SERUM LIPID LEVELS AND LIPOPROTEIN SUBCLASSES IN OBESE WOMEN RESIDING IN A RURAL AREA, LIMPOPO PROVINCE

by

NAKAMPE STANLEY MAMPEULE

[redacted]

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Supervisor : PROF M ALBERTS

Co-supervisor : MR SSR CHOMA

Co- supervisor : PROF JP VAN GEERTRUYDEN

Dedications

This study is dedicated to the following:

1. My parents Mohlatlego Hendrick and Motlatso Betty Mampeule for raising me and supporting and encouraging me through my studies.
2. My son Kagiso Daniel Mohale and his mother; your presence gave me courage for perseverance.
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Above all my heavenly father for the strength and spiritual guidance you gave me and for the courage to go on.

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Declaration

I declare that the dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Medical Sciences (Chemical Pathology) has not been submitted by me or any other person for any degree at this or any other University; and that it is my own work in design and in execution, and that all material cited herein has been duly acknowledged.

N.S MAMPEULE

SIGNITURE: _____ Date:

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List of Abbreviations

ABCA-1: ATP-binding cassette protein 1

ACAT- acyl CoA: Cholesterol acyl transferase

ACE: Angiotensin converting enzyme

AGN: Angiotensinogen

AMPK: Adenosine monophosphate protein kinase

APO A-I: Apolipoprotein A-I

APO B-100: Apolipoprotein B-100

APO C-II: Apolipoprotein C-II

APO E: Apolipoprotein E

BFM: Body fat mass

BMI: Body Mass Index

BP: Blood pressure

CETP: Cholesterol ester transfer protein

CHD: Coronary Heart Disease

CHO: Carbohydrates

CI: Confidence Interval

CM: Chylomicron

cm: Centimetre

CRP: C-reactive protein

CV: Coefficient of variance

CVD: Cardiovascular disease

DBP: Diastolic blood pressure

ER: Endoplasmic reticulum

FFA: Free fatty acid

HDL: High density lipoprotein

HDL-C: High density lipoprotein cholesterol

HDSS: Health and Demographic Surveillance System

HSL: Hormone sensitive Lipase

IASO: International Association of the study of obesity

IDL: Intermediate density lipoprotein

IL-10: Interleukin -10

IL-6: Interlukin-6

IR: Insulin resistance

IRS-1: Insulin receptor 1

Kg: Kilograms

LCAT: Lecithin-cholesterol acyl-transferase

LDL: Low density lipoprotein

LDL-C: Low density lipoprotein cholesterol

LPL: Lipoprotein lipase

MHO: Metabolically healthy obese

MHAO: Metabolically healthy abdominally obese

mmol/L: millimoles per litre

mRNA: messenger Ribonucleic acid

MTP: Monophosphate transfer protein

NEFA: None-esterified fatty acid

NHLBI: National Heart Lung and Blood institution

Nm: Nanomoles

NMR: Nuclear Magnetic Resonance

RAAS: Renin-angiotensin-aldosterone system

SAT: Subcutaneous adipose tissue

Sd-LDL: Small dense low density lipoprotein

SNS: Sympathetic nervous system

PAGE: Polyacrylamide gel electrophoresis

SPSS: Statistical Package for Social Sciences

T2DM: Type 2 Diabetes Mellitus

TBF: Total body fat

TC: Total cholesterol

TG: Triglycerides

TNF-α: Tumor necrosis factor-alpha

TRL: Triglyceride rich lipoproteins

UK: United Kingdom

USA: United States of America

VLDL: Very low density lipoprotein

WC: Waist circumference

WHO: World Health Organisation

WHR: Waist-hip ratio

Terminology

Adiponectin: A polypeptide hormone that is secreted by fat cells and which regulates glucose and lipid metabolism, especially by increasing insulin sensitivity and muscle uptake of glucose and by decreasing glucose production in the liver (Kadowaki and Yamauchi, 2005)

Dyslipidaemia: Abnormality in, or abnormal amounts of, lipids and lipoproteins in the blood (Marshall and Bangert, 2008)

Lipoproteins: Any of a group of proteins to which a lipid molecule is attached, important in the transport of lipids in the blood stream (Marshall and Bangert, 2008).

Obesity: Abnormal or excessive fat accumulation that represents a risk to health (WHO 2004).

Serum lipids: Any major lipid in the circulation (Marshall and Bangert, 2008)

Visceral obesity: A form of obesity due to excessive deposition of fat in the abdominal viscera and omentum, rather than subcutaneously (Bouchard *et al.*, 1990)

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Abstract

Background

Obesity has been associated with dyslipidaemia (increased levels of triglycerides, total cholesterol and low levels of HDL-C together with small dense lipoprotein particles) in the absence of metabolic disorders such as, type 2 diabetes mellitus and inflammation. Since community based studies in South Africa reported that obesity is more common in women, and rural Africans have a more favourable lipid profile compared to their White counterparts, the current study investigated the association of obesity in women without metabolic disorders with lipid levels and changes in proportions of small and large LDL and HDL particles.

Methods

The present study was part of the project “Prevention, Control and Integrated Management of Chronic Disease in a rural area, South Africa”. A total of 521 women participated in the above project. After excluding people with diabetes mellitus, insulin resistance and inflammation, 308 women were left and of these 67 were obese. Sixty seven ages matched, randomly selected non-obese women served as controls.

Anthropometry variables as well as systolic and diastolic blood pressures were measured and the WHO steps questionnaire was administered to collect information on medication, lifestyle and diseases. Fasting blood levels of total cholesterol, HDL-C, triglyceride, adiponectin, CRP, glucose and insulin were measured. Proportions of small and large HDL and LDL particles were determined.

Results

There was no significant difference in TC, TG and LDL-C levels (p=0.558, 0.087 and 0.948) between obese and non-obese women or between women with increased waist circumference (WC) and those with normal WC.

The HDL-C concentration was significantly lower in obese women compared to women with non- obese (p=0.001). The lipid ratios TC/HDL-C and Apo B-100/Apo A-I were significantly higher in obese women than those with non- obese (p=0.013 and p=0.006) respectively. The same phenomenon was observed in women with

increased waist circumference (p=0.001** and p=0.025* respectively). Adiponectin levels were significantly lower in obese women compared to non-obese women (p=0.004**) and in women with increased waist circumference compared to those with normal waist circumference (p=0.016*).

The proportions of small dense HDL and LDL lipoprotein particles were similar in obese and non-obese women. Both obese and abdominally obese women had significantly higher odds ratios of low levels of HDL-C and elevated Apo B-100/Apo A-I. Adiponectin was a significant predictor of elevated TC and TG in both obese and abdominally obese women while BMI was a significant predictor of low HDL-C in obese women. Waist circumference was a significant predictor of low HDL-C in abdominally obese women.

Conclusion

In the current study, obesity in women was significantly associated with lipid abnormalities such as low HDL-C levels, raised lipid ratios (TC/HDL-C and Apo B-100/Apo A-I) and low levels of adiponectin, after excluding metabolic disorders**.**

Keywords (obesity, serum lipids, lipoprotein subclasses, Adiponectin)

Chapter 1: Introduction

This chapter gives and introduction to the subject of obesity such as prevalence, health outcomes and effect on lipids and lipoprotein subclasses.

The prevalence of obesity has increased dramatically in the world, not only in high income countries but also in middle and low income countries (James, 2008, Hruby and Hu, 2015). The prevalence of overall obesity in USA is 34% while in Europe the prevalence differs among countries and ranges from 10.7 % in Sweden to 25% in Ireland (Gorber *et al.*, 2007, Ogden *et al.*, 2007, Ogden *et al.*, 2014, Gallus *et al.*, 2015). In developing countries such as Egypt, Saudi Arabia, Iran and Morocco the prevalence is 25%, 10%, 10% and 17% respectively (Nishida and Mucavele, 2005, Gorber *et al.*, 2007, Ogden *et al.*, 2007, Hruby and Hu, 2015). In South Africa the prevalence of obesity is gradually increasing mainly due to urbanization and rapid lifestyle changes (Ali and Crowther, 2009). Tibazarwa *et al.*, (2009) found that the prevalence of obesity in men and women in the townships of Soweto to be 23% and 56% respectively.

In developing countries the prevalence of obesity among women is twice that in men (James, 2008, Mitchell and Shaw, 2015), while in developed countries obesity is present at a similar level across gender or is slightly more prevalent in females (Seidell *et al.*, 2005, Mitchell and Shaw, 2015). In the USA the prevalence of obesity in women and men was 35.8 and 32.2 respectively while in Europe the prevalence is between 6.2-36.5% in women and between 4.0-28.3% in men (Berghöfer *et al.*, 2008, Flegal *et al.*, 2010, Ogden *et al.*, 2014). In South Africa 29% of men and 56% of women were reported to be either overweight or obese (Puoane *et al.*, 2002). In a rural area of Limpopo province, South Africa the prevalence of obesity was 29% in women above 30 years of age and 8% in men (Alberts *et al.*, 2005). Obesity is associated with increased risk of developing cardiovascular disease, hypertension, type 2 diabetes mellitus and abnormalities in plasma lipids (Larsson, 1991, Artham *et al.*, 2008, Lavie *et al.*, 2009, Artham *et al.*, 2009).

Not only the amount of body fat but also the distribution of fat determines the harmful effects obesity may have on health (Zhang *et al.*, 2011). Abdominal obesity measured by either waist circumference (WC) and/or waist hip ratio (W/H) identifies individuals at increased risk of developing cardiovascular disease (CVD) even if BMI is not increased (Smith Jr and Haslam, 2006, Czernichow *et al.*, 2011).

The increase in adipose tissue that occurs in obesity is associated with changes in the secretion of several adipokines including adiponectin (Flier, 1995, Kovacova *et al.*, 2009).

Adiponectin, a protein produced exclusively in visceral adipose tissue, affects carbohydrate and lipid metabolism, insulin sensitivity, and the inflammatory response (Cohen *et al.*, 2011). Several investigators have reported that Caucasians have significantly higher adiponectin levels as compared to Pima Indians, African-Americans and Africans (Weyer *et al.*, 2001, Hulver *et al.*, 2004, Schutte *et al.*, 2007).

The changes in serum lipids that occur with increases in adipose tissue are elevated triglycerides levels and decreased high density lipoprotein cholesterol (HDL-C) levels (Howard *et al.*, 1991, Burke *et al.*, 1996, Katsuki *et al.*, 2003, Miller *et al.*, 2005). These changes were found to be similar in Caucasians, Asians and African Americans.

In South Africa, a study by Kruger *et al.*, (2002) reported that black obese women had higher TG levels and lower HDL-C levels than non-obese women while Goedecke *et al.*, (2010) reported that there was no significant difference in triglyceride levels between non- obese and obese black women. In both groups HDL-C levels were low. The inverse relationship between high density lipoprotein (HDL-C) cholesterol and BMI may be important, because low HDL cholesterol contributes a greater relative risk to development of heart disease than do elevated triglycerides (Bray, 2004).

However all these studies investigated the effect of obesity in the presence of other metabolic disorders such as insulin resistance, inflammation, diabetes.

Several studies have investigated the effect of obesity or body fat in individuals without metabolic disorders (Foster *et al.*, 1986, Choi *et al.*, 2002, Magkos *et al.*, 2008, Rocha *et al.*, 2013a). Foster *et al.,* (1986) evaluated the contribution of body fat to fasting serum lipid levels in Caucasians and they found that TG levels were predicted by fat free mass (FFM), and age, LDL-C by age only and HDL-C by body build. Choi *et al.,* (2002) investigated the association between obesity and serum lipids in healthy Korean adolescents and found that, among the metrics of obesity, total body fat was most associated with serum lipids.

Magkos *et al.,* (2008) investigated the effect of obesity on serum lipids and lipoprotein subclasses in Caucasian individuals; they reported that even in the absence of

metabolic complications, obesity was associated with a pro-atherogenic lipid profile (i.e. increased TG, LDL, VLDL and decreased HDL-C).

Rocha *et al.,* (2013a) investigated the association between measurements of abdominal obesity and serum lipids in healthy elderly Brazilian people and reported that WHR contributed to a 7.3% variation in TG levels while WC explained 9.2% variations in HDL levels in women.

Waist circumference has shown a positive association with triglyceride, total cholesterol and with low density lipoprotein cholesterol and a negative association with high density lipoprotein cholesterol (Clark *et al.*, 2012, Rocha *et al.*, 2013b).
Serum lipoproteins consist of subclasses which can be identified by either polyacrylamide gradient gel electrophoresis (PAGE) or nuclear magnetic resonance (NMR) (Rainwater *et al.*, 1992, Otvos, 2001). The low density lipoprotein (LDL) comprises subclasses I, IIa, IIb, IIIa, IIIb, IVa, IVb which differ in size, density, and metabolic characteristics, with small dense LDL particles being associated with increased cardiovascular risk (Berneis and Krauss, 2002).

Different HDL subclasses that have been identified using the polyacrylamide gradient gel electrophoresis and nuclear magnetic resonance are HDL3c, HDL3b, HDL3a, HDL2a, and HDL2b (small, dense HDL particles to large HDL particles) (Pascot *et al.*, 2001). In the Québec Cardiovascular Study, although elevation of both large and small HDL particles showed improved cardiovascular risk, the cholesteryl ester rich HDL_2 particles had greater cardio- protective effects than smaller HDL_3 (Lamarche *et al.*, 1997, Williams *et al.*, 2003).
Adiponectin, a hormone produced by adipose tissue is inversely associated with obesity and is decreased in people with increased adiposity due to down regulation of adiponectin receptors (Kadowaki and Yamauchi, 2005). The low levels of adiponectin seen in obese people are associated with increased triglyceride and decreased HDL-C levels. Adiponectin has been found to be inversely associated with small LDL particle size (Weiss *et al.*, 2009).

The prevalence of obesity and its effect on the levels of serum lipids, lipoprotein particles and adiponectin is well documented in developed countries, however in developing countries these studies are limited to urban areas although it has been shown that the prevalence of obesity is also rising in the rural areas especially among

women (Walker, 1995, Organization, 2000b, Labadarios *et al.*, 2005, Pi-Sunyer, 2009, Alaba and Chola, 2014).

Obesity has been shown to be associated with abnormal lipid levels in the absence of other metabolic disorders and with the risk of developing CVD, even in the absence of these metabolic disorders. Therefore the present study will investigate the effect of obesity on the levels of serum lipids and proportions of lipoprotein particles and adiponectin in obese women residing in a rural area.

Chapter 2: Literature review

In this section details on obesity, serum lipids, lipoprotein subclasses and adiponectin are discussed.

2.1 Obesity

Obesity is characterized by an increase in adipose tissue which leads to a number of chronic diseases and early mortality (James *et al.*, 2001, Després, 2001, Bray, 2004), and has become a major public health concern throughout the world with more than 400 million people said to be obese (Lobstein *et al.*, 2004, Alwan, 2011).

It is not only a health problem in developed countries but also in developing countries, and affects people in different age groups and socioeconomic levels (Racette *et al.*, 2003, James, 2004, Kosti and Panagiotakos, 2006, Alaba and Chola, 2014). Several studies have shown obesity to be associated with adverse health consequences such as cardiovascular disease, (CVD), stroke, type 2 diabetes mellitus, dyslipidaemia, respiratory problems, hypertension, gallbladder diseases and sleep apnoea (Field *et al.*, 2001, Hu *et al.*, 2001, Chen *et al.*, 2002, Young *et al.*, 2002, Rydén and Torgerson, 2006).

However obesity is not always associated with adverse outcomes (Blüher, 2014). According to Blüher, (2014) there is an obesity phenotype referred to as metabolically healthy obese (MHO) and studies have shown that metabolically healthy obese makes up between 10-40% of obese individuals depending on the definition (Primeau *et al.*, 2011, Hinnouho *et al.*, 2013). However there is no standardized definition of metabolically healthy obese thus making it difficult to compare between populations (Phillips, 2013). Some studies lower health risk in MHO when compared to unhealthy obese subjects (Orgorodnikova *et al.,* 2012, Ortega *et al.,* 2013) while other studies have shown increased health risk in MHO when compared to healthy normal weight individuals (Hinnouho *et al.*, 2015).

2.1.1 Types of obesity

Four phenotypes of obesity have been described and they are distinguished by the fat distribution in the body, (Bouchard *et al.*, 1990). The phenotypes are as follows i) generalized fatness which is characterized by excess fat throughout the body without any specific body region being affected, ii) excess subcutaneous fat deposited in the trunk-abdominal area, iii) abdominal obesity where excess fat is deposited in the abdominal visceral region and iv) excess fat deposition in the gluteo-femoral area

mainly seen in females and referred to as gynoid obesity (Bouchard *et al.*, 1990).

Abdominal obesity, characterised by increased visceral adipose tissue, is said to be the most harmful to health since visceral adipose tissue is metabolically active (Nielsen *et al.*, 2004, Bergman *et al.*, 2007). Visceral adipose tissue has endocrine features and secretes more inflammatory cytokines than subcutaneous fat tissue, hence abdominal visceral tissue is associated with insulin resistance, type 2 diabetes mellitus and atherogenic risk (Fain *et al.*, 2004, Neeland *et al.*, 2013). Gynoid obesity, with fat accumulations in the hip area, is thought to be athero-protective (Tanko *et al.*, 2003).

2.1.2 Measurements of obesity

Laboratory measures such as dual energy x-ray absorptiometry (DEXA) and computerised tomography (CT) and isotopic dilutions of obesity are too expensive in terms of time and equipment for use in large-scale population studies (Reilly *et al.*, 2002, Goodpaster *et al.*, 2002). Instead anthropometric measurements and their associate indices are used to define obesity and identify those at risk to develop chronic health problems (Gill *et al.*, 2003b). Body mass index defined as (weight (kg)/ (height (m) ^{2}) is the accepted measure of obesity in population studies and clinical practice. Conventional BMI classifications are overweight (25.0 kg/m^{2} -≤ 29.9 kg/m^{2}) and obese (BMI ≥30.0 kg/m^{2}) (Razak *et al.*, 2007). In order to identify individuals at risk of chronic health issues, cut-off ranges for BMI have been set by World Health Organization 2000a (Table 2.1).

Table 2.1. WHO recommended definition of obesity (2000) [a].

Classification	BMI (kg/m^2)	Risk of co-morbidities
Underweight	<18.5	Low (but risk of other clinical problems increased)
Normal weight	18.5-24.9	
Overweight	25.0-29.9	Average
Obese		
Class I	30.0-34.9	Moderate
Class II	35.0-39.9	Severe
Class III	>40.0	Very severe

[a]Adapted from Obesity: preventing and managing the global epidemic. Technical Report 894. Geneva: (Organization, 2000a).

Waist circumference and waist to hip ratio are measurements used to define abdominal obesity (Caterson and Gill, 2002).

Waist measurement is taken between the lower rib and iliac crest and represents abdominal obesity (Caterson and Gill, 2002). In **Table 2.2** the waist circumference cut-off values proposed by WHO across different ethnic groups and the risk of metabolic disorders are presented. In the interpretation of anthropometric measurements the ethnicity and cultural background of populations should be taken into account (Vikram *et al.*, 2003). Studies have shown that WC coupled with BMI predicts disease risk better than BMI alone (Ardern *et al.*, 2003, Janssen *et al.*, 2004). Others have shown that WC is a better predictor of obesity related diseases than BMI (Zhu *et al.*, 2002, Dalton *et al.*, 2003, Menke *et al.*, 2007), since BMI does not take into account the distribution of fat. Current BMI cut-off points are said to underestimate obesity associated health risk factors in populations which are not Caucasian (Michels *et al.*, 1998, Razak *et al.*, 2007).

In **Table 2.2** waist circumference cut -off points in different ethnic groups are presented.

Table 2.2 Waist circumference associated with increased risk of metabolic complications of obesity.

Population	**Risk**	
	Increased	Substantially increased
Caucasian WHO		
Men	>94cm	>102cm
Women	>80cm	>88cm
Asia (IASO/IOTF/WHO)		
Men		>90cm
Women		>80cm
WGOC(China)		
Men		>85cm
Women		>80cm

[a]Adapted from Obesity: Preventing and managing the global epidemic. Technical Report 894. Geneva: (WHO, 2000a)

Two separate studies undertaken in South Africa have shown that the current WC cut-off points are not suitable for black African women. A Kwa-Zulu Natal study suggested that a cut-off of 91.5 cm would be a better indicator for WC as a risk factor for the metabolic syndrome (Motala *et al.*, 2011). Crowther and Norris, (2012) found that 92 cm for women would be a suitable cut-off point for the diagnosis of metabolic syndrome. The difference in cut-off values between black women and Caucasians was attributed to the ethnic difference in the relationship between abdominal obesity and metabolic disorder.

2.1.3 Prevalence of obesity

The rise in the obesity pandemic that has been observed in the past few decades has also been observed in low and middle income countries (Swinburn *et al.*, 2011, Mitchell and Shaw, 2015).

Kelly *et al.*, (2008) found that in 2005 the prevalence of obesity was 33% of the world's adult population, and they estimated that if prevalence of obesity continued to increase at this rate, by 2030 up to 57.8% of the world's adult population could either be obese or overweight.

Walls *et al.*, (2012) estimated that between the years 2000 and 2025 the prevalence of normal weight individuals in Australia will decrease from 40.6% to 28.1% while the prevalence of obesity will increase from 20.5% to 33.9%. A study using the WHO definition of obesity estimated that 1.1 billion people globally were overweight or obese (Deitel, 2003). According to a report by WHO (2012) between 5-75% people are obese in China, Japan, India, Indonesia and certain African countries while the prevalence of obesity in the US was 33.9%, UK 22.7%, Canada 23.1%, France 16.9 and Australia 16.4%.

Among South African women aged 15-95 years old, black women had the highest prevalence of overweight and obesity (58.5%), followed by women of mixed ancestry (52%), white women (49.2%) and Indian women (48.9%) (Steyn *et al.*, 2006). A different pattern was seen in men 15-95 years old, where the prevalence of overweight and obesity was highest in white men (54.5%), followed by Indian men (32.7%) and men of mixed ancestry (31%), with the lowest prevalence in African men (25%) (Steyn *et al.*, 2006).

This alarming rise in the obesity pandemic is not only affecting adults in both developing and developed countries but it is also extending to children and adolescents in these populations (De Onis *et al.*, 2010). Obesity in childhood is said to lead to health complications later in life and premature death (De Onis *et al.*, 2010).

In Africa the prevalence of childhood obesity was found to be 8.5% in 2010 and expected to rise to 12.7% by 2020, while in Asia it was 4.9% in 2010 while expected to rise to 6.5% by 2020 (De Onis *et al.*, 2010). Although the prevalence of obesity seems to be increasing rapidly, there are studies which are reporting the levelling off of the prevalence of obesity in both adults and children hence showing that in some countries measures are been taken to reduce the rapid rise in obesity. For example the prevalence of obesity appears to have levelled off in Swedish adults between 2000/01 and 2004/05 in both men and women (Sundquist *et al.*, 2010).

2.1.4. Genetic and Environmental factors for obesity

Obesity has a multifactorial pathogenesis and both genetic and environmental factors are involved (Lyon and Hirschhorn, 2005). Although the recent rise is said to be a result of a decrease in physical activity and increased caloric intake, these factors are said to be influenced by genetic factors (Lyon and Hirschhorn, 2005). There are several genes that have an effect on food intake (Lyon and Hirschhorn, 2005). Mutation in one of these genes combined with increased caloric intake may lead to obesity (Herrera *et al.*, 2011).

The genetic causes of obesity are due to mutations in different genes involved in energy intake and expenditure (Ochoa *et al.*, 2006, Larsen *et al.*, 2012, Moleres *et al.*, 2012, Galbete *et al.*, 2012). Although different gene mutations have been identified the most common gene defect associated with obesity is a mutation in the melanocortin 4-receptor (MC4R) gene which has been found in obese individuals in several studies (Loos *et al.*, 2008, Zobel *et al.*, 2009, Kring *et al.*, 2010, Beckers *et al.*, 2011).

Melanocortin 4-receptor is a seven transmembrane G-protein mostly found in the hypothalamic nuclei and is responsible for energy homeostasis (Lee, 2012). It is required for normal response to a high fat diet by maintaining satiety and increasing thermogenesis and metabolic rate (Lee, 2012). In the absence of this protein subjects may show obesity early in childhood (Lee, 2012). People homozygous for the MC4R mutation has a more severe phenotype than heterozygous phenotypes and are heavier and taller (Lee, 2012).

Other gene mutations such as leptin receptor and pro-opimelanocortin (POMC) genes are said to lead to obesity or uncontrolled weight increase (Cheung *et al.*, 1997, Krude *et al.*, 1998, Masuo *et al.*, 2008). Leptin operates by binding to the leptin receptor OB-Rb activating the JAK2/STAT3 pathway to control the synthesis of different neuropeptide involved in the control of food intake and energy balance (Meister, 2000, Lee *et al.*, 2013, Mercer *et al.*, 2014). Leptin is also involved in the activation of POMC neurons and increases the level of the anorectic peptide α-melanocyte-stimulating hormone while inhibiting neuropeptide Y neurons in arcuate nucleus (ARC) (Farooqi, 2011).

(i) Diet and obesity

The new dietary habits due to the nutritional transition of the 20th century have led to the gradual increase in the prevalence of obesity (Popkin *et al.*, 2006, Cecchini *et al.*, 2010). Throughout the world diets are increasingly becoming more energy dense with less fibre (Popkin, 2006). The eating patterns have also changed (Nielsen, 2002). In developed countries there is an increased intake of fast foods and snacks (Popkin, 2006). A review by Malik *et al.*, (2006) found an increase in the intake of sweetened beverages and carbohydrates in the past few decades and also that sweetened beverages were associated with overweight and obesity. The price of healthier food may also play a role (Jetter and Cassady, 2006, Temple *et al.*, 2011).

Jetter and Cassady, (2006) found that healthier food was more expensive and less available. The same trend was also observed in South Africa, a developing country, and Temple *et al.*, (2011) reported that healthy diets were unaffordable in rural South Africa.

There seems to be a controversy in regard to the recommended dietary combination which leads to preventing obesity. Shai *et al.*, (2008) suggested that the Mediterranean and low carbohydrate diets may be useful as an alternative to a low fat diet for weight loss. A review by Gaesser, (2007) indicated that weight loss was associated with a low fat, high carbohydrate diet, and that a fiber-rich carbohydrate diet may be beneficial for weight maintenance. A high protein diet has also been recommended for weight loss and weight maintenance (Halton and Hu, 2004).

A review by Mobbs *et al.*, (2007) reported that a combination of low carbohydrate/high fat/high protein diet produces obesity while a combination of high carbohydrates/low fat or a very low carbohydrate/high fat/ low protein diets reverse diet induced obesity. High fat diets in which more than 30% of the energy is from fat have been shown to cause obesity (Jequier, 2002, French and Robinson, 2003, Rosengren and Lissner, 2008, Fujita and Maki, 2016).

Studies in the USA, Canada and China have shown that increased fat diets are associated with increased incidence of obesity (George *et al.*, 1990, Popkin *et al.*, 1993, Saris *et al.*, 2000, Popkin *et al.*, 2012, Popkin, 2014). However the increasing rate of obesity cannot be explained by high intake of fat only, because obesity is still seen in populations which have reduced fat intake (Hariri and Thibault, 2010). These could be due to certain fats in their diets (Hariri and Thibault, 2010).

A low CHO diet is thought to be beneficial in that it causes weight loss and improved metabolic health (Gower and Goss, 2015). It is thought that reduced CHO intake leads to decreased insulin secretion which allows the use of fat mostly from the intra-abdominal area (Gower and Goss, 2015).

(ii) Socio-economic status and obesity

In developing countries high socio-economic status is associated with obesity, while in developed countries obesity is associated with low socio-economic status (Mclaren, 2007). However recent data suggests that obesity in developing countries has also shifted to the lower SES group showing that the rise in obesity in developing countries is not only affecting those in urban areas but also those in the rural areas (Monteiro *et al.*, 2004, Mclaren, 2007).

Blane, (2001) showed that, although health and life expectancy are improving across the world, evidence shows that SES differentials on mortality and health are maintained.

People of low SES already have severe health problems and, if the prevalence of obesity continues to rise, the health inequalities will continue to widen (Ball and Crawford, 2005). Brennan *et al.*, (2009) found that greater measures of adiposity were associated with individuals of low SES, while those of higher SES can afford healthy diets (Power, 2005, Alaba and Chola, 2014). Socioeconomic status alone does not contribute to obesity and other factors such as level of education also play a role (Mclaren, 2007, Alaba and Chola, 2014).

(iii) Physical Activity and obesity

Physical activity is another factor that can be used for prevention of obesity and weight gain in the long term (Wareham *et al.*, 2005). Physical activity (PA) has only been considered as a tool to reduce body weight however PA is much more than that and if it is managed well it can help improve body metabolism (Tremblay *et al.*, 2005). Decreased physical activity was shown to lead to decreased insulin sensitivity in skeletal muscles (Booth *et al.*, 2008). Petersen *et al.*, (2007) also showed that IR in skeletal muscle is due to decreased muscle glycogen synthesis as the energy required is used for lipogenesis. The above supports the notion that increased physical inactivity leads to development of metabolic syndromes, CVD and type 2 DM (Booth *et al.,* 2008). Studies have shown that individuals with reduced physical activity and increased energy intake have increased body weight (Pietiläinen *et al.*, 2008, Goodpaster *et al.*, 2010). In **Figure 2.1** the effect of physical inactivity on body metabolism is shown.

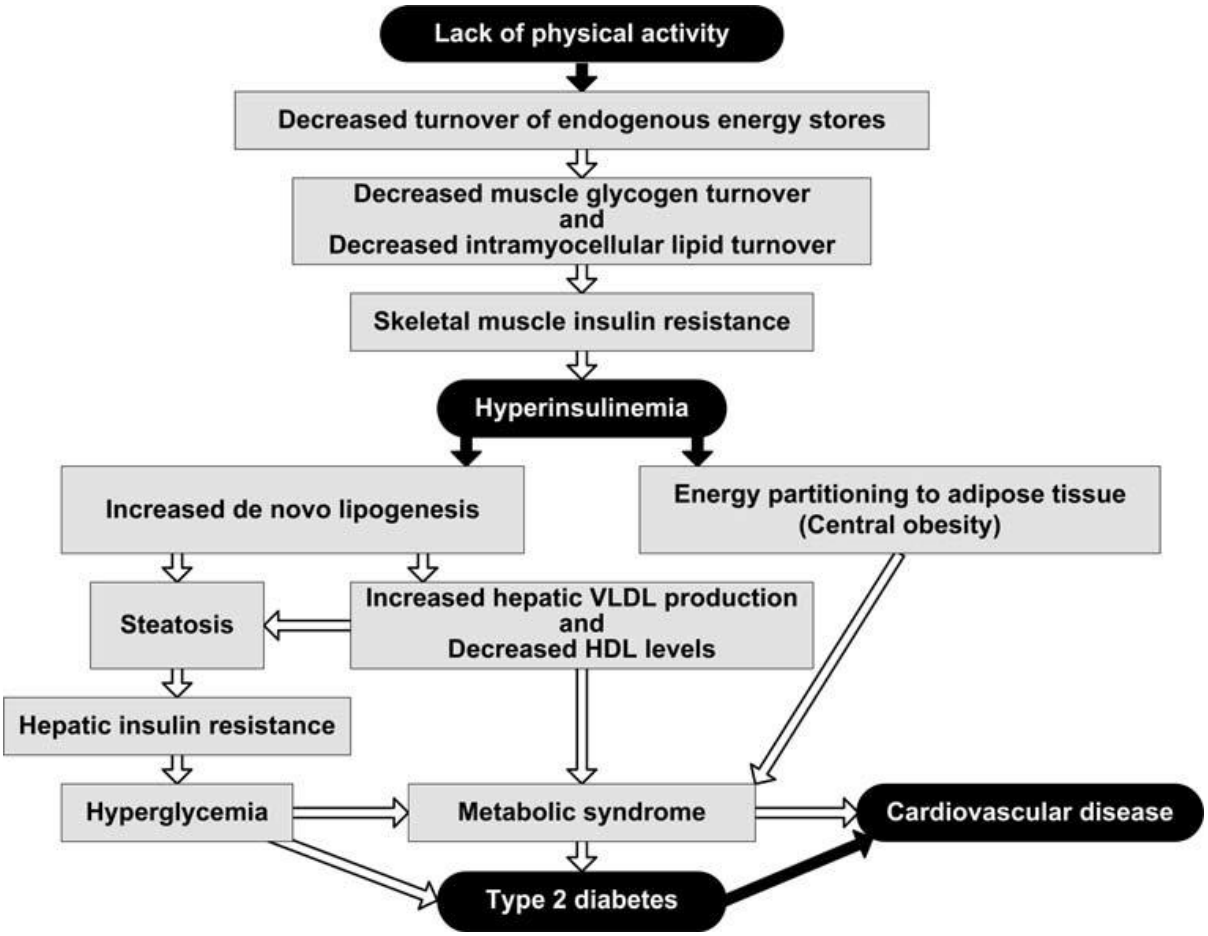

Figure 2.1: Effect of physical inactivity on body metabolism (Booth *et al.,* 2008).

There are different ways in which individuals can be physically active and these include transportation, domestic lifestyle, work and recreation, such as walking as a means of transportation instead of using automobiles, doing house chores, and manual labour at work places and for recreation (Wareham, 2007). However in order to say someone is physically active the following has to be taken into account intensity, frequency, duration and type of activity performed (Wareham, 2007). Saris *et al.*, (2003) reported that in order to lose weight one needs 60-90 minutes of moderate intensity activity daily or less time with more vigorous activity.

In order to reduce the obesity epidemic through PA there is a need to create a physically active environment (Sallis and Glanz, 2009).

Physical activity environments are places where people can be physically active such as sidewalks, play grounds, sport fields and recreation centres (Sallis and Glanz, 2009). Although obesity related genes may make a small contribution to the increase in body weight, genetic factors play an important role in how individuals respond to

environmental factors such as diet and physical activity (Lyon and Hirschhorn, 2005).

In **Figure 2.2** the interaction between genetic predisposition and environmental factors is shown.

There are a number of factors which underlie the environmental changes which result in the obesity epidemic. These include urbanization and economic restructuring which leads to a range of positive and negative consequences (Caterson and Gill, 2002). As income rises populations become more urban, employment based on farming and manual labour are replaced by more sedentary office work, thus reducing physical activity (Caterson and Gill, 2002).

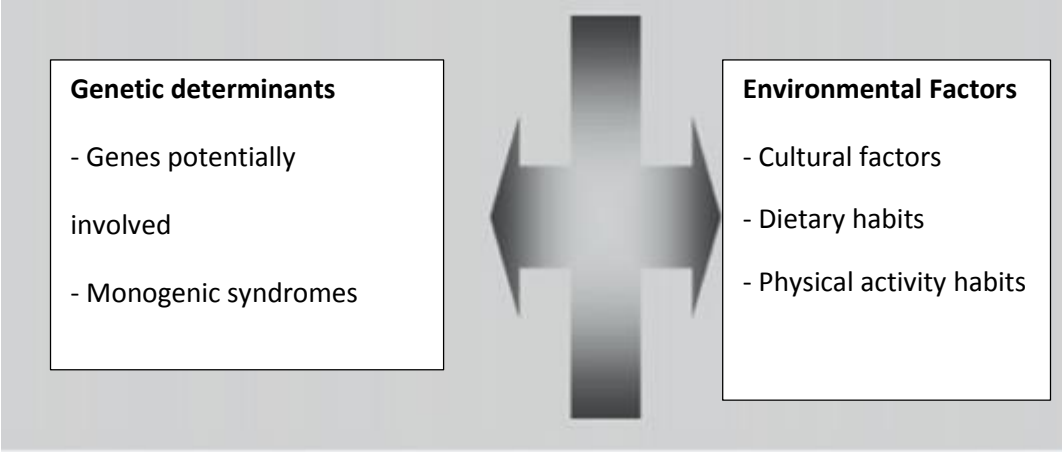

Figure 2.2 Factors involved in development of obesity. Adapted from (Martínez-Hernández *et al.*, 2007)).

2.1.5 Actions of obesity

Obesity is a chronic disorder that increases the risk of developing several metabolic conditions associated with early mortality (Bray, 2004). The disorders associated with obesity are divided into those which are associated with physical effects of excessive fat such as sleep apnoea, respiratory effects, osteoarthritis and psychological problems and those that are associated with metabolic effects of excess fat such as insulin resistance, type 2 diabetes mellitus, CVD, dyslipidaemia, hypertension, cancer, liver and gallbladder disease (Bray, 2004, Haslam and James, 2005, Kopelman, 2007, Cornier *et al.*, 2008, Bray and Wilson, 2008, Pi-Sunyer, 2009, Brown and Kuk, 2015).

2.1.5.1 Insulin resistance

Insulin is a hormone secreted by pancreatic beta cells and plays a major role in both carbohydrate and lipid metabolism in the liver, muscle and adipose tissue (Shulman, 2000, Sesti, 2006). Insulin is responsible for the transport of glucose into the cells (Dimitriadis *et al.*, 2011). Insulin also stimulates glucose metabolism by increasing the rate of glycolysis through activation of the enzyme hexokinase and 6-phosphofructokinase (Dimitriadis *et al.*, 2011). In the liver, insulin stimulates glycogen synthesis (Dimitriadis and Newsholme, 2004). Insulin inhibits glycogen phosphorylase, an enzyme responsible for the breakdown of glycogen into glucose (Dimitriadis and Newsholme, 2004).

Insulin resistance is a condition in which insulin sensitive tissues lose their response to insulin resulting in hyperglycaemia, hyperinsulineamia, hyperlipidaemia, impaired glucose tolerance and increased hepatic glucose production (Sesti, 2006).

Homoeostasis model assessment for insulin resistance is one of the most widely used indices based on fasting parameters (Matthews *et al.*, 1985, Katz *et al.*, 2000). It was described in 1985, a computer model in which fasting insulin and fasting glucose values are plotted to assess β-cell response and IR (Matthews *et al.,* 1985). The HOMA is a simplification of a mathematical model, which is a product of fasting glucose and fasting insulin divided by a constant, HOMA= ((fasting insulin)x(fasting glucose)/22.5) (Antuna-Puente *et al.*, 2011). HOMA has proved a useful tool in large epidemiological research studies (Antuna-Puente *et al.*, 2011).

The association between obesity and insulin resistance is well documented (Castro *et al.*, 2014). Studies have investigated the association of visceral adipose tissue (VAT) and subcutaneous adipose tissue with insulin resistance (Fox *et al.*, 2007, Preis *et al.*, 2010, Mclaughlin *et al.*, 2011, Castro *et al.*, 2014). There is however controversy regarding which measurement of obesity is more associated with insulin resistance. Tulloch-Reid *et al.*, (2004) found subcutaneous adipose tissue to be more closely associated with insulin resistance than visceral adipose tissue in African American women.

In contrast, other studies have found visceral adipose tissue to be strongly associated with insulin resistance (Fox *et al.*, 2007, Hayashi *et al.*, 2008, Preis *et al.*, 2010). Hayashi *et al.*, (2008) showed that visceral adipose tissue is not only associated with insulin resistance but visceral obesity was a strong predictor of future insulin resistance events.

In obesity, IR results from defects in several metabolic pathways, however the extent to which each pathway causes IR in obesity is not quite clear (Qatanani and Lazar, 2007). In obesity it has been shown that the accumulation of fat in the abdominal area is associated with a decrease in insulin sensitivity, thus causing insulin resistance (Frayn, 2000, Wiklund *et al.*, 2008, Yaturu, 2011).

2.1.5.2 Type 2 Diabetes mellitus

With the growing rise of obesity worldwide there is also an increase in the prevalence of type 2 diabetes mellitus (Avenell *et al.*, 2004, Wannamethee *et al.*, 2005, Fujimoto *et al.*, 2007). The development of type 2 diabetes in obesity is not only influenced by the degree of obesity but also by the region where most fat accumulates (Eckel *et al.*, 2011). Increased upper body fat including visceral adiposity is associated with type 2 diabetes (Yaturu, 2011).

Shai *et al.*, (2006) found the prevalence of type 2 diabetes to be higher in Hispanics, Asians and Blacks than in Whites; the reason for this being obesity and lifestyle factors such as diet and exercise. Two epidemiological studies also found that Asians, Blacks and Mexicans were less insulin sensitive than non-Hispanic Whites, suggesting that low insulin sensitivity is the key to higher risk of type 2 diabetes in some ethnic groups (Haffner *et al.*, 1999, Torréns *et al.*, 2004).

Several studies have reported an association between type 2 diabetes and body fat distribution, while others have shown a strong positive association between total body fat and type 2 diabetes (Vazquez *et al.*, 2007, Gómez-Ambrosi *et al.*, 2011).

Measures of body fat distribution such as WC and WHR are associated to type 2 diabetes although debate remains which measure of body fat distribution is the best to predict type 2 diabetes between these two (Vazquez *et al.*, 2007, Meisinger *et al.*, 2008).

A review by Freemantle *et al.*, (2008) found a strong association between measures of abdominal obesity such as WC and development of type 2 diabetes. Both BMI and WC were found to strong risk factors of type 2 diabetes in African American women (Krishnan *et al.*, 2007). **Figure 2.3** shows risk of type 2 DM with increase in BMI

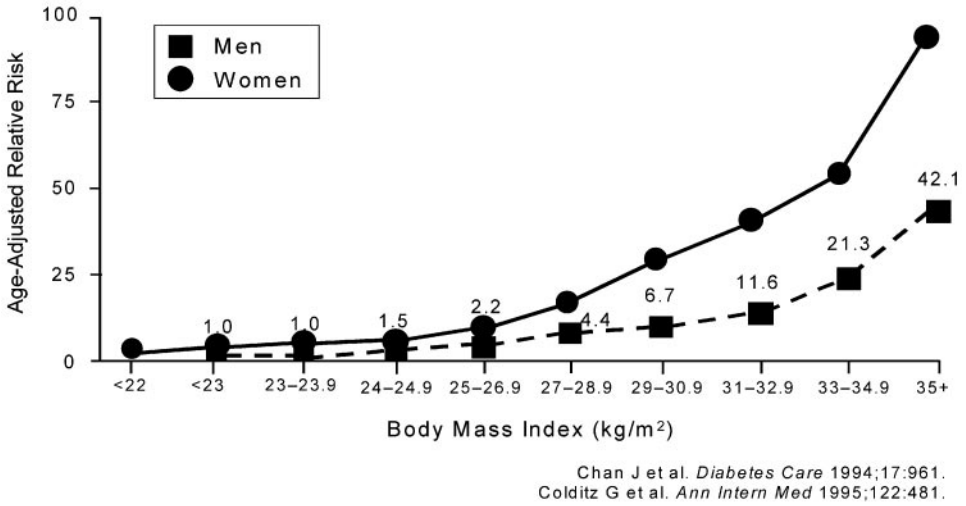

Figure 2.3: Rise in risk of type 2 diabetes with increase in body weight

2.1.5.3 Hypertension

Hypertension or increased blood pressure is defined by a systolic pressure of ≥140mmHg and/or a diastolic pressure of ≥90mmHg (Hirani *et al.*, 2008).
With the rise in the prevalence of obesity an increase in the prevalence of hypertension has also been observed (Landsberg *et al.*, 2013). Since insulin stimulates the sympathetic nervous system (SNS) and obese individuals usually have high SNS activity, insulin mediated SNS stimulation has been suggested to be the cause of hypertension in central obesity (Dibona, 2004). Insulin may also cause hypertension through stimulation of sodium retention in the kidneys (Lann and Leroith, 2007).

Another noted link between obesity and hypertension is the renin-angiotensin-aldosterone system which is activated in obesity (Engeli *et al.*, 2005, Sarzani *et al.*, 2008). Adipose tissue is thought to be a source of angiotensinogen (AGN) in addition to that which is produced by the liver and is thought to be involved in the hypertension of obesity (Mathieu *et al.*, 2009). Renin produced by the kidneys allows the transformation of angiotensinogen to angiotensin I and then, through the action of angiotensin converting enzyme (ACE), angiotensin I is transformed to angiotensin II, a powerful vasoconstrictor (Mathieu *et al.*, 2009). This leads to the rise in aldosterone concentration which leads to sodium retention and hence hypertension (Marshall and Bangert, 2008).

2.2 Serum lipids

Serum lipids including free and esterified cholesterol, triglycerides, and phospholipids circulate in plasma in spherical particles called lipoproteins that contain specific apolipoproteins (apoproteins) (Stewart and Osterman, 1998, Kwiterovich, 2000).

Lipoproteins are differentiated by their densities and include chylomicrons (CM) which transport exogenous lipids from the intestine to peripheral and liver cells. Very low density lipoproteins (VLDLs) which transport endogenous lipids from the liver to the cells, low density lipoproteins (LDL) which are the remnants of intermediate density lipoprotein (IDL) are transported back to the liver and high density lipoproteins (HDL), which are involved in reverse cholesterol transport (Marshall and Bangert, 2008, Charlton-Menys and Durrington, 2008).

2.2.1 Chylomicrons

Chylomicrons are formed in the enterocytes and are the main carriers of exogenous fat (Black, 2007). They contain three proteins which are important in the assembly and function of chylomicrons and include Apo B-48, microsomal triglyceride transporter (MTP) and Apo A-IV (Black, 2007). Apoprotein B-48 helps chylomicrons to take up digested fat are synthesized in the small intestine (Black, 2007). Apo A-IV aids chylomicrons in lipid absorption in the intestine while MTP has several functions amongst which are transport of lipids and preventing Apo B degradation (Black, 2007, Tomkin and Owens, 2012). The chylomicron apoproteins are well regulated and their production depends on the amount of fat in the meal ingested (Hussain *et al.*, 1996, Tomkin and Owens, 2012).

The assembly of chylomicrons starts with the translation of Apo B-48 in the endoplasmic reticulum (ER) lumen (Mansbach and Siddiqi, 2010). Lipidation of apoB-48 is mediated by MTP to avoid degradation. Apo A-IV is added to the surface by MTP, when chylomicrons leave the ER to the cis Golgi in a form called pre-chylomicron transport vesicles which acquires Apo A-I and exit the cell on the basolateral membrane (Kumar and Mansbach, 1999, Mansbach and Gorelick, 2007, Black, 2007).

The newly formed chylomicrons enter the lymphatic system and acquire apo C-II and apo E from HDL-C in the systemic circulation which aids in the interaction with lipoprotein lipase (LPL). Through the action of LPL, triglycerides are hydrolysed and fatty acids taken up by the adipocytes and stored as triacylglycerol (Goodman, 2010). Chylomicron remnants are removed by the hepatocytes (Ginsberg *et al.*, 2005).

2.2.2 Very Low Density Lipoprotein (VLDL)

Similar to chylomicrons the assembly of VLDL involves the synthesis of Apo B-100 by hepatocytes followed by lipidation of the apoprotein and addition of MTP to avoid degradation in the rough ER (Rustaeus *et al.*, 1998, Olofsson *et al.*, 2000, Olofsson and Boren, 2005, Stillemark-Billton *et al.*, 2005).

The VLDL particle is released as a pre-VLDL particle similar to chylomicrons and, through further lipolysis by lipoprotein lipase, is converted into a TG-poor VLDL (Rustaeus *et al.*, 1998, Olofsson *et al.*, 2000, Olofsson and Boren, 2005, Stillemark-Billton *et al.*, 2005). Maturation of the TG-poor VLDL particle takes place in the liver through the incorporation of TG into the VLDL particle hence resulting in a TG-rich VLDL particle. The maturation step takes place in the ER (Yamaguchi *et al.*, 2003, Stillemark-Billton *et al.*, 2005).

After its maturation, the TG rich VLDL is released into the circulation were it acquires apo C- II from circulating HDL-C which activates lipoprotein lipase an enzyme which removes TG from VLDL. There is further removal of Apo E, C and cholesterol from this particle which are taken up by the circulating HDL (Olofsson *et al.*, 2000, Adiels *et al.*, 2008a, Nguyen *et al.*, 2008). The VLDL particle becomes smaller and is converted to intermediate density lipoprotein (IDL).

The cholesterol taken up by HDL is esterified and converted into cholesterol ester which is exchanged with IDL for TG (Olofsson *et al.*, 2000, Adiels *et al.*, 2008a, Marshall and Bangert, 2008).

Some of the IDL is taken up by the liver via the Apo B/E receptor and the hepatic lipase further removes triglycerides from this particle to form a LDL particle rich in cholesterol (Olofsson *et al.*, 2000, Adiels *et al.*, 2008a, Marshall and Bangert, 2008, Nguyen *et al.*, 2008).

2.2.3 LDL-C metabolism

Low density lipoprotein is a cholesterol-rich, TG-poor particle (Tomkin and Owens, 2012). It is made of a hydrophilic surface layer of phospholipids, free cholesterol and Apo B-100 to stabilize the molecule (Tomkin and Owens, 2012). The LDL particle enters the cell through the LDL receptor found on the surface of the cells (Charlton-Menys and Durrington, 2008). These receptors have an amino end which binds Apo E and Apo B (Charlton-Menys and Durrington, 2008). The binding site of the receptor is lined with negatively charged amino acids which complement the positive charges in the binding site of Apo B and Apo E (Charlton-Menys and Durrington, 2008). The synthesis of these receptors is suppressed when cells have enough cholesterol (Charlton-Menys and Durrington, 2008).

The LDL receptor is present in almost all the cells in the body (Goldstein and Brown, 2009). That is because LDL supplies cholesterol all over the body to maintain cell

viability and cholesterol for the synthesis of hormones (Tomkin and Owens, 2012).

The metabolism of LDL is closely regulated and a reduction in endogenous cholesterol synthesis stimulates the LDL receptor causing LDL clearance (Tomkin and Owens, 2012) .

Familial hypercholesterolaemia is caused by a defect in the LDL receptor gene (Tomkin and Owens, 2012). A gene which results in increased LDL in the circulation is the proprotein convertase subtilisin kexin type 9 (PCSK9); which regulates the degradation of liver LDL receptor (Abifadel *et al.*, 2003,Tomkin and Owens, 2012).

It binds tightly to the receptor and transports it to the lysosome for degradation. This leads to reduced LDL receptor and increased LDL in the circulation (Tomkin and Owens, 2012).The estimation of LDL-C does not give information on the LDL particle size because a patient can have normal LDL-C value only to find that most of these are small dense particles (Otvos, 2001, Jaiswal *et al.*, 2014, Ridker, 2014).Low density lipoprotein is formed when TG is removed from VLDL and its main function is to carry cholesterol to the liver for excretion (Marshall and Bangert, 2008).

The liver removes LDL-C from the circulation through the LDL receptor and excretes the cholesterol through bile salts (Carpentier and Sobotka, 2008). After being taken up into the hepatocytes, LDL-C is broken down by lysosomal action releasing free cholesterol which may be esterified in a step catalysed by the enzyme acyl CoA: cholesterol transferase (ACAT) (Adiels *et al.*, 2008a).

2.2.4 HDL-C metabolism

The HDL particle has two major apolipoproteins which are apo A-I and apo A-II (Rader, 2006). Apolipoprotein A-I is produced in the liver and intestine while apo A-II is only produced in the liver (Rader, 2006). Apo A-I makes up 70% of the HDL particle while apo A-II makes up 20% (Rader, 2006).

The newly secreted nascent HDL acquires phospholipids and cholesterol for maturation, mediated by ATP-binding cassette protein 1(ABCA1) which transports cholesterol from an external source into the nascent HDL particle (Huuskonen *et al.*, 2001, Barter *et al.*, 2006, Naik *et al.*, 2006). The incorporation of cholesterol, apo C and apo A from other lipoproteins by the nascent HDL results in a change in the conformation of the particle from disc shaped into a spherical HDL particle (Adiels *et al.*, 2008b, Movva and Rader, 2008). Through further development of the HDL particle, the free cholesterol in the particle is esterified to form cholesteryl ester, catalysed by

the enzyme lecithin: cholesterol acyltransferase (LCAT).

Hence the HDL particle becomes denser and more mature (Jonas, 2000, Marshall and Bangert, 2008).

The efflux of cholesterol from macrophages and reverse cholesterol transport to the liver and bile is how HDL-C protects against the development of atherosclerosis (Movva and Rader, 2008).

The complete lipoprotein metabolism from the absorption and transport of dietary lipids, production and secretion of VLDL by the liver, uptake of LDL by the liver and macrophages and reverse cholesterol transfer by HDL-C to the liver for excretion is presented in **Figure 2.4**.

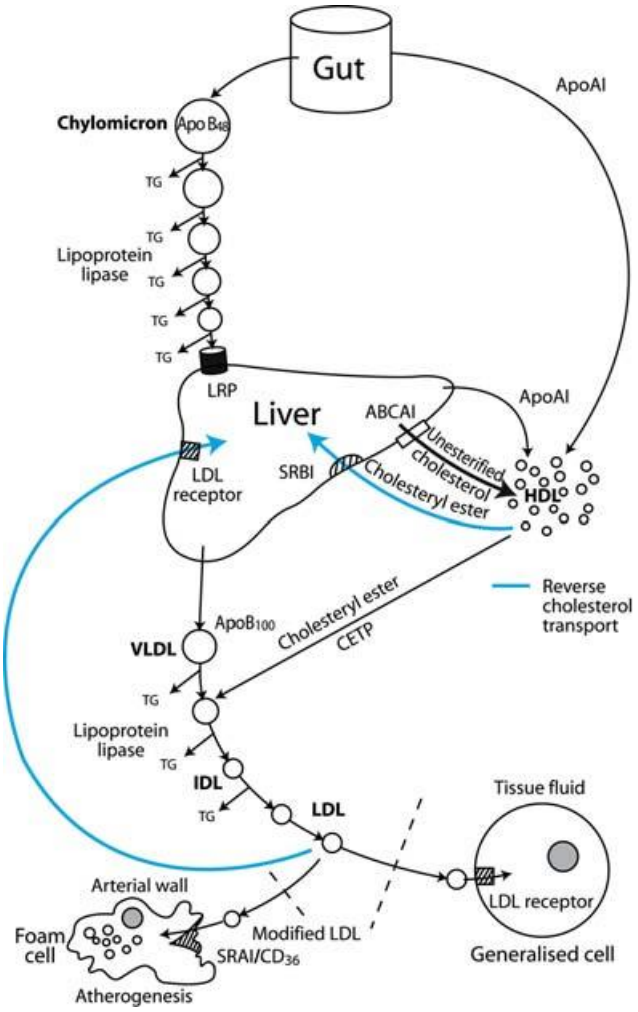

Figure 2.4: Lipoprotein mechanism adapted from Charlton-Menys and Durrington, 2008.

2.3 Lipoprotein subclasses

There are many cases of CVD that cannot be explained by the level of serum lipids, which has led to the investigation of other factors leading to the development of CVD (Gazi *et al.*, 2007). Lipoprotein subclasses have emerged as strong determinants of risk for CVD (Parish *et al.*, 2012).

High-density lipoprotein (HDL-C), LDL-C, and very low-density lipoprotein (VLDL) can be divided into subclasses based on size or density, and some subclasses appear to have stronger associations with coronary artery disease than others (Mackey *et al.*, 2002).

Very low density lipoprotein (VLDL) subclasses are subdivided into two subclasses namely, that are large TG-rich $VLDL_1$ and small cholesterol rich $VLDL_2$ particles (Packard and Shepherd, 1997, Gill *et al.*, 2003a). The production and regulation of these two VLDL subclasses is independent of each other.

The large VLDL1 accounts for plasma TG concentration, while the smaller VLDL2 particle determines plasma LDL concentration (Packard and Shepherd, 1997, Packard *et al.*, 2000). Large VLDL particles contain Apo E and Apo C-III which inhibits LPL, keeping the large particle in the circulation longer (Bjorkegren *et al.*, 1996, Jong *et al.*, 1999).

The large VLDL is involved in the production of small dense LDL particles which are known to be risk factors for development of atherosclerosis. This pathway involves the conversion of the TG-rich, large VLDL particles by cholesterol ester transfer protein to TG-rich LDL. In the liver this particle undergoes further lipolysis by hepatic lipase resulting in a small dense LDL particle (Zimmet *et al.*, 2001, Taskinen, 2003, Verges, 2005, Ginsberg *et al.*, 2006).

2.3.1 LDL subclasses

LDL particles differ in size, density and metabolic characteristics and are made up of at least four distinct subclasses (large LDL-I, medium LDL-II, small LDL-III and very small LDL-IV) (Berneis and Krauss, 2002). The difference in size and density among LDL subclasses is mainly due to the difference in lipid content and structural changes in apoB-100 (Segrest *et al.*, 2001, Charlton-Menys and Durrington, 2008, Tomkin and Owens, 2012).

In comparison with the large more buoyant LDL particles, small dense-LDL particles are easily taken up by arterial tissue and show less affinity for the LDL receptor (Rizzo and Berneis, 2006, Superko and Gadesam, 2008). Small dense LDL has a longer half-life in plasma thus greater oxidation and glycation susceptibility therefore increasing the risk of atherogenesis (Rizzo and Berneis, 2006, Superko and Gadesam, 2008).

Evidence suggests that coronary heart disease (CHD) risk is influenced by plasma lipoprotein particle concentration and the lipoprotein subclass distribution(Carmena *et al.*, 2004). The predominance of smaller LDL particles is associated with increased CHD risk (Carmena *et al.*, 2004) and increased numbers of intermediate-density lipoprotein (IDL) particles. Small HDL particles and large very low-density lipoprotein (VLDL) particles are associated with increased incidence or progression to atherosclerosis (Hodis *et al.*, 1997, Freedman *et al.*, 1998, Carmena *et al.*, 2004).

2.3.2 HDL subclasses

Previously two major HDL subclasses were recognized based on the apolipoprotein they contained (Fruchart and Ailhaud, 1992, Skinner, 1994). Those with only apo A-I and those with both Apo A-I and apo A-II (Fruchart and Ailhaud, 1992, Skinner, 1994). Recently however through more advanced techniques such as polyacrylamide gradient gel electrophoresis and nuclear magnetic resonance HDL subclasses identified are HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a}, and HDL_{2b} (from small, dense HDL particles to large HDL particles) (Pascot *et al.*, 2001). The HDL-2 particles (large HDL particles) are composed of Apo A-I while HDL-3 particles (smaller HDL particles) are composed of Apo A-I and Apo A-II (Camont *et al.*, 2011).

The small HDL particle achieves its antiatherogenic function through pre-β-1 mediating cell-cholesterol efflux through the ATP-binding cassette transporter 1 (ABCA1) (Khera *et al.*, 2011).

However, high levels of pre-β-1are associated with increased CVD risk showing that small HDL does not always perform its function positively (Sethi *et al.*, 2010, Asztalos *et al.*, 2011). The HDL subclasses are responsible for several functions reducing risk of CVD (De La Llera-Moya *et al.*, 2010).

The small HDL particle plays an important role in cellular cholesterol efflux by mobilisation of cellular cholesterol to the plasma membrane (Adorni *et al.*, 2007, De La Llera-Moya *et al.*, 2010).

The large HDL particle is a bigger ligand of cellular cholesterol efflux and is more effective than the smaller HDL particle (Rothblat and Phillips, 2010). The small HDL particle also inhibits LDL particle oxidation and inflammation (Kontush *et al.*, 2003).

2.4 Obesity and serum lipids

Obesity has been reported to be associated with a change in serum lipid levels independent of other metabolic disorders (Magkos *et al.*, 2008). The changes in serum lipids observed in obesity are increased plasma TG, LDL-C and decreased HDL-cholesterol concentrations (Dixon and O'brien, 2002, Howard *et al.*, 2003).

The abnormalities of serum triglycerides and HDL cholesterol in obesity are due to changes in the activities of the key lipoprotein metabolizing enzymes such as increased hepatic lipase (Ginsberg *et al.*, 2006). Not all obese individuals that present with abnormal lipids levels (Wildman *et al.*, 2008).

An earlier study by Brown *et al.*, (2000) found a strong association between BMI and abnormal lipids and also emphasised that cholesterol measurements and control was vital in obese people. Shamai *et al.*, (2011) found that elevated BMI was inversely associated with HDL-C and directly with TG, but no association between BMI and LDL-C was observed. Choi *et al.*, (2002) found that total body fat (TBF) had a stronger association with serum lipids than with BMI.

Abdominal obesity measured by WC has also been reported to be associated with adverse lipid levels (Rezende *et al.*, 2006, Lara *et al.*, 2012). Rocha *et al.*, (2013b) found an association between WC and serum lipids in elderly women. Waist circumference has also been shown to be strongly associated with elevated TG and LDL-C and low HDL-C, however both BMI and WC have been shown to be equally useful in predicting most lipid levels in obese individuals (Chan *et al.*, 2004, Lara *et al.*, 2012).

The abnormal serum lipids observed in obesity result from release of large amounts of non-esterified free fatty acids (NEFAs) into the circulation due to increased activity of hormone sensitive lipoprotein lipase (HSLPL) due to insulin resistance (Grundy, 2000, Grundy, 2004, Ginsberg *et al.*, 2006). When NEFAs reach the liver they are used as building blocks in the synthesis of TGs which are incorporated into VLDL by the liver (Grundy, 2000, Grundy, 2004, Ginsberg *et al.*, 2006).

The resulting hypertriglyceridemia may be the major cause of lipid abnormalities in obesity since it leads to delayed clearance of TG-rich lipoproteins and formation of sd-

LDL (Capell *et al.*, 1996, Ginsberg *et al.*, 2006).

The inability to remove TG from the circulation is largely due to impaired LPL activity in skeletal muscles and competition for LPL between CM and VLDL (Clemente-Postigo *et al.*, 2011, Klop *et al.*, 2013). When LPL remains attached to VLDL and IDL this leads to further TG depletion and further exchange of TG from these remnants for cholesterol ester by CETP to HDL which is lipolysed by hepatic lipase (HP) results in the formation of small dense LDL (Capell *et al.*, 1996, Ginsberg *et al.*, 2006).

High density lipoprotein metabolism is affected by obesity due to the increase in chylomicrons and VLDL remnants and impaired lipolysis (Klop *et al.*, 2013). The increased number of TG-rich lipoproteins results in increased CETP activity, which exchanges cholesterol esters from HDL for TG from VLDL and LDL (Subramanian and Chait, 2012).

Moreover, lipolysis of these TG-rich HDL occurs by hepatic lipase resulting in small HDL with a reduced affinity for Apo A-I, which leads to dissociation of apo A-I from HDL (Deeb *et al.*, 2003). This will ultimately lead to lower levels of HDL-C and a reduction in circulating HDL particles with impairment of reverse cholesterol transport (Deeb *et al.*, 2003).

Obesity has been shown to be associated with unfavourable lipid levels in the absence of metabolic disorders (Magkos *et al.*, 2008). Obesity was associated with 50-100% increase in VLDL, IDL and LDL; also obesity was shown to favour a more atherogenic lipid profile in the absence of metabolic disorders (Magkos *et al.*, 2008). However there are studies which have investigated apparently healthy obese individuals without any chronic illnesses which have found these individuals have abnormal lipids (Foster *et al.*, 1986, Choi *et al.*, 2002, Magkos *et al.*, 2008, Rocha *et al.*, 2013a).

One study found that in apparently healthy obese adolescents total body fat was associated with elevated serum lipids than BMI Choi *et al.*, (2002), while another study which excluded all chronic illness in the elderly, found WC and WHR were associated with elevated TG and low HDL-C levels (Rocha *et al.*, 2013a).

Obese people with no other metabolic disorders, also referred to as metabolically healthy obese (MHO), have normal HDL-C and TG levels despite their obesity due to their normal insulin levels and adipokines (Soriguer *et al.*, 2013, Blüher and Schwarz, 2014, Gonçalves *et al.*, 2016).

In abdominally obese people without metabolic disorders also known as metabolically

healthy abdominally obese (MHAO), TC, HDL-C and TG concentrations have all been found to be normal, although with age they tend to develop dyslipidaemia (Eshtiaghi *et al.*, 2015).

2.5 Obesity and lipoprotein subclasses

Obesity is associated with different lipoprotein particle sizes such as small LDL and also small HDL particles (Cali *et al.*, 2007). Magkos *et al.*, (2008) showed that obesity is associated with an unfavourable lipoprotein particle distribution.

A study by Hirooka *et al.*, (2013) in three different ethnic groups found that WC and BMI were associated with small LDL particles suggesting that obesity, whether abdominal or overall obesity, is associated with a pro-atherogenic lipoprotein particle size. Goedecke *et al.*, (2010) found that black women had more small dense LDL particles than their white counterparts.

Hirooka *et al.*, (2013) found that VAT was strongly associated with small LDL and large HDL particles across three different ethnic groups. While (Neeland *et al.*, 2013) found that VAT was more closely associated with small LDL and HDL particle size than subcutaneous adipose tissue (SAT).

The high level of TG in the liver leads to increased production of TG-rich VLDL particles which carry TG in the circulation. In obesity there is reduced LPL activity due to reduced mRNA expression in adipose tissue which leads to accumulation of TG-rich lipoproteins (Howard *et al.*, 2003).

Furthermore VLDL and chylomicrons compete for LPL, hence the accumulation of TG-rich lipoproteins (Clemente-Postigo *et al.*, 2011, Klop *et al.*, 2013). In people with obesity there is delayed clearance of VLDL which leads to an exchange of cholesterol ester in LDL with TG in VLDL mediated by cholesterol ester transfer protein (CETP) (Howard *et al.*, 2003).

This exchange results in TG-rich LDL particles that are rapidly lipolyzed by hepatic lipase to form smaller, denser LDL particles, Furthermore these small dense LDL particles are prone to oxidation and glycation, which may lead to an immune-response against the modified apoB-100 and formation of immunocomplexes (Howard *et al.*, 2003).

The reduced diameter of these particles increases the chances of moving through endothelial spaces into the sub-endothelial space where inflammation, leukocyte

ingestion, and transformation into plaque takes place (Kwiterovich, 2002).

This impaired TG-rich lipoprotein lipolysis leads to reduced HDL particle concentration by decreasing the transfer of apolipoproteins and phospholipids from TG-rich lipoprotein to the HDL particle (Howard *et al.*, 2003). The delayed clearance of TG-rich lipoproteins from the circulation allows for the exchange of cholesterol ester in HDL to the TG in VLDL which is mediated by CETP (Howard *et al.*, 2003). In obesity, the activity of hepatic lipase is raised, with HDL exchanging cholesterol for TG. These TG-rich HDL particles are lipolyzed by hepatic lipase into smaller HDL particles hence a predominance of small dense LDL particles and smaller HDL particles in obesity (Frenais *et al.*, 2001).

2.6. Adiponectin

Adipose tissue is not only a reservoir of fat energy but also functions as an endocrine tissue that secretes several bioactive molecules (Juge-Aubry *et al.*, 2005, Chang *et al.*, 2009). Adiponectin is a peptide secreted by adipose tissue and is found in large quantities in the plasma (Meilleur *et al.*, 2010).

It performs several functions amongst them are insulin sensitizing, anti-inflammatory and anti-atherosclerotic properties (Kern *et al.*, 2003, Yamauchi *et al.*, 2002, Kadowaki *et al.*, 2006, Yamauchi *et al.*, 2014). However unlike other adipose tissue hormones such as leptin and resistin, adiponectin is inversely associated with BMI (Weyer *et al.*, 2001, Kern *et al.*, 2003, Kadowaki *et al.*, 2006, Madsen *et al.*, 2008).

Human adiponectin gene codes for a 244 amino acid polypeptide and the primary sequence contains a signal peptide at the N-terminal which differs among different species, a collagenous region and C-terminal globular domain (Tsao *et al.*, 2002, Haluzik *et al.*, 2004).

It is mainly produced in the adipocytes and performs its action through two receptors which are AdipoR1 and AdipoR2 (Frayn *et al.*, 2003, Yamauchi *et al.*, 2014). Adiponectin structurally belongs to the complement 1q family known to form homomultimers (Shapiro and Scherer, 1998, Wong *et al.*, 2004). Adiponectin can be found in various forms and can be present as trimers, hexamers, high molecular weight oligomers and as full length adiponectin multimers (Singla *et al.*, 2010).

However in the plasma it seems to occur in its full length structure (Kadowaki and Yamauchi, 2005).

The formation of oligomer adiponectin depends on the formation of disulfide bonds mediated by Cys-3. In a mutation where there is substitution of Cys by Ser on codon 39 a trimer is formed which readily undergoes proteolytic cleavage and this trimer reduces glucose output in hepatocytes (Pajvani *et al.*, 2003, Kadowaki and Yamauchi, 2005). Wang *et al.*, (2002) showed that the hydroxylation and glycosylation of four lysine residues in the collagenous domain of adiponectin has an influence on the action of insulin on gluconeogenesis.

2.6.1 Actions of adiponectin

2.6.1.1 Adiponectin Receptors

The actions of adiponectin are all exerted through two adiponectin receptors adipoR1 and adipoR2 (Frayn *et al.*, 2003, Yamauchi *et al.*, 2014). AdipoR1 is expressed mainly in the skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver and both of them are also expressed widely in peripheral tissues and the brain (Hug *et al.*, 2004, Yamauchi *et al.*, 2014).The receptors have different affinities for globular and full length adiponectin (Yamauchi *et al.*, 2014).

AdipoR1 receptor has high affinity for globular adiponectin and low affinity for full length adiponectin while adipoR2 has intermediate affinity for both globular and full length adiponectin (Hug *et al.*, 2004, Yamauchi *et al.*, 2014). The binding of adiponectin to its receptor mediates increased adenosine monophosphate activated protein kinase (AMPK) and phosphorylation thus activating fatty acid oxidation and glucose uptake. The action of adiponectin on insulin sensitivity in muscle is mediated through AMPK and in the liver adiponectin activates glucose uptake by stimulating gluconeogenesis through AMPK (Fasshauer *et al.*, 2003).

2.6.1.2 Adiponectin and Insulin

Adiponectin sensitizes the body to insulin and it exerts this function through different mechanisms (Combs *et al.*, 2004). Adiponectin can achieve this by reducing triglyceride content in skeletal muscles by increasing the expression of molecules involved in fatty acid transport and also by oxidation of fatty acid (Yamauchi *et al.*, 2002). Increased TG content in the tissue interferes with insulin signalling and glucose transporter4 translocation, hence reduced glucose uptake and insulin resistance (Yamauchi *et al.*, 2002). By reducing of TG content, adiponectin improves insulin signal transduction (Yadav *et al.*, 2013).

2.6.1.3 Antiatherogenic effect of insulin

Adiponectin modulates communication between endothelial cells, smooth muscle cells,

leukocytes and platelets to protect against vascular injury and atherogenesis (Ouchi *et al.*, 2004, Sprague and Khalil, 2009).

Adiponectin achieves its cardio-protective action through various mechanisms which include its anti-inflammatory effects, stimulation of nitric oxide production, attenuation of pro-atherogenic mediators and modulation of coronary plaque vulnerability (Yadav *et al.*, 2013). The inhibition of atherosclerosis by adiponectin is shown in Figure 2.5.

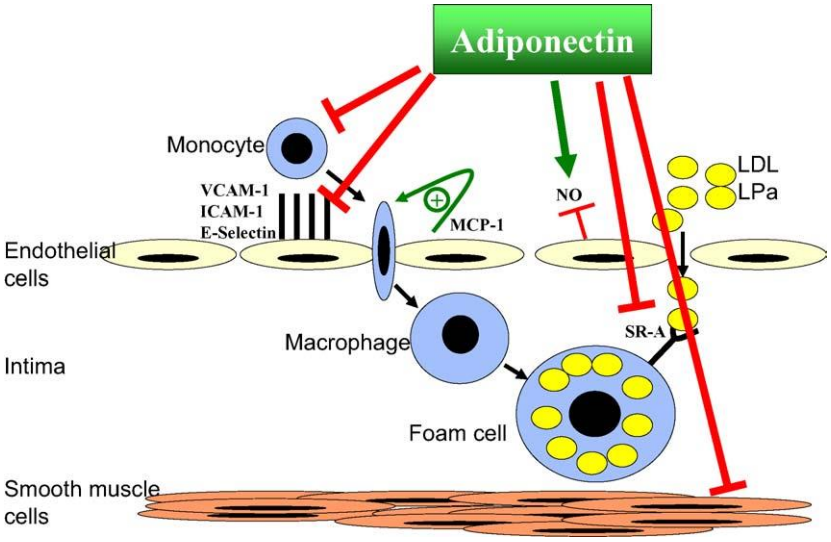

Figure 2.5: Mechanisms by which adiponectin inhibits atherosclerosis (Fasshauer *et al.*, 2003).

Another function of adiponectin is the suppression of inflammatory markers which are associated with its anti-atherogenic mechanisms. Low grade Inflammation is present in cardiovascular disorders and is also associated with low levels of adiponectin, the anti-inflammatory effect of adiponectin has been shown to be important (Hotta *et al.*, 2000, Villarreal-Molina and Antuna-Puente, 2012).

A study by Ouchi *et al.*, (2003) showed an inverse relationship between CRP and adiponectin. Further studies on adiponectin have shown it to suppress inflammatory markers such as TNFα and IL-6 while it increased the production of IL-10 (Wulster-Radcliffe *et al.*, 2004, Yamaguchi *et al.*, 2008).

2.6.1.4 Effect of Adiponectin on serum lipids

A positive association has been reported between adiponectin and HDL-C concentration and a negative association with TC, LDL-C concentrations and low

density lipoprotein particle size (LDL) (Nakamura *et al.*, 2004, Kazumi *et al.*, 2004).

Adiponectin has an independent role on triglyceride rich lipoprotein metabolism (Chan *et al.*, 2005). The role of adiponectin on lipid metabolism is through intrinsic changes in skeletal muscle lipid metabolism and it also has an effect on lipoprotein lipase (Yamauchi *et al.*, 2002, Havel, 2004, Combs *et al.*, 2004). Adiponectin decreases the accumulation of TG in skeletal muscle promoting fatty acid oxidation (Yamauchi *et al.*, 2002). In obesity this function is not maintained due to the low levels of adiponectin, hence the release of free fatty acid into the circulation (Chan *et al.*, 2005).

In skeletal muscles, binding of adiponectin to its receptor increases peroxisome proliferative activated receptor-α (PPAR-α) activity which stimulates fatty acid oxidation in myocytes (Yamauchi *et al.*, 2003).

Adiponectin further activates AMPK which in turn restores energy balance in muscle cells by increasing glucose uptake and oxidation of fatty acids (Kahn *et al.*, 2005). In the liver adiponectin acts via its AdipoR2 receptor to inhibit the liver's uptake of fatty acids from the circulation thus reducing TG production in the liver (Yamauchi *et al.*, 2003).

2.7 Adiponectin and obesity

Although secreted in abundance into the circulation by adipose tissue, the concentration of adiponectin is decreased in obesity which is in contrast to other adipokines secreted by adipose tissue (Juge-Aubry *et al.*, 2005, Matsuzawa, 2006).

Obesity seems to have a negative effect on the levels of adiponectin although the full mechanism of this is not fully understood. It is believed that the increased secretion of TNFα or other adipose tissue products in obesity may inhibit adiponectin secretion (Matsuzawa, 2006).

The distribution of body fat has shown to be related to adiponectin levels with visceral fat being more associated to adiponectin levels than subcutaneous fat (Cnop *et al.*, 2003, Staiger *et al.*, 2003). There is also a gender difference in the levels of adiponectin with women having higher adiponectin levels than men (Cnop *et al.*, 2003, Yatagai *et al.*, 2003).

Hulver *et al.*, (2004) found that adiponectin levels were higher in Caucasians than in African Americans (12.2vs7.1µg/ml) although there was no significant difference in adiponectin levels between obese and non-obese African Americans. A study by Weyer

et al., (2001) showed that adiponectin levels in Caucasians were higher than in Pima Indians and also that adiponectin levels had a negative relationship to measures of adiposity. In South Africa normal weight whites had higher adiponectin levels than normal weight blacks however there was no difference in adiponectin in obese people of both ethnic groups (Schutte *et al.*, 2007).

Chapter 3 Purpose of study

In this section detail of the purpose of the study such aims, objectives and research question are listed.

3.1 Aim

To determine and compare serum lipid concentrations and lipoprotein subclasses in obese and non-obese women without metabolic disorders, residing in a rural area

3.2 Objectives

- To measure waist circumference, weight and height and to calculate BMI.
- To identify and measure proportions of LDL and HDL subclasses.
- To measure Apo A-1 and Apo B levels.
- To measure serum lipid (HDL-C, triglycerides and total cholesterol) levels.

3.3 Research Question

What are the serum lipid levels and proportions of small dense LDL and HDL subclasses in obese women residing in a rural area?

Chapter 4 Methodology

In this section all aspects of methodology that were followed for the current study such as study design, study area, study population, sampling, ethical consideration, data collection, reliability and validity of the study and data analysis untaken in the study are discussed.

4.1Study design

The study design for this project was cross-sectional in which obese and non-obese subjects were selected from the Dikgale health demographic surveillance system (HDSS) database. A cross-sectional study is carried out once over a short period (Levin, 2006). It is conducted to estimate the prevalence of any factor in a given population for public health planning (Levin, 2006). The present study assessed the effect of obesity on serum lipids and lipoprotein subclasses in obese women without any other metabolic disorders to determine if there is risk of CVD. A cross-sectional study design was used because it allowed the investigator to assess the lipid levels and estimate the prevalence of abnormal lipid levels due to obesity and to assess the risk of CVD with serum lipids and lipoprotein subclasses in this area. A quantitative correlational study design will allow the investigator to observe the parameters at a single point in time and compare subjects with obesity and those without obesity.

4.2 (i) Study area

The study was undertaken at Dikgale Health and Demographic Surveillance System (HDSS) centre. Dikgale is a rural area in South Africa which is situated about 30-45km from Polokwane city, Limpopo province and approximately 15- 40km from the University of Limpopo (Turfloop Campus). Dikgale HDSS consists of 15 villages with a total population of approximately 35000 people. The dwellings in Dikgale villages are made up of shacks and brick houses. Electricity is available in all the dwellings in the villages and there is access to clean water. Figure 4.1 shows the location of Limpopo Province within South Africa and of Dikgale HDSS within the Province. Due the recent rise in prevalence of obesity both in urban and rural areas, the present study sought to investigate the effect of obesity in a rural area hence the current study area.

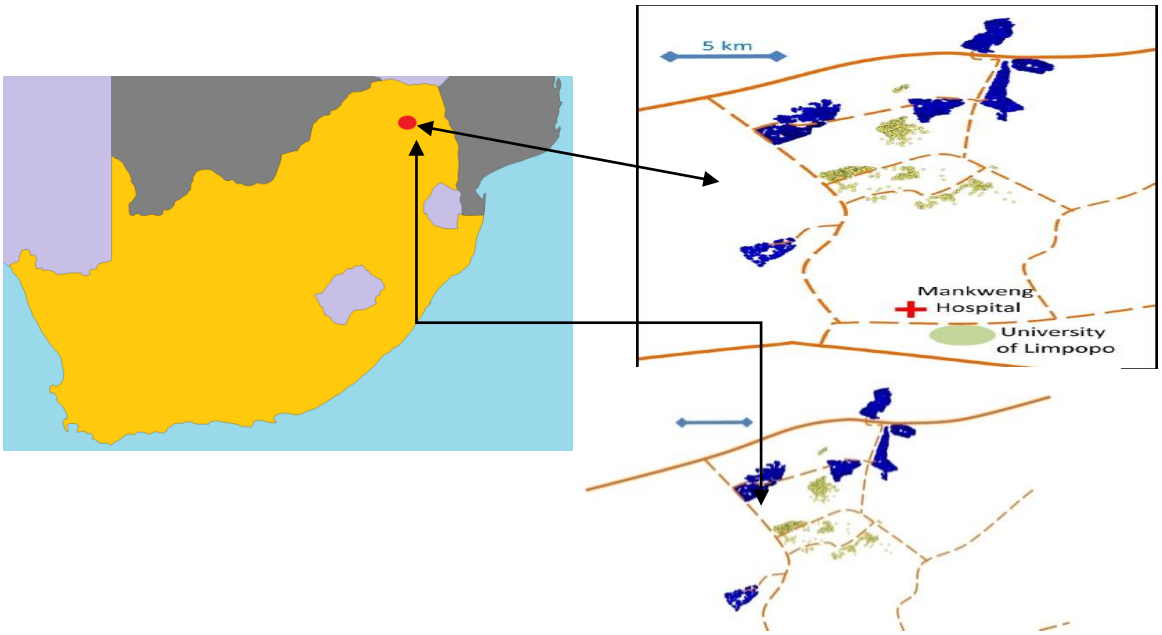

Figure 4.1: Maps of South Africa and Limpopo Province (Dikgale)(Alberts *et al.*, 2015).

(ii) Study population

Studies in South Africa, including those conducted in the Dikgale HDSS, have reported that obesity is more prevalent in women not only in this study population but also in South Africa compared to men (Alberts *et al.,* 2005, Steyn *et al.,* 2006). The present study focused on women because previous studies in this area reported women to be more obese and at increased risk of cardiovascular disease than men (Alberts *et al.,* 2005, Steyn *et al.,* 2006). These studies also showed that there is a rapid rise in prevalence of obesity in women in rural areas (Alberts *et al.,* 2005, Steyn *et al.,* 2006).

The other reasons for choosing to use women only is that previous studies in the Dikgale HDSS have shown that women are more willing to participate in health research than men and furthermore a number of men are migrant workers and thus not available for data collection (Alberts *et al.,* 2005, Steyn *et al.,* 2006).

4.3 Sampling

Sample size for the current study was calculated using the following formula.

$N= (Z_{\alpha/2}\sqrt{2p\,(1-p)} + Z_{\beta}\sqrt{p_1\,(1-p_1)\,p2\,(1-p_2)})^2 / (p_1-p_2)^2$

Where $Z_{\alpha/2}$ is the critical value of the normal distribution at α/2 (for a confidence level of 95%, α is 0.05 and the critical value is 1.96),

Z_{β} is the critical value of the normal distribution at β (for a power of 80%, β is 0.2 and

the critical value is 0.84)

p= (p1+p2)/2

p_1= 0.48 was the expected sample proportion in the control group

p_2 =0.71 was the expected sample proportions in the obese groups.

This calculation gave a minimum of 45 cases and 45 controls were also selected. However the current study used a sample size of 67 which would give enough statistical difference between obese and non-obese women.

The present study is part of the project “Prevention, Control and Integrated Management of Chronic Diseases in a rural area, South Africa” for which ethical approval has been obtained (MRECHS/06/2011: IR). In this project random sampling was performed which allowed everyone in the Dikgale HDSS to have equal chance of participating in that study. Therefore, the present study is also a random sample size because participants were selected from a random sample of the larger project.

After excluding women with diabetes mellitus, IR and inflammation, 308 women were left. Of these 308 women, 67 obese women were selected for the current study. Age matched (67) non-obese women were randomly selected from the remaining women and served as controls. Therefore the current study employed random sampling as women were selected from a randomly selected sample size.

- Exclusion criteria

Since the main focus of the research in the current study was to investigate changes in serum lipids and lipoprotein subclasses in obese women without metabolic disorders. women with metabolic disorders such as diabetes mellitus (fasting glucose≥7mmol/L), or reported being on treatment for diabetes mellitus, insulin resistance (HOMA-IR≥2.7), inflammation (hs-CRP≥10) and HIV were excluded from the study population. A report by Ombeni and Kamuhabwa, (2016) has shown that HIV patients develop dyslipidaemia. Women who were also on treatment that could affect lipid levels were also excluded from this study; the reason being that the current study was looking at the effect of obesity on lipid levels independent of any other factors that could affect lipid levels.

4.4 Ethical considerations

The present study was approved by the Medunsa Ethics Committee of the University of Limpopo (Medunsa campus) MREC/HS/271/2012:PG. All participants received counselling prior to giving blood by qualified counsellors, consent forms were also signed by participants before they could take part in the study. For the bigger study blood collection was done by qualified nurses. For the present study, stored samples from the main project were used for laboratory analysis and no new samples were collected. An information leaflet was distributed to the participants for the VLIR project (attached in appendix II). In the data base each participant has a specific ID number in order to maintain confidentiality and anonymity. Those who participated in the study received results on biochemical parameters such as glucose, insulin, serum lipids and also results on blood pressure, weight, height and BMI.

4.5 Data collection

The study was part of the VLIR project "Prevention, Control and Integrated Management of Chronic Disease in a rural area, South Africa". During my fourth year of study I was involved in training the field workers for data collection and I was also involved in the collection of data such as anthropometry and in the performance of all laboratory tests. Data collected for VLIR project included blood samples, lifestyle factors and anthropometry. All these data was used in the present study and no further data collection was done for the present study.

- Determination of lifestyle factors

The WHOSTEP questionnaire which is available at www.who.int/chp/steps translated into Sepedi (Northern Sotho) was administered by trained field workers. Questions on tobacco use, alcohol consumption and medical conditions were extracted from the WHOSTEP questionnaire.

- Anthropometric measurements

i. Weight (kg)

The body weight was measured to the nearest 0.1 kg with the subjects wearing light clothes and without shoes using an OMRON BF-400 digital scale which was calibrated against a beam balance and it was placed on a flat surface when taking the measurements.

ii. Height (cm)

Body height was measured to the nearest 0.1cm using stadiometer. The subjects were

standing vertically on the stadiometer without shoes, with the head straightened and the hands vertically at the side of the body.

iii. Calculation of Body Mass Index (BMI)

$$\mathrm{BMI} = \frac{weight(\mathrm{kg})}{height^2(\mathrm{m}^2)}$$

Reference ranges

<18.5kg/m^2-underweight

18.5-24.9 kg/m^2 normal weight

25-29.9 kg/m^2-overweight

≥30 kg/m^2 obese

iv. Waist circumference

Waist circumference was measured to the nearest 0.1cm using a measuring tape between the lowest rib and iliac crest. Reference range (<88cm normal WC) and (≥88cm increased WC)

v. Blood pressure (mmHg)

Blood pressure was measured using Omron M2 Compact sphygmomanometer (sp) (Omron Healthcare). Subjects had to relax for up to five minutes before being measured. Three measurements of BP were taken 3-5min apart and the mean of the last two measurements recorded. Reference ranges (120/80mm/Hg normal BP) and (≥140/90mm/Hg high BP)

- Blood collection and Laboratory analysis

Blood samples were taken by professional nurses for the VLIR project, serum and plasma separated and stored at -80^{o}C.

Laboratory methods

(a) **Determination of lipoprotein subclasses**

Lipoprotein particle sizes were determined using Polyacrylamide Gradient Gel Electrophoresis on a mini vertical gel electrophoresis (Amersham Biosciences UK).

Determination of the proportions of small and large HDL and LDL lipoprotein particles.

Principle of test

Electrophoretic mobility of lipoprotein subclasses is a function of macromolecular charge, size and shape. In non-denaturing native polyacrylamide gradient gel electrophoresis (N PAGE), Colloidal Blue G-250 dye coats proteins, masking the intrinsic protein charge. Thus G-250-coated lipoproteins of different sizes are sieved by the acrylamide gel with uniform electric strength, and the mobility of lipoproteins is inversely proportional to their size. In N-PAGE, lipoproteins migrate from a lower to a higher concentration of a polyacrylamide gradient in a slab gel. As the pores in the gel decrease in size, the migration rate of the lipoproteins also decreases. The lipoproteins reach their respective pore limits determined by their molecular sizes and form sharp bands.

Lipoprotein profiles were analysed using an Image Scanner III Labscan 6.0 (GE Healthcare) at 632nm with Image Master Software.

Materials

- Gloves
- Power supply
- Electrophoresis chamber
- Gel holders
- Pipettes
- Gel cutter
- Image Scanner III Labscan 6.0 (GE Healthcare)
- Measuring cylinder
- 4-16% non-denaturing polyacrylamide gradient gel

- Volumetric flask.
- Allegra X-22 Centrifuge (Beckman Coulter, Inc)

Reagents

All reagents were supplied by (Amersham Biosciences) and (Invitrogen by Life Technologies USA).

The running buffer was prepared by adding 50 ml of the 20X supplied running buffer (50mM BisTris and 50mM Tricine) to 950 ml distilled water and mixed. The supplied sample buffer (50mM BisTris, 6N HCl, 50mM NaCl, 10% w/v Glycerol, 0.001% Coomasie) was ready to use. Sudan black was prepared by dissolving 0.5g Sudan Black B in 20ml acetone and into this mixture 15ml of acetic acid and 85ml of distilled water were added. Then the mixture was stirred for 30 minutes using the magnetic stirrer and centrifuged for 20 minutes at 2000 rpm/448g to remove the precipitate. Lipid destain solution was prepared in three 100ml volumetric flasks where 15ml of acetic acid and 20ml of acetone were mixed with 65ml of distilled water in each flask. Fixation solution was prepared in three 100ml volumetric flasks where 15ml of acetic acid and 20ml of acetone were mixed with 65ml of distilled water in each flask. Colloidal Blue stain (protein stain) was prepared with colloidal blue staining kit containing stainer A and stainer B. Twenty millitre of stainer A, 5ml of stainer B and 20ml of methanol were added to 55ml of distilled water to make up 100ml protein stain in a 100ml volumetric flask. Protein destain was prepared by adding 8ml of acetic acid into a 100ml volumetric flask and distilled water was added to the mark. Protein standard which includes proteins such as (IgM Hexamer,IgM Pentamer, Apoferritin band , Apoferritin band 2, B-phycoerythrin, Lactate dehydrogenase, Bovine serum albumin, and Soybean trypsin inhibitor) was prepared from 5µl of Native Mark™ unstained protein standard, mixed with 2.5µl of sample buffer and 2.5µl of distilled water in a microtiter plate well. Sample was prepared by mixing 5µl of serum with 2.5µl sample buffer and 2.5µl of distilled water in a microtiter plate well to make up 10µl of the sample.

Gel preparation (NativePAGE Novex 4-16% Bis-Tris Gels)

The gel cassette was cut out of the plastic holder and dried with a paper towel. The comb was gently removed from the cassette and the wells were rinsed with running buffer three times to displace all air bubbles from the wells and then the buffer was removed through shaking of the gel cassette. The wells were filled with running buffer

to the halfway mark.

Procedure

LDL and HDL particle separation procedure

Lipoprotein subclass distribution and size were measured using the 4 to 16% non-denaturing polyacrylamide gradient gel electrophoresis of serum at 4°C. The 10µl participant serum sample was loaded using gel loading tips into each well containing running buffer and the 10µl of the protein standard was the added last into a dedicated well. After loading the samples and standards the tape at the bottom of the gel cassette was peeled and the cassette was placed in the Mini-Cell. The upper chamber of the Mini-Cell was filled with small amount of running buffer to check for tightness of seal as there is not supposed to be a leak from the Upper to the Lower buffer chamber and if there was no leakage the Upper chamber was filled with running buffer until it exceeded the level of the wells and the Lower chamber was filled to the level of the buffer in the Upper chamber.

The Mini-Cell lid was firmly placed on the corresponding electrodes and the electrode cords were connected to the power supply and the gel was subjected to electrophoresis at 150V for 60 minutes and then for last 30 minutes at 250V. After electrophoresis the gel cassette was taken out of the Mini-Cell and the gel knife was gently pushed in between the cassette to separate the plates and this was repeated on the sides of the cassette until plates are separated. The top plate was carefully removed and discarded and the gel remained on the bottom plate. The side of the gel with the well containing the standard was cut off from the wells containing the sample and the plate was held with the gel facing downwards over the container containing running buffer. The part of gel with protein standard was taken out and placed in a container with fixation solution and was placed in a microwave at high for 45 seconds and shaken on the orbital shaker for 15 minutes and this step was repeated and then the fixation solution was discarded and the colloidal blue staining solution was poured over the standard gel and the container was placed in a plastic bag to prevent evaporation and shaken on the orbital shaker overnight. The gel containing lipids was placed in a container with Sudan Black solution and was placed on the orbital shaker overnight as well. The staining solutions were discarded out of both containers the next morning and the gels were destained using the respective destaining solutions until the background of the gels was clear. The gels were read on the Image Scanner III Lab-scan 6.0 (GE Healthcare).

The coefficient of variance for this procedure ranged between 4.9%-10.3% for inter-

assay and between 0.78%-7.3% for intra-assay.

(b) **<u>Determination of serum lipids</u>**

I. Determination of serum Total Cholesterol (T-Chol) level

Cholesterol was measured on Instrumentation Laboratory analyser (ILab 300) from Instrumentation Laboratory Company (Italy).

Principle of the test

End point analysis based on the a modification of the method by Allain *et al.,* (1974)

Cholesterol esterase

Cholesterol esters → cholesterol + fatty acids

Cholesterol oxidase

Cholesterol $+0_2$ → cholest-4-en-3-one $+H_2O_2$

Peroxidase

$2H_2O_2$ + phenol +4-aminoantipyrine → red dye + 4 H_2O

Cholesterol esters in the blood reacted with water to form cholesterol and fatty acids, the reaction was catalysed by cholesterol esterase, and the cholesterol produced reacts with oxygen to form cholest-4-en-3-one and H_2O_2 in the presence of the enzyme cholesterol oxidase. $2H_2O_2$, phenol and 4-aminoantipyrine react together to produce a red dye and 4 H_2O, absorbance measurements was taken at 510 nm

The increase in absorbance as generated by the red dye is proportional to the cholesterol concentration in the sample

Materials

- Gloves
- Pipettes
- Pipette tips

- Sample cups
- 100 ml glass Beaker

Reagents

All reagents were supplied by Beckman Coulter (USA).

The Cholesterol reagent (chol reagent) contains (Cholesterol esterase, cholesterol oxidase, Peroxidase, Phenol, 4-aminoantripyrine and Tris buffer) was prepared using a volumetric pipette, 23 mL of distilled water was pipetted into one bottle of cholesterol reagent, the bottle was inverted several times to mix the solution, and it was then placed in the refrigeration compartment of ILab 300. SeraChem level 1 and seraChem level 2 reagents (used in monitoring the accuracy and precision of automated clinical chemistry methods) were prepared by adding 5ml of distilled water into each vial of reagent then mixed and allowed to stand for 30 minutes.

Procedure

In the ILab 300 4 µl of the patient sample and 320 µl of the cholesterol reagent are mixed and incubated at 2-8°C for 512 seconds and absorbance was then read at 510 nm.

Performance characteristics of the method according to the manufacturer were as follows, precision, within run-Coefficient of variation is 1.6%, and sensitivity is 1mg/dL. Method is not subjected to interference by lipemia up to sample absorbance of 2.3/cm at 660 nm. According to the manufacturer haemoglobin concentration of up to 100 mg/dL and bilirubin concentration of up to 14 mg/dL do not interfere with the method.

(ii) **Determination of high density lipoprotein cholesterol (HDL-C) level**

High density lipoprotein cholesterol was measured on Instrumentation Laboratory analyser (ILab 300) from Instrumentation Laboratory Company (Italy).

Principle of the test

This HDL-C assay is a homogeneous, direct method for measuring levels of HDL-C without the need for sample pre-treatment. Anti-human β-lipoprotein antibody in R1 binds to lipoproteins (LDL-C, VLDL-C AND Chylomicrons) other than HDL-C. The antigen-antibody complexes formed block enzyme reaction with all lipoproteins except HDL-C when R2 is added. Cholesterol esterase and cholesterol oxidase react only with HDL-C. Hydrogen peroxidase produced by enzyme reactions with HDL-C yields a blue

coloured complex upon oxidative condensation of the chromogen. Absorbance measurement was read at 620 nm. The concentration of the blue coloured complex is proportional to the concentration of HDL-C

Materials

- Gloves
- Pipettes
- Pipette tips
- Sample cups

Reagents

All reagents were supplied by Beckman Coulter (USA).

HDL-R1 and HDL-R2 reagents were ready to use. . SeraChem level 1 and seraChem level 2 reagents (used in monitoring the accuracy and precision of automated clinical chemistry methods) were prepared by adding 5ml of distilled water into each vial of reagent then mixed and allowed to stand for 30 minutes.

Procedure

In the ILab 300 300 µl of HDL R1 and 100 µl HDL R2 reagents and 3 µl of participant serum were mixed and incubated at 2-8°C for 242 seconds and the absorbance was then read at 620 nm.

Performance characteristics according to the manufacturer were as follows; Precision, within run-Coefficient of variation is 1.7% and Sensitivity is 1 mg/dL. Interferences, no interference from lipaemia up to sample absorbance of 2.7/cm at 660 nm. According to the manufacturer haemoglobin concentration of up to 100 mg/dL and bilirubin concentration of up to 14 mg/dL do not interfere with the method.

(iii) **Determination of Triglyceride (TG) level**

Triglyceride was measured on Instrumentation Laboratory analyser (ILab 300) from Instrumentation Laboratory Company (Italy).

Principle of test

End point analysis, based on the following reactions

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein lipase}} \text{glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-phosphate + fatty acids}$$

$$\text{Glycerol-3-phosphate + } 0_2 \xrightarrow{\text{Glycerophosphate oxidase}} \text{dihydroxyacetone phosphate+} H_20_2$$

$$H_2O_2 \text{ + 4-chlorophenol +4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{red quinoneimine + } 2H_20$$

Triglycerides in the sample are broken down into glycerol and fatty acids by lipoprotein lipase; glycerol then reacts with ATP to produce glycerol-3-phosphate and fatty acids. The reaction is catalysed by the enzyme glycerol kinase. Glycerol-3-phosphate reacts with oxygen in the presence of the enzyme glycerophosphate oxidase to produce dehydroxyacetone and $2H_20_2$. $2H_2O_2$, 4-chlorophenol and 4-aminoantipyrine react together to produce a red quinoneimine and $2H_20$. The absorbance measurements were read at 510 nm.

The increase in absorbance due to the red dye is proportional to the triglyceride concentration in the sample

Materials

- Gloves
- Pipettes
- Pipette tips
- Sample cups

Reagents

All reagents were supplied by Beckman Coulter (USA).

Triglyceride reagent is ready to use. SeraChem level 1 and seraChem level 2 reagents (used in monitoring the accuracy and precision of automated clinical chemistry methods) were prepared by adding 5ml of distilled water into each vial of reagent then mixed and allowed to stand for 30 minutes.

Procedure

In the ILab 300 300 µl of the triglyceride reagent and 3 µl of the participant serum were mixed and incubated at 2-8°C for 350 seconds and the absorbance was then read at 510 nm

Performance characteristics of the method according to the manufacturer were as follows; Precision, within run-Coefficient of variation is 2.3% and Sensitivity is 4 mg/dL. According to the manufacturer haemoglobin concentration of up to 100 mg/dL and bilirubin concentration of up to 14 mg/dL do not interfere with the method.

(c) **Determination of Apolipoproteins**

Apolipoprotein A-I was measured on IMMAGE instrument Beckman Coulter (USA).

Principle of the test

The IMMAGE apolipoprotein A test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during antigen-antibody reaction.

Apolipoprotein A-1 (Sample) + antibody→ [Apolipoprotein A-1 (sample)-antibody (aggregates)]

Materials

- Gloves
- Pipettes
- Pipette tips
- Sample cups

Reagents

All reagents were supplied by Beckman Coulter (USA).

Apolipoprotein A reagents are ready to use. Vigil control levels 1-4 (intended for monitoring the reliability of manual and automated in vitro diagnostic assays of lipid analytes) are also ready to use.

Procedure

In the IMMAGE 0.58 µl of the participant serum sample and 341.42 µl Apolipoprotein A reagent that is made up of 21 µl of antibody, 300 µl of buffer 1 and 20.42 µl of diluent are mixed.

Performance characteristics of the method according to the manufacturer were as follows; precision, within run-coefficient of variation is 4.0% and Sensitivity is 25 mg/dL. The method can detect concentrations between 25-225 mg/Dl. Method not subjected to interferences by bilirubin, lipids and haemoglobin

Participants' serum was assayed together with controls (Vigil lipid control level 1-4) to see if the results obtained are reliable.

(ii) **Determination of Apolipoprotein B-100 levels**

Apolipoprotein B-100 was measured on IMMAGE Beckman Coulter (USA).

Principle

The IMMAGE apolipoprotein B test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction

Apolipoprotein B (sample) +antibody→ [Apolipoprotein B (sample)-antibody (aggregates)

Materials

- Gloves
- Pipettes
- Pipette tips
- Sample cups

Reagents

All reagents were supplied by Beckman Coulter (USA).

Apolipoprotein B-100 reagents are ready to use. Vigil control levels 1-4 (intended for monitoring the reliability of manual and automated in vitro diagnostic assays of lipid analytes) are also ready to use.

Procedure

In the IMMAGE 0.58 µl of the participant serum sample with 341.42 µl Apolipoprotein B-100 reagent that is made up of 21 µl of antibody, 300 µl of buffer 1 and 20.42 µl of diluent are mixed.

Performance characteristics of the method according to the manufacturer were as follows; precision, within run-coefficient of variation is 4.0% and Sensitivity is 35 mg/dL. The method can detect concentrations between 35-225 mg/dL. Method not subjected to interferences by bilirubin, lipids and haemoglobin

Participants' serum was assayed together with controls (Vgil lipid control level 1-4) to see if the results obtained are reliable.

(iii) **Determination of high sensitive C-reactive protein level**

High sensitivity C-reactive protein (hs-CRP) was measured on IMMAGE Beckman Coulter (USA).

Principle of the test

SYNCHRON System(s) CRPH reagent is based on the highly sensitive near Infrared Particle Immunoassay rate methodology. An anti-CRP antibody-coated particle binds to CRP in the patient sample resulting in the formation of insoluble aggregates causing turbidity. The SYNCHRON System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 26 parts reagent. The system monitors the change in absorbance at 940 nanometres. This change in absorbance is proportional to the concentration of C-reactive protein in the sample and is used by the System to calculate and express C-reactive protein concentration based upon a single-point adjusted, pre-determined calibration curve

C-reactive protein (sample) +Particle bound anti-CRP→[C-reactive protein+antibody

complex]

Materials

- Gloves
- Pipettes
- Pipette tips
- Sample cups

Reagents

All reagents were supplied by Beckman Coulter (USA).

- Two CRPH Reagent Cartridges (2 x 200 tests)
- One lot-specific Parameter Card
- Reagent Buffer
- SYNCHRON® Systems CAL 5 Plus

Procedure

In the IMMAGE 10µL of patient serum and CRP reagent which contains 17.3L antibody and 47.8mL reagent Buffer and 185µL cartridge A and 70µL cartridge B are mixed.

Performance characteristics of the method according to the manufacturer were as follows; precision, within run-coefficient of variation is 5.0% and analytic sensitivity is 0.011mg/dL. The method can detect concentrations between 0.22-79.20 mg/dL. Method not subjected to interferences by bilirubin, lipids, haemoglobin and Rheumatoid factor

Participants' serum was assayed together with two controls to see if the results obtained were reliable.

(d) **Determination of Adiponectin level**

Adiponectin concentration was measured using the DTX 800 Multimode Detector Beckman Coulter (USA)

Principle of the test

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for high molecular weight (HMW) Adiponectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any HMW Adiponectin present is bound by the immobilized antibody. After washing away any unbound substance, an enzyme-linked monoclonal antibody specific for HMW Adiponectin is added to the wells.

Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of HMW Adiponectin bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

Materials

- Gloves
- Pipettes
- Pipette tips
- Plate shaker
- 96 microtiter plate
- 100 ml glass beaker
- Adhesive strip
- Paper towel

Reagents

Human HMW Adiponectin/Acrp 30 Immunoassay kit from R&D Systems (USA).

All reagents were brought to room temperature before use. If crystals had formed in the wash buffer concentrate it was warmed to room temperature and mixed gently until the crystals were completely dissolved. Twenty millitres of concentrated wash buffer

(concentrated solution of buffered surfactant with preservatives) was diluted with distilled water to prepare 500mL of Wash buffer. Substrate solution colour reagents A (stabilized hydrogen peroxide) and B (stabilized chromogen (tetramethylbenzidine) were mixed together in equal volumes within 15min of use. Mixture was protected from light. Two hundred microliters of the resultant mixture was required per well. High molecular weight adiponectin standard (Recombinant human Adiponectin in a buffered protein base with preservatives; lyophilized) was reconstituted with distilled water. This reconstitution produced a stock solution of 250ng/mL. The standard was mixed to ensure complete reconstitution and the standard was allowed to sit for a minimum of 15min before, it was then mixed well prior to making dilutions. Two hundred microlitres of Calibrator diluent RD6-39 (buffered protein base with preservatives) (ready to use) was pipetted into each tube. Six test tubes were labelled with concentrations of 125ng/ml, 62.5ng/ml, 31ng/ml, 15.6ng/ml, 7.8ng/ml and 3.9ng/ml respectively. In each of these test tubes 200µl of distilled water was added. From the standard stock solution of 250ng/ml 200µl was pipetted into the first tube labelled 125ng/ml and mixed; from the first tube 200µl was pipetted into the second test tube labelled 62.5ng/ml and mixed; from the second test tube 200µl was pipetted into the third test tube labelled 31ng/ml and mixed; from the third test tube 200µl was pipetted into the fourth test tube labelled 15.6ng/ml and mixed; from the fourth test tube 200µl was pipetted into the fith test tube labelled 7.8ng/ml and mixed and lastly from the fith test tube 200µl was pipetted into a test tube labelled 3.9ng/ml and mixed. Stop solution reagent (2 N sulfuric acid) was ready to use.

Sample Preparation

Serum/plasma samples required 100-fold dilution to reduce the concentration of adiponectin. 10µL of sample was added to 990µL of Calibrator Diluent RD6-61

Procedure

All reagents, working standards and samples were prepared as directed. Excess microplate strips from the plate frame were removed and returned the foil pouch containing the desiccant and resealed. One hundred microlitres of Assay Diluent RD1W (buffered protein base with preservatives) was added into each well. Fifty microlitres of Standard, control or sample were added per well. This was covered with the adhesive strip provided and incubated for 3 hours at room temperature. A plate layout was provided to record the standards and samples assayed. Each well was aspirated and washed for a total of four washes. Each well was aspirated and washed with wash

buffer (400µL) using a 1000µL pipette. Complete removal of liquid at each step was essential for good performance. After the last wash, any remaining wash buffer was removed by decanting. The plate was inverted and blotted against clean paper towels. Two hundred microlitres of HMW Adiponectin Conjugate (monoclonal antibody specific for human Adiponectin globular domain conjugated to horseradish peroxidase with preservatives) was added to each well to bind to the adiponectin present in patient sample. The plate was covered with a new adhesive strip. The plate was incubated for 1 hour at room temperature. Each well was washed four times. Two hundred microlitres of Substrate solution was added to each well and the substrate binds to the conjugate bound on adiponectin. The plate was incubated for 30 minutes at room temperature protected from light because light interferes with the reaction. Fifty microlitres of Stop solution (2 N sulfuric acid) was added to each well to stop enzyme activity. The colour in the well should change from blue to yellow to show that a reaction has occured. If the colour in the wells was green or the colour change did not appear uniform, the plate was gently tapped to ensure thorough mixing. The optical density of each well was determined within 30 minutes using a microplate reader (DTX 800 Multimode Detector Beckman Coulter (USA) set at 450nm.

Three samples of known concentration were tested in twenty separate assays to asses both intra-assay and inter-assay. Coefficient of Variance (%) for intra-assay was 2.6, 3.7 and 2.8 respectively and for inter assay 8.6, 8.5 and 8.3 respectively.

(e) **Determination of Glucose concentration**

Glucose was measured on Instrumentation Laboratory analyser (ILab 300) from Instrumentation Laboratory Company (Italy).

Principle of the test

End point analysis.Trinder methodology: glucose oxidase/peroxide

$$\text{B-D glucose} + 0_2 + H_20 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{red quinoneimine} + 4H_20$$

Glucose in the blood reacted with oxygen and water in the presence of the enzyme glucose oxidase to produce gluconic acid and hydrogen peroxide (H_2O_2). $2H_2O_2$,

phenol and 4-aminoantipyrine reacted together in the presence of peroxidase to produce a red quinoneimine and $4H_20$.

The absorbance was measured at 510nm. The increase in the absorbance generated by the red dye is proportional to the concentration in the sample.

Materials

- Gloves
- Pipettes
- Pipette tips
- Sample cups
- ILAB 300 Instrumentation Laboratory Company (Italy)

Reagents

All reagents were supplied by Beckman Coulter (USA).

GLUC OX reagent was prepared with a volumetric pipette, 12 ml of distilled water was mixed with one bottle of GLUC OX reagent, the bottle was inverted several times to mix the solution, it was then placed in the refrigeration compartment of I Lab 300. . SeraChem level 1 and seraChem level 2 reagents (used in monitoring the accuracy and precision of automated clinical chemistry methods) were prepared by adding 5ml of distilled water into each vial of reagent then mixed and allowed to stand for 30 minutes.

Procedure

In the ILab 300 4µl of the participant serum sample and 300 µl of the Gluc OX reagent were mixed and incubate for 80 seconds at 2-8°C and absorbance was read at 510nm

Performance characteristics of the method according to the manufacturer was as follows, Precision, Within run-Coefficient of variance is 2.2%, Sensitivity-0mg/dL and the method is not subjected interference from lipemia up to sample absorbance of 6.8/cm 660 nm. According to the manufacturer haemoglobin concentration of up to 100 mg/dL and bilirubin concentration of up to 14 mg/dL do not interfere with the method.

4.6 Reliability, Validity and Objectivity

To ensure accuracy controls were run simultaneously with the samples as indicated in protocol (Appendix I). Good analytical specificity of the method was further indicated by no interference by other blood constituents as outlined under each method (Appendix I). The reliability of each method was explained by the analytical co-efficient of variation (CV) outlined under each method (Appendix I) and field workers were trained for the current study. This work was validated through piloting during training. The WHO STEPS questionnaire has been previously validated (http://www.who.int/chp/steps/instrument/en/index.html).

4.7 Data analysis

In the present study obesity was diagnosed by BMI of ≥ 30kg/m^2. Abdominal obesity was diagnosed by WC≥88cm. Diabetes was diagnosed by fasting glucose of ≥7mmolL or by history on health collected by WHO questionnaire. Insulin resistance was diagnosed by HOMA of ≥2.7 and inflammation by hs-CRP of ≥10mg/L.

Body mass index was calculated using collected data on weight and height using the formula $\mathrm{BMI} = \frac{weight(\mathrm{kg})}{height^2(\mathrm{m}^2)}$. In the present study low density lipoprotein cholesterol concentration was also calculated using the formula, LDL-C=TOTAL CHOL-(HDL-CHOL+TRIG/2.2) (Marshall and Bangert, 2008).

The Statistical Package for Social Science (SPSS) version 22 was used for analysis. The distribution of variables was checked using a histogram plot. The variables that were normally distributed were expressed as the mean and 95% confidence interval (CI) and those that were not normally distributed were expressed as median (interquartile range) and logarithmically transformed before further analysis. Results of the current study were analysed to evaluate the effect of overall obesity on serum lipids and lipoprotein subclasses using BMI and to evaluate effect of abdominal obesity on serum lipids and lipoprotein subclasses using WC.

The student unpaired t-test was used to compare continuous variables between the obese group and the non-obese group. Pearson's correlation was used to test for correlations of measures of obesity, serum lipids, and adiponectin and lipoprotein subclasses. Backward multivariate linear regression was used for reporting standardized beta coefficient and P-value. The variables in the linear regression model included age, BMI, hs-CRP, adiponectin, tobacco use and alcohol use as predictors of serum lipids levels. Bivariate regression was used to investigate the odds of obese

women to have elevated serum lipid levels. The variable in the binary logistic regression model included serum lipids (TC, LDL-C, HDL-C and TG) lipid ratios (ApoB-100/Apo A-I and TC/HDL-C) with BMI and WC. Crosstabs were used to check the prevalence of abnormal lipids in both generally obese and women with abdominal obesity.

4.8 Bias

To minimise bias random sampling was used to select the subjects. Bias resulting from contribution of confounding factors was addressed by using partial correlation and regression analysis. Statistical bias was addressed by calculating the minimum sample size. Bias that may result from methodology was addressed by using validated methods.

Chapter 5: Results

The results of the current study are presented through tables and figures.

In the present study 134 women were investigated of which 67 were obese and 67 non-obese. The results of the present study were reported as mean and 95% confidence interval for normally distributed variables and as median and interquartile range for variables that are not normally distributed. The average age for an obese woman was 50 years ranging from (45.78-54.5) while for non-obese it was 48yrs ranging from (43.76-53.9). They were all of African origin and resided at Dikgale HDSS. **Table 5.1** shows mean (95%CI) of serum lipids, lipoprotein subclasses and other biochemical parameters among obese and non-obese women. **Table 5.2** shows the prevalence of abnormal serum lipids, lifestyle factors and other biochemical parameters among obese and normal weight women. **Table 5.3** shows the odds ratio for BMI in predicting abnormal lipids and **Table 5.4** shows predictors of elevated lipid levels in obese women.

5.1 Body mass index and serum lipids, lipoprotein subclasses, lifestyle factors and other biochemical parameters

Table 5.1: Mean (95% CI) of serum lipids, lipoprotein subclasses, lifestyle factors and other biochemical parameters among obese and non-obese women

	Non-Obese (18.5 kg/m^2≤BMI≤24.99 kg/m^2)	Obese (BMI≥30kg/m^2)	P-value
Population	67	67	
Age(yrs.)	48.83(43.76-53.9)	50.1(45.78-54.5)	0.609
SBP mm/Hg †	124.7(115.0-145.0)	131.0(114.5-155.0)	0.091
DBP mm/Hg †	82.1(78.5-85.7)	88.5(85.2-91.8)	0.004**
WC(cm)	79.2(76.4-82.0)	99.9(97.2-100.1)	0.000**
BMI (kg/m2)	23.33(22.29-24.36)	33.59(31.95-35.23)	0.000**

Total Chol(mmol/L)	4.77(4.45-5.10)	4.49(4.19-4.80)	0.558
LDL-C(mmol/L)	2.77(2.53-3.02)	2.66(2.42-2.90)	0.948
HDL-C(mmol/L) †	1.41(1.12-1.66)	1.15(0.99-1.42)	0.001**
Trig(mmol/L)†	0.98(0.63-1.73)	1.22(0.94-1.69)	0.087
TC/HDL-C	3.45(3.19-3.70)	3.82(3.55-4.09)	0.013*
Apo A-1(mg/dL) †	151.5(142.0-161.0)	141.1(133.4-148.6)	0.069
Apo B-100(mg/dL) †	86.2(79.7-92.7)	94.8(87.5-102.0)	0.067
ApoB-100/ApoA-I †	0.56(0.46-1.21)	0.65(0.54-0.86)	0.006**
LDL-small (%)†	19.12(13.38-21.76)	15.87(13.50-23.87)	0.489
LDL-large (%)	80.88(78.6-86.6)	84.13(76.1-86.5)	0.357
HDL-small (%)	43.97(39.75-48.20)	45.94(40.66-51.2)	0.666
HDL-large (%)	56.03(52.6-60.4)	54.06(48.77-59.3)	0.521
Glucose (mmol/L)	4.98(4.74-5.22)	5.26(5.04-5.49)	0.032**
Insulin(ulU/mL)†	6.20(3.65-13.41)	7.54(3.84-15.60)	0.224
HOMA-IR†	1.81(1.50-2.12)	2.27(1.92-2.64)	0.097
Adiponectin(mg/ml) †	3.99(2.34-6.69)	2.38(1.22-3.72)	0.004**
CRP(mg/L)†	1.14(0.57-2.67)	3.48(0.82-6.97)	0.003**
Tobacco use (%)	4.5(3)	13.4(9)	0.064
Alcohol (%)	7.5(5)	19.4(13)	0.037*

**Significant at p-value≤ 0.001, *Significant at P-value≤0.05, † Median (interquartile range)

There was no difference in systolic blood pressure between obese and normal weight women (p=0.091). Diastolic blood pressure and WC were significantly higher in obese women than in the normal weight women (p=0.005 and p= 0.000) respectively.

Total cholesterol and LDL-C were not significantly different between normal weight and obese women (p=0.558 and p=0.958 respectively).

HDL-C levels were significantly lower in obese women than normal weight women (p=0.001), while the TC/HDL-C ratio was significantly higher in obese women than in non- obese women (p=0.013). There was no significant difference in TG levels between the two groups (p=0.087). There was no difference in the proportions of lipoprotein subclasses between the two groups.

There was no significant difference in Apo B-100 l and Apo A-I levels while the Apo B-100/Apo A-I ratio was significantly higher in obese than non- obese women (p=0.006). Obese women showed significantly increased fasting glucose levels (p=0.032) as compared to non-obese women.

Adiponectin levels were significantly lower in obese than non- obese women (p= 0.005). Obese women had significantly higher hs-CRP levels than non- obese women (p=0.013). A higher percentage of obese women consumed alcohol as compared to non- obese women (p= 0.037), while there was no difference in tobacco use between the two groups (p=0.065).

5.2 Body mass index and prevalence of abnormal parameters

Table 5.2: The prevalence of abnormal serum lipids and biochemical parameters among obese and non- obese women

Abnormal lipids	Non-obese %(n)	Obese %(n)	P-value
High TC (≥5.0mmol/L)	43.3(29)n=67	33.3(22)n=66	0.286
High LDL-C (≥3.0mmol/L)	46.3(31) n=67	44.6(29)n=65	0.295
Low HDL-C (≤1.3 mmol/L)	13.4(9)n=67	27.6(18)n=65	0.053
High TG(≥1.7mmol/L)	26.9(18)n=67	23.9(16)n=67	0.853
High TC/HDL-C(≥5)	7.5(5)n=67	16.9(11)n=65	0.115
High Apo B-100/Apo A-I (≥0.85)	9.8(6)n=61	26.2(17)n=65	0.021*
		54(27)n=50	0.012*
Low Adiponectin (≤2.52mg/ml)	30.2(16)n=53	53.7(36)n=67	0.000*

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05, † Log transformed variables.

The prevalence of hypertension was significantly higher in obese women as compared to non-obese women (p= 0.009). 76.1% of obese women were hypertensive while 37.3% non-obese women were hypertensive. The prevalence of abnormal levels of TC, TG, HDL-C and LDL-C were not significantly different between the two groups (p=0.286, p=0.853, p=0.053 and p=0.295 respectively).

There was also no significant difference in the prevalence of high TC/HDL-C ratio (≥5) between obese and non-obese women (p=0.115), while there was a significant difference in the prevalence of high Apo B-100/Apo A-I (≥0.85) between obese and non-obese women (p=0.021). 26.2% obese women had high ApoB-100/Apo A-I ratio as compared with 9.8% of non-obese women. The prevalence of low Adiponectin (≤2.52mg/ml) was significantly higher in obese women compared to non-obese women (p=0.012).

Over 55% of obese women had low adiponectin levels compared to 30.2% of non-obese women. The prevalence of elevated hs-CRP was also significantly higher in obese women compared to non-obese women (p=0.000). Over 50% of obese women

had high levels of hs-CRP (≥3mg/L) as compared to 22.5% of non-obese women.

5.3 Binary logistic regression and BMI

Binary logistic regression was performed to determine the odds ratio of having abnormal serum lipid levels in obese women. The results are presented in Table 5.3.

Table 5.3: The odds ratio of abnormal serum lipids in obese compared to non-obese women

	High TC Odds(CI)	High LDL-C Odds(CI)	High TG Odds(CI)	Low HDL-C Odds(CI)	High ApoB-100/Apo A-I Odds(CI)
Non-obese	1	1	1	1	1
Obese	0.66(0.32-1.33)	0.68(0.35-1.36)	0.85(0.39-1.86)	3.30(1.62-6.76)**	3.25(1.19-8.90)*

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

The likelihood that obese women will have elevated total cholesterol, LDL-C and triglyceride levels was not significant (0.66, 0.68 and 0.85) respectively. Obese women were 3 times more likely to develop low HDL-C levels (p=0.001) and obese women were also three times more likely to have high Apo B-100/Apo A-I ratio (p=0.022)

5.4 Linear regression and BMI

Backward multivariate linear regression was performed to determine predictors of TC and LDL-C. Those variables with p-value ≤0.05 in the last module were predictors of TC and LDL-C. There were no significant predictors for large and small LDL subclasses hence they were not included in regression analysis.

Table 5.4.1: Predictors of TC and LDL-C in obese women.

TC	LDL-C

Variables	1ST model β(P-value)	last model β (P-value)	1ST model β (P-value)	last model β (P-value)
Age	0.032(0.000)	0.033(0.000)**	0.026(0.000)	0.023(0.000)**
BMI	-0.031(0.035)	-0.030(0.030)*	-0.019(0.102)	
hs-CRP	-0.055(0.836)	-0.712(0.013)*	0.035(0.850)	
Adiponectin	-0.659(0.030)		-0.356(0.151)	
Tobacco use	-0.050(0.927)		0.059(0.870)	
Alcohol	-0.252(0.030)		-0.377(0.261)	

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Age showed significant positive association with both Total cholesterol and Low density lipoprotein cholesterol in obese women. Adiponectin and BMI showed a significant negative association with total cholesterol (Table 5.4.1).

Backward multivariate linear regression was performed to determine predictors of HDL-C and TG. Those variables with p-value ≤0.05 in the last module were predictors of HDL-C and TG.

Table 5.4.2: Predictors of HDL-C and TG in obese women.

	HDL-C			TG
Variables	1ST model	last model	1ST model	last model
Age	β(P-value)	β (P-value)	β (P-value)	β (P-value)
BMI	0.0000016(0.982)	-0.005(0.000)**	0.005(0.005)	0.005(0.000)**
Hs-CRP	-0.005(0.003)		0.002(0.550)	
Adiponectin	-0.017(0.509)		-0.016(0.751)	
Tobacco use	0.006(0.853)		-0.173(0.013)	
Alcohol	-0.025(0.588)		0.025(0.810)	
	0.002(0.9560		0.061(0.505)	-0.198(0.002)*

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Body mass index showed a significant negative association with HDL-C. Age was positively associated with TG levels and adiponectin negatively associated with TG levels. (Table 5.4.2)

Backward multivariate linear regression was performed to determine predictors of TC/HDL-C and Apo B/Apo A. Those variables with p-value ≤0.05 in the last module were predictors of TC/HDL-C and Apo B/Apo A. (Table 5.4.3)

Table 5.4.3: Predictor of TC/HDL-C and Apo B/Apo A in obese women.

	TC/HDL-C		Apo B-100/Apo A-I	
Variables	1ST model	last model	1ST model	last model
Age	β(P-value)	β (P-value)	β (P-value)	β (P-value)
BMI	0.028(0.000)	0.022(0.000)**	0.003(0.001)	0.003(0.001)**
hs-CRP	0.015(0.226)		0.006(0.001)	0.006(0.000)**
Adiponectin	0.156(0.519)		0.053(0.080)	-0.076(0.029)*
Tobacco use	-0.579(0.027)		-0.069(0.053)	
Alcohol	0.175(0.652)		0.028(0.583)	
	-0.262(0.555)	-0.681(0.005)*	-0.030(0.053)	

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Age showed significant positive association with both TC/HDL-C and Apo B-100/Apo A-I ratio, while BMI was significant associated with Apo B-100/Apo A-I ratio (P=0.000) in obese women. Adiponectin showed a significant negative association with TC/HDL-C and Apo B-100/Apo A-I ratios (p= 0.005 and 0.029 respectively) (Table 5.4.3)

Table 5.4.4: Predictors of large and small HDL particles in obese women.

	HDL large particle		HDL small particle	
Variables	1ST model	last model	1ST model	last model
	β(P-value)	β (P-value)	β (P-value)	β (P-value)
Age	-0.133(0.237)		0.122(0.285)	
BMI	-0.093(0.0692)		0.063(0.790)	
hs-CRP	-0.313(0.925)		0.325(0.923)	
Adiponectin	7.352(0.130)		-7.732(0.115)	
Tobacco use	-11.196(0.111)	-	11.083(0.117)	
Alcohol	-13.155(0.051)	13.761(0.019)*	13.559(0.037)	13.380(0.023)*

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Alcohol use was significantly negatively associated with large HDL particles and positively associated with small HDL particles. (Table 5.4.5)

5.5 Waist circumference and serum lipids, lipoprotein subclasses, lifestyle factors and biochemical parameters

The women in the present study were divided into two groups dependent on waist circumference. Those with a WC of 88cm and above were regarded as abdominally obese. The average age for abdominally obese women was 53yrs while for those without abdominal obesity it was 45yrs. **Table 5.8** shows predictor of elevated lipid levels in women with large WC.

The characteristics of women with large and normal waist circumferences are shown in **Table 5.5.**

Table 5.5: Mean (95%CI) of serum lipids, lipoprotein subclasses, lifestyle factors and biochemical parameters among women with normal and large waist circumference

	Normal WC<88cm	Large WC≥88cm	P-value age adjusted
Population F	65	69	
Age(yrs.)	45.54(40.72-50.37)	53.23(48.82-57.64)	0.018**
SBP mm/Hg †	124.00(112.25-145.50)	130.00(117.75-155.00)	0.048*
DBP mm/Hg	81.84(78.22-85.46)	88.61(85.33-91.88)	0.012*
WC(cm)	79.2(76.4-82.0)	99.9(97.2-100.1)	0.000**
BMI (kg/m^2)	23.33(22.29-24.36)	33.59(31.95-35.23)	0.000**
Total Chol(mmol/L)	4.69(4.37-5.01)	4.58(4.26-4.89)	0.756
LDL-C(mmol/L)	2.73(2.49-2.97)	2.70(2.45-2.95)	0.553
HDL-C(mmol/L) †	1.41(1.13-1.64)	1.12(0.99-1.44)	0.000**
Trig(mmol/L)†	0.88(0.61-1.70)	1.26(1.00-1.87)	0.002**
TC/HDL-C	3.35(3.12-3.59)	3.90(3.63-4.18)	0.000**

Apo A-1(mg/dL) †	149.33(139.94-158.72)	143.24(135.36-151.12)	0.408
Apo B-100(mg/dL) †	86.47(80.02-92.92)	94.40(87.13-101.67)	0.039*
ApoB-100/ApoA-I †	0.58(0.47-0.62)	0.65(0.51-0.85)	0.025*
HDL-large (%)	58.59(54.17-62.49)	52.61(47.42-57.80)	0.136
HDL-small (%)	42.41(38.20-46.63)	47.39(42.20-52.58)	0.201
LDL-large (%)	80.93(78.00-86.00)	84.14(76.31-87.59)	0.229
LDL-small (%)†	19.07(14.01-22.66)	15.86(12.42-23.12)	0.772
Glucose(mmol/L)	4.89(4.64-5.15)	5.34(5.13-5.55)	0.004**
Insulin(ulU/mL)†	7.33(4.35-13.51)	7.11(3.42-15.60)	0.759
HOMA-IR†	1.89(1.58-2.20)	2.19(1.83-2.55)	0.319
Adiponectin(mg/ml) †	3.6(2.25-6.75)	2.41(1.24-4.61)	0.016*
CRP(mg/L)†	1.24(0.70-2.97)	3.21(0.73-6.84)	0.048*
Tobacco use (%)	6.2(4)	11.6(8)	0.213
Alcohol (%)	12.3(8)	14.5(10)	0.454

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05, † Log transformed variables.

Since women with high WC were significantly older than women with normal WC, the p-values were age adjusted. Diastolic blood pressure was found to be significantly higher in women with high WC than normal WC while SBP was not significantly different between the two groups (p=0.012 and p=0.058). There was no significant difference in TC and LDL-C levels between the two groups (p=0.756 and p=0.553 respectively).

HDL-C levels were significantly lower in women with large WC than in women with normal WC (p=0.000). TG levels were significantly higher in women with large WC than those with normal WC (p=0.002). The TC/HDL-C and ApoB-100/Apo A-I ratio were significantly higher in women with large WC than those with normal WC (p=0.000 and p=0.025 respectively).

There was no significant difference in the levels of Apo A-I (p=0.508), while Apo B-100 levels were significantly higher in women with large WC than those with normal WC (p=0.039).

There was no difference in the proportions of either large or small HDL particles or small or large LDL particles (p=0.136 and 0.201, P=0.229 and p=0.772 respectively). Adiponectin levels were significantly lower in women with large WC than those with normal WC, while hs-CRP levels were significantly higher in women with large WC than those with normal WC (p=0.016 and p=0.048).

There was no difference in tobacco use and alcohol consumption between the two groups (p= 0.213 and p=0.454).

5.6 Waist circumference and prevalence of abnormal parameters

In **Table 5.6** the prevalence of abnormal parameters in women with high waist circumference and those with normal waist circumference is presented

Table 5.6: The prevalence of serum lipids and abnormal parameters in women with normal and large waist circumference.

Abnormal Variables	Normal WC %(n)	Large WC %(n)	P-value
Hypertension(≥140/90mmHg)	35.4(23)n=65	76.8(53)n=69	0.002*
High TC (≥5mmol/L)	38.5(25)n=65	37.7(26)n=69	1.000
High LDL-C (≥3mmol/L)	41.5(27)n=65	41.2(28)n=68	1.000
Low HDL-C (≤1.3 mmol/L)	12.3(8)n=65	27.9(19)n=68	0.032*
High TG(≥1.7mmol/L)	24.6(16)n=65	26.1(18)n=69	1.000
High TC/HDL-C(≥5)	7.7(5)n=65	17.6(12)n=68	0.061
	10(6)n=60	25.8(17)n=66	0.036*
High Apo B-100/Apo A-I (≥0.85)	32.7(17)n=52	51(26)n=51	0.075
Low Adiponectin (≤2.52mg/ml)	24.6(16)n=65	50.7(35)n=69	0.002*
High CRP (≥3mg/L)			

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05, † Log transformed variables.

The prevalence of hypertension was significantly higher in women with high WC compared to those with normal WC (p=0.002). Over 60% of women with high WC were hypertensive compared to 35.5% of women with normal WC.

Although the prevalence of high TC, LDL-C and TG were higher in women with large WC than those with normal WC the difference was not significant (p=1.000, p=1.000

and p=1.000) respectively. The prevalence of low HDL-C levels was significantly higher in women with high WC than those with normal WC (p=0.032). 27.9% of women with large WC had low HDL-C levels compared to 12.5% in normal WC women.

While there was no significant difference in the prevalence of high TC/HDL-C ratio (p=0.061) the prevalence of high ApoB-100/Apo A-I ratio was significantly higher in women with high WC than in women with normal waist circumference (p=0.036). There was also no significant difference in the prevalence of low adiponectin levels between women with large WC and normal WC (p=0.075).

There was however a significant difference in the prevalence of high hs-CRP levels between women with large and normal WC (p=0.002). Over 50% of women with large WC had high levels of hs-CRP compared to 25.6% in women with normal WC.

5.7 Binary logistic regression and WC

Binary logistic regression was also performed to determine the odds ratio of women with increased WC to have abnormal lipid levels.

Table 5.7: The odds ratio of abnormal serum lipids in women with large waist circumference compared to women with normal waist circumference.

	High TC Odds(CI)	High LDL-C Odds(CI)	High TG Odds(CI)	Low HDL-C Odds(CI)	High ApoB-100/Apo A-I Odds(CI)
Normal WC	1	1	1	1	1
Large WC	0.95(0.57-1.90)	0.96(0.58-1.92)	1.08(0.50-2.36)	3.05(1.50-6.21)*	3.12(1.15-8.56)*

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Similar to overall obese women, the odds of having elevated TC, TG and LDL-C (0.95, 1.08 and 0.96) respectively were not significant p (0.870, 0.906 and 0.855) respectively (table 5.7.).

Women with abdominal obesity were also 3 times more likely to have a low HDL-C concentration and elevated ApoB-100/ApoA-I ratio. There was no significant difference

in the odds ratio of lipoprotein subclasses, hence they are excluded.

5.8 Linear regression and WC

Backward multivariate linear regression was performed to determine predictors of TC and LDL-C. Those variables with p-value ≤0.05 in the last module were predictors of TC and LDL-C. (Table 5.8.1)

Table 5.8.1: Predictors of TC and LDL-C in abdominally obese women.

	TC		LDL-C	
Variables	1ST model β(P-value)	last model β (P-value)	1ST model β (P-value)	last model β (P-value)
Age	0.036(0.000)	0.036(0.000)**	0.028(0.000)	0.023(0.000)**
WC	-0.015(0.093)	-0.655(0.021)*	-0.009(0.170)	
Hs-CRP	-0.056(0.795)		0.029(0.868)	
Adiponectin	-0.609(0.055)		-0.327(0.186)	
Tobacco use	-0.059(0.895)		0.057(0.897)	
Alcohol	-0.255(0.551)		-0.378(0.262)	

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Age was positively associated with both TC and LDL-C levels in women with abdominal obesity (p=0.000 and p=0.001 respectively). While adiponectin was negatively associated with TC levels in obese women

Backward multivariate linear regression was performed to determine predictors of HDL-C and TG. Those variables with p-value ≤0.05 in the last module were predictors of HDL-C and TG. (Table 5.8.2)

Table 5.8.2: Predictors of HDL-C and TG in abdominally obese women.

	HDL-C		TG	
Variables	1ST model	last model	1ST model	last model
Age	β(P-value)	β (P-value)	β (P-value)	β (P-value)
WC	0.001(0.826)	-0.002(0.002)*	0.005(0.015)	0.005(0.000)**
Hs-CRP	-0.002(0.006)		0.003(0.138)	-0.198(0.002)*
Adiponectin	-0.018(0.500)		-0.025(0.611)	
Tobacco use	0.012(0.698)		-0.168(0.015)	
Alcohol	-0.027(0.555)		0.025(0.805)	
	0.002(0.962)		0.067(0.558)	

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Age showed a significant positive association with TG levels in women with abdominal obesity (p=0.000). While WC had a significant negative association with HDL-C levels and adiponectin also showed a significant negative association with TG levels in women with abdominal obesity (Table 5.8.2)

Backward multivariate linear regression was performed to determine predictors of TC/HDL-C and Apo B/Apo A. Those variables with p-value ≤0.05 in the last module were predictors of TC/HDL-C and Apo B/Apo A. (Table 5.8.3)

Table 5.8.3: Predictors of TC/HDL-C and Apo B/Apo A in abdominally obese women.

	TC/HDL-C		Apo B/Apo A	
Variables	1ST model	last model	1ST model	last model
Age	β(P-value)	β (P-value)	β (P-value)	β (P-value)
WC	0.005(0.023)	0.005(0.005)*	0.002(0.011)	0.002(0.000)**
hs-CRP	0.005(0.017)	0.005(0.013)*	0.002(0.078)	-0.093(0.010)*
Adiponectin	-0.019(0.702)	-0.195(0.005)*	0.058(0.065)	
Tobacco use	-0.185(0.010)		-0.083(0.022)	
Alcohol	-0.001(0.995)		0.025(0.637)	
	0.128(0.182)		-0.025(0.612)	

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Age had a significant positive association with both TC/HDL-C and Apo B-100/Apo A-I ratios (p=0.005 and 0.000 respectively). Adiponectin showed a significant negative association with both TC/HDL-C and Apo B-100/Apo A-I ratios (p=0.005 and 0.010 respectively). While in women with abdominal obesity WC was positively associated with TC/HDL-C levels.

Backward multivariate linear regression was performed to determine predictors of large and small HDL particle. Those variables with p-value ≤0.05 in the last module were predictors of large and small HDL particle. (Table 5.8.4)

Table 5.8.4: Predictors of large and small HDL particle in abdominally obese women.

	HDL large particle		HDL small particle	
Variables	1ST model	last model	1ST model	last model
	β(P-value)	β (P-value)	β (P-value)	β (P-value)
Age	-0.085(0.452)	-0.275(0.018)*	0.075(0.511)	0.261(0.026)*
WC	-0.236(0.065)	-	0.231(0.073)	12.377(0.034)*
Hs-CRP	0.690(0.834)	13.386(0.044)*	-0.727(0.827)	
Adiponectin	6.270(0.185)	-	-6.573(0.168)	
		12.708(0.028)*	11.281(0.105)	
Tobacco use	-11.385(0.099)		14.056(0.028)	
Alcohol	-13.685(0.031)			

**Significant at p-value≤ 0.001, *Significant at p-value≤0.05

Tobacco use and alcohol consumption were significant negative predictors of the large HDL particle (p=0.028 and p=0.044 respectively) and also alcohol consumption was again significant positively associated with small HDL particle size (p=0.034) in women with abdominal obesity. Waist circumference also showed a significant positive association with small HDL particle, while it had a significant negative association with large HDL particle size.

Figure 5.1 below shows the migration distribution of large and small LDL and HDL subclasses on a 4-16% polyacrylamide gel. The lipoprotein subclasses are separated according to their size. As seen in Figure 5.1 the small HDL subclasses migrate the furthest on the gel while the larger LDL subclasses migrate the least.

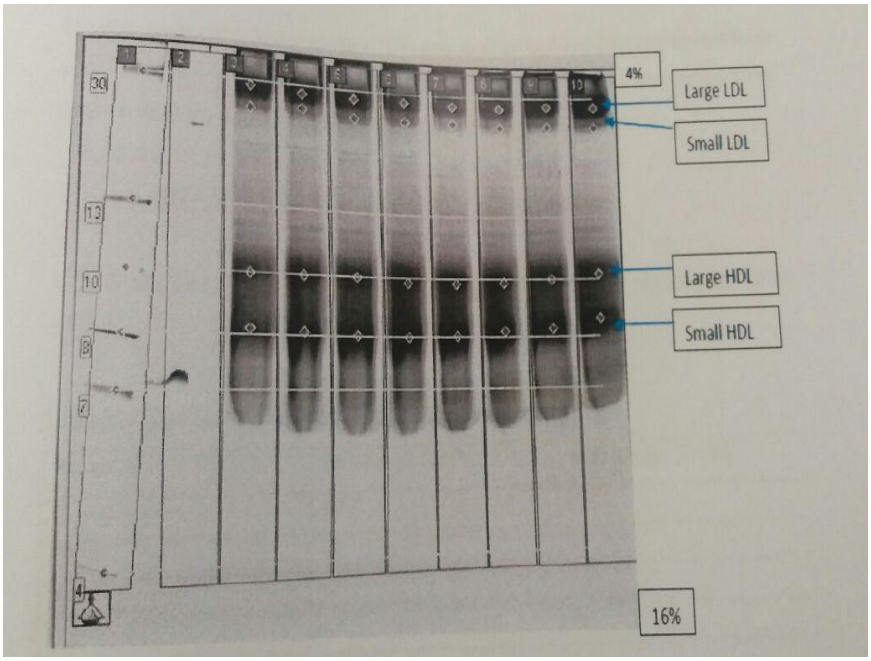

Figure 5.1: Image of Lipoprotein subclasses on gel electrophoresis

Chapter 6 Discussion

6.1 Introduction

The aim of the current study was to assess CVD risk relating to lipid metabolism in obese black women residing in rural areas. It was hypothesised that obesity is associated with dyslipidaemia including an increase in the proportion of small dense lipoprotein particles independent of metabolic disorders.

There is limited information on the association between obesity and lipid levels independent of other metabolic disorders in African women from rural areas. A study by Magkos *et al.*, (2008) investigated the association between obesity and lipoprotein concentrations independent of other metabolic disorders in Caucasians, in order to assess whether obesity on its own is associated with changes in serum lipoprotein levels. They found that obesity was associated with atherogenic lipoprotein alterations such as increases in plasma concentrations of VLDL, IDL and LDL together with increases in small LDL and HDL particles in the absence of classical metabolic risk factors. In this chapter the findings of the present study and the association between obesity, serum lipids, adiponectin and apolipoproteins are discussed.

6.2 Obesity, Total Cholesterol, LDL-C and apoB-100 levels

In the current study there was no significant difference in TC and LDL-C concentrations between obese and non-obese women. Similarly no differences were observed in total cholesterol and LDL-C levels between women with abdominal obesity and women without abdominal obesity in the present study. These results are in contrast to other studies which demonstrated significantly elevated total cholesterol and LDL-C levels in obese women (Maksvytis and Stakisaitis, 2004, Chehrei *et al.*, 2007, Lavie *et al.*, 2009). It is however important to note that in these studies women with metabolic disorders were not excluded.

A study by Després *et al.*, (2001) showed that abdominal obesity was associated with elevated TG and low HDL-C concentrations, with LDL-C in the normal range and an increase in proportions of small dense LDL particles. While Shamai *et al.*, (2011) suggested that it is LDL particle size that is affected by obesity rather than LDL-C concentration and they showed that BMI was associated with LDL particle size and not with LDL-C concentration.

In the current study there was also no difference in the prevalence of elevated cholesterol and elevated LDL-C concentrations in obese and non-obese women.

These results are in contrast to other studies in rural settings which have found an elevated prevalence of cholesterol in obese individuals (Brown *et al.*, 2000, Control and Prevention, 2010, Sengwayo *et al.*, 2012).

The current study also investigated the association between obesity and Apo B-100 concentration. ApoB-100 represents the atherogenic lipids and is found in low, intermediate and very low density lipoprotein particles (Marcovina and Packard, 2006, Thompson and Danesh, 2006).

In the current study there was no difference in the levels of Apo B-100 between obese and non-obese women. This finding is in contrast to previous studies which have found elevated Apo B-100 in obese individuals (Van Gaal *et al.*, 2006, Lamon-Fava *et al.*, 2007). The lack of difference in Apo B-100 levels in the current study may be attributed to the similar proportion of small dense LDL particles among obese and non-obese women (Grundy, 2000). The exclusion of metabolic disorders may also be attributed to the lack of difference in Apo B-100 between obese and non-obese women in this study.

When comparing women with abdominal obesity with women without abdominal obesity, it was observed that Apo B-100 levels were significantly higher in women with abdominal obesity as compared to women without abdominal obesity. These results are similar to studies which have shown elevated Apo B-100 levels in abdominally obese individuals (Batista *et al.*, 2004).

Although metabolic disorders such as insulin resistance, DM, inflammation and HIV have been excluded in this current study, abdominal obesity still showed an association with elevated Apo B-100 independent of these disorders, this shows that abdominal obesity may have an athorogenic component despite exclusion of metabolic disorders.

Visceral adipose tissue releases FFAs into the circulation which, when they reach the liver, are used for the production of TG leading to increased Apo B-100 particles, such as (VLDL, IDL and LDL) hence increasing Apo B-100 concentration (Grundy, 2000, Ginsberg *et al.*, 2006, Bamba and Rader, 2007).

Although the use of anthropometric measures such as BMI and WC as predictors of CVD are well documented there is still debate on which one is the better measure of predicting CVD risk (Iwao *et al.*, 2001, Tankó *et al.*, 2005, Taylor *et al.*, 2010, Schneider *et al.*, 2007). In the current study neither BMI nor WC were predictors of TC and LDL-C. These results suggest that obesity has no association with total cholesterol and LDL-C in the absence of metabolic disorders in this population.

However adiponectin showed a significant negative association with TC in the current study.

6.3 Adiponectin and serum lipid concentrations

Adiponectin showed a significant negative association with cholesterol levels among generally obese women and women with abdominal obesity in this study.

The current study also investigated the association between adiponectin and lipid levels because adiponectin is known to be associated with lipid metabolism. Adiponectin concentration was found to be significantly lower in obese women as compared to non-obese women, and the prevalence of low adiponectin concentration was also significantly higher in obese than in non-obese women. Adiponectin is decreased in obesity due to down regulation through its receptors (Kadowaki and Yamauchi, 2005). Furthermore logistic or linear regression analysis showed that adiponectin was negatively and significantly associated with TC levels in both obese women and in women with abdominal obesity. These results are similar to other studies which have shown a similar relationship (Staiger *et al.*, 2003, Meilleur *et al.*, 2010).

Adiponectin is thought to decrease cholesterol accumulation by reducing its uptake by macrophages or through increase HDL cholesterol efflux by macrophages to the liver (Tian *et al.*, 2009). It is thought to have an inverse relationship with atherogenic lipids, by promoting FFA catabolism and increased TG uptake in skeletal muscle and increased cholesterol clearance through increased reverse cholesterol transport (Qiao *et al.*, 2008, Kazumi Tsubakio-Yamamoto 2008).

In the present study adiponectin was found to have a negative association with TC, and its concentration was significantly different between obese and non-obese women, however there was no significant association between obesity and TC. This lack of association between obesity and TC may be due to lack of metabolic disorders in these obese women since disorders such as insulin resistance and diabetes mellitus are known to affect cholesterol levels (Castro *et al.*, 2014, Wu and Parhofer, 2014, Jaiswal *et al.*, 2014).

6.4 Obesity, serum triglycerides and HDL-C

In the current study the concentration of serum triglycerides was similar among obese and non-obese women, while women with abdominal obesity were found to have significantly higher TG levels than those without abdominal obesity. Although there are studies in South Africa which have shown low TG concentrations in black women (Van Der Merwe *et al.*, 2000, Punyadeera *et al.*, 2001, Motala *et al.*, 2011), there are other

studies which have found TG elevated in both black and white generally obese and abdominally obese women (Misra and Vikram, 2003, Chan *et al.*, 2004, Crowther *et al.*, 2006, Chapman *et al.*, 2011).

A study by Goedecke *et al.*, (2010) found lower TG and HDL-C levels in obese black women when compared to Caucasian women.The lower TG levels in black women may be explained by the higher lipoprotein lipase activity the enzyme responsible for clearance of TG in the circulation which is thought to be higher in black people (Sumner and Cowie, 2008, Goedecke *et al.*, 2010). Another reason for the lack of association between obesity and serum lipids in Black obese women may be the lower visceral fat in Black women compared to their white counterparts (Goedecke *et al.*, 2010).

These results support the suggestion that WC is a better indicator of abnormal lipid concentrations than is BMI in obese women. Obese women were found to have significantly lower HDL-C concentrations compared to non-obese women. These results are in agreement with other studies which have shown similar associations (Goedecke *et al.*, 2010, Barter, 2011, Chapman *et al.*, 2011).

The concentration of HDL-C was found to be significantly lower in women with abdominal obesity as compared with women with normal waist circumference. These results are also in agreement with previous studies which have also reported low concentrations of HDL-C in abdominal obesity (Misra and Vikram, 2003, Chan *et al.*, 2004, Crowther *et al.*, 2006, Chapman *et al.*, 2011). These low HDL-C levels are testimony to show that despite being metabolically health obese women still had increased risk of CVD.

The metabolism of TG and HDL-C are inter-related, and the concentration of TG in the circulation affects HDL-C concentration (Tenkanen *et al.*, 1994, Rashid *et al.*, 2002).

In obesity it is hypothesised that increased production of TG and VLDL by the liver leads to increased cholesterol exchange due to increased hepatic lipase activity which leads to increased removal of HDL-C in the circulation, hence low HDL-C concentrations observed in obese individuals (Barter 2011).

Results from the current study showed that obese women were three times more likely to have low HDL-C levels. Similar results were observed in women with abdominal obesity. Adiponectin showed a significant negative association with TG concentrations in both generally obese and abdominally obese women. These results are similar to other studies which have shown negative association of adiponectin with TG

concentrations (Yamauchi *et al.*, 2001, Meilleur *et al.*, 2010, Kishida *et al.*, 2011).

Adiponectin, a hormone secreted by adipose tissue, is known to be negatively associated with TG and positively with HDL-C (Van Der Vleuten *et al.*, 2005). Yamauchi *et al.*, (2001) investigated the effect of adiponectin on triglyceride concentration. They showed that adiponectin reduced muscle TG content thus increasing FFA combustion in obesity. In skeletal muscle adiponectin increases the expression of VLDL receptor and also the activity of lipoprotein lipase enzyme therefore increasing the removal of TG in the circulation (Qiao *et al.*, 2008).

One study suggested that adiponectin increases cholesterol efflux by increasing the expression of ATP-binding cassette transporters (ABCA1) which is mainly expressed in the liver, peripheral tissues and small intestine (Kazumi Tsubakio-Yamamoto 2008). This results in increased cholesterol efflux by macrophages through reverse cholesterol transfer. It was also found that in cases of low adiponectin the expression of ABCA1 was low and reverse cholesterol transfer was affected leading to accumulation of cholesterol in the circulation (Kazumi Tsubakio-Yamamoto 2008).

Apo A-I present in HDL can be a useful tool in assessing CVD risk (Barter and Rye, 2006). In the current study there was no significant difference in the Apo A-I concentration between obese and non-obese women. Similar results were observed between women with abdominal obesity and those without.

These results are in contrast to previous studies which have demonstrated a low concentration of Apo A-I in obese individuals (Walldius *et al.*, 2001, Walldius and Jungner, 2005). The removal of TG-rich HDL by hepatic lipase results in small HDL particles which have low affinity for Apo A-I hence dissociation from HDL resulting in increased small HDL particle size which are not associated with Apo A-I (Deeb *et al.*, 2003).

6.5 Obesity and Lipid ratios

In the current study Apo B-100/Apo A-I and TC/HDL-C ratios were significantly higher in obese women than in the non- obese women demonstrating increased CVD risk associated with obesity. These results are in agreement with several studies which have shown that lipid ratios are elevated in obese women hence favouring a more atherogenic lipid profile (De Backer *et al.*, 2003, Shai *et al.*, 2004).

The current study also showed significantly higher TC/HDL-C and Apo B-100/Apo A-I ratios in women with abdominal obesity as compared to those without abdominal

obesity.

Both obese and abdominally obese women were three times more likely to have an elevated Apo B-100/Apo A-I ratio than non-obese women. These results are similar to results from previous studies which have shown that obesity is associated with a more atherogenic lipid profile (Agoston-Coldea *et al.*, 2008, Mcqueen *et al.*, 2008).

Both these lipid ratios are potent CVD risk factors as they represent atherogenic lipids over non-atherogenic lipids (Agoston-Coldea *et al.*, 2008, Mcqueen *et al.*, 2008) and their elevation in obese women show an increased risk of developing CVD in this population.

6.6 Obesity and lipoprotein particle size

In the current study non- obese women had a similar proportion of small dense LDL particles as obese women. There was also no significant difference in the proportions of small and large HDL particles between the two groups although obese women showed a non-significant lower proportion of large HDL particles and higher proportion of small HDL particles.

Women with and without abdominal obesity had a similar proportion of lipoprotein subclasses. Although the trends are similar to what other studies have reported with large HDL particle proportions being reduced in obesity and small dense HDL particles being increased there was no significant difference in the proportions of large and small HDL particles among obese and non-obese women in the current study.

There was also no difference in the proportions of small and large LDL particles among women with abdominal obesity and those without abdominal obesity. These results are not in agreement with studies which demonstrated that abdominal obesity is associated with increased small dense LDL particles (Tchernof *et al.*, 1996, Magkos *et al.*, 2008, Goedecke *et al.*, 2010).

The size of the lipoprotein particles is partially depended on circulating TG concentrations, that is elevated circulating TG concentrations favour production of small dense LDL particles and small HDL particles (Krauss and Ronald, 1998, Chapman *et al.*, 2011).

The lack of difference in TG concentrations among obese and non-obese women in the current study may thus be responsible for the lack of difference in lipoprotein particle proportions between obese and non-obese women (Krauss and Ronald, 1998,

Carmena *et al.*, 2004, Talayero and Sacks, 2011).

The similar prevalence of elevated TG levels among obese and non-obese women further supports the lack of difference in proportion of lipoprotein particle size in this population.

Limitations of the study

The current study only estimated visceral obesity using waist circumference. Therefore the results of the current study could not show the association between visceral fat content with serum lipids because WC estimate the overall size of the abdominal area and not the fat content of the abdominal area.

Conclusion

The current study assessed changes in serum lipids and lipoprotein subclasses in metabolically healthy obese women residing in a rural area. This study is a first of its kind in this area. Results of the current study showed that although obese and non-obese women had similar total cholesterol and LDL-C levels, obese women had lower HDL-C and adiponectin levels, elevated TG levels and increased Apo B-100/Apo A-I ratio which are risk factors for CVD.

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Appendix I. Ethics approval

UNIVERSITY OF LIMPOPO
Medunsa Campus

UNIVERSITY OF LIMPOPO

MEDUNSA RESEARCH & ETHICS COMMITTEE

CLEARANCE CERTIFICATE

MEETING:	09/2012
PROJECT NUMBER:	MREC/HS/271/2012: PG

PROJECT:

Title:	Levels of serum lipid and lipoprotein subclasses in obese women residing in a rural area Limpopo Province
Researcher:	Mr NS Mampeule
Supervisor:	Prof M Alberts
Co-supervisor:	S Choma
	Prof JP van Geertruyden
Department:	Medical Sciences, Public Health & Health Promotion
School:	Health Sciences
Degree:	MSc Medical Science (Chemical Pathology)

DECISION OF THE COMMITTEE:

MREC approved the project.

DATE: 08 November 2012

PROF GA OGUNBANJO
CHAIRPERSON MREC

The Medunsa Research Ethics Committee (MREC) for Health Research is registered with the US Department of Health and Human Services as an International Organisation **(IORG0004319)**, as an Institutional Review Board **(IRB00005122)**, and functions under a Federal Wide Assurance **(FWA00009419)**
Expiry date: **11 October 2016**

Note:
i) **Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.**
ii) **The budget for the research will be considered separately from the protocol.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

PROUDLY SOUTH AFRICAN

Appendix II: WHO Steps Questionnaire

WHO STEPS Instrument

(Core and Expanded / Dipotšišo tše bohlokwa le tša tlaleletšo)

The WHO STEPwise approach to chronic disease risk factor surveillance (STEPS)

World Health Organization

20 Avenue Appia, 1211 Geneva 27, Switzerland

For further information: **www.who.int/chp/steps**

STEPS Instrument

Overview

Introduction **This is the generic STEPS Instrument which sites/countries will use to develop their tailored instrument. It contains the:**

CORE items (unshaded boxes)

EXPANDED items (shaded boxes).

Core Items **The Core items for each section ask questions required to calculate basic variables. For example:**

current daily smokers

mean BMI.

Note: All the core questions should be asked, removing core questions will impact the analysis.

Expanded items **The Expanded items for each section ask more detailed information. Examples include:**

use of smokeless tobacco

sedentary behaviour.

Guide to the **The table below is a brief guide to each of the columns in the**

columns Instrument.

Column	Description	Site Tailoring
Number	**This question reference number is designed to help interviewers find their place if interrupted.**	**Renumber the instrument sequentially once the content has been finalized.**
Question	**Each question is to be read to the participants**	**Select sections to use.** **Add expanded and optional questions as desired.**
Response	**This column lists the available response options which the interviewer will be circling or filling in the text boxes. The skip instructions are shown on the right hand side of the responses and should be carefully followed during interviews.**	**Add site specific responses for demographic responses (e.g. C6).** **Change skip question identifiers from code to question number.**
Code	**The column is designed to match data from the instrument into the data entry tool, data analysis syntax, data book, and fact sheet.**	**This should never be changed or removed. The code is used as a general identifier for the data entry and analysis.**

ument

Chronic Disease Risk Factor Surveillance

<SOUTH AFRICA, LIMPOPO PROVINCE / DIKGALE HEALTH AND DEMOGRAPHIC SURVEILLANCE SITE>

Survey Information / TshedimoŠo ka ga dinyakiŠiŠo

Location and Date / Lefelo le Letšatši		Response / Dikarabo	Code
1	Cluster/Centre/Village ID / Nomoro ya Motse (go ya ka DHDSS)	⌊__⌋⌊__⌋⌊__⌋	I1
2	Cluster/Centre/Village name / Leina la Motse		I2
3	Interviewer ID or Initials / Nomoro ya Mmotšišiši	⌊__⌋⌊__⌋⌊__⌋	I3
4	Date of completion of the instrument / Letšatši la go tlatsa form ye	⌊__⌋⌊__⌋ ⌊__⌋⌊__⌋ ⌊__⌋⌊__⌋⌊__⌋⌊__⌋ dd mm year Letšatši Kgwedi Ngwaga	I4

Nomoro ya Motšeakarolo └─┴─┴─┘ └─┴─┴─┘ └─┴─┴─┘			
Consent, Interview Language and Name / Tumelelo ya go tšea karolo, Leleme la poledišano, Le Leina		**Response / Karabo**	**Code**
5	**Consent has been read and obtained / Kwano ya go tšea karolo e badilwe ebile e hweditšwe**	**Ee 1** **Aowa 2 Ge eba Aowa, GONA FETŠA POLEDIŠANO**	**I5**
6	**Interview Language *[Insert Language]* / Leleme la Poledišano**	**English / Sekgowa 1** ***Pedi / Sepedi* 2**	**I6**
7	**Time of interview (24 hour clock) / Nako ya Poledišano** **(go ya ka di-iri tše masomepedinne tša letšatši)**	└─┴─┘ : └─┴─┘ **hrs mins** **Di-Iri Metsotso**	**I7**
8	**Family Surname / Sefane**		**I8**
9	**First Name / Leina la Mathomo**		**I9**
Additional Information that may be helpful / Tlaleletšo yeo e ka thušago			
10	**Contact phone number where possible / Nomoro ya mogala ge ele gona**		**I10**

Record and file identification information (I5 to I10) separately from the completed questionnaire.

Step 1 Demographic Information

Legato la pele TshedimoŠo ya tŠa bodudi

Nomoro ya Motšeakarolo ⌊__⌋⌊__⌋⌊__⌋ ⌊__⌋⌊__⌋⌊__⌋ ⌊__⌋⌊__⌋⌊__⌋

TŠE BOHLOKWA: Tshedimošo ya tša bodulo			
Potšišo		**Karabo**	**Code**
11	**Sex (*Record Male / Female as observed) /***	**Male / Monna 1** **Female / Mosadi 2**	**C1**
12	**What is your date of birth? /** **Letšatši la Matswalo?** ***Don't Know 77 77 7777 /*** ***Ga ke tsebe 77 77 7777***	⌊__⌋⌊__⌋ ⌊__⌋⌊__⌋ ⌊__⌋⌊__⌋⌊__⌋⌊__⌋ ***If known, Go to C4 / Ge a tseba, E ya go C4*** **dd mm year** **Letšatši Kgwedi Ngwaga**	**C2**
13	**How old are you? O na le mengwaga e me kae?**	**Years / Mengwaga** ⌊__⌋⌊__⌋	**C3**
14	**In total, how many years have you spent at school or in full-time study (excluding pre-school)? /** **Ka moka, o tsere mengwaga ye mekae o le sekolong goba o ithuta se sengwe (Nako ya Pre-school ga e balwe)?**	**Years** **/Mengwaga** ⌊__⌋⌊__⌋	**C4**

Step 1 Behavioural Measurements / Legato la pele Kelo ya maitshwaro

Nomoro ya Motšeakarolo

|_|_|_| |_|_|_| |_|_|_|

CORE: Tobacco Use / DIPOTŠISO TŠE BOHLOKWA: Tšhumišo ya Motsoko

Now I am going to ask you some questions about various health behaviours. This includes things like smoking, drinking alcohol, eating fruits and vegetables and physical activity. Let's start with tobacco. /

Bjale ke tlo go botšiša dipotšišo mabapi le tsa maphelo. Go akaretša go kgoga, go nwa bjala, go ja dienywa le merogo le go thobolla mmele. A re thome ka ka motsoko.

	Question / Potšišo	Response / Karabo		Code
15	**Do you currently smoke any tobacco products, such as cigarettes, cigars or pipes? *(USE SHOWCARD) /*** **Naa gona bjale o kgoga e mengwe ya metšoko ya go swana le sekerete, sikara goba peipe? *(ŠOMIŠA KARATA YA GO HLALOŠA)***	**Yes / Ee** **No / Aowa**	**1** **2** ***If No, go to T6 /*** ***Ge eba aowa gona fetela go T6***	**T1**
16	**Do you currently smoke tobacco products daily? /**	**Yes /Ee** **No / Aowa**	**1**	**T2**

	Naa le kgoga mehuta ye tšatši le tšatši?		**2** ***If No, go to T6 /*** ***Ge eba aowa gona fetela go T6***	
17	**How old were you when you first started smoking daily?** **If not known probe by asking questions such as when you started was your first child**	**Age (years) / Mengwaga** **Don't know / Ga ke tsebe 77**	└┴┘ ***If Known, go to T5a /*** ***Ge a tseba eya go T5a***	**T3**
18	**Do you remember how long ago it was?** ***(RECORD ONLY 1, NOT ALL 3) /***	**In Years /**	└┴┘ ***If Known, go to***	**T4a**
		OR in Months /	└┴┘ ***If Known, go to***	**T4b**
		OR in Weeks /	└┴┘	**T4c**
18	**On average, how many of the following do you smoke each day?** ***(RECORD FOR EACH TYPE, USE SHOWCARD) /*** **Ka palogare, naa o kgoga e me kae metšoko ye e latelago?** ***(TLATŠA MEHUTA KAMOKA, MMONTŠHE KARATA)***	**Manufactured cigarettes / Sekerete**	└┴┘	**T5a**
		Hand-rolled cigarettes / Sekerete sa go bofiwa goba go tatwa	└┴┘	**T5b**
		Pipes full of tobacco / Peipe ye e tletšego motšoko	└┴┘	**T5c**
		Cigars, cheroots, cigarillos / **Sikara go ya ka mehutahuta**	└┴┘	**T5d**

	Don't Know / Ga ke tsebe 77	**Other / E mengwe**	***Ge eba ye mengwe e ya go T5other goba T9*** └─┴─┘	**T5e**
		Other (please specify) / E mengwe(Hlaloša):	└─┴─┴─┴─┴─┴─┴─┘ ***Go to T9 / E ya go T9***	**T5other**

EXPANDED: Tobacco Use / DIPOTŠIŠO TŠA TLALELETŠO: Tšhomišo ya Motsoko				
Question / Potšišo		**Response Karabo**		**Code**
Please note that questions 27 – 29 and 33 are for people who are not currently smoking. (That is those who have never smoked or those who had smoked but stopped smoking). **Lemoga gore dipotšišo 27 -29 le 33, ke tša bao ba sa folego gona bjale. (Bao ba sakago ba fola goba bao ba bego ba fola feela ba emiša go fola).**				
19	**In the past, did you ever smoke daily? /**	**Yes / Ee** **No / Aowa**	**1** **2** ***If No, go to T9***	**T6**
20	**How old were you when you stopped smoking daily? /** **Naa o be ona le mengwaga e me kae ge o tlogela go kgoga tšatši ka tšatši?**	**Age (years) / Megwaga** **Don't Know / Ga ke tsebe 77**	└─┴─┘ ***If Known, go to T9 /*** ***Ge ba tseba fetela go T9***	**T7**
22	**How long ago did you stop smoking daily?** ***(RECORD ONLY 1, NOT ALL 3)***	**Years ago / Mengwaga ya go feta**	└─┴─┘ ***If Known, go to T9 /*** ***Ge ba tseba fetela go T9***	**T8a**
		OR Months ago	└─┴─┘ ***If Known, go***	**T8b**

	Naa ekaba lebaka le le kae o tlogetše go kgoga tšatši ka tšatši? ***(TLATŠA KARABO E TEE FELA E SEGO KAMOKA)*** ***Don't Know / Ga ke tsebe 77***	***/ to T9 /*** **GOBA Dikgwedi tša go feta**	***Ge ba tseba fetela go T9***	
		OR Weeks ago / **GOBA Dibeke tša go feta**	⌊__⌋⌊__⌋	**T8c**
23	**Do you currently use any smokeless tobacco such as *[snuff, chewing tobacco,***	**Yes / Ee** **No / Aowa**	**1** **2** ***If No, go to T12/*** **Go eba aowa**	**T9**
24	**Do you currently use smokeless tobacco products**	**Yes / Ee** **No / Aowa**	**1** **2** ***If No, go to T12 /***	**T10**
25	**On average, how many times a day do you use ….** ***(RECORD FOR EACH TYPE, USE SHOWCARD)*** **Ka palogare/ average, naa ka letšatsi o o šomiša ga kae? ….** ***(TLATŠA MEHUTA KAMOKA, ŠOMIŠA KARATA GO HLALOŠA)***	**Snuff, by mouth /** **Sneife, Ka**	⌊__⌋⌊__⌋	**T11a**
		Snuff, by nose / **Sneife ka nkong**	⌊__⌋⌊__⌋	**T11b**
		Chewing tobacco / **Motšoko wa go jewa**	⌊__⌋⌊__⌋	**T11c**
		Other / O mongwe	⌊__⌋⌊__⌋	**T11e**

	Don't Know / Ga ke tsebe 77	**Other (specify) /** **O mongwe (Hlaloša)**	***If Other, go to T11other,*** └┴┘ ***else go to T13*** ***Ge eba o mongwe fetela goT11other, goba go T13***	**T11other**
26	**In the past, did you ever use smokeless tobacco such as snuff, chewing tobacco daily. /** **Mo lebakeng le le fetilego, naa o ile wa šomiša motšoko wa go hloka muši bjalo ka sneife goba motšoko wa go jewa?**	**Yes / Ee** **No / Aowa**	**1** **2**	**T12**
27	**During the past 7 days, on how many days did someone in your home smoke when you were present/ /** **Mo matšatšing a 7 a go feta, naa go na le matšatši a makae mo go ilego gwa ba le yo mongwe ka lapeng yo a ilego a fola o le gona?**	**Number of days / Matšatši** **Don't Know / Ga ke tsebe**	___ **77**	**T13**
28	**During the past 7 days, on how many days did someone smoke**	**Number of days / Matšatši**	___	**T14**

	in closed areas in your workplace when you were present? **/** **Mo matšatšing a 7 a go feta, naa go na le matšatši a makae mo go ilego gwa ba le yo mongwe ka mošomong wa gago yo a ilego a fola o le gona?**	**Don't Know or do't work in closed areas / Ga ke tsebe goba ga ke šome mafelong a tšwaletšego**	**77**	

CORE: Alcohol Consumption / DIPOTŠIŠO TŠE BOHLOKWA: Tšhomišo ya Bjala				
The next questions ask about the consumption of alcohol./ Dipotšišo tša go latela di amana le tšhomišo ya bjala.				
Question / Potšišo		**Response / Karabo**		**Code**
29	**Have you ever consumed an alcoholic drink such as beer, wine, spirits, fermented cider or *traditionally fermented beer*?**	**Yes / Ee** **No / Aowa**	**1** **2 *If No, go to D1*** ***Ge eba aowa fetela go D1***	**A1a**
30	**Have you consumed an alcoholic drink within the past 12 months? /** **Naa o ile wa nwa bjala mo kgweding tše lesome pedi (12) tša go feta?**	**Yes / Ee** **No / Aowa**	**1** **2 *Ge eba aowa fetela go D1***	**A1b**
31	**During the past 12 months, how**	**Daily / Tšatši ka**	**1**	**A2**

	frequently have you had at least one alcoholic drink? ***(READ RESPONSES, USE SHOWCARD) /*** **Mo dikgweding tše lesome pedi (12) tša go feta, naa o ile wa nwa bjala makga a ma kae?** ***(BALA DIKARABO, O ŠOMIŠE KARATA)***	**tšatši** **5-6 days per week / Matšatši a 5-6 ka beke** **2** **1-4 days per week / Matšatši a 1-4 ka beke** **3** **1-3 days per month / Matšatši a 1-3 Ka kgwedi** **4** **Less than once a month / Ka tlase ga ga-tee ka kgwedi** **5**		
32	**Have you consumed an alcoholic drink within the past**	**Yes / Ee** **1** **No / Aowa** **2**	***If No, go to D1/***	**A3**
33	**During the past 30 days, on how many occasions did you have at least one alcoholic drink? /** **Mo matšatšing a masome tharo (30) a go feta, naa o ile wa nwa bjala makga a ma kae?**	**Number / Nomoro** **Don't know / Ga ke tsebe 77** └─┴─┘		**A4**
34	**During the past 30 days, when you drank alcohol, on average, how many standard alcoholic drinks did you have during one drinking occasion?** ***(USE SHOWCARD) /***	**Number / Nomoro** **Don't know / Ga ke tsebe 77** └─┴─┘		**A5**

	Mo matšatšing a masome tharo (30) a go feta moo o ilego wa nwa bjala, naa ka palogare o ile wa nwa bjala bjo bo kaakang lekgeng le tee? ***(ŠOMIŠA KARATA GO HLALOŠA)***		
35	**During the past 30 days, what was the largest number of standard alcoholic drinks you had on a single occasion, counting all types of alcoholic drinks together? /** **Mo matšatšing a masome tharo (30) a go feta, naa nomoro e kgolo ya bjala bjo o bo nwelego mo lebakeng le tee ge o akaretša dino kamoka ke eng?**	**Nomoro** **Don't know / Ga ke tsebe 77** ⌊__⌋⌊__⌋	**A6**
36	**During the past 30 days, how many times did you have** **for men: five or more** **for women: four or more** **standard alcoholic drinks in a single drinking occasion? /** **Mo matšatšing a masome tharo (30) a go feta, ke ga kae mo o ilego wa nwa dino tša go feta tše:-** **Hlano goba go feta (ge**	**Number of times / Ga** **Don't know / Ga ke tsebe 77** ⌊__⌋⌊__⌋	**A7**

	mmotšišwa e le monna) **Nne goba go feta (ge mmotšišwa e le mosadi** **Lebakeng le tee?**		

EXPANDED: Alcohol Consumption / DIPOTŠISO TŠA TLALELETŠO: Tšhomišo ya bjala			
37	**During the past 30 days, when you consumed an alcoholic drink, how often was it with meals? Please do not count**	**Usually with 1** **Sometimes with meals / Le 2** **Rarely with meals / Ga se 3** **Never with meals / Ntle le dijo 4**	**A8**
38	**During each of the past 7 days, how many standard alcoholic drinks did you have each day?** ***(USE SHOWCARD) /*** **Mo matšatšing a šupa(7) ago feta, naa o nwele bjala bjo bo kaakang tšatši le lengwe le le lengwe?** ***(ŠOMIŠA KARATA GO HLALOŠA)*** ***Don't Know / Ga ke tsebe 77***	**Monday / Mosupologo** ⌊_⌋⌊_⌋	**A9a**
		Tuesday / Labobedi ⌊_⌋⌊_⌋	**A9b**
		Wednesday / Laboraro ⌊_⌋⌊_⌋	**A9c**
		Thursday / Labone ⌊_⌋⌊_⌋	**A9d**
		Friday / Labohlano ⌊_⌋⌊_⌋	**A9e**
		Saturday / Mokibelo ⌊_⌋⌊_⌋	**A9f**
		Sunday / Lamorena ⌊_⌋⌊_⌋	**A9g**

CORE: Diet / DIPOTŠIŠO TŠA BOHLOKWA: Tša Dijo
The next questions ask about the fruits and vegetables that you usually eat. I have a nutrition card here that shows you some examples of local fruits and vegetables. Each picture represents the size of a serving. As you answer these questions please think of a

typical week in the last year /

Dipotšišo tše di latelago ke tša mabapi le dienywa le merogo yeo o ejago. Ke nale karata ya dijo yeo e laetšago dienywa le merogo e tlwaelegilego ya gae. Seswantšho se sengwe le se sengwe se laetša kalo ya dijo. Ge o araba dipotšišo ka kgopelo nagana ka beke ye e tlwaelegilego mo ngwageng wa go feta.

Question / Potšišo		Response / Karabo	Code
39	**In a typical week, on how many days do you eat fruit?** ***(USE SHOWCARD) /***	**Number of days / Palo ya matšatši** **Don't Know / Ga ke tsebe 77** ⌊__⌋__ ***If Zero***	**D1**
40	**How many servings of fruit do you eat on one of those days?** ***(USE SHOWCARD) /***	**Number of servings / Palo ya go ja** ⌊__⌋__⌋	**D2**
41	**In a typical week, on how many days do you eat vegetables?** ***(USE SHOWCARD)***	**Number of days / Palo ya matšatši** **Don't Know / Ga ke** ⌊__⌋__ ***If Zero days, go to D5***	**D3**
42	**How many servings of vegetables do you eat on one of those days?** ***(USE***	**Palo ya go ja** **Don't Know / Ga ke** ⌊__⌋__⌋	**D4**

EXPANDED: Diet / DIPOTŠIŠO TŠA TLALELETŠO: Tša Dijo					
43	**What type of oil or fat is most often used for meal preparation in your household?** ***(USE SHOWCARD)*** ***(SELECT ONLY ONE)*** **Naa o šomiša makhura a mohuta mang ge o apea?**	**Vegetable oil / Makhura a merogo**	**1**		**D5**
		Lard or suet	**2**		
		Butter or ghee / Botoro	**3**		
		Margarine / Margarine	**4**		
		Other / A mangwe	**5**	***If Other, go to***	

	(ŠOMIŠA KARATA GO HLALOŠA) *(KGETA YE TEE FEELA)*		*D5 other /* *Ge eba se sengwe fetela go* *D5other*	
		None in particular	**6**	
		None used / Ga ke šomiše Makhura	**7**	
		Don't know / Ga ke tsebe	**77**	
		Other / Se sengwe	⌊__⌊__⌊__⌊__⌊__⌊__⌊__⌋	**D5other**
44	**On average, how many meals per week do you eat that were not prepared at a home? By meal, I mean breakfast, lunch**	**Number / Palo** **Don't know / Ga ke tsebe 77**	⌊__⌊__⌋	**D6**

CORE: Physical Activity / DIPOTŠITŠO TŠE BOHLOKWA: Thobollo ya mmele
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. /**

Bjale ke ile go go botšiša ka nako yeo o e tseyago o thobolla mmele mo bekeng. Ka kgopelo araba dipotšišo le ge o ša ipone o le motho wa go fela a ithobolla mmele. Nagana pele ka nako yeo o etšeago o soma. Nagana ka mošomo ele dilo tšeo o di dirago go swana le mošomo wa go lefšwa goba wa go se lefšwe, go ithuta, mešomo ya ka gae, go lema, go thea dihlapi goba go nyakana le mošomo.[Tsentšha mehlala ye mengwe ge go hlokega]. Ge o fetola dipotšišo tše latelago;tseba gore ge re bolela ka mošomo o boima re ra gore mošomo woo o dirago gore o hemele godimo le pelo e kibela godimo, mola mošomo o boleta e le woo o dirago gore o se hemele godimo kudu le pelo e se kibela godimo kudu.

	Question / Potšišo	Response / Karabo	Code
Work / Go šoma			
Please note that in this case work means all paid and unpaid working activities. This sections should be filled by even those who are unemployed			
45	**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**	**Yes / Ee** **1** **No / Aowa** **2** ***Ge eba aowa fetela go P 4***	**P1**
46	**In a typical week, on how many days do you do vigorous-intensity activities as part of**	**Number of days / Palo ya matšatši** ⌊__⌋	**P2**
47	**How much time do you spend doing vigorous-intensity activities at work on a typical day? /** **Naa o tšea nako e kaakang ge o šoma boima mo letšatšng le le tee?**	**Hours : minutes / Di-iri : metsotso** ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ **hrs** **mins** **Di-iri metsotso**	**P3 (a-b)**

48	**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 / Ee** **No / Aowa**	**1** **2** ***If No, go to P 7 / Ge eba aowa fetela go P 7***	**P4**
49	**In a typical week, on how many days do you do moderate-intensity activities as part of**	**Number of days / Palo ya matšatši**	⌊__⌋	**P5**
50	**How much time do you spend doing moderate-intensity activities at work on a typical day? / Naa o tšea nako e kaakang ge o šoma mošomo o bofefo, mo letšatšing le le tee?**	**Hours : minutes / Di-iri : metsotso**	⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ **hrs mins** **Di-iri metsotso**	**P6 (a-b)**
Travel to and from places / Go ya le go boa mafelong a go fapana				
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] /*** **Dipotšišo tše di latelago di akaretša mošomo o boima wo re šetšego re boletše ka ona. Bjale ke rata go go botšiša mabapi le go ya le go boa mafelong a go fapana. Mohlala: Go sepela go ya mošomong, mabenkeleng mmarakeng goba mafelong a go rapela.[TŠENTŠHA MEHLALA E MENGWE GE GO HLOKEGA].**				
51	**Do you walk or use a bicycle *(pedal cycle)* for at least 10 minutes continuously to get to and from places? / Naa o a sepela goba o šomisa**	**Yes / Ee** **No / Aowa**	**1** **2** ***Ge eba aowa fetela go P 10***	**P7**

	paesekela (ya materapo) go lekana nako ya metsotso e lesome goba go feta ge o eya goba o bowa mafelong?		
52	**In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from**	**Number of days / Palo ya matšatši** ⌊__⌋	**P8**
53	**How much time do you spend walking or bicycling for travel on a typical day? /** **Naa o tšea nako e kaakang mo letšatšing go sepela goba o šomiša paesekela?**	**Hours : minutes / Di-iri : metsotso** ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ **hrs mins** **Di-iri Metsotso**	**P9 (a-b)**

CORE: Physical Activity, Continued / DIPOTŠITŠO TŠE BOHLOKWA: Thobollo ya			
Question / Potšišo		**Response / Karabo**	**Code**
Recreational activities / Maitapološo			
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]*. /** **Dipotšišo tše di latelago a di akaretše mošomo le mokgwa wa dinamelwa tšeo o šetšego o di boletše.** **Bjale ke rata go go botšiša mabapi le tša dipapadi, tša boitekanelo le tša boitapološo (boiketlo) [tsentšha tšeo di swanetšego)**			
54	**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**	**Yes / Ee** **No / Aowa**	**1** **2 *If No, go to P 13 /*** ***Ge e le aowa eya go P 13***	**P10**
55	**In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational *(leisure)* activities? /** **Mo bekeng a naa o raloka papadi ye e thata, goba o tsenela tša boitekanelo, goba tša boitapološo (boiketlo) ga kaakang?**	**Number of days / Matšatši**	⌊__⌋	**P11**
56	**How much time do you spend doing vigorous-intensity sports, fitness or recreational**	**Hours : minutes / Di-iri: metsoso**	⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ **hrs mins**	**P12** **(a-b)**

	activities on a typical day? / **A naa o tšea nako e kaakang o raloka papadi yeo ye boima, goba o tsenetše tša boitekanelo goba tša boitapološo mo letšatšing?**		**di-iri mets**	
57	**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 / Ee** **No / Aowa**	**1** **2** ***If No, go to P16 /*** ***Ge ele aowa eya go P16***	**P13**
58	**In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational *(leisure)* activities? /** **Mo bekeng a naa o tsenela tše tša dipapadi, tsa botekanelo goba tša boitapološo (boiketlo) tšeo di sego boima kudu ga kae?**	**Number of days /** **Matšatši**	⌊__⌋	**P14**
59	**How much time do you spend doing moderate-intensity sports, fitness or recreational *(leisure)* activities on a typical day? /** **A naa o tšea nako e kaakang o**	**Hours : minutes /** **Diiri:metsotso**	⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ **hrs mins** **di-iiri metsotso**	**P15 (a-b)**

	tsenetše tšeo tša dipapadi, tša boitekanelo goba tša boitapološo (boiketlo) tšeo di sego boima kudu ka letšatši ?		

EXPANDED: Physical Activity / DIPOTŠIŠO TSA TLALELETŠO: Thobollo ya Mmele			
Sedentary behaviour / Mokgwa wa go dula felo go tee			
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] (USE SHOWCARD) /*** **Potšišo e e latelago e mabapi le go dula fase goba go ithekga ka sengwe mosomong, ka gae, go ya le go bowa mafelong a itseng, goba le bagwera go akaretšwa nako eo o e tšerego o dutše setulong, o dutše le bagwera, o sepela ka sefatanaga, pese, setimela, o bala, o raloka dikarata, goba o bogetše thelebišene, e fela ga e akaretše nako yeo o e tsšrego o robetše** **[TSENTŠHA MEHLALA] [BONTŠHA KA KARATA]**			
60	**How much time do you usually spend sitting or reclining on a typical day? /** **A naa o fela o tšea nako e kaakang ka letšatši o dutše fase goba o ithekgile ?**	**Hours : minutes /** **Di-iri:metsotso** └─┴─┘: └─┴─┘ **hrs mins** **di-iri** **mets**	**P16** **(a-b)**

CORE: History of Raised Blood Pressure / DIPOTŠITŠO TŠE BOHLOKWA: Tša madi a magolo					
Question / Potšišo		**Response / Karabo**			**Code**
61	**Have you ever had your blood pressure measured by a doctor or other health worker? /**	**Yes / Ee** **No / Aowa**	**1** **2**		**H1**
62	**Have you ever been told by a doctor or other health worker that you have raised blood**	**Yes / Ee** **No / Aowa**	**1** **2** **/**	***If No, go to H6***	**H2a**
63	**Have you been told in the past 12 months? /** **A naa o boditšwe mo dikgweding tše lesome-pedi tša go feta?**	**Yes / Ee** **No / Aowa**	**1** **2**		**H2b**

EXPANDED: History of Raised Blood Pressure / DIPOTŠIŠO TŠA TLALELETŠO: Tša madi a magolo				
64	**Are you currently receiving any of the following treatments/advice for high blood pressure prescribed by a doctor or other health worker? /** **A naa ga bjale o nwa dihlare tše/o fiwa maele ka tša madi a magolo tšeo o di/ao o a fiwago ke ngaka goba mošomedi o mongwe wa tša maphelo?**			
	Drugs (medication) that you have taken in the past two weeks / **Diokobatši (dihlare) tšeo o di nwelego mo dibekeng tše pedi tša gofeta**	**Yes / Ee** **No / Aowa**	**1** **2**	**H3a**
	Advice to reduce salt intake /	**Yes / Ee**	**1**	**H3b**

	Keletšo ya go fokotša letswai dijong	**No / Aowa**	**2**	
	Advice or treatment to lose weight / **Keletšo goba kalafo ya go fokotša boima bja mmele**	**Yes / Ee** **No / Aowa**	**1** **2**	**H3c**
	Advice or treatment to stop smoking / **Keletšo goba kalafo ya go tlogela go kgoga motšoko**	**Yes / Ee** **No / Aowa**	**1** **2**	**H3d**
	Advice to start or do more exercise / **Keletšo ya go thoma go itšhidulla kudu**	**Yes / Ee** **No / Aowa**	**1** **2**	**H3e**
65	**Have you ever seen a traditional healer for raised blood pressure or hypertension? /** **A naa o ile wa bonwa ke ngaka ya setšo mabapi le madi a magolo?**	**Yes / Ee** **No / Aowa**	**1** **2**	**H4**
66	**Are you currently taking any herbal or traditional remedy for your raised blood pressure? /**	**Yes / Ee** **No / Aowa**	**1** **2**	**H5**

CORE: History of Diabetes / DIPOTŠITŠO TŠE BOHLOKWA: Tša mabapi le bolwetši bja swikiri					
Question / Potšišo		**Response / Karabo**			**Code**
67	**Have you ever had your blood sugar measured by a doctor or other health worker? /**	**Yes / Ee** **No / Aowa**	**1** **2**		**H6**
68	**Have you ever been told by a doctor or other health worker that you have raised blood**	**Yes / Ee** **No / Aowa**	**1** **2** **/**	***If No, go to M1***	**H7a**
69	**Have you been told in the past 12 months? /** **A naa o boditšwe mo dikgweding tše lesomepedi tša go feta ?**	**Yes / Ee** **No / Aowa**	**1** **2**		**H7b**

EXPANDED: History of Diabetes / DIPOTŠIŠO TSA TLALELETŠO: Tša bolwetsi bja swikiri					
70	**Are you currently receiving any of the following treatments/advice for diabetes prescribed by a doctor or other health worker? /** **A naa ga bjale o hwetša kalafo/keletšo ya mabapi le bolwetši bja swikiri tšeo o di fiwago ke ngaka goba mošomedi o mongwe wa tša maphelo?**				
	Insulin / **Tšhwaana ya taolo ya swikiri**	**Yes / Ee** **No / Aowa**	**1** **2**		**H8a**
	Drugs (medication) that you have taken in the past two weeks / **Diokobatši (dihlare) tšeo o di nwelego mo dibekeng tše pedi tša go feta**	**Yes / Ee** **No / Aowa**	**1** **2**		**H8b**

	Special prescribed diet / **Dijo tšeo o di kgethetšwego ke ngaka ?**	**Yes / Ee** **No / Aowa**	**1** **2**	**H8c**
	Advice or treatment to lose weight / **Keletšo goba kalafo ya go fokotša boima bja mmele**	**Yes / Ee** **No / Aowa**	**1** **2**	**H8d**
	Advice or treatment to stop smoking / **Keletšo goba kalafo ya go tlogela go kgoga motšoko**	**Yes / Ee** **No / Aowa**	**1** **2**	**H8e**
	Advice to start or do more exercise / **Keletšo goba kalafo ya go itšhidulla kudu**	**Yes / Yes** **No / No**	**1** **2**	**H8f**
71	**Have you ever seen a traditional healer for diabetes or raised blood sugar? /** **A naa o ile wa bonwa ke ngaka ya setšo mabapi le bolwetši bja swikiri goba swikiri e ntši mo mading ?**	**Yes / Ee** **No / Aowa**	**1** **2**	**H9**
72	**Are you currently taking any herbal or traditional remedy for your diabetes? /** **A naa ga bjale o nwa**	**Yes / Ee** **No / Aowa**	**1** **2**	**H10**

	mešunkwane goba dihlare tša setšo go alafa bolwetši bjoo bja swikiri ?		

Step 2 Physical Measurements

Nomoro ya Motšeakarolo

|_|_|_| |_|_|_| |_|_|_|

CORE: Height and Weight		
Question	**Response**	**Code**

73	**Interviewer ID**		└─┘└─┘└─┘	**M1**
74	**Device IDs for height and weight**	**Height** **Weight**	└─┘└─┘ └─┘└─┘	**M2a** **M2b**
75	**Height**	**in Centimetres (cm)**	└─┘└─┘└─┘ . └─┘	**M3**
76	**Weight** ***If too large for scale 666.6***	**in Kilograms (kg)**	└─┘└─┘└─┘ . └─┘	**M4**
77	**For women: Are you pregnant?**	**Yes** **No**	**1** ***If Yes, go to M 8*** **2**	**M5**
CORE: Waist				
78	**Device ID for waist**		└─┘└─┘	**M6**
79	**Waist circumference**	**in Centimetres (cm)**	└─┘└─┘└─┘ . └─┘	**M7**
CORE: Blood Pressure				
80	**Interviewer ID**		└─┘└─┘└─┘	**M8**
81	**Device ID for blood pressure**		└─┘└─┘	**M9**
82	**Cuff size used**	**Small** **Medium** **Large**	**1** **2** **3**	**M10**
83	**Reading 1**	**Systolic (mmHg)**	└─┘└─┘└─┘	**M11a**
		Diastolic (mmHg)	└─┘└─┘└─┘	**M11b**
84	**Reading 2**	**Systolic (mmHg)**	└─┘└─┘└─┘	**M12a**
		Diastolic (mmHg)	└─┘└─┘└─┘	**M12b**
85	**Reading 3**	**Systolic (mmHg)**	└─┘└─┘└─┘	**M13a**
		Diastolic (mmHg)	└─┘└─┘└─┘	**M13b**
86	**During the past two weeks,**	**Yes**	**1**	**M14**

	have you been treated for	No 2	

EXPANDED: Hip Circumference and Heart Rate			
87	Hip circumference	in Centimeters (cm) ⌊_⌊_⌊_⌋.⌊_⌋	M15
88	Heart Rate		
	Reading 1	Beats per minute ⌊_⌊_⌊_⌋	M16a
	Reading 2	Beats per minute ⌊_⌊_⌊_⌋	M16b
	Reading 3	Beats per minute ⌊_⌊_⌊_⌋	M16c

Step 3 Biochemical Measurements

Nomoro ya Motšeakarolo

⌊_⌊_⌊_⌋ ⌊_⌊_⌊_⌋ ⌊_⌊_⌊_⌋

CORE: Blood Glucose			
Question		Response	Code
89	During the past 12 hours have you had anything to eat or drink, other than water?	Yes 1 No 2	B1
90	Technician ID	⌊_⌊_⌊_⌋	B2
91	Device ID	⌊_⌊_⌋	B3
92	Time of day blood specimen taken (24 hour clock)	Hours : minutes ⌊_⌊_⌋ : ⌊_⌊_⌋ hrs mins	B4
93	Fasting blood glucose *Choose accordingly: mmol/l or*	mmol/l ⌊_⌊_⌋.⌊_⌊_⌋ mg/dl ⌊_⌊_⌊_⌋.⌊_⌋	B5

94	**Today, have you taken insulin or other drugs (medication) that have been prescribed by a doctor or other health worker for raised blood glucose?**	**Yes** **No**	**1** **2**	**B6**
CORE: Blood Lipids				
95	**Device ID**		☐☐	**B7**
96	**Total cholesterol** ***Choose accordingly: mmol/l or***	**mmol/l** **mg/dl**	☐☐.☐☐ ☐☐☐.☐	**B8**
97	**During the past two weeks, have you been treated for raised cholesterol with drugs (medication) prescribed by a doctor or other health worker?**	**Yes** **No**	**1** **2**	**B9**

EXPANDED: Triglycerides and HDL Cholesterol				
98	**Triglycerides** ***Choose accordingly: mmol/l or***	**mmol/l** **mg/dl**	☐☐.☐☐ ☐☐☐.☐	**B10**
99	**HDL Cholesterol** ***Choose accordingly: mmol/l or***	**mmol/l** **mg/dl**	☐.☐☐ ☐☐☐.☐	**B11**

Oral health


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