *do not consent* to computed tomography scan (CT-scan) that accurately assess the fat content that is dangerous for cardiovascular diseases

AND

I *consent* to Dual-energy X-ray absorptiometry (DXA) used to study body composition.

I do not consent Dual-energy X-ray absorptiometry (DXA) used to study body composition

Signed at (*place*) ..... on (*date*).....

Finger print

....

Signature of participant Signature of witness

16. Declaration by investigator

I *(name)* …… declare that:

I explained the information in this document to ……..……..

I encouraged him/her to ask questions and took adequate time to answer them.

I am satisfied that he/she adequately understands all aspects of the research as discussed above.

I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…….. on (*date*) ……...…… 2016.

.....…. ……..

Signature of investigator Signature of witness

17. Declaration By Interpreter

I *(name)* …… declare that:

I assisted the investigator (*name*) …….. to explain the information in this document to (*name of participant*) …….. Using the language medium of Afrikaans/Xhosa.

We encouraged him/her to ask questions and took adequate time to answer them.

I conveyed a factually correct version of what was related to me.

I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....….....……on (*date*) ……...…….. 2016.

..... ……...

Signature of interpreter Signature of witness