ANTHROPOMETRIC MEASUREMENTS VERSUS COMPUTED TOMOGRAPHY FOR THE ASSESSMENT OF METABOLIC SYNDROME IN THE BELLVILLE SOUTH MIXED ANCESTRY COMMUNITY, SOUTH AFRICA

by

SAAIGA ISMAIL

Student no: 205222714

Thesis submitted in fulfilment of the requirements for the degree

Master of Science in Radiography

in the Faculty of Health and Wellness Sciences

at the Cape Peninsula University of Technology

Supervisor: Prof T. Matsha
Co-supervisor: Mr A. Speelman

Bellville

15 November 2017

DECLARATION

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

Signed **Date**

ABSTRACT

Background: Metabolic syndrome (MetS) is a clustering of cardiovascular disease (CVD) risk factors which include abdominal obesity, hyperglycaemia, hyper-triglyceridaemia, low HDL-cholesterol and hypertension. This cluster of metabolic deviations is believed to be the primary driver of the double global epidemic of diabetes (DM) and CVD, conditions which account for much of the social and economic global burden of disease. Similar to the global trend, a high prevalence of MetS (62%) and type 2 Diabetes Mellitus (T2DM) (28.2%) has been reported for the South African mixed ancestry population, with CVD risk significantly higher in subjects with DM. The increase in MetS prevalence is driven by the obesity epidemic and anthropometric cut-off values to define MetS for this particular component of the disease has been reported to differ widely between different populations and ethnicities. Currently the waist circumference (WC) cut-off value for MetS diagnosis needs to be determined and validated per ethnic group as opposed to the previously used European derived cut-off values (Alberti et al., 2006; 2009). Validation of the WC cut-off value needs to be against one of the so called gold standards of central obesity measurement, such as Computer Tomography (CT). This technique can measure subcutaneous fat (SAT) and visceral fat (VAT) individually, which is important because VAT has been reported to be associated with MetS and CVD. The current study forms part of a large research group, investigating the prevalence and risk factors for MetS and T2DM in the South African mixed ancestry population.

Aims: This thesis focused on the imaging segment of the study in order to validate the previously derived 90 cm WC cut-off for MetS diagnosis in the mixed ancestry population of South Africa. Furthermore, to validate the use of a portable digital scale, using Bioelectrical impedance analysis (BIA) against CT-obtained VAT as an inexpensive substitute for VAT evaluation. The current study further aimed to investigate the relationship between CT-obtained VAT and SAT against known MetS components, including anthropometric and biochemical components.

Methods: Ethical approval for the larger Diabetes and Cardiovascular Disease cohort study as well as for the current study was obtained from the Cape Peninsula University of Technology, Health and Wellness Sciences Research Ethics Committee. Written informed consent was obtained from participants who formed part of this study. The current study population consisted of individuals of mixed ancestry residing in Bellville-South with the sample size overall 401, consisting of 93 males and 308 females. All participants were evaluated with respect to anthropometric measurements, blood pressure measurements,

biochemical analyses and CT scans of abdominal obesity. MetS classification was done according to the JIS MetS criteria, but adapted for the WC cut-off (≥90 cm for both men and women). Statistical analysis was done using the software program Statistica; Breakdown and one-way ANOVA and the Kruskal-Wallis ANOVA & Median test were used respectively for descriptive statistics and significance testing ($P<0.05$), correlation and association analysis with the Spearman Rank R test and linear regression analysis respectively. Primarily, CT-obtained SAT and VAT (cm^2) were assessed against the other components of MetS, all anthropometric and biochemical measurements as well as additional risk factors for Mets such as age and gender. To validate the 90 cm WC cut-off value previously reported, receiver operating characteristic (ROC) curve analysis has been used to determine cut-off values for CT-obtained VAT (cm^2) and BIA-obtained VAT (levels) in MetS diagnosis and which was then used for evaluation.

Results: The prevalence of MetS was high overall (55.6%), with a higher prevalence for females (57.1%) than for males (50.5%). The overall prevalence of obesity was 51.8% and for Type 2 Diabetes Mellitus (T2DM) it was 24.3% (known and screen detected). CT-measured VAT and SAT (cm^2) were significantly higher in females than in males, median and range, VAT (cm^2) in males 76.8 (39.5; 131.2) and in females 97.9 (62.3; 138.1); $P=0.0153$ and SAT (cm^2) in males 158.2 (66.9; 254.3) and in females 378.6 (270.6; 492.6); $P<0.0001$. The BMI ($P<0.0001$), WC (cm) ($P=0.0033$), hip (cm) ($P<0.0001$), post 2HR BG (mmol/L) ($P=0.0056$), fasting insulin (mIU/L) ($P=0.0001$), post 2HR insulin (mIU/L) ($P=0.0002$) and HDL-chol (mmol/L) ($P=0.0008$) were all significantly higher in females than in males. The SBP (mmHg) ($P=0.0074$) and Gamma glutamyltransferase (GGT) (IU/L) ($P=0.0043$) were significantly higher in males than females. Results showed significant increases in CT-obtained SAT (cm^2), CT-obtained VAT (cm^2) and in BIA-obtained VAT (levels) in both male and female subjects with MetS (All, $P<0.0001$). Anthropometric and glycaemic measurements, the lipids, GGT (IU/L) and the SBP (mmHg) and DBP (mmHg) were significantly increased in both male and female subjects with MetS ($P<0.02$) except HDL-chol (mmol/L) which was significantly decreased in subjects with MetS in both genders ($P=0.0001$). CT-measured SAT (cm^2) and VAT (cm^2) showed a highly significant positive correlation with each other in subjects without MetS ($R=0.6128$; $P< 0.0001$) as well as with other components of MetS, but in general with correlations of higher strength in subjects without MetS than in those with MetS.

Of the anthropometric measurements, the WC (cm) showed the highest and most significant association with CT-measured VAT (cm^2); the regression equation for method agreement in the total number of participants was $y = 79.1217 + 0.1854*x$; $R = 0.7519$; $P < 0.0001$; $R^2 =$

0.5654, indicating 56.54% method agreement between the two measuring modalities. Method agreement between BIA-measured VAT (levels) and CT-measured VAT (cm^2) was lower at 49.12% in the total group. Cut-off values for MetS diagnosis derived from ROC curve analysis for CT-measured VAT (cm^2) was >88.40 (cm^2) {sensitivity, specificity and area under the curve (AUC) respectively 72.2%, 70.8% and 0.81} and for BIA-measured VAT (levels) >10.50 (levels) {sensitivity, specificity and AUC respectively 66.2 %, 71.1 % and 0.79) which compared well with those of CT-measured VAT (cm^2). The % sensitivity and % specificity of the cut-off value of the WC (previously derived 90 cm) showed a higher % sensitivity as either CT-measured VAT (cm^2) or BIA-measured VAT (levels) did, but with the % specificity lower as compared to that of the other two measuring modalities.

Discussion: Results from the current study showed a high prevalence of MetS as well as T2DM, with overweight and obesity prevalence very high. These findings are similar to the previously reported for the mixed ancestry population.

The diagnostic performance of CT-measured VAT (cm^2) as tested for by ROC curve analysis was acceptable and that of BIA-measured VAT (levels) compared well with that of CT-measured VAT (levels). The WC (previously derived cut-off of 90 cm) showed a very high % sensitivity as compared to the other two measuring modalities, indicating a higher level of the correct diagnosis of MetS positive cases, but with the % specificity lower in the total group as compared to that of the other two measuring modalities. The need to identify subjects at risk for MetS has been reported and similarly results from the current study have shown that both overweight and obesity were equally associated with T2DM prevalence, highlighting the need to have a WC (cm) cut-off value to identify the maximum number of MetS positive cases, suggesting that the current cut-off value of 90 (cm) be accepted as a screening tool for MetS identification in the predominantly overweight/obese mixed ancestry population. The cut-off value of CT-measured VAT (cm^2) was >88.40 (cm^2) for MetS prediction in the total group of participants which differed from published data.

Conclusion: The South African mixed ancestry population as tested for in the current study has a high prevalence of obesity, MetS as well as T2DM, with results indicating obesity associated with both MetS as well as T2DM prevalence. Evaluation of these results suggested that both the WC (cm) cut-off value of 90 cm as well as the derived cut-off value of BIA-measured VAT (levels) of >10.50 (levels) can be accepted as sufficiently comparable to that of the CT-measured VAT (cm^2) and can be used as substitute markers for abdominal obesity in MetS diagnosis. Furthermore, that the derived cut-off value of >88.40 (cm^2) for CT-

measured VAT (cm^2) is recommended in the mixed ancestry population as specific for MetS diagnosis in the mixed ancestry population.

KEY WORDS

Bioelectrical impedance analysis, central obesity, computed tomography, Metabolic Syndrome, subcutaneous abdominal tissue, visceral abdominal tissue, waist circumference

ACKNOWLEDGEMENTS

I wish to thank:

- God Almighty for carrying me through this journey and for blessing me with the much-needed health and strength.
- Professor Tandi Matsha, for allowing me to be part of this study. Her encouragement, advice and expertise in this field of study empowered me with new knowledge and skills. I would also like to express my thanks to her for her willingness to guide me during my study period.
- Mr Aladdin Speelman for his suggestions, assistance and continuous support.
- Dr Gloudina Hon for assisting with the statistical analysis.
- Ms Saarah Davids for her practical assistance.
- The Bellville South community.
- The Radiology, Radiography and admin staff at Morton and Partners Radiology Practice.
- My colleaques and the students at the Department of Medical Imaging and Therapeutic Sciences for their patience.
- Ms Estelle Krige, the librarian at the Groote Schuur Hosptal campus for her assistance.

The financial assistance of the National Research Foundation towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the National Research Foundation.

DEDICATION

I dedicate this thesis to my late parents who has encouraged me throughout my life.

I would also like to dedicate this thesis to my sister, Wardah for her constant support and love.

TABLE OF CONTENTS

	GLOSSARY OF RELATED TERMS
Anthropometry	The use of body measurements such as weight and height; it measures the physical aspects of the body
Bioelectrical impedance analysis	An analysis used to calculate an estimate of body water by measuring its resistance to the flow of an electric current through the body tissues
Body mass index	Calculated as weight (kg) of the patient divided by height^2 (m^2)
Central adiposity/obesity	The term that is used clinically to define abdominal adiposity/obesity
CT number or Hounsfield unit	The numeric information defining the density of tissue within a pixel on a CT image
Field of view	The diameter of image reconstruction in CT
Pixel	Picture element which is a 2-dimentional representation of a corresponding tissue volume within a CT image
Subcutaneous fat	Fat which is located beneath the skin (in the context of this study this fat will be within the abdominal cavity)
Visceral fat	Fat that surrounds the organs, also called visceral adipose tissue (in the context of this study this fat will be within the abdominal cavity)
Waist circumference	Anthropometric measurement of the waist; measured midway between the lower border of the rib cage and the iliac crest
Waist to hip ratio	The waist circumference divided by the hip circumference
Window width	The absorption measurement range in CT that determines the maximum number of shades of grey that can be displayed on the CT monitor
Window level	The centre of the range of CT numbers

ABBREVIATIONS	
AACE	American Association of Clinical Endocrinology
AHA	American Heart Association
ATP III	Adult Treatment Panel III
AUC	Area under the curve
BFAM ratio	Brachio-femoral adipomuscular ratio
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM	Diabetes Mellitus
EGIR	European Group for the Study of Insulin Resistance
FBG	Fasting blood glucose
FOV	Field of view
GGT	Gamma GT-S: Gamma-glutamyl transferase
HbA1c	Glycated haemoglobin
HDL-chol	High-density lipoprotein cholesterol
HPT	Hypertension
HU	Hounsfield units
IDF	International Diabetic Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
L4-L5	Inter-vertebral disc space of lumbar vertebrae 4 and 5
LDL-chol	Low-density lipoprotein cholesterol
MetS	Metabolic Syndrome
NCEP	National Cholesterol Education Program
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
Post 2HR BG	Post load blood glucose
ROC	Receiver operating characteristic
T2DM	Type 2 Diabetes Mellitus
VAT	Visceral adipose tissue
WHO	World Health Organisation
WHR	Waist to hip ratio
WL	Window level
WW	Window width

CHAPTER ONE
LITERATURE REVIEW

1.1 Introduction

Metabolic Syndrome (MetS) is a metabolic disorder used to identify high risk individuals for type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Kang et al. 2012; Roberts et al., 2013; Dragsbæk et al., 2016). High prevalence rates have been reported globally for MetS as well as for T2DM and CVD, with the prevalence of MetS ranging between 10% and 84% (Byrne & Wild, 2011). The prevalence of MetS was significantly higher in diabetic individuals (69% vs. 34%) (Won et al., 2014), with estimated numbers for diabetes mellitus (DM) 346 million globally of which 90% was T2DM {(The World Health Organization (WHO, 2011)} and which has been reported as a major risk factor for CVD (Bradshaw et al., 2007). The WHO described CVD as the number one cause of death globally and the disease has been reported as the second leading cause of death in South Africa (Bradshaw et al., 2003). Reports from South African investigators showed similarly high prevalence rates for MetS and T2DM. Erasmus et al. (2012) reported a high prevalence of T2DM (28.2%) in the South African mixed ancestry population, with a high percentage of undiagnosed T2DM (18.1%) and a very high prevalence for MetS (62.0%). Due to the global epidemic of T2DM and CVD the need for optimal guidelines on the early diagnosis of MetS have been recognised (Grundy, 2008).

Components to identify MetS include abdominal or central obesity, hyperglycaemia, hyper-glyceridaemia, low HDL-cholesterol and hypertension (Alberti et al., 2006; Sutton & Raines, 2007; Handelsman, 2009). The high prevalence of obesity requires accurate non-invasive measurements of body fat as biomarkers in risk assessment (Hu et al., 2011). One of the markers to assess abdominal obesity is the waist circumference (WC), a rapid method which is simple and convenient for epidemiological studies (Atkinson & Uwaifo, 2005). However, recommended reference values on WC cut-off in Mets diagnosis derived from studies conducted in Europe and North-America (Motala et al., 2009; Crowther & Norris, 2012) have in most cases been inadequate when applied to other race or ethnic groups, including populations in Asia and Africa (Seidell et al., 2001; Misra et al., 2005). Local environmental and genetic influences may have an impact on the reference values when validating diagnostic criteria and reference values should therefore be race or ethnic specific and can only be generalised to the population where the study is being conducted (Seidell et al., 2001; Misra et al., 2005). In this regard, there is a lack of data available to define WC reference values for MetS diagnosis, particularly in African populations (Alberti et al. 2009).

It was within this context that Erasmus et al. (current) and Matsha et al. (current) conducted a second cross-sectional study in the mixed ancestry population of Bellville South, a suburb of Cape Town, South Africa. Reports from their first study, conducted from 2008 to 2011 in the mixed ancestry population (Erasmus et al., 2012; Matsha et al., 2012), showed a high prevalence of MetS (62%) and T2DM (28.2%), with CVD risk significantly higher in subjects with T2DM. The group reported central obesity (as measured by anthropometrically measured WC) as the most common diagnostic criterion for MetS and identified 90 cm for both males and females as the optimum WC cut-off value for the diagnosis of MetS. Due to the high prevalence of MetS and T2DM reported in the mixed ancestry population (Erasmus et al., 2012; Matsha et al., 2012), the group commenced on a second study in the same population, from 2014 to 2016 to investigate additional risk factors for T2DM and CVD and additionally to validate the waist cut-off for MetS diagnosis reported by them during their earlier study. The current study forms part of this second study conducted by Erasmus et al. (current) and Matsha et al. (current) and primarily aimed to investigate abnormal fat distribution by assessing central obesity with the use of CT scanning and to validate the newly identified 90 cm WC cut-off for the diagnosis of MetS reported by Erasmus et al. (2012) against CT measurements of visceral fat (VAT). In this regard, in order to have consensus and to allow further modification of the definition of MetS, the International Diabetic Federation (IDF) advocated that new research studies should investigate abnormal fat distribution by assessing central obesity with the use of Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) (IDF, 2006). The abdomen contain both VAT and subcutaneous abdominal fat (SAT) and both CT and MRI is capable of differentiating between the two fat types (Van der Kooy & Seidell, 1993; Mattsson & Thomas, 2006). Consensus on which modality is better remains open, but reports suggest a preference for CT and this techniques is considered as the gold standard for SAT and VAT measurements (Mattsson & Thomas**,** 2006; Hu et al., 2011; Shuster et al., 2012).

However, due to the usage of radiation during the acquisition of CT images, alternative assessments have been used to evaluate central obesity, including anthropometric measurements, including the WC and more currently Bioelectrical Impedance Analysis (BIA) which assess VAT per se. The current research study also investigated the use of BIA, using a portable digital scale for VAT measurements, to evaluate its usefulness as a possible substitute for CT measurements of VAT. The last two decades have seen a growing trend in the use of the portable digital scale as a non-invasive assessment method of VAT (Jebb et al., 2000; Maligie et al., 2012), however, using BIA in VAT estimation in Africa, including the mixed ancestry population needs verification (Dehghan & Merchant, 2008).

1.2. Metabolic syndrome (MetS)

1.2.1. MetS prevalence

1.2.1.1 MetS prevalence reported globally

An increase in MetS prevalence has been shown globally. In 2004, MetS afflicted 20-25% of the world's population (Stern et al., 2004), by 2006 it was reported increased to approximately 25% (IDF, 2006) and to 35% in 2007 (Sutton & Raines, 2007). Recent data suggested that the global prevalence of MetS has further increased and now ranges between 10% and 84% (Byrne & Wild, 2011). Evaluation of the reported prevalence rates showed that the appropriate criteria have been applied in these epidemiological studies (Byrne & Wild, 2011). MetS has been reported in all ethnic and age groups (Handelsman, 2009) but prevalence varied between various ethnicities, including differences reported for the American Non-Hispanic blacks, Non-Hispanic whites and Mexican-Americans (Ervin, 2009).

1.2.1.2 MetS prevalence reported in South Africa

Studies from South-Africa have shown a higher prevalence of MetS in white South Africans than black South Africans, 74% and 46% respectively (Kalk & Joffe, 2008). Motala et al. (2011) reported a lower MetS prevalence in a rural black community at 26.5% overall and demonstrated a peak prevalence in the age group between 45-54 years. In 2011, a study by Hoebel et al. showed that black African women presented with fewer MetS risk factors than their counterparts. Results from an epidemiological study, investigating the prevalence of MetS in a mixed ancestry population in Bellville-South, South Africa, showed a 62% and 60.6% prevalence of Mets, using the JIS and IDF criteria respectively (Erasmus et al., 2012). In this regard, early detection of MetS is important as it is the MetS-afflicted individual who has an increased risk of developing T2DM and CVD (Stern et al., 2004; Alberti et al., 2006; Galassi et al., 2006) and similar to the high prevalence of MetS reported by Erasmus et al. (2012), the group also reported a high prevalence of T2DM (28.2%) and of undiagnosed T2DM (18.1%).

1.2.2. The patho-physiology of MetS

MetS has originally been named 'Syndrome X', a term used by Professor Reaven in his Banting lecture in 1988; in 1989 Kaplan rephrased it to 'The Deadly Quartet' which then became known as 'The Insulin Resistance Syndrome'. However, currently the term 'metabolic syndrome' is preferred. MetS is not a disease, but rather a constellation of CVD risk factors which arises from obesity. Components to identify MetS include abdominal or central obesity, hyperglycaemia, hyper-glyceridaemia, low HDL-cholesterol and hypertension (Alberti et al., 2006). Abdominal fat, which contributes to abdominal obesity, is divided into

SAT and VAT and it is the VAT measurement which corresponds mostly to the WC (Després, 2006). WC as a measurement criterion for the confirmation of MetS refers therefore more to the metabolically active visceral fat region of the abdomen. VAT is central to the pathogenesis of MetS as it is essentially the accumulation of this fat that plays a role in the development of risk factors of T2DM and CVD (Matsuzawa et al., 2011; Unno et al., 2012). Measurement of the WC has been reported to be simple and convenient for epidemiological studies (Atkinson & Uwaifo, 2005).

1.2.3 MetS associated diseases, including diabetes and cardiovascular disease

MetS with its cluster of metabolic deviations is believed to be the primary predictor of the double global epidemic of T2DM and CVD, conditions which account for much of the social and economic global burden of disease (Grundy et al., 2004; Stern et al., 2004; Esteghamati et al., 2008; Alberti et al., 2009). MetS has been reported to increase the risk of future T2DM (3.6-fold) and CVD (1.3-fold) in elderly Caucasian woman and similarly for women with only some risk factors for MetS as compared to women with no MetS risk factors (Dragsbæk et al., 2016). The MetS-afflicted population who is at high risk of developing DM and CVD are predisposed to myocardial infarcts, cerebral vascular accidents and even premature mortality (Stern et al., 2004; Alberti et al., 2006; Galassi et al., 2006). Studies have shown that the prevalence of MetS was significantly higher in diabetic individuals than in those individuals without diabetes (69% vs. 34%, $P < 0.001$) when the association between MetS and coronary artery disease in the presence of diabetes was investigated (Won et al., 2014). Dudina et al. (2011) reporting on 12 European cohort studies, showed a graded increase in both genders in the risk of CVD death with increasing body weight.

Globally, reports from the WHO showed that 346 million people already have DM of which 90% is T2DM (WHO, 2011) and reports from South Africa showed that CVD is the second leading cause of death amongst South Africans (Bradshaw et al., 2003), with DM a major risk factor for CVD (Bradshaw et al., 2007). Erasmus et al. (2012) reported a high rate of undiagnosed T2DM (18.1%) in their study, leading the authors to predict a huge increase in T2DM as well as in CVD cases in the South African mixed ancestry population (Erasmus et al., 2012).

1.2.4. Diagnostic criteria for MetS

The clinical manifestations for MetS include abdominal obesity, dyslipidaemia, hyperglycaemia and hypertension. MetS is confirmed if 3 of the 5 diagnostic criteria are present (Sutton & Raines, 2007; Handelsman, 2009). Prior to 2009, there were various definitions of MetS and research studies were conducted to elucidate the physiological

processes which led to the development of the disease. A world-wide definition for MetS was needed that would facilitate its diagnosis in a clinical setting so that it would be easy to identify patients at risk for T2DM and/or CVD. The proposed definition had to be easy to use in different clinical settings, such that data could easily be compared across the world (IDF, 2006). Various expert groups investigating MetS have suggested criteria for diagnosing MetS. The most widely used criteria were those that were devised by the World Health Organisation (WHO), the European Group for the Study of Insulin Resistance (EGIR), the National Cholesterol Education Program -Third Adult Treatment Panel (NCEP-ATP III), the American Association of Clinical Endocrinology (AACE), the International Diabetic Federation (IDF) (Alberti et al., 2006) and the Joint Interim Statement (JIS) (Alberti et al., 2009).

1.2.4.1. Diagnostic criteria for MetS, WHO

The WHO defined MetS in 1998 as insulin resistance together with any two of the following criteria, obesity which is measured using the BMI, dyslipidaemia, hypertension or microalbuminuria (Alberti & Zimmet, 1998). The BMI, however only measures the weight and the height of an individual and does not take the distribution of fat in the abdomen into account. There is therefore no indication of the degree of intra-abdominal adiposity (Després, 2006).

1.2.4.2. Diagnostic criteria for MetS, EGIR

In 1999, EGIR determined that MetS required the presence of insulin resistance plus any two of the following criteria, a WC of ≥94 cm and ≥80 cm in men and women respectively, dyslipidaemia, hypertension or a raised fasting blood glucose (FBG) for the diagnosis of MetS (Balkau,1999).

1.2.4.3. Diagnostic criteria for MetS, NCEP-ATPIII

In 2001, NCEP-ATPIII, determined the diagnoses of MetS as the presence of any three or more of the following criteria were present: a WC >102 cm in men and >88 cm in women, hypertension, elevated triglycerides, reduced high-density lipoprotein cholesterol (HDL-chol) and a raised FBG (Cleeman, 2001).

1.2.4.4. Diagnostic criteria for MetS, AACE

In 2003, the AACE proposed that the MetS definition should rely more on clinical judgement rather than on a specific criteria, but the group did specify some abnormalities which included elevated triglycerides, reduced HDL-chol, elevated blood pressure, elevated FBG and post load blood glucose (Post 2HR BG). They further confirmed that obesity and hypertension were associated with MetS prevalence.

1.2.4.5. Diagnostic criteria for MetS, IDF

The IDF held a workshop in 2004 where the group discussed the features that would confirm the presence of MetS. These included abnormal fat distribution, insulin resistance, atherogenic dyslipidaemia, elevated blood pressure, pro-inflammatory state and a pro-thrombotic state (Alberti et al., 2006).

1.2.4.6. Diagnostic criteria for MetS, IDF/AHA

An attempt was made in 2005 by the IDF and AHA to merge the different clinical definitions. The IDF however, made abdominal obesity a required component for the diagnosis of MetS, using the WC as the screening tool. The group recommended that the threshold WC for Europeans should be ≥94 cm for men and ≥80 cm for women (Alberti et al., 2005). As has subsequently been reported, the WC is gender and ethnic specific, but Sub-Saharan Africans together with Eastern Mediterranean and Middle East (Arab) populations were to use the proposed WC reference values of the Europeans because a WC cut-off value for these populations had not yet been established (Alberti et al., 2005). In contrast to the IDF's position, the AHA did not require the abdominal obesity component but its threshold recommendation for WC was ≥102 cm for men and ≥88 cm for women (Grundy et al., 2005).

1.2.4.6. Diagnostic criteria for MetS, JIS

The IDF task team, together with representatives from other scientific groups released a Joint Interim Statement (JIS) in 2009, which proposed to reduce the controversies surrounding the definitions of MetS. An agreement was reached between these groups which stated that neither abdominal obesity nor any other diagnosing criterion would be a required component for the diagnosis of MetS. Their decision was that any 3 of the 5 diagnostic criteria would confirm MetS, but suggested that WC measurements should still be used as a screening tool.

1.2.4.6.1. MetS diagnostic criteria with WC reference per ethnic group (JIS) (Table 1.1)

The need to determine MetS criteria, specifically with regards to obesity measures was becoming known and furthermore that MetS prevalence should be investigated per ethnic group (Grundy, 2008). The panel on JIS similarly acknowledged the importance of investigating reliable WC cut-off points for different ethnic groups, but that either IDF or AHA WC cut-off points should be used in the interim for non-Europeans, until appropriate data becomes available (Alberti et al., 2009). The consensus diagnostic criteria would therefore result in an improvement in applicability and in positive predictive values for the diagnosis of MetS (Kaur, 2014).

Table 1.1. Diagnostic criteria for MetS as defined by JIS (Alberti et al., 2009)

	Criterion	Measurement cut-off points
1.	Waist circumference (WC)	Population- and country-specific definitions (IDF recommendation for cut-off points to be used for non-Europeans until more data available)
2.	Triglycerides	≥150 mg/dL (1.7 mmol/L)
	(if on drug therapy due to elevated reading)	(a different cut off point)
3.	High density lipoprotein cholesterol (HDL-chol)	<40 mg/dL (1.0 mmol/L) in males <50 mg/dL (1.3 mmol/L) in females
	(if on drug therapy for reduced HDL-C)	(a different cut off point)
4.	Blood pressure	Systolic (SBP) ≥130 and/or diastolic (DBP) ≥85 mmHg
	(if on drug therapy due to elevated reading)	(a different cut off point)
5.	Fasting blood glucose (FBG)	≥100 mg/dL (≥5.6mmol/L)
	(if on drug therapy due to elevated reading)	(a different cut off point)

1.2.5. MetS diagnostic components

Metabolic syndrome is thought to be caused by insulin resistance and adipose tissue dysfunction. Even though the pathophysiological sequence of metabolic abnormalities resulting in MetS remains uncertain, experts agree that insulin resistance and abdominal obesity due to abnormal adipose deposition and function are the main risk factors for the syndrome (Esteghamati et al., 2008; Olufadi & Byrne, 2008). Currently, the specific criteria used to diagnose MetS, include abdominal or central obesity, hyperglycaemia, hyperglyceridaemia, low HDL-chol (dyslipidaemia) and hypertension (Alberti et al., 2006).

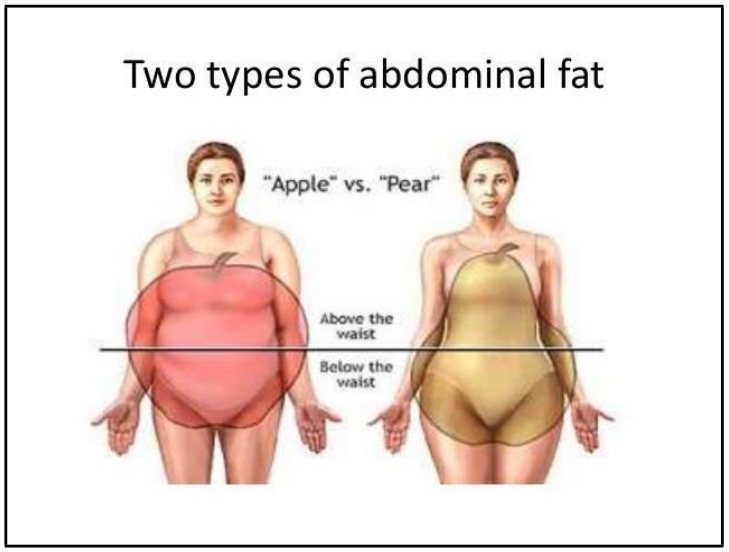
1.2.5.1. Abdominal or central obesity

Abdominal obesity has been identified by the IDF in 2005 as one of the criteria that define MetS (Hansen, et al., 2006). Obesity is a consequence of overeating and a sedentary lifestyle which causes a chronic imbalance between energy intake and energy expenditure (Misra & Khurana, 2008). MetS is said to be the primary predictor of CVD and T2DM, which are the most prevalent obesity-related diseases, especially when associated with dyslipidaemia and hypertension (Misra et al., 2005).

Globally, the United States has the highest prevalence of obesity (32%) (WHO, 2004). Reports from South Africa are showing similar and/or higher trends as the obesity levels reported in well developed countries, in that 29% of South African men and 56% of women were classified as either overweight or obese (Puoane et al., 2002; Lokuruka, 2013). Goedecke, (2005) reported that the highest prevalence of overweight and obesity was found amongst black women (58.5%), but that the prevalence of obesity was similarly high in mixed ancestry (52%) white (49.2%) and Indian (42.8%) women.

1.2.5.1.1 Abdominal or central obesity classification, android and gynoid shapes

Abdominal obesity is caused by the accumulation of excess VAT and excess SAT) (Freedland, 2004; Shuster et al., 2012). When abdominal fat is more centrally distributed, an obese individual is classified as either android or 'apple' shaped which infers a greater risk for MetS; when the fat is predominantly in the gluteo-femoral area, the obese individual is described as gynoid or 'pear' shaped (Hansen et al., 2006). In as early as 1947 Vague addressed the distribution of fat and suggested that upper-body fat, also known as android-type excess weight, was more dangerous than the lower risk gynoid-type obesity (Figure 1.1). The classification of fat by Vague was substantiated by the brachio-femoral adipomuscular (BFAM) ratio whereby subjects with a higher BFAM ratio were described as the android type, which is associated with more metabolic complications. However, obesity is a heterogeneous condition and not every obese person has co-morbidities (Wang, 2012).



(a) **(b)**

Figure 1.1. Demonstrates (a) the android or 'apple' shaped individual, with abdominal fat more centrally distributed and (b) the gynoid-type or 'pear' shaped individual, with the fat predominantly in the gluteo-femoral area (Hansen et al., 2006; New York Times, http://www.nytimes.com/imagepages/2007/08/01/health/adam/19265Differenttypesofweightgain.html) [Accessed 15 February 2017].

1.2.5.1.2 Abdominal or central obesity classification, VAT and SAT

In the 1980's, obesity was divided into central and peripheral obesity using the anthropometric measurement of waist to hip ratio (WHR) to show that upper-body obesity is related to obesity-related diseases, more so than lower-body obesity (Bjorntorp, 1985). Individuals with higher levels of central adiposity are at a greater risk of developing lipid and glucose abnormalities than those with fat distributed more peripherally (Lamarche, 1998). Obesity was then further classified into VAT and SAT obesity, when the difference in fat distribution could be demonstrated with CT scans (Tokunaga et al., 1983; Matsuzawa et al., 1987). CT scanning of the abdomen of obese individuals with excess VAT showed a positive association with some of the diagnostic criteria of MetS, while the association with these risk factors in obese individuals with less VAT was absent (Després, 2012).

Although abdominal adipose tissue is described as metabolically active, the VAT has different metabolic qualities than SAT (Misra & Vikram, 2003). VAT has been classified as an endocrine organ because it produces hormones such as leptin and adiponectin and has a pathogenic role (Freedland, 2004; Kershaw & Flier, 2004).

1.2.5.1.2.1 VAT and SAT distribution in gender

Fat distribution differs in males and females. Men have more VAT than women and women have more SAT than men. Matsuzawa (2008) suggested that the sex hormones may be associated with fat distribution; an association between low testosterone levels and visceral adiposity in males has been reported and in females oestrogen may contribute to an increase in subcutaneous deposition which would increase insulin sensitivity (Crowther & Ferris, 2010). Excess intra-abdominal fat therefore infers an increased risk of developing a metabolic disorder (Misra & Vikram, 2003). However, women may be more obese than men but are less prone to metabolic diseases as a result of the distribution of their fat (Lokuruka, 2013).

1.2.5.2. Hyperglycaemia

1.2.5.2.1. Fasting blood glucose (FBG)

The current recommendations (WHO, 2016) for the diagnosis of diabetes and intermediate hyperglycaemia are a FBG of ≥7.0 mmolL or post 2HR BG ≥11.1 mmolL for DM classification, a FBG of <7.0 mmolL and a post 2HR BG between ≥7.8 mmolL and <11.1 mmolL for impaired glucose tolerance (IGT) classification, a FBG between 6.1 and 6.9 mmolL and if measured, a post 2HR BG of <7.8 mmolL for impaired fasting glucose (IFG) classification. That is, levels below 6.1 mmol/L is classified as normal, levels between 6.1 and 6.9 mmol/L is classified as FBG and levels above 7.0 mmol/L are confirmation for DB.

The IDF classified a lower level of FBG of ≥5.6 mmol/L (>100 mg/dL) as a diagnostic criterion for MetS (Janghorbani & Amini, 2011), but Bjornholt et al. (1999) showed that a FBG of more than 85 mg/dL already poses a 40% higher risk of mortality than a FBG of less than 85 mg/dL. Al Kadi and Alissa (2011) reported an increase in the prevalence of IFG with an increase in the WC.

1.2.5.2.2. Insulin resistance

Insulin resistance appears to be the primary mediator of MetS. Insulin promotes glucose uptake in muscle, fat and liver cells and can influence lipolysis and the production of glucose by hepatocytes (Wang, 2012). Insulin is the hormone that regulates metabolism and plays a vital role in maintaining glucose and lipid levels in the blood as well as controlling carbohydrates during metabolism. The clinical significance of elevated plasma glucose, particularly in the obese person, may be indicative of insulin resistance. The pancreatic beta cells in the insulin-resistant individuals secrete more insulin to overcome hyperglycaemia (Gill et al., 2005). In insulin resistance, tissue has a diminished ability to respond to the action of insulin. Insulin resistance in fat cells results in hydrolysis of stored triglycerides, which elevates free fatty acids in the blood plasma. Insulin resistance in muscle reduces glucose uptake, whereas insulin resistance in the liver reduces glucose storage, with both effects serving to elevate circulating blood glucose. Raised serum glucose levels precede the development of essential hypertension (Reaven, 2003). In addition to developing essential hypertension and glucose intolerance, these insulin-resistant patients tend to also develop elevated plasma triglyceride levels and low HDL-C. All of these findings are consistent with the diagnosis of metabolic syndrome (Sutton & Raines, 2007).

1.2.5.3. Dyslipidaemia and hyperglyceridaemia

The lipid profile includes HDL-chol, low-density lipoprotein cholesterol (LDL-chol) and triglycerides (blood fats) of which in general a higher level of the first and lower levels of the last two are predictive of better health status (Toth, 2005). HDL-chol is said to protect against atherosclerosis by removing cholesterol and thereby preventing build-up of cholesterol in the blood vessels (Toth, 2005). Dyslipidaemia is characterised as either elevated LDL-chol levels, or as low levels of HDL-chol levels, which may be present in insulin-resistant patients (Sutton & Raines, 2007; Kaur, 2014), while hyperglyceridaemia is characterized by elevated triglyceride levels. The lipid profile changes in MetS subjects consist of decreased levels of HDL-chol, increased levels of triglycerides as well as increased levels of LDL-chol. Low levels of HDL-chol are also a risk factor for both DM and CVD (Wierzbicki, 2006).

1.2.5.4. Hypertension

Hypertension plays an important role in the development of CVD and is currently described as the leading cause of morbidity and mortality (Kearney et al., 2005). Hypertension is defined as having persistent elevated systemic blood pressure, with diagnostic criteria from the WHO set at a SBP of 160 mmHg and/or a DBP of 95 mmHg (WHO, 2003). The condition can be either primary, with no underlying cause or secondary of which the most common cause is chronic renal failure. The persistent elevated systemic blood pressure leads to the development of atherosclerosis which thickens and narrows the walls of the arteries and which lead to ischemia of affected organs. MetS may increase the risk of cardiovascular morbidity and mortality also in hypertensive patients, which are signs of end organ damage (Mulè et al., 2014).

The blood pressure stipulated by the IDF in order to qualify as a diagnostic criterion of MetS is 130 mmHg for SBP and 85 mmHg for DBP and lower if nephropathy is present (IDF, 2006; Duvnjak et al., 2008). Some authors have reported that the association of hypertension with MetS is still not clear; while others believe that this pathological condition may develop as a result of hyperglycaemia and hyperinsulinemia. In this regard, hyperglycaemia and hyperinsulinemia have been reported as risk factors for hypertension and atherosclerosis (Sowers et al., 1993; Duvnjak et al., 2008). Some experts are of the opinion that all diagnostic criteria of MetS contribute to hypertension (Franklin et al., 2006).

1.2.6 Factors affecting MetS diagnostic components

Several factors are known to contribute to an increase in MetS prevalence, mostly by increases/decreases in the components that are used to identify MetS with. The world-wide increase in obesity and sedentary lifestyle have been suggested as contributors to the high prevalence (25%) of MetS world-wide (Alberti et al., 2009; Wang, 2012). Other contributing risk factors to MetS include weight, genetics, aging, stress, inadequate exercise and poor diet (Wang, 2012) with reports showing an increased health risks with high WC values in both males and females (Janssen et al., 2002).

1.2.6.1. Gender in MetS prevalence

MetS affects both genders but the difference in prevalence reported is not consistent. An overall high prevalence (66.1%) was reported for an indigenous population of Brazil (Soares et al., 2015) with a higher prevalence for women (76.2%) than for men (55.6%). The investigators of the study concluded that the increase in prevalence was due to excess weight and a sedentary lifestyle. However, while some reports showed a higher prevalence of MetS in females than in males (Handelsman, 2009), others have reported no gender

differences (Borch-Johnsen, 2010, Motala, et al., 2011). Reports from South Africa showed that black African women presented with fewer MetS risk factors than their counterparts (Hoebel et al., 2011), while Erasmus et al. (2012) reported much higher MetS prevalence rates in mixed ancestry females (68.4%) than in males (35.2%). Gender differences in MetS prevalence may be due to the different criteria used to define MetS (Borch-Johnsen, 2010).

1.2.6.2. Age in MetS prevalence

The incidence of obesity and insulin resistance, which are strongly linked to MetS, increases with age, resulting in a higher prevalence of MetS in the elderly (Figure 1.2). MetS have been reported in subjects at age 30, but peaks at age 60 to 75 years, with a 40 % prevalence reported in the over 60 year age group (Ford et al., 2004; Borch-Johnsen, 2010).

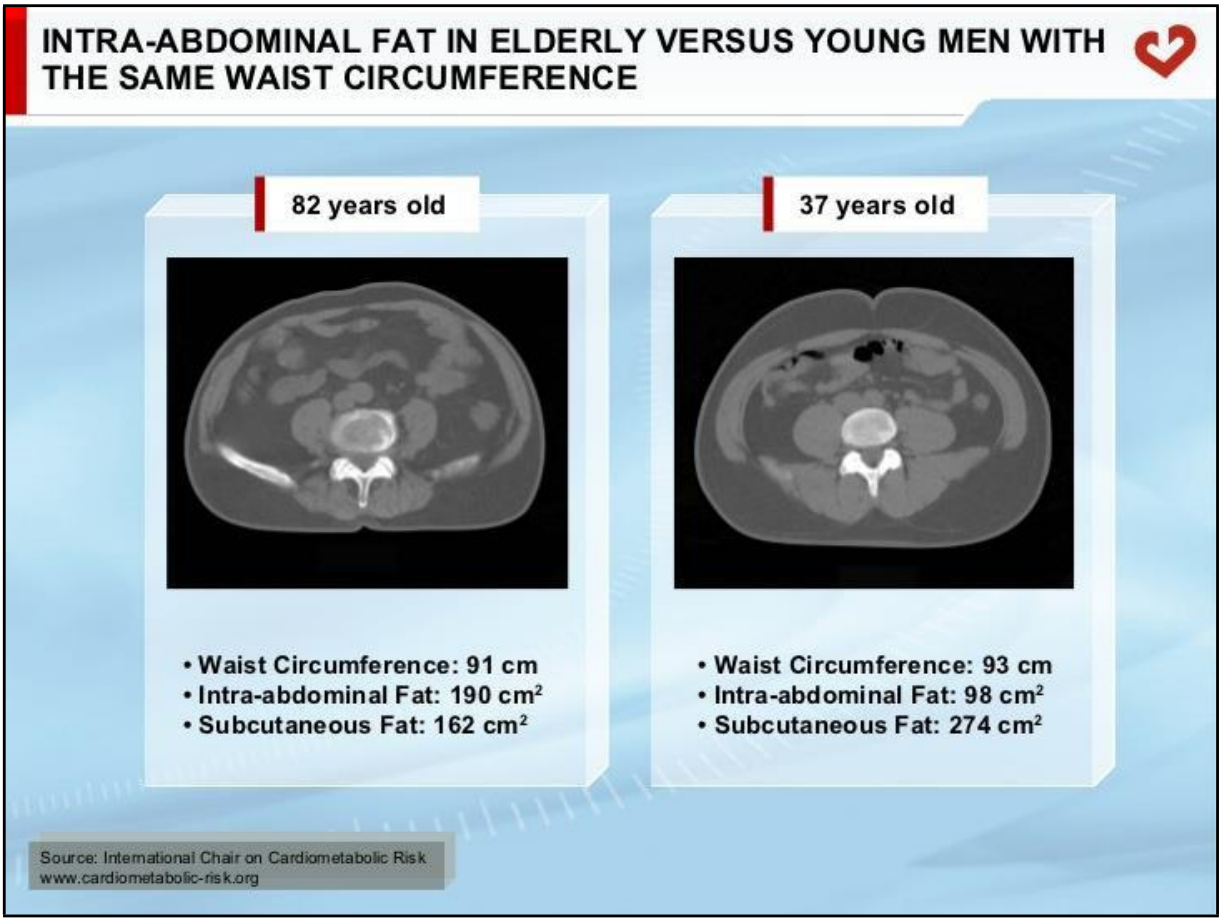


Figure 1.2. The increase in both intra-abdominal and subcutaneous fat in an aging subject as compared to that of a younger subject with a similar WC. Reference: International Chair on Cardiometabolic Risk, www.cardiometabolic-risk.org [Accessed 15 February 2017].

1.2.7. Evaluating measures of abdominal or central obesity in MetS determination

Due to the increase in the prevalence of MetS, early identification of individuals at risk was necessary to enable appropriate preventative measures to reduce the development of

related diseases, including DB and/or CVD in the population (IDF, 2006). In this regard, early identification of and then prevention of the major risk factors is crucial because these are likely to become apparent as the disease progresses (Grundy, et al., 2005). Obesity, which is strongly linked to MetS, is one of the risk factors and an important evaluation tool in the diagnosis of MetS and as such establishing optimal cut-off points for obesity in different ethnicities was and remains a priority. According to Ayvaz and Çimen (2011) quantification of body fat tissue and its distribution are important in the determination of disease risk in clinical research. There are two ways of diagnosing obesity, either anthropometrically, which includes body mass index (BMI), waist to hip ratio (WHR) and the WC or imaging modalities, including Magnetic Resonance Imaging (MRI) amongst others.

1.2.7.1. Measures of obesity, anthropometric measurements

1.2.7.1.1. st circumference, measuring tape

An increase in central obesity predicts health risk (WHO, 1998). Initially, central or abdominal obesity has been suggested by the IDF (2006) as a specific requirement for the diagnosis of MetS, but was later declared by JIS (2009) to be one of five criteria of which the presence of any three was sufficient to confirm MetS (Alberti et al., 2006; Alberti et al., 2009). The anthropometrically measured WC has been suggested as a surrogate marker for abdominal obesity, with the threshold set between 94 cm and 104 cm and between 80 cm and 88 cm for men and women respectively. Due to differences in WC in ethnicity and gender, prescribed criteria for WC cut-off for MetS evaluation established in European subjects needs re-evaluation for different ethnicities. Currently available WC cut-off values that have been defined according to ethnicity and gender are summarized in Table 1.2 (IDF, 2006).

However, extrapolation of these WC cut-off values for MetS diagnosis is not optimal. For example, the reference value as stipulated by the IDF requires a tape-measured WC of ≥90 cm for men and ≥80 cm for women in MetS evaluation, while the WC cut-off as a risk factor for MetS for Japanese Americans were established at between 87.1 cm and 90 cm for men and between 80.8 cm and 89 cm for women (Hayashi et al., 2007). Similarly, Motala et al. (2011) reported a WC cut-off of 86 cm and 92 cm for men and women respectively in a Zulu rural South African community which were not in accordance with the IDF WC values for Sub-Saharan Africa whose WC threshold recommendations for men are higher (≥94 cm) and lower for women (≥80 cm) (IDF, 2006). The report by Motala et al. (2011) also highlighted gender differences in WC cut-off for MetS diagnosis, with the cut-off point for males much lower than the cut-off point for females as opposed to those reported in European settings {Table 1.2 (IDF, 2006)}.

A disadvantage of using anthropometric measurements is that it only measures VAT indirectly (Shuster et al., 2012) and that the WC measurement is an estimate of central obesity only, as it represents the accumulation of VAT and SAT which may challenge the accuracy of this measurement (Misra & Vikram, 2003; Unno et al., 2012). However, Hayashi et al. (2007), in addition to establishing cut-off values for WC for MetS diagnosis in Japanese Americans, reported a similar discrepancy in VAT cut-off values for this group compared to established cut-off points. The authors reported VAT cut-offs for MetS to be between 88.6 cm^2 and 96.1 cm^2 for men and between 51.5 cm^2 and 86.3 cm^2 for women in the Japanese Americans, which differed from the prescribed cut-off points of 130 cm^2, a cut-off point which according to Després and Lemieux (2006) is associated with a high risk of CVD. Therefore, establishing obesity cut-off values using WC measurements to comply with differences in ethnicity may also be needed for VAT specifically.

Table 1.2. Ethnic-specific WC as a threshold for central obesity (IDF, 2006)

Population	Waist circumference	
	Males	**Females**
Europe	≥94 cm	≥80 cm
United States	≥102 cm	≥88 cm
Asian	≥90 cm	≥80 cm
Middle East, Mediterranean	≥94 cm	≥80 cm
Sub-Saharan Africa	≥94 cm	≥80 cm

1.2.7.1.2. st to hip ratio, measures relationship between waist and hip

The waist to hip ratio (WHR) was introduced as a comparative type of measurement for obesity. The WHR and BMI as assessment of fat distribution have been widely used to predict risks of CVD (Qiong et al., 2017). The WC is measured at the navel, while the hip circumference is measured at the widest circumference of the hips and buttocks (WHO, 2011). The threshold values for identifying abdominal obesity is a WHR of >0.95 for men and >0.85 for women (Wajchenberg, 2000). However, the WHR also does not explain the differences in fat distribution, similar to tape measurements of the waist and hip (Atkinson & Uwaifo, 2005).

1.2.7.1.3. Body mass index, measures relationship between weight and height

The body mass index (BMI) indicates overall obesity, although it only takes the individual's weight and height into consideration and does not measure abdominal adiposity and the extend of excess VAT may therefore be missed by using BMI only (Janssen et al., 2004; Paniagua et al., 2008; Shuster et al., 2012). Therefore, although the BMI is generally used for the classification of obesity, it also does not explain the differences in fat distribution, similar to tape measurements of waist and hip (Atkinson & Uwaifo, 2005). However, studies have reported a positive association between BMI and MetS (St-Onge et al., 2004; Lear et al., 2007), but the pattern of fat distribution as well as muscular tone may be of higher importance than BMI status when evaluated as a risk factor for CVD (Janssen et al., 2004; Shuster et al., 2012). Individuals with higher levels of central adiposity are at greater risk of developing lipid and glucose abnormalities than those with fat distributed more peripherally (Lamarche, 1998). Comparing the BMI and the WC, Janssen et al. (2004) reported the WC a better marker of health risk than the BMI as assessed against metabolic variables which included plasma glucose, serum cholesterol and blood pressure and suggested that the WC should be included in the classification of obesity together with BMI (Janssen et al., 2004).

1.2.7.2. Imaging Techniques

The increase in the recognition of the usefulness of imaging in identifying central obesity in MetS diagnosis has led to studies investigating the reliability of the different modalities available. In this regard, an imaging modality that would allow a reliable and practical method to measure visceral obesity would be valuable as a diagnostic criteria. In this regard, the use of CT-imaging in assessing SAT and VAT has been described.

1.2.7.2.1. Computer Tomography (CT)

CT is a preferred method for efficient evaluation of the human body composition, with high accuracy and reproducibility (Mazonakis & Damilakis, 2016). CT can accurately separate and quantitative various tissue types, including adipose tissue. It is considered as the gold standard for assessment of abdominal fat because of its ability to distinguish between VAT and SAT (Wajchenberg, 2000; Shuster et al., 2012) and represents a direct method of assessing VAT (Shuster et al., 2012).which is of great value in clinical studies (Mazonakis & Damilakis, 2016). CT has been used to estimate SAT and VAT in order to quantify abdominal adiposity (Irlbeck et al., 2010) and that in their own study, results supported the finding that single-slice CT measurements provide a good estimate of SAT and VAT volumes (Irlbeck et al., 2010). Brandberg et al. (2008) describes CT as a reliable measuring tool because tissue characterization is based on characteristic CT numbers (HU) for adipose tissue that eliminates errors which are normally caused by manual operator-dependent techniques.

1.2.7.2.1.1. CT in abdominal fat assessment

CT measurement of VAT and SAT is an accurate method and although a time-consuming process (Roos et al., 2002), the CT image acquisition time is still shorter than that of MRI (Klopfenstein et al., 2012) and can be corrected for by training of technicians, state-of-the-art workstations, and fast networking (Roos et al., 2002). The measurement of visceral adiposity by CT and MRI vary in accessibility, accuracy and reproducibility (Shuster et al., 2012). Although both modalities are reported to be very accurate, both are similarly not easily accessible. The advantage of MRI is that it uses no radiation (Shuster et al., 2012), is a non-invasive and accurate imaging modality for measuring both VAT and SAT, does not expose the individual to ionizing radiation (Klopfenstein et al., 2012) and can be safely used on children and pregnant females (Hu et al., 2011). However, MRI scanners are less available than CT scanners and in addition, it is costly and the examination is time consuming (Shuster et al., 2012). Sequence-related artifacts with MRI, such as chemical shift and blood flow artifacts may also give rise to inaccurate visceral adipose tissue estimates (Poll et al., 2003; Shen et al., 2003). Compared to other measures of obesity, including surrogate markers BMI and WC, Brandberg (2009) and Berker et al. (2010) reported CT measurements to be superior, with CT furthermore able to exhibit differences by gender. Berker et al. (2010) also reported CT measured fat superior to BMI levels when it is associated with MetS.

However, the need to identify and quantify VAT specifically is highlighted by a report from Després et al. (2008) which stated that reducing the WC may be more clinically relevant than weight loss. Després et al. (2008) conducted a lifestyle modification program at the Quebec Heart Institute and found that with minor weight loss, the WC could be reduced by 1 cm which indicated a loss of VAT, with an increase in muscle mass and which improved the metabolic profile of the patients.

1.2.7.2.1.2. CT; radiation dose

The use of CT imaging is not always a preferential method due to the radiation exposure that the individual will experience (Shuster et al., 2012) and which remains a concern (Mazonakis & Damilakis, 2016). However, according to the Commission on Radiological Protection, the risk that is associated with the radiation dose level in CT is ‘trivial’ when compared to the dose delivered by natural background radiation over a few weeks and that a single slice of the abdomen will render a dose of <0.1mSv (Brandberg et al., 2008).

There are furthermore several methods of reducing radiation dose to a patient undergoing CT examinations. The use of CT single-slice measurements of SAT and VAT would reduce radiation exposure to the participant as compared to a volumetric measurement (Irlbeck et al., 2010; Mazonakis & Damilakis, 2016). Furthermore, by using the automatic exposure

control that is inherent in the CT scanner (Bushong, 2008), the desired image can be produced by adapting the tube current according to the specific patient attenuation needs, that is, the tube current would automatically be decreased in smaller individuals and similarly increased for larger individuals.

However, the need to use a minimal radiation dose should be balanced by the need to maintain image quality (Brandberg et al., 2008). In order to assess image quality the image has to be assessed in terms of its spatial resolution, contrast resolution, noise, linearity and uniformity. Image noise, an important determinant of CT image quality, is inversely related to the x-ray beam energy and in this regard, Brandberg et al. (2008) reported between 0% and 2% differences in area measurements when using adjusted versus standard clinical scan parameters.

1.2.7.2.1.3. CT; operating principles

CT works on the principle of a collimated x-ray beam that is directed on the patient, where the low attenuation factor of fat makes it easily identifiable (Klopfenstein et al., 2012). The beam that is attenuated by the anatomical part is measured by a detector, which is transmitted to a computer for analysis. CT images are therefore computer reconstructions of different separate determinations of radiation beam attenuation of which the data is analyzed statistically by using CT software. A cross-sectional image of the anatomy is reconstructed by means of mathematical equations adapted for computer processing. This reconstructed image is then displayed on a television monitor (Bushong, 2008), where the different fat areas in the abdomen can be identified and measured (Borkan, et al., 1982). The image can be enhanced to suit the needs of the viewer by adjusting the window width and window level (Seeram, 2001). Hounsfield units (HU) are used to express CT numbers and these are proportional to the degree of x-ray attenuation by the tissue (Razi et al., 2014). The cut-off point to define VAT has been established at 130 cm^2 (Ross et al., 1996 Morricone et al., 2002).

1.2.7.2.1.4. CT; region of choice in the measurement of abdominal fat

The abdominal fat is usually assessed at a single CT slice at the preferred anatomical level chosen (Mazonakis & Damilakis, 2016). Yoshizumi et al. (1999) conducted a study to standardize a method for measuring abdominal fat using CT. The study subjects were 60 males and 60 females with a BMI between17.3 and 39.1 kg/m^2 and CT scans were obtained at the umbilicus in order to calculate the adipose tissue. A region of interest was identified by tracing the SAT and VAT contour independently on each scan. Thereafter the attenuation range was calculated in HU using a standard reference of -190 to -30 HU for adipose tissue.

The authors tested for inter-scanner reproducibility as well as for intra- and inter-observer reproducibility of VAT measurements and reported a high intra- and inter-observer reproducibility. Yoshizumi et al. (1999) concluded that their methodology was the most practical method for determining abdominal fat distribution using CT. Other studies have also used the technique of delineating SAT from VAT by having an analyst trace the abdominal wall muscle layer which separates SAT from VAT (Borkan et al., 1982; Klopfenstein et al., 2012).

1.2.7.2.1.4.1. CT; anatomical level of measurement

VAT is associated with an increased incidence of MetS (Goodpaster et al., 2005), however, there is a lack of consensus with regards to the anatomical level chosen for VAT and SAT measurements (Mazonakis & Damilakis, 2016). Kuk et al. (2006) reported L1-L2 or L2-L3 as the preferred site to measure VAT and SAT to predict MetS, with L1-L2 showing stronger association with MetS evaluation than L4-L5. O'Connor et al. (2015) reported VAT measurements at L2-L3 in females and L1-L2 or L5-S1 in males more accurate, while Irlbeck et al. (2010) reported measurements at level L3-L4 to be strongly associated with SAT and VAT measurements. Irlbeck et al. (2010) reported that all single-slice area-based CT measurements obtained at L1/2, L2/3, L3/4, the umbilicus, L4/5, L5/S1 were highly correlated with SAT and VAT volumes, but that SAT and VAT obtained at the L3/4 level were strongest associated with SAT and VAT volumes and furthermore associated with cardio-metabolic risk factors. Consensus about the optimal level to use in CT scan measurements of abdominal fat remains unresolved, but various research studies have used the level of L4/L5 intervertebral disc space (Tokunaga et al., 1983; Sjostrom et al., 1986, Kvist, et al., 1988; Piernas et al., 2009) and in the current study, abdominal fat was measured at the L4-5 level according to the protocol followed by the Radiology Practice where the CT scans were done.

1.2.7.2.3. Bioelectrical Impedance Analysis (BIA) (digital scale)

1.2.7.2.3.1. Principles of BIA

Bioelectrical Impedance Analysis (BIA) is the technology used in body fat scales to measure body fat and is becoming popular due to its non-invasive nature. The Omron Body Composition Monitor (BF511) (Omron, Japan) (used in the current study) (Figure 1.3) can be used to measure the following body composition parameters, body fat (in %), VAT (levels), BMI (kg/m^2), skeletal muscle (in %) and calculate the resting metabolism rate (in kcal). Personal data, including age, gender and height can be adjusted for on the scale monitor before weight is measured (subjects up to 150 kg). The body fat, VAT, BMI and skeletal muscle are measured and the resting metabolism rate calculated. The rational used for

measuring body fat is that while muscle, blood vessel and bone tissue have a high water content which conducts electricity easily, body fat has little electric conductivity. The Omron technology uses weak electrical currents to do the body measurements, using sensors to both the hands and feet. This is to reduce the influence of water distribution in the body, for example, the ratio of water in the upper body and lower body differ according to the time of day (adapted from the Omron manual, Omron, Japan). Under normal conditions, 45% of extra-cellular water and 55% of intra-cellular water is compartmentalised within the body (Coppini et al., 2005). Lean tissue which is rich in water and electrolytes has minimal impedance and allows for the calculation of differences in conductivity between lean and fat mass (Pietrobelli & Heymsfield, 2002), while body fat, which is a non-conducting material, provides resistance to the flow of an electric current and is less conductive than muscle and bone (Scharfetter et al., 2001).

In contrast to the Omron Body Composition Monitor (BF511), others, including the Tanita scale measures the voltage drop from foot to foot only, when a small current is applied through contact with 2 metal plates. Jebb et al. (2000) reported that although the scale was practical, it was inaccurate compared to other BIA techniques. In an attempt to overcome this, Kyle et al. (2004) in a review on BIA stated that there is a need for BIA measurements to be validated in the population studied which would include the race, age and gender.

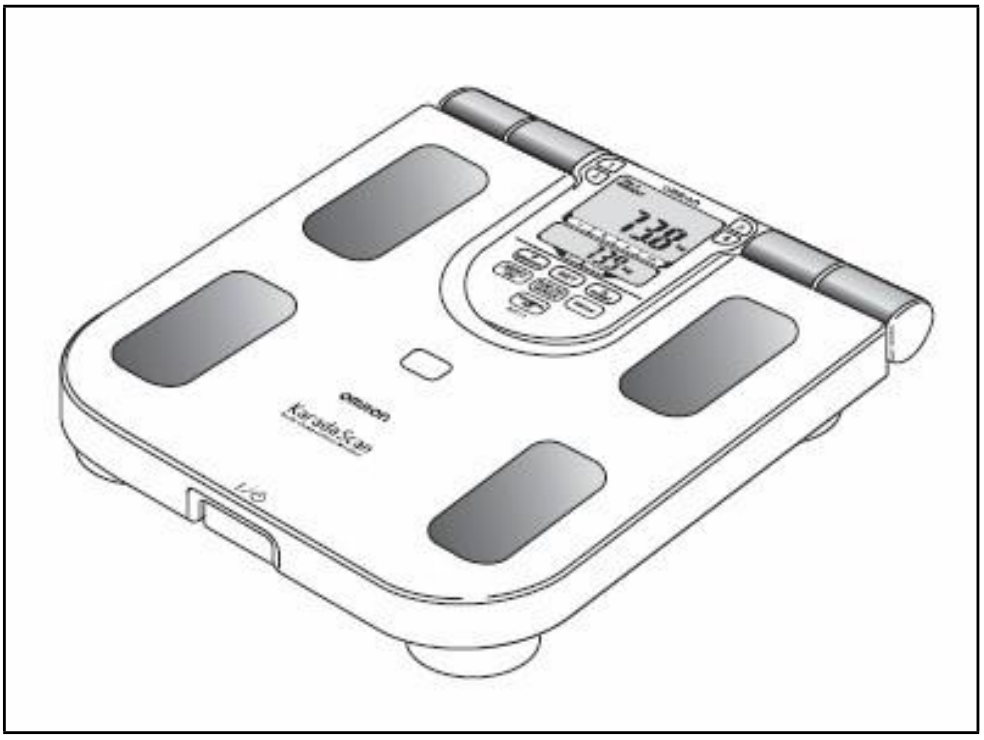


Figure 1.3. The Omron Body Composition Monitor (BF511) using Bioelectrical Impedance Analysis (BIA). Reference: THE Manufacturer, Omron Healthcare Co. Ltd., 53 Kunotsubo, Terado-cho, Muko, Kyoto, 617-0002 Japan.

1.2.7.2.3.2. Advantages and disadvantages of BIA

The advantages in using an instrument capable of doing Bioelectrical Impedance Analysis is that it is non-invasive, relatively inexpensive, does not use ionising radiation and can be used with healthy and chronically diseased individuals (Kyle et al., 2004). However, there are certain limitations in the use of this type of equipment, e.g. an upper level in weight and/or BMI for some of these scales have been recorded, Kyle et al. (2004) reported it as a BMI of 34 kg/m^2 and for the Omron BF511 scale (used in the current study) the manufacturers stated 150 kg as the upper weight limit (Omron, Japan). Significantly altered hydration states, body shape and pregnancy can also affect measurements (Kyle et al., 2004) and the Omron manual (Omron) also stated age (older than 81 years), fever, pregnancy, swelling and dialysis as limiting factors in accuracy, possible due to changing ratios of body fluid and/or body composition. Kyle et al. *(*2004) therefore recommended that BIA measurements have to be validated in the population studied and which should include the race, age and gender.

1.2.7.2.3.3. BIA: validation reports, including against CT

Nagai et al. *(*2010) conducted a study to compare measurement of VAT obtained using BIA to VAT obtained using CT. They reported that the BIA method was simple to use and convenient for the estimation of VAT, a limitation identified was that their results were only applicable to the Japanese population (Nagai et al., 2010). Lee et al. (2015), similarly

reported a good correlation between BIA and CT in Korean subjects, especially in females, but that a more accurate formula is needed to match CT data in different sub-classifications, including subjects with a high BMI. Ozhan et al. (2012) validated the use of BIA in VAT assessment for MetS diagnosis in a large Turkish adult cohort. The authors reported the use of BIA as an easily applicable and inexpensive method as a screening tool for evaluating VAT in MetS diagnosis and reported a cut-off value specific for the Turkish population. Literature supports moreover the usefulness of a reliable BIA instrument in assessing/monitoring lifestyle changes as it can detect a weekly change of abdominal fat (Ida et al., 2013). The above findings confirms the usefulness of BIA, but similarly support the need for verification of BIA measures against that of known modalities, such as CT.

1.3. Research rationale

The recommended reference values for MetS diagnosis have been derived from studies that were conducted in Europe and North America (Motala et al., 2009; Crowther & Norris, 2012). These values have then been applied to populations in Asia and Africa without due consideration for local environmental and genetic influences. Currently, there is general agreement that diagnostic criteria for MetS are race and/or ethnic specific and can therefore only be generalised to the population where the study is being conducted (Seidell et al., 2001; Misra et al., 2005). In this regard, WC, one of five MetS risk factors specifically needs to be optimised (Alberti et al., 2009) as the WC is used as a surrogate for visceral adiposity, which has shown to vary substantially between ethnicities as well as gender.

In order to have consensus and to allow further modification of the definition of MetS, the IDF advocated that new research studies should investigate abnormal fat distribution by assessing central obesity with the use of CT or MRI (IDF, 2006). The current study therefore aimed to investigate and report on this aspect by investigating abdominal fat distribution in the South African mixed ancestry population with the use of CT scans and to evaluate these findings against anthropometric measurements, focusing on MetS and its components. Furthermore to validate the newly established WC cut-off of 90 cm in both genders for MetS diagnosis reported in the same population group (Erasmus et al., 2012) against VAT measurements (CT). In this regard, CT is considered as the gold standard for fat measurement because of its ability to easily differentiate between and quantify VAT and SAT separately (Van der Kooy & Seidell, 1993; Mattsson & Thomas, 2006; Shuster et al., 2012), while anthropometric measurements only measures VAT indirectly (Shuster et al., 2012) hence the need for a validation study.

A further aim was to investigate the use of BIA as a non-invasive assessment method of VAT measurement and to evaluate this against CT-obtained VAT measurements. The advantages

in using a BIA scale is that it is non-invasive, relatively inexpensive, does not use ionising radiation and can be used with healthy and chronically diseased individuals (Kyle et al., 2004), but similar to anthropometric measurements needs to be validated against a reliable standard.

1.4. Significance of the study

A high prevalence of MetS and T2DM has been reported in the South African mixed ancestry population, with obesity and MetS implicated in the onset of DM. These reports therefore necessitate the diagnosis of MetS to be simplified as much as possible. In this regard, the diagnostic criteria for MetS as defined by JIS (Alberti et al., 2009) include biochemical measurements (FBG, HDL-chol and triglycerides) which may not be practical to include in routine testing in the South African Health Care System, but two of the risk factors for MetS are easily tested for in a clinical setting, both WC and blood pressure can be tested for on a regular basis. Using some of the MetS risk factors as initial screening tools may be a feasible option in developing countries so that appropriate preventive measures can be taken to reduce the risk of T2DM and/or CVD in the population.

Outcomes from this study may therefore be useful in clinical practice to identify subjects at risk at an early stage of MetS, or even elevation in individual symptoms of MetS, specifically as an early screening tool of obesity (WC) of which the cut-off value has been scientifically validated. Similarly, a simple, portable digital scale could be used for VAT estimation of which the cut-off point has been validated in MetS diagnosis. As MetS can be controlled by lifestyle changes, it would allow for preventative measures to be put in place to reduce the development of DM and/or CVD (Lorenzo et al., 2003; Handelsman, 2009).

CHAPTER TWO

RESEARCH FOCUS

2.1. Statement of the research problem

A prevalence of 62% of subjects with MetS has been reported in the mixed ancestry population of Bellville-South, South Africa (Erasmus et al., 2012). The study also reported a prevalence of 28.2% of T2DM which, together with MetS would be challenging as both are risk factors for CVD (Erasmus et al., 2012). T2DM and CVD have been estimated to escalate to such an extent that these diseases would become a global social and economic burden by 2030 (Shaw et al., 2010).

An increased WC has been identified as one of the risk factors for MetS and is included as one of the components for the diagnosis of MetS. The WC is used to measure central adiposity and is used in clinical practice as a surrogate marker for VAT measurement. In their 2012 study, Erasmus et al. reported that the most common MetS criterion identified with the high prevalence of MetS in the mixed ancestry population was central adiposity, but that their results did not support the IDF WC reference value. The group reported a 90 cm anthropometrically measured WC to be an optimal cut-off value to include in the panel for MetS diagnosis in the mixed ancestry population. However, the recommended cut-off value needs to be validated against a known measurement of abdominal fat, such as CT, before it could be supported as a recommended value for the South African mixed ancestry population. This thesis focused on the imaging segment (CT) in the validation of the previously identified 90 cm WC cut-off to be used in the panel of risk factors to identify the presence of MetS in the mixed ancestry population reported by Erasmus et al. (2012).

2.2. Hypotheses

2.2.1. Null hypothesis

The 90 cm anthropometric WC is not reliable to use as an optimal cut-off value for the diagnosis of MetS in the South African mixed ancestry population

2.2.2. Opposing hypothesis

The 90 cm anthropometric WC is reliable to use as an optimal cut-off value for the diagnosis of MetS in the South African mixed ancestry population

2.3. Aims and Objectives

2.3. 1. Aims

- To validate the 90 cm WC cut-off for the diagnosis of MetS, derived by Matsha et al. (2013) in the mixed ancestry population of South Africa, against CT-measured VAT as the gold standard
- To investigate the association of known risk factors for MetS against CT-measured VAT in the mixed ancestry population
- To validate the use of BIA as a substitute VAT measurement in the mixed ancestry population against CT-measured VAT as a gold standard

2.3.2. Objectives

The aims were accomplished by fulfilling the following research objectives:

- "To validate the 90 cm WC cut-off for the diagnosis of MetS, derived by Matsha et al. (2013) in the mixed ancestry population of South Africa, against CT-measured VAT as the gold standard"
    - To measure the abdominal fat of research participants, using CT scans
    - To measure the anthropometric parameters of research participants
    - To measure the blood pressure of research participants
    - To determine biochemical measurements of research participants
    - To determine MetS status in the study population, using JIS MetS criteria
    - To derive a cut-off value for CT-measured VAT for MetS diagnosis
    - To validate the previously identified 90 cm WC cut-off used in MetS diagnosis against the CT-measured VAT cut-off value for MetS diagnosis
- "To investigate the association of known risk factors for MetS against CT-measured VAT in the mixed ancestry population"
    - To determine correlation and/or association between CT-measured VAT and SAT and the components used to identify MetS.
    - To determine correlation and/or association between CT-measured VAT and SAT with additional anthropometric and biochemical MetS risk factors
- "To validate the use of BIA as a substitute VAT measurement in the mixed ancestry population against CT-measured VAT as a gold standard"
    - To measure the VAT of participants, using a portable digital scale
    - To validate the BIA-obtained VAT against CT-obtained VAT in the diagnosis of MetS in the South African mixed ancestry population

CHAPTER THREE
RESEARCH METHODOLOGY

3.1. Introduction

The current study is a sub-study of the larger Diabetes and Cardiovascular Disease cohort study which commenced in 2014 in Bellville-South. A population of predominantly mixed ancestry individuals resides within this area which when investigated previously (2008 to 2011) to establish risk factors for T2DM and CVD showed high prevalence rates of MetS (62%) and T2DM (28,2%) (Erasmus et al., 2012). One of the study aims of the group was to identify individuals at risk for MetS at an early stage and the group reported abdominal obesity as the most common diagnostic criterion to confirm MetS. The group furthermore reported a specific cut-off value for the WC (90 cm for males and females) to identify MetS in the mixed ancestry population. One of the aims of the current study therefore was to validate the WC cut-off value reported by Erasmus et al. (2012) against CT measurements of VAT in the mixed ancestry population.

3.2. Ethics approval

Prior to the collection of data, ethics approval for the larger Diabetes and Cardiovascular Disease cohort study was sought from and granted by the Cape Peninsula University of Technology, Health and Wellness Sciences Research Ethics Committee {CPUT/HW-REC 2015/H01 (renewal)} (Appendix 1). Ethics approval for the current study, which forms part of the larger Diabetes and Cardiovascular Disease cohort study was similarly granted by the Cape Peninsula University of Technology, Health and Wellness Sciences Research Ethics Committee {(CPUT/HW-REC 2015/H18 (extension)} (Appendix 2). Written informed consent was obtained from participants who formed part of this study. Any potential dangers and complications, including that of the imaging segment of the study were explained by the researcher to the participant prior to obtaining consent. The study was done in accordance with the guidelines of The Declaration of Helsinki (2008). After an explanation of the study was given to the participants, the consent form was signed which completed the process of voluntary recruitment. The autonomy of the participants was respected as they were given the opportunity to consent or withdraw from the imaging segment after the initial examinations had taken place. Each participant was assigned a number which was used throughout the study, also as identification on the CT scan to maintain anonymity. The obtained data were furthermore stored on a secure password protected computer and the CT digital video disks (DVD's) were locked in a cupboard in the office of the manager of the cohort. After analyses the CT data was removed from the workstation in the Radiology Department.

3.3. Research design

The study was an observational study which adopted a cross-sectional design. The overall methodology for the present study was quantitative as numeric data was gathered.

3.4. Study population

The current study population consisted of individuals of mixed ancestry residing in Bellville-South and who were a subset of the cohort that is being investigated for T2DM and CVD. A randomized sampling method was used to generate a pool of 2000 participants for the cohort which was achieved when suitably trained research assistants recruited in the designated area. The sample size for the current study (with CT scan results) was overall 401, consisting of 93 males and 308 females.

3.4.1. clusion criteria

The participants for the current research study met with the following inclusion criteria:

- Consenting adults (both male and female) aged 20 years and older
- Individuals of mixed ancestry origin

3.4.2. Exclusion criteria

Individuals were excluded from the current study if they met with the following criteria:

- Acutely ill individuals
- Female individuals that were pregnant, as radiation poses a potential risk to the unborn baby
- Individuals that weighed more than 200 kg as that is the maximum weight capacity that the CT couch will allow
- Individuals with breathing problems, i.e. unable to hold their breath
- Individuals that suffered from claustrophobia
- Individuals that had a differential diagnosis of ascites as it may mimic central obesity
- Individuals who may have suffered from any abdominal distension due to suspected bowel obstruction, such as liver cirrhosis or other related abdominal pathologies
- Any individual with a pacemaker or internal electronic medical equipment
- The medical history of the participants was self-reported

3.4.3. Sample size

The sample size was an important factor as the study used radiation to achieve one of its objectives. In order to obtain a representative sample, the following formula was used to calculate the sample size using the prevalence of MetS (62%) in the area:

$$n = \frac{z^2 (p\ q)}{e^2} \quad \rightarrow \quad n = \frac{1.96^2 (62 \times 38)}{5^2} \quad \rightarrow \quad n = 362 \text{ participants needed}$$

n = Sample size

z = Standard error association with chosen level of confidence

p = Estimated percentage in population affected

q = 100-p

e = Acceptable sample error

3.4.4. Data collection

Data was collected prospectively. All the participants were evaluated with respect to anthropometric measurements, blood pressure measurements, CT assessments and biochemical analyses in order to fulfil the study objectives. The VAT and SAT was measured using CT and the VAT also using BIA technology. The CT scan of the abdomen took place during the same week as the anthropometric measurements and biochemical analyses were done. The CT examinations were conducted at a Private Radiology Practice at a remote site from the research site. The assessment of the anthropometric and blood pressure measurements as well as blood sample collection were done at the research site, based at the Cape Peninsula University of Technology which was specifically set up for the Diabetic and Cardiovascular cohort study. The collected blood samples were sent to an accredited pathology practice for biochemical analysis of samples.

3.4.4.1. Biochemical Analysis

Blood samples were collected after an overnight fast as well as after an oral glucose tolerance test (2 HRs) by a qualified registered nursing sister. The collected samples were sent to an accredited pathology practice, Pathcare Reference Laboratory, Cape Town, South Africa for biochemical analysis. Tests (method; instrument used) included testing for FBG (mmol/L) (Hexokinase; Beckman AU or DXC), serum insulin (mIU/L) (Paramagnetic particle; Chemiluminescence), triglycerides (mmol/L) (GPO-POD Endpoint; Beckman AU), LDL-chol (mmol/L) (Enzymatic Selective Protection -Endpoint; Beckman AU), HDL-chol (mmol/L) (Enzymatic Immunoinhibition - Endpoint; Beckman AU), glycated haemoglobin (HbA1c) (%) (HPLC; Biorad Variant Turbo) and C-reactive protein (CRP) (mg/L) (Latex Particle immunoturbidimetric; Beckman AU).

3.4.4.2. Blood pressure

The blood pressure was measured using an automatic digital blood pressure monitor (Omron M6 Comfort-preformed Cuff Blood Pressure Monitor, Omron) while the participant was at rest in a sitting position with his/her back supported. The size of the cuff was selected according to the size of the circumference of the individual's arm. The SBP and DBP (mmHg) readings were measured 3 times by a trained professional nurse at 5 minute intervals according WHO (1999) guidelines. All three readings were recorded and for the purposes of this study, the lowest SBP reading and the corresponding DBP reading were used for statistical analysis.

3.4.4.3. Anthropometric assessment

Anthropometric measurements were conducted by suitably trained research assistants to ensure consistency. These measurements were done on the same day as the CT examinations. The measurements were conducted in a venue especially assigned for this purpose to protect the participant's privacy.

3.4.4.3.1. Anthropometric assessment by tape measure, waist and hip

The anthropometric measurements were taken with the participants wearing light clothing and no shoes. The WC was measured midway between the lowest rib and the top of the iliac crest at the narrowest part of the abdomen with a soft measuring tape all around the body with the participant in a standing position. The hip circumference was measured at the widest circumference of the hips and buttocks, with the participant in a standing position.

3.4.4.3.2. Anthropometric assessment by stadiometer, height

The height was measured to the nearest 0.5 cm using a stadiometer with the participant in a vertical position.

3.4.4.3.3. Anthropometric assessment, using BIA

Anthropometric assessment using BIA was done using the Omron Body Composition Monitor (BF511) scale and these measurements were done on the same day as CT scans were done. The participant details, including age, gender and height were entered into the analyser of the Omron BF511 scale. Weight was measured in kilograms to the nearest 0.5 kg, which allowed for the BMI to be calculated as well as the determination of VAT (recorded in levels, which ranged from 1 to 30) (Table 3.1), with weight allowance up to 150 kg. For these measurements the participants were standing facing forward with their feet placed on the electrodes embedded in the scale. Their arms were extended at ninety degrees to the base of the scale while holding onto the handles of the analyser which are attached to the scale by means of an extension cord (Fig 3.1). Prior to the commencement of data collection, the scale was calibrated for the sample of participants of the current study. The limitation of the Omron BF511 scale is an age range of 18 to 80 years; in the current study, only 4

participants were older than 81 years. Similarly, the weight range was up to 150 kg and similarly only 3 participants from the current study weighed more than 150 kg.

Table 3.1. Level classification of BIA (Omron BF511) VAT

VAT levels	**Level classification**
1 to 9	0 (normal)
10 to 14	+ (high)
15 to 30	++ (very high)

Figure 3.1. The Omron Body Composition Monitor (BF511), model HBF-511B-E / HBF-511T-E was used in the current study. Reference: Manufacturer, Omron Healthcare Co. Ltd., 53 Kunotsubo, Terado-cho, Muko, Kyoto, 617-0002 Japan.

3.4.4.4. CT scan of the abdomen

CT is considered one of the gold-standards for measuring abdominal fat due to its ability to differentiate between VAT and SAT and its reliable and reproducible capabilities. The CT imaging segment of the current study started towards the end of March 2015 and took place at a private radiology practice in Rondebosch, South Africa. Between 6 and 12 participants were scanned every Monday and Friday until the end of July 2016. The scans took place after the blood pressure, anthropometric assessments and blood samples were obtained. The CT scans of the abdomen for the assessment of abdominal fat were performed by a qualified radiographer that is registered with the Health Professional Council of South Africa.

The scans were all performed without intravenous or oral contrast. Before measuring the fat on the CT images of the study participants, measurement of the abdominal fat was piloted. The scanning radiographer and the reporters of the images could therefore familiarise themselves with the measuring tool of the CT scanner and with the time frame of the fat measuring process. To test for reliability of the instrument, the fat of individuals who did not form part of the research study (volunteers) was measured at the designated measurement level of the abdomen prior to the commencement of the study.

3.4.4.4.1. CT scan of the abdomen; GE 16 slice Lightspeed CT scanner

Abdominal imaging was performed using a GE 16 slice Lightspeed CT scanner which was used in axial mode. See Table 3.2 for a detailed description of the CT protocol for the present study. The equipment offered a 40 cm FOV with an image reconstruction matrix of 512x512. The table of the scanner can accommodate a maximum weight of 205 kg, therefore participants with weight in excess of 205 kg and similarly those with an abdominal size in excess of the size of the FOV of 40 cm used for the study, were excluded from the CT segment of the study.

The scanner is equipped with an automatic exposure control system (AEC) which allowed the scan to be performed with the lowest possible exposure. Only a scout image and one slice through the abdomen, using a low dose protocol was employed in this study, enhancing the relative safety of the study. The scan was performed with arrested inspiration. The CT equipment conformed to the quality assurance requirements of the Department of Radiation Control with respect to servicing and maintenance regulations.

3.4.4.4.2. CT scan of the abdomen; Imaging

The level of L4/L5 intervertebral disc space was selected on the scout image (Figure 3.2) as suggested by literature (Tokunaga et al., 1983; Sjostrom et al., 1986, Kvist, et al., 1988; Piernas et al., 2009). A 5 mm single axial slice was then performed at the L4/L5 level as a valid predictor for total abdominal fat. On the cross-sectional scan produced, SAT was separated from VAT by a line drawn manually with a cursor which demarcated the abdominal muscular wall. SAT is defined as the layer between the skin and the muscles of the abdominal wall (Figure 3.3.a), while VAT was enclosed by the visceral cavity which was measured by tracing around the inner margin of the abdominal musculature as per Yoshizumi et al. (1999) (Figure 3.3.b). The delineation of fat on the CT images was completed by the radiographer and was then recorded on DVD's.

3.4.4.4.3. CT scan of the abdomen; software program, Vitrea Core Version 6.4.4083.268

For VAT and SAT analysis, the abdominal CT images were transferred to a CT work station in order to use a dedicated fat assessment software program, Vitrea Core Version 6.4.4083.268. The abdominal protocol of the software generated an image of blue for SAT

and red for VAT in the abdominal images (Figure 3.4). The software uses thresholding methods to identify fat. For the present study, the threshold was set at WW of -150 and a WC of -70, as was stipulated by the manufacturer, which then generated a fat density mask, thus identifying adipose tissue. Pixels within the threshold that were not anatomically one of the two adipose tissue depots were removed automatically. The resultant image demonstrated the variation of abdominal fat distribution at the L4/5 level which was evaluated and recorded automatically. Both SAT and VAT fat size was measured in cm^2. The images obtained were reviewed by consultant radiologists at the practice who were blinded to the clinical information of the participants. A CT report was then generated for each participant.

The fat assessment software also allowed editing of the image. For the current study, it was not necessary as only the pre-set HU was used as was specified by the manufacturer and due to only one slice being performed at the level of L4/5 no further manipulation of the image was required.

Table 3.2. The CT protocol of the private radiology practice used in the current study

		CT protocol
1.	Preparation of the participant before the scan	• An explanation of the scanning procedure given to the participant to ensure maximum cooperation • All radio opaque clothing was removed and the a night gown was given to participant to wear to prevent any artifacts
2.	Scanning protocol	• The participant lie in a supine position on the scanning table with both arms placed in a comfortable position above the head. • To ensure comfort a soft pad will be placed under the knees which may minimize movement. • The scan area ranged from lower border of the xiphisternum to top of symphysis pubis (for the topogram). • The participant was instructed to hold the breath in arrested inspiration to prevent movement of the bowel. After the topogram the level for the axial scan was selected at L4-L5 intervertebral disc space
3.	Accessories/additional requirements	• Soft pad for the knees for comfort Pillow and blanket for the participant
4.	Imaging parameters	An axial scan was performed by using the following parameters: • 120kv/auto mAs • 5mm thickness • Field of view = 40cm • Image reconstruction matrix of 512x512. Window settings: width = 350 HU; Level= 50HU (which were adjusted for fat when the measurements were gathered. (Klopfenstein et al., 2012)

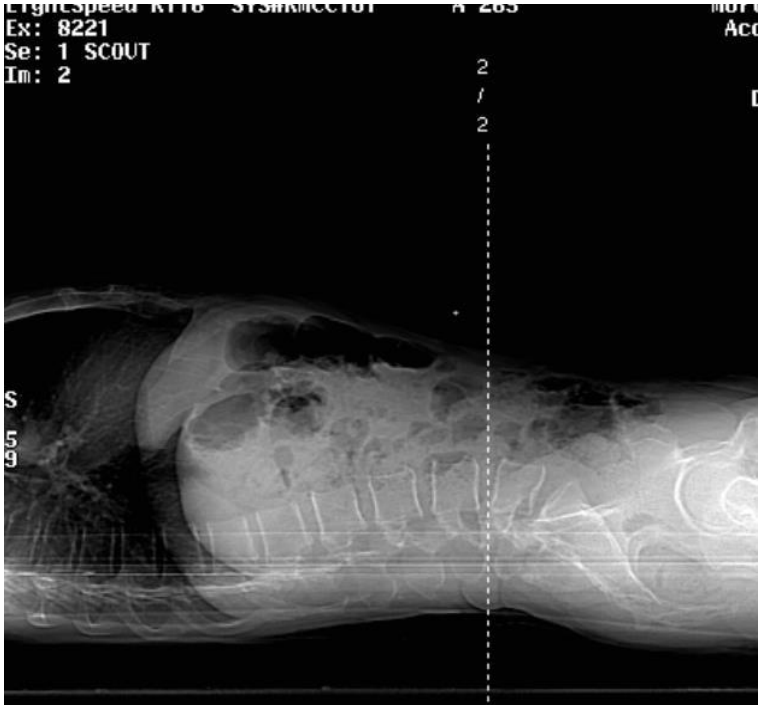
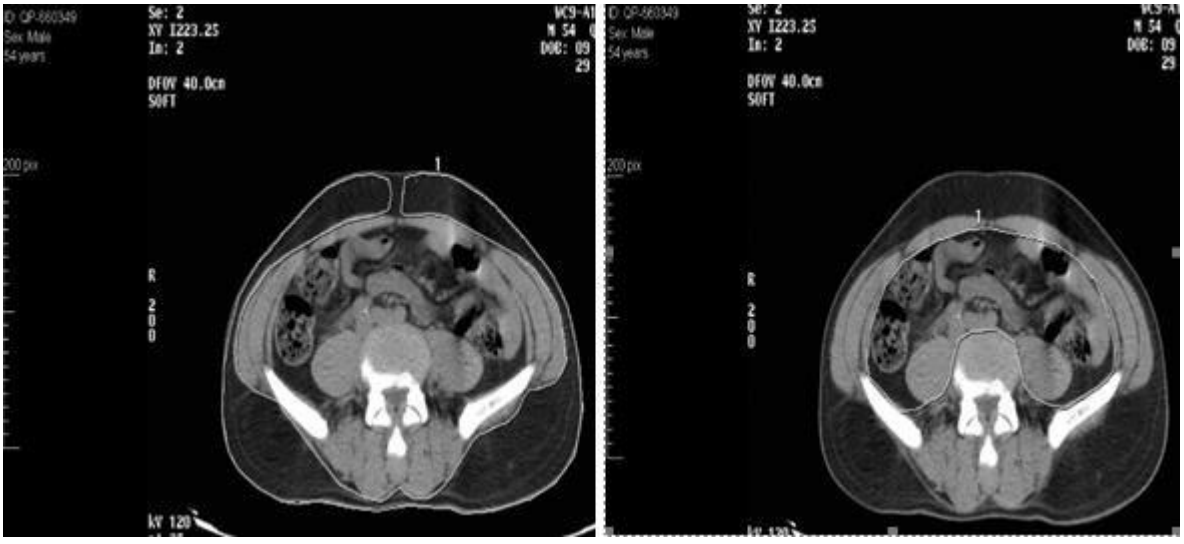


Figure 3.2. Lateral topogram**,** demonstrating the level of L4/L5 inter-vertebral disc space which was selected on the scout image for the cross-sectional slice in the current study



(a) **(b)**

Figure 3.3. Cross-sectional CT images depicting manual demarcation of (a) SAT, which is defined as the layer between the skin and the muscles of the abdominal wall (b) the white line outlines (for the purpose of this study) VAT of a participant.

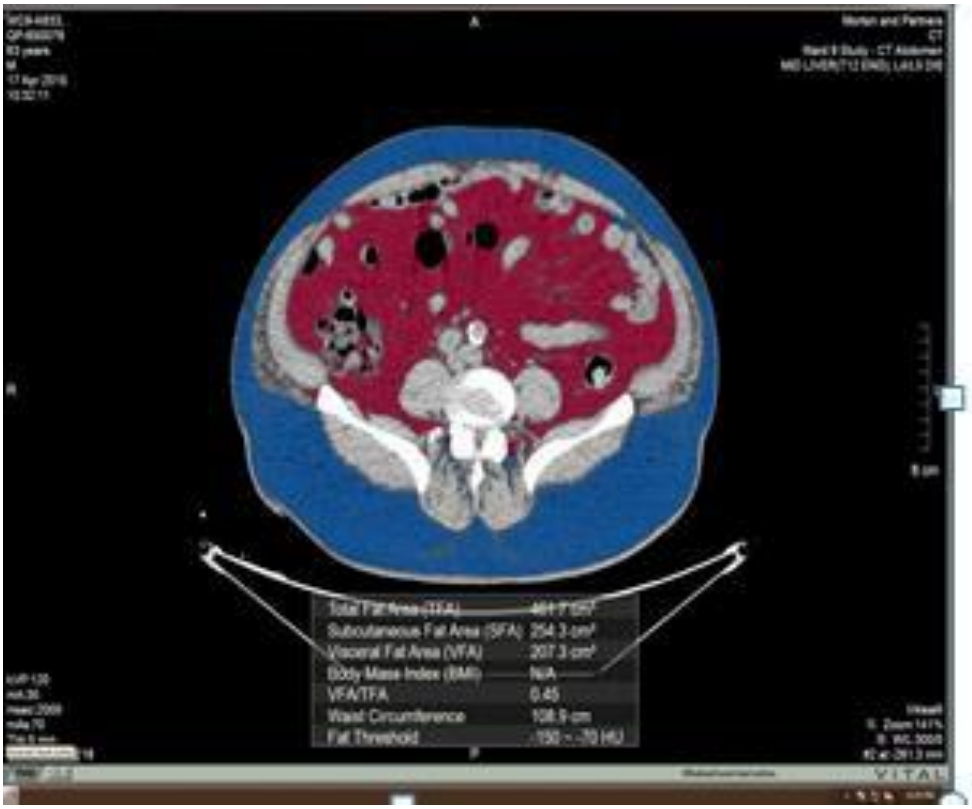


Figure 3.4. Image acquired in the current study, using the fat assessment software program Vitrea Core Version 6.4.4083.268, showing the SAT in blue and the VAT in red.

3.4.4.5. Formulae used in the current study

3.4.4.5.1. Obesity classification, according to BMI (kg/m^2) values

BMI (kg/m^2) is calculated from the formula body weight (kg)/(height (m)^2. The International classification of adult obesity according to the BMI is as follow (WHO, 2004, updated 2016):

- Underweight: < 18.50 kg/m^2
- Normal range: 18.50 to 24.99 kg/m^2
- Overweight: ≥ 25.00 to 29.99 kg/m^2
- Obese: ≥ 30.00 kg/m^2

3.4.4.5.2. MetS classification, adapted from JIS MetS criteria

JIS MetS includes the presence of any three of the following conditions, with population- and country-specific definitions for WC recommended (Alberti et al., 2009):

- WC: ≥90 cm for both men and women Matsha et al. (2013)
- FBG: ≥5.6 mmol/L
- SBP: ≥130 mmHg
- DBP: ≥85 mmHg
- Low HDL-chol: men ≤1 mmol/L and women ≤1.3 mmol/L
- Triglycerides: ≥1.7 mmol/L

3.4.4.5.3. T2DM classification

The current recommendations (WHO, 2016) for the diagnosis of diabetes and intermediate hyperglycaemia are summarized below:

- T2DM: if FBG is ≥7.0 mmolL or post 2HR BG ≥11.1 mmolL
- IGT: if FBG is <7.0 mmolL and post 2HR BG is between ≥7.8 mmolL and <11.1 mmolL
- IFG: if FBG is between 6.1 and 6.9 mmolL and if measured, post 2HR BG is <7.8 mmolL

3.4.4.6. Statistical analysis

A database was prepared on an Excel spread sheet including all demographic details, relevant clinical measurements, biochemical tests results, blood pressure readings, anthropometric measurements, the CT-obtained VAT and SAT (cm^2) measurements as well as the BIA-obtained VAT (levels) measurements. The MetS status of participants were determined according to the JIS MetS criteria, adapted for a WC cut-off of 90 cm for both genders as previously reported for by Matsha et al. (2013) for the mixed ancestry population.

Data was analysed using the software program Statistica (StatSoft, Southern Africa). Distribution testing was done using the Shapiro-Wilk W test; the main variables tested for in the current study, VAT (cm^2), SAT (cm^2) and WC (cm) showed skewed distributions (Results; Table 4.6), therefore descriptive statistics were done using Breakdown and one-way ANOVA (median and range 25Q; 75Q) and the Kruskal-Wallis ANOVA & Median test was used for statistical significance testing, with a P-value of less than 0.05 considered to indicate statistical significance between groups (gender and MetS status). The Spearman Rank R test was used for correlation analysis (R and P-values) between appropriate variables and regression analysis was used to establish association between these variables. Primarily, CT-obtained SAT and VAT (cm^2) were assessed against the other components of MetS, all anthropometric and biochemical measurements as well as additional risk factors for Mets such as age and gender and similarly also against BIA-obtained VAT (levels).

Receiver operating characteristic (ROC) curve analysis was used to determine gender specific CT-obtained VAT (cm^2) cut-off values for MetS diagnosis. This cut-off value was used to validate the 90 cm WC cut-off value in the mixed ancestry population reported by Matsha et al. (2013). Similarly, ROC curve analysis was used to validate the use of BIA in VAT (levels) assessment for MetS diagnosis against that of CT-obtained VAT (cm^2) measurements.

CHAPTER FOUR

RESULTS

4.1. MetS prevalence: The sample size for the current study with CT scan results was overall 401 participants, consisting of 93 males and 308 females. Individuals whose weight exceeded the maximum table weight (205 kg) of the CT equipment were excluded from the study. The prevalence for MetS (JIS criteria) was high overall (55.6%), with a higher prevalence for females (57.1%) than for males (50.5%) (Table 4.1, Figure 4.1). The overall prevalence of obesity in the current study population was 51.8% and when overweight subjects were included it was 74.5% (Table 4.2). The prevalence of T2DM in the total group was 24.3% (known and screen detected) and 41.4% in the total group when all hyperglycaemic cases were included (Table 4.2).

Table 4.1. Number and prevalence of MetS in the current study population (Figure 4.1.)

	Total group, N401		**Males, N93**		**Females, N308**	
	MetS		**MetS**		**MetS**	
MetS	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
N, (%)	178 (44.4%)	223 (55.6%)	46 (49.5%)	47 (50.5%)	132 (42.9%)	176 (57.1%)

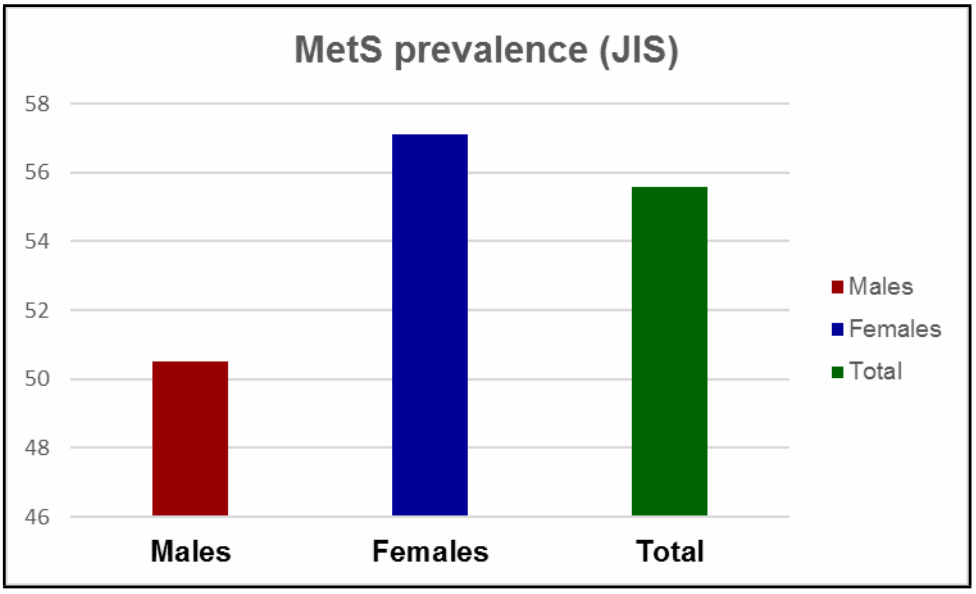


Figure 4.1 The percent prevalence of MetS was high overall (55.6%), with a higher prevalence for females (57.1%) than for males (50.5%).

4.2. The clinical characteristics, anthropometric and biochemical measurements as well as CT and BIA VAT analysis of the study population, categorised by gender are summarized in Table 4.2, while CT Images 1 and 2 illustrate the difference in SAT and VAT distribution in male and female participants from the current study. The prevalence of obesity and T2DM has been included in Table 4.2: The number of

participants with CT-measured VAT (cm^2) was 401 of which 363 had measurements for the BIA-measured VAT (levels) as well (78 males and 285 females). Of the 38 participants not measured with BIA, the following errors were displayed that resulted in non-measurements: including “values of body composition out of measurable range”, “palms or soles are too dry”, “not firm contact”, an “error” code which was not followed up by the on-site technician.

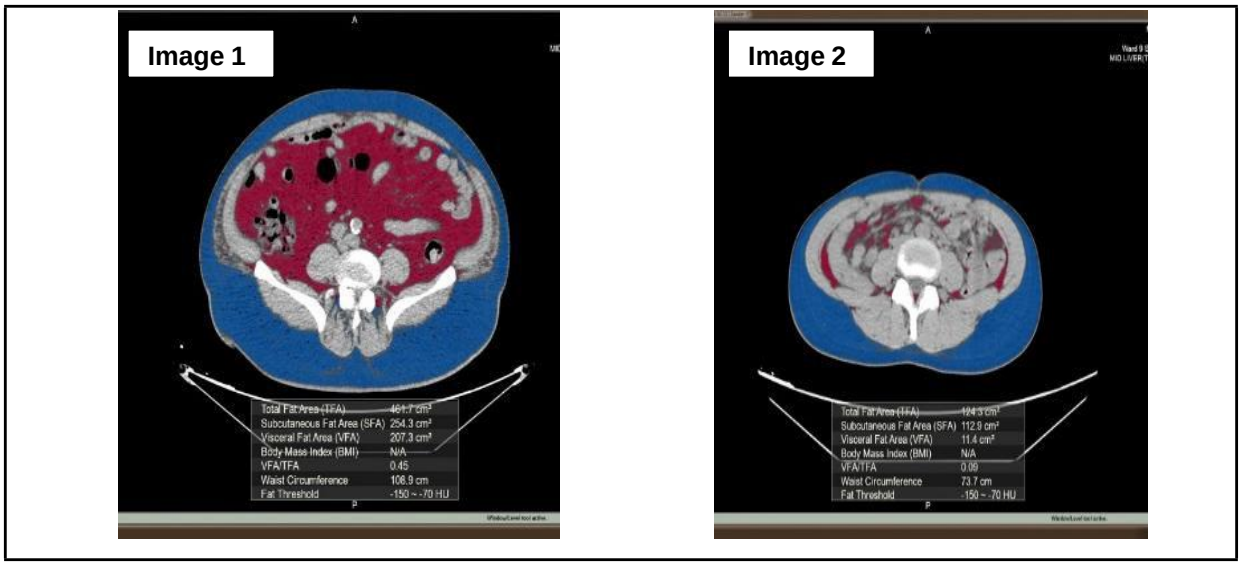
There was no difference in age between the genders, P=0.3224. CT-measured VAT and SAT (cm^2) were significantly higher in females than in males, measures are given for median and range, VAT (cm^2) in males 76.8 (39.5; 131.2) and in females 97.9 (62.3; 138.1); P=0.0153 and SAT (cm^2) in males 158.2 (66.9; 254.3) and in females 378.6 (270.6; 492.6); P<0.0001. Although both VAT and SAT were higher in females than in males, the VAT to SAT ratio was significantly higher in males than in females, 0.52 (0.36; 0.77) and 0.26 (0.17; 0.35) (P<0.0001) respectively, showing that males had higher VAT levels in relation to SAT levels as compared to that of females, even though both VAT and SAT were higher in females than in males. In contrast BIA-measured VAT (levels) was near-significantly higher in males: 12.0 (7.0; 16.0) than in females, 10.0 (8.0; 13.0); P=0.0599. The BMI (P<0.0001), WC (cm) (P=0.0033), hip (cm) (P<0.0001), post 2HR BG (mmol/L) (P=0.0056), fasting insulin (mIU/L) (P=0.0001), post 2HR insulin (mIU/L) (P=0.0002) and HDL-chol (mmol/L) (P=0.0008) were all significantly higher in females than in males. The SBP (mmHg) (P=0.0074) and Gamma GT-S (IU/L) (P=0.0043) were significantly higher in males than females.

The prevalence of obesity was high; in the total group it was 51.8%, in males 28.0% with that of females significantly higher at 59.0% (P<0.0001). Combined obesity and overweight subjects showed extremely high prevalence rates, in the total group 74.5%, in males 57.0% and again significantly higher in females at 79.8% (P<0.0001). There was no difference in the prevalence of T2DM or hyperglycaemia between males and females (P=0.9654 and P=0.5544 respectively). However the prevalence nearly doubled when T2DM was assessed against total hyperglycaemia; in females from 24.2% to 43.25% (Chi-square 11.24; P = 0.0008; data not shown elsewhere).

Table 4.2 The clinical characteristics, anthropometric and biochemical measurements, CT and BIA measurements, as well as obesity and T2DM prevalence of the study population categorised by gender

	Total, N401	Males, N93 Median (25Q; 75Q)	Females, N308	P-value
Age (years)	55.0 (45.0; 64.0)	56.0 (47.0; 65.0)	55.0 (44.5; 64.0)	0.3224
Menopause age (years)	NA	NA	45.0 (40.0; 50.0)	NA
CT and BIA measurements				
Total fat (cm^2)	431.9 (284.5; 597.2)	264.9 (99.7; 418.2)	492.0 (354.6; 628.0)	**<0.0001**
SAT (cm^2)	322.5 (202.1; 461.2)	158.2 (66.9; 254.3)	378.6 (270.6; 492.6)	**<0.0001**
VAT (cm^2)	94.2 (52.6; 136.8)	76.8 (39.5; 131.2)	97.9 (62.3; 138.1)	**0.0153**
VAT to SAT ratio	0.29 (0.21; 0.46)	0.52 (0.36; 0.77)	0.26 (0.17; 0.35)	**<0.0001**
VAT (levels) (BIA)*	10.0 (8.0; 14.0)	12.0 (7.0; 16.0)	10.0 (8.0; 13.0)	0.0599
Anthropometric measurements				
Weight (kg)	75.8 (63.3; 91.3)	72.3 (58.5; 87.4)	76.9 (64.2; 92.6)	**0.0482**
Height (cm)	159.4 (154.5; 165.0)	168.5 (164.0; 173.6)	156.7 (153.0; 161.6)	**<0.0001**
BMI (kg/m^2)	29.9 (24.8; 36.2)	26.0 (20.4; 30.1)	31.3 (26.1; 37.5)	**<0.0001**
Obesity prevalence, N (%)	207/400 (51.8%)	26/93 (28.0%)	181/307 (59.0%)	**<0.0001****
Obesity and overweight combined, N (%)	298/400 (74.5%)	53/93 (57.0%)	245/307 (79.8%)	**<0.0001****
WC (cm)	98.5 (86.7; 110.2)	93.5 (81.3; 105.5)	99.5 (88.5; 110.5)	**0.0033**
Hip (cm)	107.5 (99.0; 118.8)	99.5 (92.3; 106.5)	110.8 (102.5; 122.8)	**<0.0001**
Waist/hip ratio	0.89 (0.83; 0.94)	0.93 (0.87; 0.98)	0.88 (0.82; 0.92)	**<0.0001**
Hypertension measurements				
SBP (mmHg)	127.0 (113.0; 142.0)	132.0 (116.0; 150.0)	125.0 (112.0; 140.0)	**0.0074**
DBP (mmHg)	83.0 (74.0; 90.0)	83.0 (75.0; 96.0)	83.0 (73.0; 89.0)	0.1365
Pulse (bpm)	70.0 (62.0; 79.0)	70.0 (62.0; 77.0)	70.0 (62.0; 80.0)	0.6908
Glycaemic measurements				
FBG (mmol/L)	5.10 (4.60; 5.80)	5.00 (4.60;.70)	5.10 (4.60; 5.90)	0.4390
Post 2HR BG (mmol/L)	6.50 (5.30; 8.00)	5.95 (4.60; 7.75)	6.70 (5.50; 8.10)	**0.0056**
HbA1c (%)	5.90 (5.50; 6.50)	5.70 (5.40; 6.20)	5.90 (5.60; 6.60)	**0.0455**
Fasting insulin (mIU/L)	7.80 (5.00; 12.10)	6.00 (2.90; 10.60)	8.40 (5.60; 12.20)	**0.0001**
Post 2HR insulin (mIU/L)	49.2 (27.7; 84.6)	31.4 (17.5; 68.0)	51.9 (31.6; 90.7)	**0.0002**
Glucose/insulin ratio	0.71 (0.46; 1.14)	1.00 (0.56; 1.52)	0.66 (0.45; 1.02)	**0.0003**
T2DM prevalence, N (%)	97/399 (24.3%)	23/93 (24.7%)	74/306 (24.2%)	0.9654*
Hyperglycaemia, N (%)	165/399 (41.4%)	36/93 (38.7%)	129/306 (43.25)	0.5544*
Lipid measurements				
LDL-chol (mmol/L)	3.30 (2.70; 4.00)	3.35 (2.60; 3.90)	3.30 (2.70; 4.00)	0.5547
HDL-chol (mmol/L)	1.20 (1.10; 1.40)	1.20 (1.00; 1.30)	1.30 (1.10; 1.50)	**0.0008**
Chol (mmol/L)	5.30 (4.60; 6.00)	5.20 (4.45; 5.85)	5.35 (4.60; 6.00)	0.1825
Chol/HDL-ratio	4.20 (3.50; 5.10)	4.30 (3.40; 5.60)	4.20 (3.50; 4.90)	0.2483
Triglycerides (mmol/L)	1.32 (0.96; 1.81)	1.25 (0.94; 1.74)	1.33 (1.00; 1.84)	0.4134
Diverse measurements				
CRP (mg/L)	4.72 (2.05; 9.75)	4.37 (1.65; 7.67)	5.00 (2.30; 10.07)	0.1170
Cotinine (ng/mL)	10.0 (10.0; 223.0)	10.0 (10.0; 269.0)	10.0 (10.0; 195.0)	0.0589
Gamma GT-S (IU/L)	28.5 (21.0; 46.0)	34.5 (24.0; 55.5)	28.0 (20.0; 43.0)	**0.0043**

Key: VAT: Visceral fat; SAT: Subcutaneous fat; CRP: C-reactive protein; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HbA1c: Glycated Haemoglobin; LDL-chol: Low-density lipoprotein; HDL-chol: High-density lipoprotein; Gamma GT-S: Gamma-glutamyl transferase, *The number of participants with BIA measurements was 363 (78 males and 285 females), **Pearson Chi-square has been used to determine statistical analysis.



Images 1 and 2. CT images illustrating the difference in SAT and VAT distribution in male and female participants from the current study. The SAT and VAT in Image 1 (male participant) is 254.3 cm^2 and 207.3 cm^2 respectively (that is more SAT than VAT), while the SAT and VAT in Image 2 (female participant) is 112.9 cm^2 and 11.4 cm^2 respectively (that is also more SAT than VAT). Results in the current study showed similar findings in the total group of participants, that is, more SAT than VAT in both males and females.

4.3.A. The clinical characteristics, anthropometric and biochemical measurements, CT and BIA measurements of the study population, categorised by both gender and MetS status are summarized in Table 4.3.A: Differences in measurements between subjects with and without MetS are given per gender due to the highly significant differences between the gender groups in most variables tested for (see Table 4.2). Age was significantly increased in subjects with MetS in both genders (P=0.0146 and P=0.0011 for males and females respectively). Results showed significant increases (median and range) in CT-obtained SAT (cm^2), CT-obtained VAT (cm^2) and in BIA-obtained VAT (levels) in both male and female subjects with MetS (All, P<0.0001). However, the CT-obtained VAT to SAT ratio showed differences between the genders in the MetS sub-categories, in males the ratio stayed similar: in male subjects without MetS: 0.50 (0.36; 0.79) and in male subjects with MetS: 0.54 (0.33; 0.76); P=0.9387, while in females the ratio increased in female subjects with MetS: without and with MetS respectively: 0.22 (0.14; 0.30) and 0.29 (0.22; 0.41); P<0.0001.

Anthropometric measurements including weight (kg), BMI (kg/m^2), WC (cm), hip (cm) and waist to hip ratio were also increased in both genders in subjects with MetS, (All, P<0.0001). SBP (mmHg), DBP (mmHg) and pulse (bpm) were significantly increased in both male and female subjects with MetS (All, P<0.0001). Glycaemic measurements, including FBG (mmol/L), post 2HR BG (mmol/L), HbA1c (%), fasting insulin (mIU/L) and post 2HR insulin (mIU/L) were significantly increased in male and female subjects with MetS (All, P<0.02). HDL-chol (mmol/L) was significantly lower in subjects with MetS in both genders (P=0.0001,

P<0.0001 respectively). LDL-chol (mmol/L) was significantly higher in female subjects with MetS than in female subjects without MetS (P=0.0050), but this difference was not found in male subjects. Triglycerides (mmol/L) were significantly increased in both male and female subjects with MetS (Both, P<0.0001). The CRP (mg/L) was significantly increased in female subjects with MetS (P<0.0001), but not in male subjects with MetS (P=0.2101), while Gamma GT-S (IU/L) was significantly increased in both male and female subjects with MetS, but of higher significance in female subjects (P=0.0343, P=0.0001 respectively).

The prevalence of obesity was high in both male and female subjects, but highest in female subjects with MetS; without MetS it was 34.4% which increased highly significantly to 77.3% in female subjects with MetS (P<0.0001). When overweight and obesity were combined the prevalence in females without MetS increased from 61.8% to 93.2% in females with MetS (P<0.0001).

The prevalence of T2DM was non-significantly increased in male subjects and significantly in female subjects with MetS to a similar prevalence; in male subjects with MetS 36.2% and in female subjects with MetS 36.6%. These values increased significantly per gender to 51.1% (P=0.0134) and 57.7% (P<0.0001) in males and females respectively when evaluated in overall hyperglycaemic cases in subjects with MetS.

Table 4.3.A. The clinical characteristics, anthropometric and biochemical measurements, CT and BIA measurements as well as the prevalence of obesity and T2DM of the study population, categorised by both gender and MetS status

	Males			Females		
	MetS/No, N46	MetS/Yes, N47		MetS/No, N132	MetS/Yes, N176	
	Median (25-75Q)		P-value	Median (25-75Q)		P-value
Age (years)	53.0 (44.0; 59.0)	60.0 (52.0; 66.0)	**0.0146**	51.0 (38.5; 62.5)	56.5 (48.0; 64.0)	**0.0011**
Menopause age (years)	NA	NA	NA	45.0 (40.0; 50.0)	46.0 (40.0; 50.0)	0.9907
CT and BIA measurements						
Total fat (cm^2)	137.3 (42.1; 266.2)	384.7 (245.3; 483.9)	**<0.0001**	362.9 (267.7; 487.3)	562.9 (455.9; 700.3)	**<0.0001**
SAT (mm^2)	88.7 (30.5; 175.2)	217.9 (149.7; 324.7)	**<0.0001**	298.2 (199.9; 405.3)	434.5 (325.4; 532.3)	**<0.0001**
VAT (mm^2)	40.7 (11.9; 66.9)	125.0 (76.8; 190.8)	**<0.0001**	65.2 (37.0; 103.5)	124.8 (86.0; 171.1)	**<0.0001**
VAT to SAT ratio	0.50 (0.36; 0.79)	0.54 (0.33; 0.76)	0.9387	0.22 (0.14; 0.30)	0.29 (0.22; 0.41)	**<0.0001**
VAT (levels) (BIA)*	7.0 (3.0; 12.0)	14.0 (12.0; 19.0)	**<0.0001**	8.0 (6.0; 11.0)	11.0 (10.0; 14.0)	**<0.0001**
Anthropometric measurements						
Weight (kg)	58.9 (54.8; 72.8)	80.7 (71.2; 94.9)	**<0.0001**	66.8 (56.7; 77.2)	83.6 (72.7; 100.6)	**<0.0001**
Height (cm)	168.3 (163.8; 174.5)	169.5 (164.0; 173.3)	0.7504	157.5 (154.0; 160.8)	156.3 (152.5; 163.0)	0.6870
BMI (kg/m^2)	21.1 (19.6; 26.3)	28.5 (25.6; 32.8)	**<0.0001**	26.5 (22.9; 31.3)	34.5 (30.2; 39.3)	**<0.0001**
Obesity prevalence, N (%)	4/46 (8.7%)	22/47 (46.8%)	**<0.0001****	45/131 (34.4%)	136/176 (77.3%)	**<0.0001****
Obesity and overweight combined, N (%)	15/46 (32.6%)	38/47 (80.9%)	**<0.0001****	81/131 (61.8%)	164/176 (93.2%)	**<0.0001****
WC (cm)	84.1 (77.5; 88.1)	102.5 (93.5; 112.5)	**<0.0001**	88.5 (80.1; 99.5)	106.8 (96.8; 114.5)	**<0.0001**
Hip (cm)	93.5 (90.8; 100.5)	104.5 (97.5; 109.5)	**<0.0001**	104.8 (96.5; 113.7)	115.5 (106.5; 127.7)	**<0.0001**
Waist/hip ratio	0.88 (0.84; 0.91)	0.97 (0.93; 1.03)	**<0.0001**	0.84 (0.80; 0.89)	0.90 (0.87; 0.95)	**<0.0001**
Hypertension measurements						
SBP (mmHg)	120.5 (107.0; 136.0)	138.0 (130.0; 164.0)	**<0.0001**	114.0 (104.0; 125.0)	134.0 (122.0; 148.0)	**<0.0001**
DBP (mmHg)	76.0 (72.0; 86.0)	92.0 (80.0; 102.0)	**<0.0001**	75.0 (70.0; 82.0)	87.0 (81.0; 93.0)	**<0.0001**
Pulse (bpm)	69.5 (62.0; 76.0)	70.0 (63.0; 78.0)	0.5590	67.0 (59.0; 76.0)	71.5 (65.0; 81.0)	**0.0006**
Glycaemic measurements						
FBG (mmol/L)	4.80 (4.40; 5.20)	5.60 (4.80; 6.50)	**0.0006**	4.80 (4.40; 5.20)	5.50 (4.80; 7.60)	**<0.0001**
Post 2HR BG (mmol/L)	5.20 (4.20; 7.10)	6.30 (5.40; 9.30)	**0.0075**	6.10 (5.00; 7.25)	7.40 (6.10; 9.50)	**<0.0001**
HbA1c (%)	5.60 (5.40; 6.00)	6.00 (5.50; 6.90)	**0.0174**	5.70 (5.35; 5.95)	6.20 (5.80; 7.50)	**<0.0001**
Fasting insulin (mIU/L)	3.05 (2.30; 6.10)	8.50 (6.00; 14.40)	**<0.0001**	6.20 (4.10; 8.60)	10.60 (7.20; 15.60)	**<0.0001**
Post 2HR insulin (mIU/L)	23.1 (14.2; 40.1)	61.1 (24.7; 92.6)	**0.0006**	40.6 (27.3; 64.9)	66.1 (40.5; 116.1)	**<0.0001**
Glucose/insulin ratio	1.47 (0.87; 2.14)	0.75 (0.44; 1.00)	**<0.0001**	0.78 (0.56; 1.22)	0.55 (0.39; 0.91)	**<0.0001**
T2DM prevalence, N (%)	6/46 (13.0%)	17/47 (36.2%)	0.0654**	10/131 (7.6%)	64/175 (36.6%)	**<0.0001****
Hyperglycaemia, N (%)	12/46 (26.1%)	24/47 (51.1%)	**0.0134****	28/131 (21.4%)	101/175 (57.7%)	**<0.0001****
Lipid measurements						
LDL-chol (mmol/L)	3.20 (2.40; 3.80)	3.55 (2.90; 4.00)	0.1376	3.20 (2.50; 3.70)	3.40 (2.90; 4.10)	**0.0050**
HDL-chol (mmol/L)	1.25 (1.10; 1.50)	1.00 (0.90; 1.20)	**0.0001**	1.40 (1.20; 1.70)	1.20 (1.10; 1.30)	**<0.0001**
Chol (mmol/L)	5.10 (4.30; 5.70)	5.25 (4.70; 6.10)	0.2968	5.20 (4.50; 5.90)	5.40 (4.80; 6.20)	0.1055
Chol/HDL-chol ratio	3.85 (3.30; 4.60)	5.10 (4.10; 6.10)	**0.0001**	3.70 (3.00; 4.40)	4.60 (3.90; 5.30)	**<0.0001**
Triglycerides (mmol/L)	1.01 (0.78; 1.26)	1.70 (1.17; 2.38)	**<0.0001**	1.05 (0.76; 1.34)	1.65 (1.27; 2.13)	**<0.0001**
Diverse measurements						
CRP (mg/L)	2.45 (1.32; 8.11)	4.72 (2.30; 6.56)	0.2101	3.40 (1.23; 7.52)	6.64 (3.24; 12.03)	**<0.0001**
Cotinine (ng/mL)	10.0 (75.8; 299.0)	10.0 (10.0; 226.0)	0.0825	10.0 (10.0; 228.0)	10.0 (10.0; 176.0)	0.2033
Gamma GT-S (IU/L)	28.0 (21.0; 50.0)	36.5 (27.0; 66.0)	**0.0343**	24.0 (18.0; 36.0)	30.0 (22.0; 46.0)	**0.0001**

*The number of participants with BIA results was 363 (35 and 43 males; 124 and 161 females without and with MetS respectively)

4.3.B. The predictive value of obesity in MetS and T2DM prevalence, **categorised by both gender and obesity status are summarized in Table 4.3.B:** The results clearly showed that MetS prevalence increased highly significantly through normal weight subjects to obese subjects, in both males and females (both P<0.0001), with very high MetS prevalence rates in the obese subjects; MetS prevalence in males of normal weight 22.5%, overweight 59.3% and in obese males 84.6%; in females respectively 19.4%, 43.8% and 75.1%. MetS positivity was also shown in 22.5% and 19.4% of normal weight males and females respectively.

T2DM prevalence increased from normal weight subjects to a similar prevalence in overweight and obese subjects in both genders; T2DM prevalence in normal weight, overweight and obese males males respectively 12.5%, 37.0% and 30.8% (P=0.0346) and in females 8.2%, 27.0% and 28.7% (P=0.0302).

Table 4.3.B. The predictive value of obesity in MetS and T2DM prevalence, categorised by both gender and obesity status

	Males				Females			
	Normal weight	**Overweight**	**Obese**	**P-value**	**Normal weight**	**Overweight**	**Obese**	**P-value**
MetS prevalence, N (%)	9/40 (22.5%)	16/27 (59.3%)	22/26 (84.6%)	**<0.0001**	12/62 (19.4%)	28/64 (43.8%)	136/181 (75.1%)	**<0.0001**
T2DM prevalence, N (%)	5/40 (12.5%)	10/27 (37.0%)	8/26 (30.8%)	**0.0346**	5/61 (8.2%)	17/63 (27.0%)	52/181 (28.7%)	**0.0302**

4.4.A. Correlations between CT-obtained SAT and VAT (cm^2) with the clinical characteristics, anthropometric and biochemical measurements and BIA-obtained VAT (levels), categorized according to MetS status are summarized in Table 4.4: CT-measured SAT (cm^2) and VAT (cm^2) showed a highly significant positive correlation with each other in subjects without MetS (R=0.6128; P< 0.0001) and a weaker, but significant correlation in subjects with MetS (R=0.2373; P=0.0004). BIA-measured VAT (levels) showed highly significant correlations with CT-measured SAT and VAT (cm^2) but with a higher correlation strength with CT-measured VAT; with CT-measured VAT in subjects without MetS R=0.7959; P<0.0001 and with MetS R=0.6036; P<0.0001. Similarly, CT-measured VAT (cm^2) showed significant correlations with anthropometric measurements in subjects without and with MetS (but weaker in subjects with MetS); respectively with the BMI (kg/m^2) R=0.7298; P<0.0001 and R=0.4905; P<0.0001; with the WC (cm) R=0.7730; P<0.0001 and R=0.6194; P<0.0001 and the hip (cm) R=0.6587; P<0.0001 and R=0.3513; P<0.0001.

Overall, CT-measured VAT (cm^2) showed correlations of higher strength in subjects without MetS than in those with MetS when correlated with other measurements as well; it correlated

positively respectively with FBG (mmol/L (R=0.4318; P<0.0001 and R=0.1716; P=0.0106), with post 2 HR BG (mmol/L) (R=0.3276; P<0.0001 and R=0.2933; P=0.0001), with fasting insulin (mIU/L) (R=0.5072; P<0.0001 and R=0.3304; P<0.0001) and with post 2HR insulin (mIU/L) (R=0.4419; P<0.0001 and R=0.3313; P<0.0001).

CT-measured VAT (cm^2) correlated positively with LDL-chol (mmol/L) in subjects without MetS (R=0.2922; P=0.0001), but not in subjects with MetS (R=-0.0357; P=0.5985) and similarly correlated with cholesterol (mmol/L) and triglycerides (mmol/L) in subjects without MetS only, respectively R=0.2426; P=0.0011 and R=0.4675; P<0.0001. The correlation of CT-measured VAT (cm^2) with the CRP (mg/L) was near-significant in subjects without and significant in subjects with MetS, R=0.1408; P=0.0617 and R=0.1370; P = 0.0424 respectively.

Figures 4.2, 4.3, 4.4, 4.5, 4.6, and 4.7 demonstrate the correlation profile between measures of central obesity in the total study population: Figures 4.2, 4.3, 4.4 and 4.5 demonstrate the difference in correlation strengths between CT-measured VAT and SAT (cm^2) when correlated with the weight (kg), BMI (kg/m^2), WC (cm) and hip (cm). Figure 4.6 demonstrates the difference in correlation strength of CT-measured VAT (cm^2) when correlated with the weight (kg), BMI (kg/m^2), WC (cm) and hip (cm). Figure 4.7 demonstrates the difference in correlation strength between BIA-measured VAT (levels) when correlated with CT-measured VAT and SAT (cm^2) (N363).

Table 4.4. Correlation of CT-measured SAT and VAT (cm^2) with the clinical characteristics, anthropometric and biochemical measurements and BIA-obtained VAT (levels), categorized according to MetS status

	Correlation with CT-measured SAT (cm^2)							Correlation with CT-measured VAT (cm^2)					
	Total, N401		**MetS No, N178**		**MetS Yes, N223**			**Total, N401**		**MetS No, N178**		**MetS Yes, N223**	
	R	**P-value**	**R**	**P-value**	**R**	**P-value**		**R**	**P-value**	**R**	**P-value**	**R**	**P-value**
Age (years)	-0.0097	0.8460	0.0367	0.6263	**-0.2132**	**0.0014**		**0.3938**	**<0.0001**	**0.4670**	**<0.0001**	**0.2545**	**0.0001**
Menopause age (years)	0.0387	0.6196	0.0569	0.6606	0.0076	0.9387		0.0226	0.7715	0.1023	0.4288	-0.0339	0.7317
CT and Omron measurements													
Total fat (cm^2)	**0.9482**	**<0.0001**	**0.9757**	**<0.0001**	**0.9214**	**<0.0001**		**0.7200**	**<0.0001**	**0.7392**	**<0.0001**	**0.5240**	**<0.0001**
SAT (cm^2)	NA	NA	NA	NA	NA	NA		**0.5220**	**<0.0001**	**0.6128**	**<0.0001**	**0.2373**	**0.0004**
VAT (cm^2)	**0.5220**	**<0.0001**	**0.6128**	**<0.0001**	**0.2373**	**0.0004**		NA	NA	NA	NA	NA	NA
VAT (levels) (BIA)*	**0.5803**	**<0.0001**	**0.7081**	**0.0000**	**0.3138**	**<0.0001**		**0.7709**	**<0.0001**	**0.7959**	**<0.0001**	**0.6036**	**<0.0001**
Anthropometric measurements													
Weight (kg)	**0.8012**	**<0.0001**	**0.7716**	**<0.0001**	**0.7293**	**<0.0001**		**0.6768**	**<0.0001**	**0.6721**	**<0.0001**	**0.4351**	**<0.0001**
Height (cm)	**-0.2369**	**<0.0001**	**-0.3424**	**<0.0001**	**-0.1685**	**0.0129**		-0.0995	0.0503	**-0.1800**	**0.0185**	-0.0509	0.4553
BMI (kg/m^2)	**0.8956**	**<0.0001**	**0.9036**	**<0.0001**	**0.8523**	**<0.0001**		**0.7064**	**<0.0001**	**0.7298**	**<0.0001**	**0.4905**	**<0.0001**
WC (cm)	**0.7663**	**<0.0001**	**0.7521**	**<0.0001**	**0.6960**	**<0.0001**		**0.7796**	**<0.0001**	**0.7730**	**<0.0001**	**0.6194**	**<0.0001**
Hip (cm)	**0.8936**	**<0.0001**	**0.8849**	**<0.0001**	**0.8653**	**<0.0001**		**0.5936**	**<0.0001**	**0.6587**	**<0.0001**	**0.3513**	**<0.0001**
Waist/hip ratio	0.0829	0.0982	0.1144	0.1305	**-0.2604**	**0.0001**		**0.5188**	**<0.0001**	**0.4704**	**<0.0001**	**0.3556**	**<0.0001**
Hypertension measurements													
SBP (mmHg)	0.0186	0.7109	-0.0840	0.2650	**-0.2584**	**0.0001**		**0.2880**	**<0.0001**	**0.1478**	**0.0490**	0.0138	0.8376
DBP (mmHg)	0.0867	0.0828	-0.1407	0.0610	-0.0885	0.1879		**0.1364**	**0.0062**	-0.1205	0.1092	**-0.1833**	**0.0061**
Pulse (bpm)	0.0825	0.0990	-0.0512	0.4977	0.0975	0.1468		0.0539	0.2818	-0.0860	0.2536	0.0578	0.3907
Glycaemic measurements													
FBG (mmol/L)	**0.1921**	**0.0001**	0.1459	0.0527	-0.0225	0.7392		**0.4287**	**<0.0001**	**0.4318**	**<0.0001**	**0.1716**	**0.0106**
Post 2 HR BG (mmol/L)	**0.2074**	**0.0002**	**0.2354**	**0.0023**	-0.0280	0.7217		**0.4183**	**<0.0001**	**0.3276**	**<0.0001**	**0.2933**	**0.0001**
HbA1c (%)	**0.2112**	**<0.0001**	**0.1682**	**0.0248**	0.0014	0.9836		**0.4384**	**<0.0001**	**0.3897**	**<0.0001**	**0.2242**	**0.0008**
Fasting insulin (mIU/L)	**0.5280**	**<0.0001**	**0.5477**	**<0.0001**	**0.3207**	**<0.0001**		**0.5477**	**<0.0001**	**0.5072**	**<0.0001**	**0.3304**	**<0.0001**
Post 2HR insulin (mIU/L)	**0.3630**	**<0.0001**	**0.4922**	**<0.0001**	0.0531	0.5020		**0.4750**	**<0.0001**	**0.4419**	**<0.0001**	**0.3313**	**<0.0001**
Glucose/insulin ratio	**-0.4679**	**<0.0001**	**-0.5273**	**<0.0001**	**-0.3130**	**<0.0001**		**-0.4282**	**<0.0001**	**-0.4187**	**<0.0001**	**-0.2666**	**0.0001**
Lipid measurements													
LDL-chol (mmol/L)	**0.1340**	**0.0075**	0.1331	0.0775	0.0690	0.3080		**0.1683**	**0.0008**	**0.2922**	**0.0001**	-0.0357	0.5985
HDL-chol (mmol/L)	**-0.1910**	**0.0001**	-0.1426	0.0583	-0.0013	0.9853		**-0.2191**	**<0.0001**	-0.1194	0.1133	0.0198	0.7705
Chol (mmol/L)	0.0837	0.0955	0.0657	0.3852	0.0628	0.3529		**0.1266**	**0.0115**	**0.2426**	**0.0011**	-0.0154	0.8203
Chol/HDL-chol ratio	**0.2593**	**<0.0001**	**0.2284**	**0.0022**	0.0696	0.3038		**0.2986**	**<0.0001**	**0.3255**	**<0.0001**	-0.0224	0.7408
Triglycerides (mmol/L)	**0.2619**	**<0.0001**	**0.2202**	**0.0032**	0.0166	0.8068		**0.4612**	**<0.0001**	**0.4675**	**<0.0001**	0.1136	0.0927
Diverse measurements													
CRP (mg/L)	**0.2939**	**<0.0001**	**0.1619**	**0.0313**	**0.2629**	**0.0001**		**0.2571**	**<0.0001**	0.1408	0.0617	**0.1370**	**0.0424**
Cotinine (ng/mL)	**-0.2982**	**<0.0001**	**-0.3561**	**<0.0001**	**-0.2040**	**0.0023**		**-0.2668**	**<0.0001**	**-0.3289**	**<0.0001**	**-0.1889**	**0.0047**
Gamma GT-S (IU/L)	0.0641	0.2021	-0.0418	0.5805	-0.0339	0.6163		**0.2837**	**<0.0001**	**0.2177**	**0.0036**	**0.1872**	**0.0053**

*The number of participants with BIA results was 363 (159 without and 204 with MetS respectively)

Figures 4.2 to 4.5 highlights the difference in correlation strengths (R) of CT-measured SAT and VAT (cm^2) with anthropometric measurements in the total group of participants

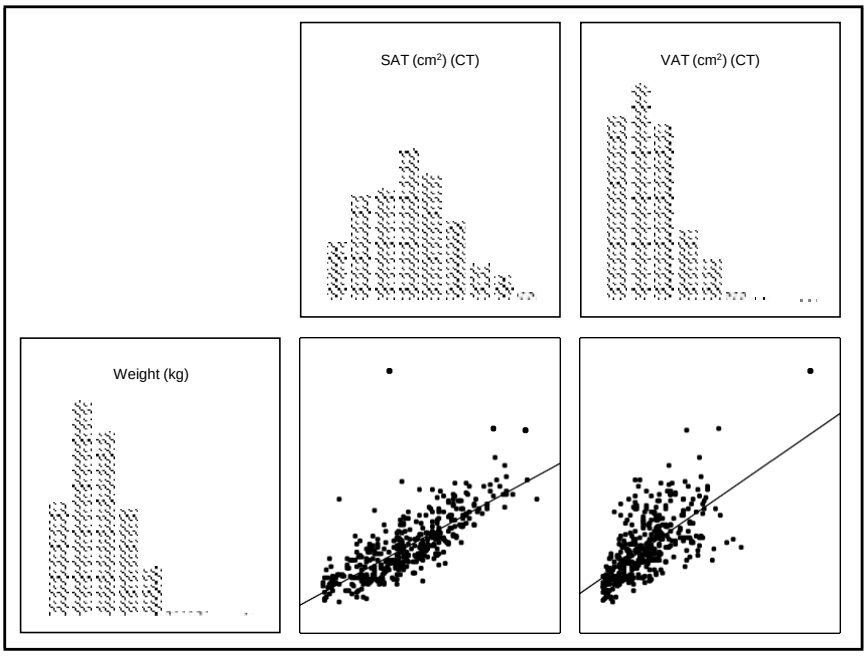


Figure 4.2. The correlation of the weight of the total participant group (N401) was stronger with CT-measured SAT (cm^2) than VAT (cm^2), R=0.8012, P<0.0001 and R=0.6768; P<0.0001 respectively.

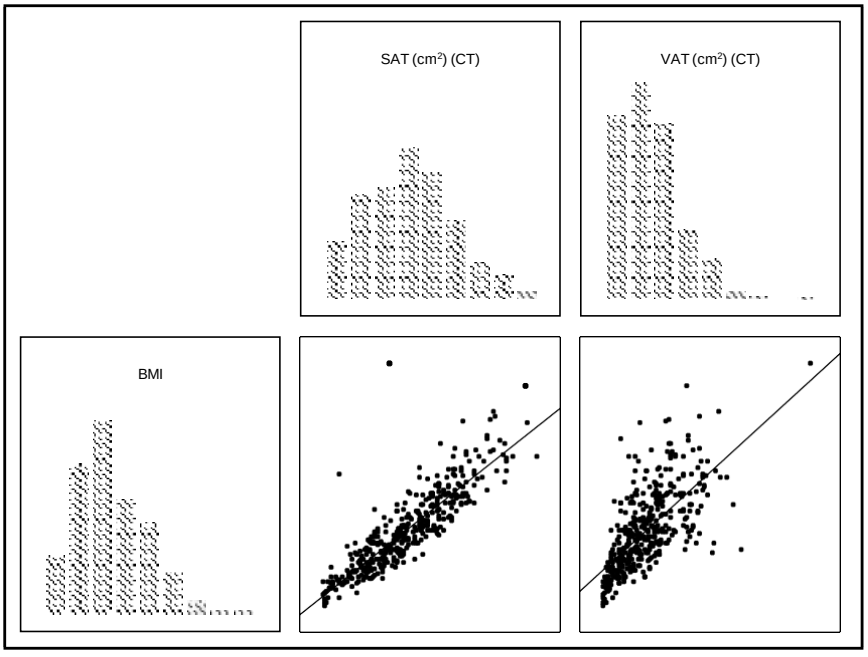


Figure 4.3. The correlation of the BMI (kg/m^2) of the total participant group (N401) was stronger with CT-measured SAT (cm^2) than VAT (cm^2), R=0.8956, P<0.0001 and R=0.7064; P<0.0001 respectively.

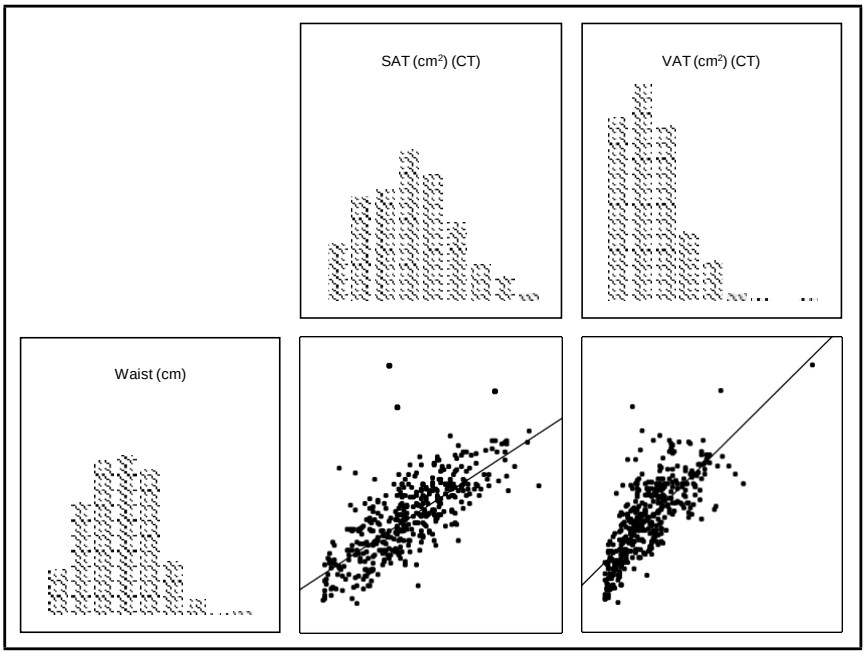


Figure 4.4. The correlation of the WC (cm) of the total participant group (N401) was stronger with CT-measured VAT (cm^2) than SAT (cm^2), R=0.7796; P<0.0001 and R=0.7663, P<0.0001 respectively,

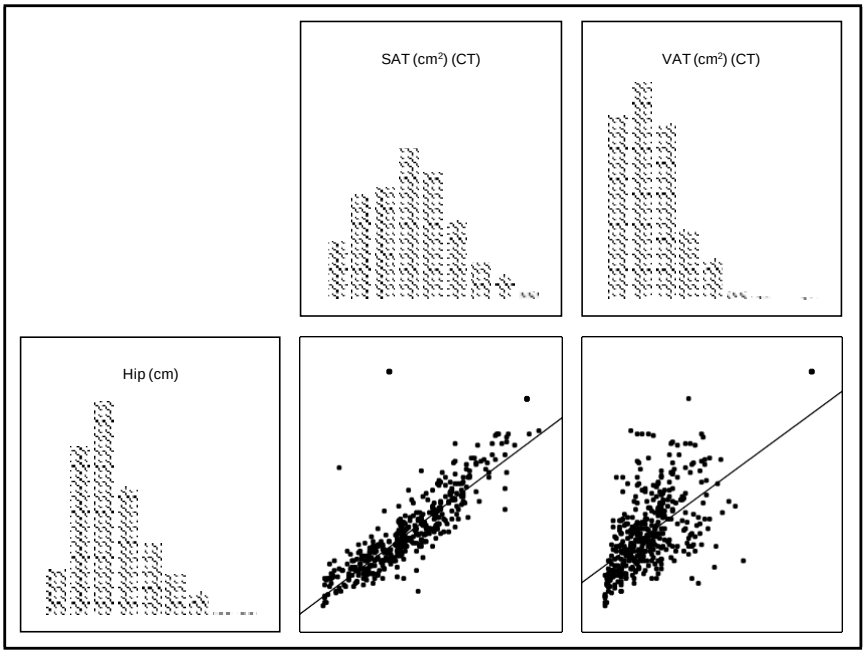


Figure 4.5. The correlation of the hip (cm) of the total participant group (N401) was stronger with CT-measured SAT than VAT (cm^2), R=0.8936, P<0.0001 and R=0.5936; P<0.0001 respectively.

Figure 4.6 highlights the difference in correlation strength (R) of CT-measured VAT (cm^2) with the anthropometric measurements

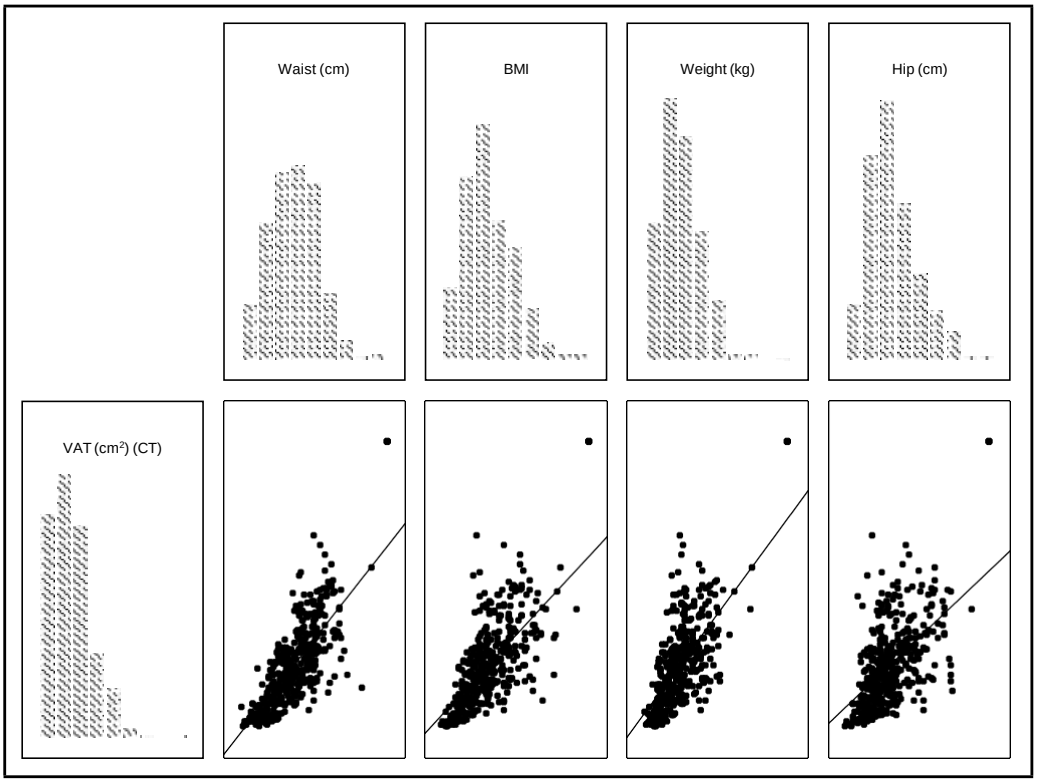


Figure 4.6. The correlation of CT-measured VAT (cm^2) with anthropometric measurements of the total participant group (N401), from highest correlation strength: the WC (cm) (R=0.7796; P<0.0001); the BMI (kg/m^2), (R=0.7064, P<0.0001), the weight (kg) (R=0.6768; P<0.0001) and the hip (cm) (R=0.5936; P<0.0001).

Figure 4.7 highlights the difference in correlation strengths (R) of CT-measured SAT and VAT (cm^2) with BIA-measured VAT (levels)

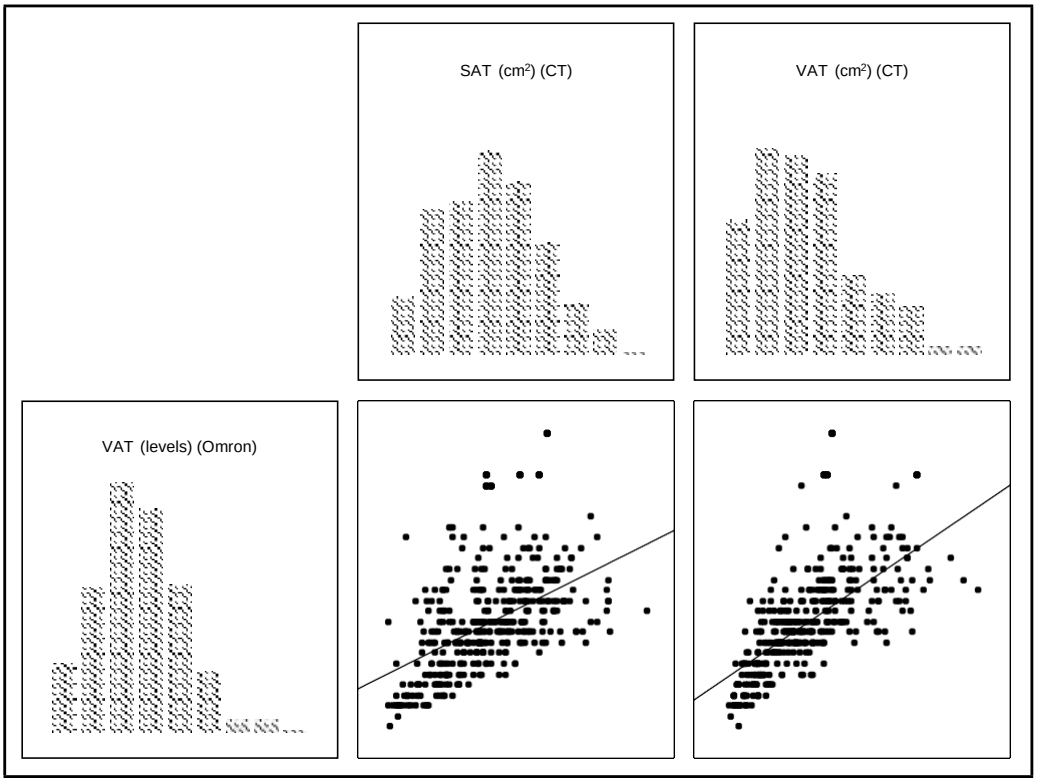


Figure 4.7. The correlation of BIA (Omron)-measured VAT (levels) of the total participant group with both types of measurements available (N363) was stronger with CT-measured VAT than SAT (cm^2), R=0.7709; P<0.0001 and R=0.5803, P<0.0001 respectively.

4.4.B. Results from the correlation study (Table 4.4) between CT-measured SAT and VAT (cm^2) with the SBP and DBP (mmHg) suggested co-contributors to these correlation coefficients (results were unexpected). Therefore regression analysis has been used to determine association between MetS components and the SBP and DBP (mmHg); these are summarized in Table 4.4.B: When adjusted for age and gender, results showed that there was no association between the SBP (mmHg) and CT-measured VAT or SAT (cm^2) when evaluated together with MetS risk factors, but that the SBP (mmHg) showed a significant association with the triglycerides (mmol/L) when evaluated together with CT-measured SAT (cm^2) and near-significantly when evaluated together with CT-measured VAT (cm^2). The DBP (mmHg) in contrast, when adjusted for age and gender showed a significant association with CT-measured SAT (cm^2) when evaluated together with the MetS risk factors, but not with CT-measured VAT (cm^2).

Table 4.4.B. Association of the MetS risk profile with blood pressure

CT measurements	SBP (mmHg)				DBP (mmHg)			
	VAT (cm^2)		SAT (cm^2)		VAT (cm^2)		SAT (cm^2)	
	b*	P-value	b*	P-value	b*	P-value	b*	P-value
Age (years)	0.4023	<0.0001	0.4121	<0.0001	-0.2878	<0.0001	-0.2821	<0.0001
Gender	-0.0709	0.0378	-0.0453	0.2603	-0.0038	0.9233	-0.0594	0.1918
CT-measured VAT (cm^2)	0.0267	0.4914	NA	NA	0.0168	0.7045	NA	NA
CT-measured SAT (cm^2)	NA	NA	-0.0418	0.3098	NA	NA	0.1091	0.0188
SBP (mmHg)	NA	NA	NA	NA	0.7498	<0.0001	0.7472	<0.0001
DBP (mmHg)	0.5792	<0.0001	0.5843	<0.0001	NA	NA	NA	NA
FBG (mmol/L)	-0.0047	0.8933	-0.0026	0.9403	0.0363	0.3604	0.0358	0.3620
Triglycerides (mmol/L)	0.0615	0.0995	0.0738	0.0461	0.0111	0.7939	-0.0033	0.9364
HDL-chol (mmol/L)	0.0148	0.6815	-0.0003	0.9944	0.0228	0.5786	0.0451	0.2758

4.5. Results from regression analysis to demonstrate the association strengths of risk factors for MetS against CT-measured VAT and SAT (cm^2) and BIA-measured VAT (levels), adjusted for age and gender are summarized in Table 4.5: Weight, height, age and gender were programmed into the Omron scale (BIA) measurement calculations, which may have biased the results of the analysis in this section. The WC (cm) and hip (cm) measurements were done independently from the Omron scale (BIA) measurement calculations. The WC (cm) showed a stronger association with CT-measured VAT (cm^2) (b*=0.4174; P<0.0001) than with SAT (cm^2) (b*=0.1441; P=0.0052). The BMI (kg/m^2) showed similar strength of association with CT-measured VAT (cm^2) (b*=0.3934; P=0.0002) and SAT (cm^2) (b*=0.3789; P<0.0001). The hip (cm) showed an inverse association with CT-measured VAT (cm^2) (b*=-0.2006; P=0.0242) and a stronger, more significant positive association with CT-measured SAT (cm^2) (b*=0.3568; P<0.0001). Neither the WC (cm) nor the hip (cm) measurements showed any association with BIA-measured VAT (levels), but the BMI (kg/m^2) showed a highly significant association with BIA-measured VAT (levels) (b*=0.7253; P<0.0001).

Of the anthropometric measurements, the WC (cm) showed the highest and most significant association with CT-measured VAT (cm^2) (b*=0.4174; P<0.0001), followed by the BMI (kg/m^2) (b*= 0.3934; P=0.0002), the hip (cm) (inverse association) (b*=-0.2006; P=0.0242) and post 2 HR BG (mmol/L) (b*=0.1512; P=0.0137).

Table 4.5. Regression analysis was used to highlight strength of associations between CT-measured SAT and VAT (cm^2) and BIA-measured VAT (levels) against risk factors for MetS and adjusted for age and gender; detailed from highest level of association of CT-measured VAT (cm^2) with these measurements.

	CT-measured SAT (cm^2) N363		**CT-measured VAT (cm^2) N363**		**BIA-measured VAT (levels) N363**	
	b*	**P-value**	**b***	**P-value**	**b***	**P-value**
WC (cm)	**0.1441**	**0.0052**	**0.4174**	**< 0.0001**	0.0708	0.2240
BMI (kg/m^2)	**0.3789**	**< 0.0001**	**0.3934**	**0.0002**	**0.7253**	**< 0.0001**
Age (years)	**-0.0906**	**0.0033**	**0.2864**	**< 0.0001**	**0.3758**	**< 0.0001**
Hip (cm)	**0.3568**	**< 0.0001**	**-0.2006**	**0.0242**	-0.0964	0.1300
LDL-chol (mmol/L)	0.0189	0.9196	-0.1786	0.5471	-0.1829	0.3904
Post 2 HR BG (mmol/L)	0.0237	0.5384	**0.1512**	**0.0137**	-0.0343	0.4340
Cholesterol (mmol/L)	-0.0049	0.9801	0.1508	0.6318	0.1676	0.4579
FBG (mmol/L)	-0.0375	0.3344	-0.0938	0.1272	0.0593	0.1786
Post 2HR insulin (mIU/L)	-0.0361	0.2379	0.0931	0.0549	0.0336	0.3334
HDL-chol (mmol/L)	-0.0288	0.6439	-0.0537	0.5860	-0.0295	0.6770
DBP (mmHg)	0.0173	0.5968	-0.0294	0.5702	-0.0356	0.3380
Gamma GT(IU/L)	-0.0013	0.9619	0.0275	0.5159	-0.0274	0.3667
Fasting insulin (mIU/L)	0.0052	0.8860	0.0212	0.7121	0.0445	0.2806
Cotinine (ng/mL)	-0.0146	0.5583	-0.0204	0.6064	-0.0350	0.2184
SBP (mmHg)	0.0055	0.8837	-0.0130	0.8273	0.0298	0.4862
Gender	**0.2213**	**< 0.0001**	-0.0127	0.7693	**-0.2657**	**< 0.0001**
CRP (mg/L)	0.0018	0.9405	-0.0091	0.8155	-0.0241	0.3901
Triglycerides-S (mmol/L)	-0.0073	0.8486	-0.0008	0.9899	0.0813	0.0614

4.6. Method agreement testing

4.6.1. Distribution profile of CT-measured VAT (cm^2), WC (cm) and BIA-measured VAT (levels): The degree of agreement between two measuring units can be affected by a skewed distribution in one or both types of measurements. Results from the current study highlight the skewed distribution of VAT (cm^2) (CT) measurement: Shapiro-Wilk W=0.94; P<0.0001 (Figure 4.8), the WC (cm): Shapiro-Wilk W=0.99; P=0.0170 (Figure 4.9) and BIA-measured VAT (levels): Shapiro-Wilk W=0.97546, P<0.0001 (Figure 4.10) as determined in the total group of participants. Results in Table 4.6 summarized the distribution profile for the total group as well as per MetS classification in the above measuring modalities. Figure 4.11 summarizes the different central tendency and range profiles of the three measuring modalities, CT-measured VAT (cm^2), the WC (cm) and the BIA-measured VAT (levels). CT-measured VAT (cm^2) and the WC (cm) showed similar measurement profiles between the lower and upper quartiles, with a cross-over at the median; however, the WC (cm) over-reads to some extent from the lower quartile to the median and under-reads from the median to the upper quartile. There are however, substantial differences between the distribution profiles of CT-measured VAT (cm^2) and the WC (cm) between the minimum reading and the lower quartile, with the WC (cm) over-reading substantially, as well as

between the upper quartile and the maximum readings where the WC (cm) under-reads substantially. These results showed that the increases over the WC (cm) quartiles were in effect linear, while that of CT-measured VAT (cm^2) showed a sharp increase at the upper quartile towards the maximum reading (possibly in obese subjects). The BIA-measured VAT (levels) profile compared better with the WC (cm) profile than with the CT-measured VAT (cm^2) profile. These readings suggest that method agreement between the CT-measured VAT (cm^2) and the WC (cm) may be compromised at both lower and higher readings.

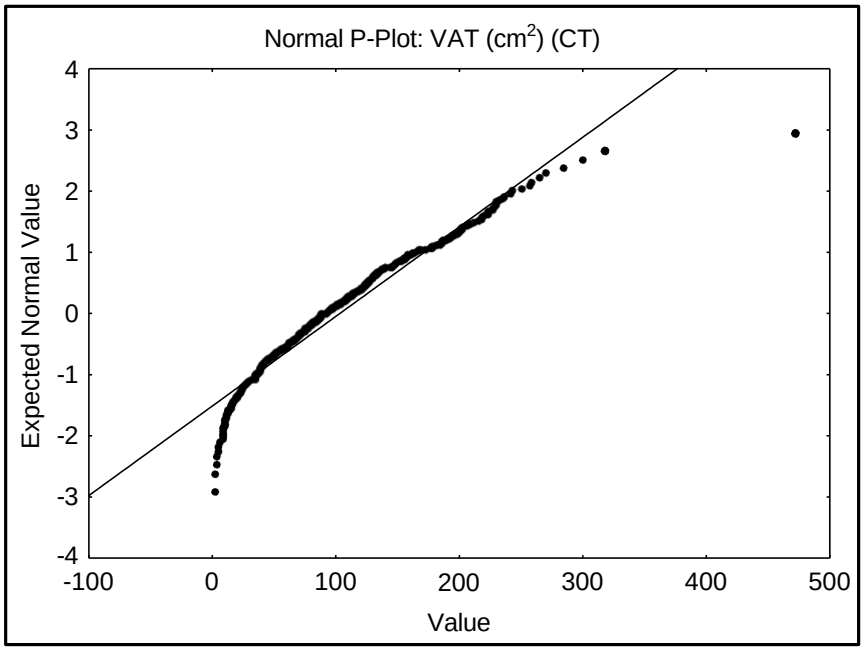


Figure 4.8. The Shapiro-Wilk W Test showed the skewed distribution of CT-measured VAT (cm^2) as tested for in the total group of participants: Shapiro-Wilk W=0.94; P<0.0001.

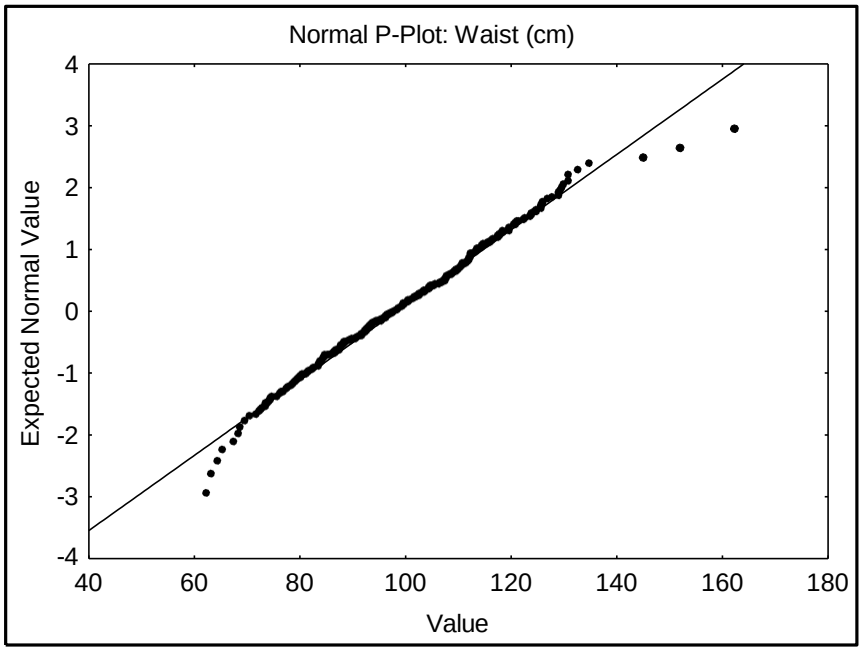


Figure 4.9. The Shapiro-Wilk W Test showed the skewed distribution of the WC (cm) as tested for in the total group of participants: Shapiro-Wilk W=0.99; P=0.0170.

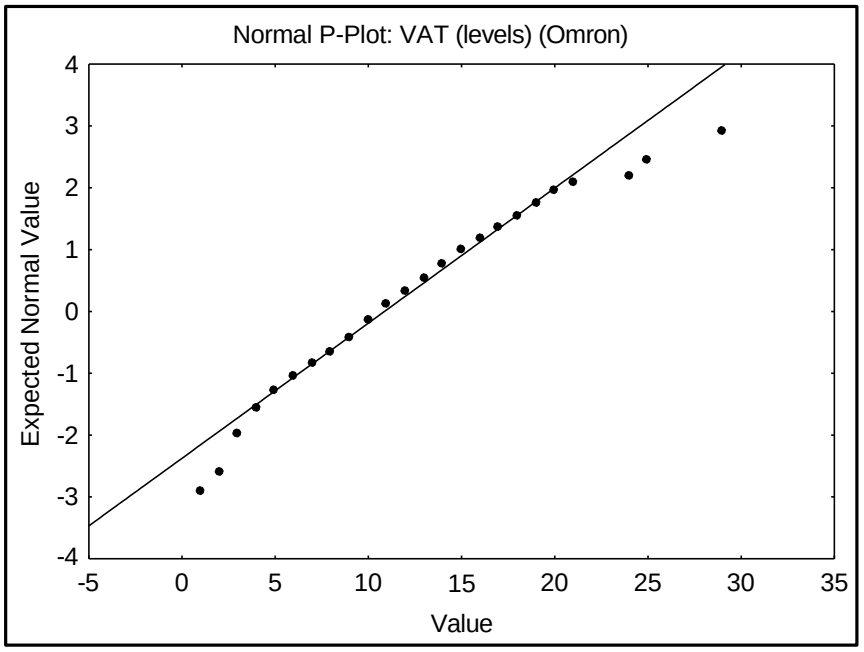


Figure 4.10. The Shapiro-Wilk W Test showed the skewed distribution of the BIA-measured VAT (levels) as tested for in the total group of participants: Shapiro-Wilk W=0.97546, P<0.0001.

Table 4.6. Summary statistics for distribution testing, categorized according to MetS subgroups

	CT-measured VAT (cm^2)			**WC (cm)**			**BIA-measured VAT (levels)**		
	Total	**MetS No**	**MetS Yes**	**Total**	**MetS No**	**MetS Yes**	**Total**	**MetS No**	**MetS Yes**
Shapiro-Wilk W	0.94	0.80	0.98	0.99	0.94	0.99	0.98	0.96	0.95
P-value	< 0.0001	< 0.0001	0.0012	0.0170	< 0.0001	0.5847	< 0.0001	0.0001	< 0.0001
Number	401	178	223	401	178	223	363	159	204
Mean	103.29	67.57	131.79	98.31	89.13	105.55	10.84	8.38	12.75
SD	65.99	53.68	60.90	16.29	15.49	12.92	4.48	3.91	3.93
Median	94.20	54.80	124.80	98.50	87.13	105.50	10.00	8.00	12.00
Lower quartile	52.60	28.90	84.70	86.70	79.25	96.50	8.00	5.00	10.00
Upper quartile	136.80	97.50	176.80	110.20	97.67	113.50	14.00	11.00	15.00
Quartile range	84.20	68.60	92.10	23.50	18.42	17.00	6.00	6.00	5.00
Minimum	3.50	4.30	3.50	62.50	62.50	70.70	1.00	1.00	4.00
Maximum	473.30	473.30	318.80	162.50	162.50	152.00	29.00	25.00	29.00
Variance	4354.13	2881.80	3708.65	265.30	240.00	166.98	20.04	15.30	15.43
Coef.Variance	63.89	79.44	46.21	16.57	17.38	12.24	41.30	46.66	30.81
Skewness	0.99	2.84	0.48	0.24	1.20	0.08	0.54	0.77	0.91
SE Skewness	0.12	0.18	0.16	0.12	0.18	0.16	0.13	0.19	0.17
Kurtosis	2.03	17.57	-0.24	0.08	2.92	0.25	0.76	1.11	1.52
SE Kurtosis	0.24	0.36	0.32	0.24	0.36	0.32	0.26	0.38	0.34

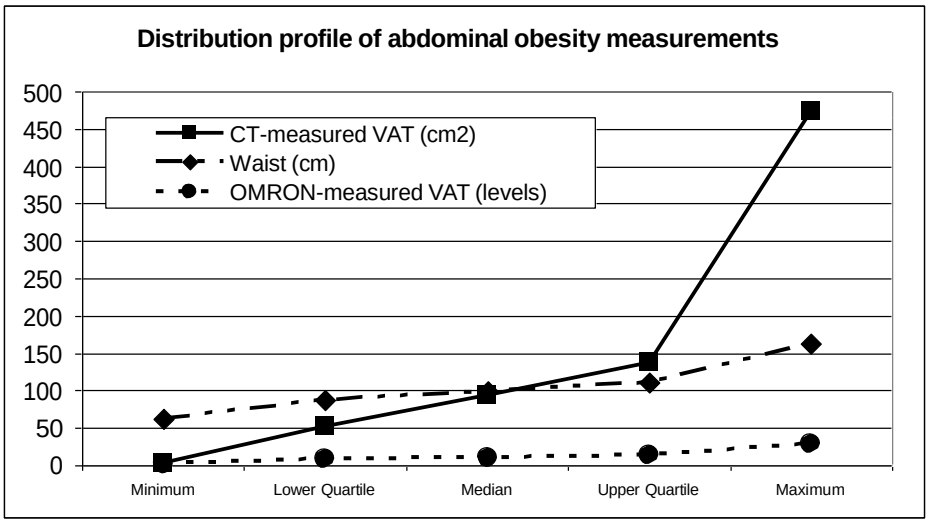


Figure 4.11. Demonstrates the different central tendency and range profiles of the three measuring modalities; CT-measured VAT (cm^2), the WC (cm) and the BIA-measured VAT (levels).

4.6.2. Regression equations were used to demonstrate the level of agreement between the measuring modalities of abdominal obesity; between the WC (cm) and CT-measured VAT (cm^2) (Figure 4.12) and between BIA-measured VAT (levels) and CT-measured VAT (cm^2) (Figure 4.13). Results for the total group as well as MetS sub-groups are summarized in Table 4.7: The regression (fit) equation for method agreement testing between the WC (cm) and CT-measured VAT (cm^2) for the total number of participants was $y = 79.1217 + 0.1854*x$; $R = 0.7519$; $P < 0.0001$; $R^2 = 0.5654$. Therefore, method agreement between CT-measured VAT (cm^2) and the WC (cm) on abdominal obesity assessment was 56.54% in the total group (58.49% in subjects without MetS and 37.23% in subjects with MetS). For results on further sub-grouping (gender), see Table 4.7. The regression (fit) equation for method agreement testing between the BIA-measured VAT (levels) and CT-measured VAT (cm^2) for the total number of participants was $y = 5.5774 + 0.0511*x$; $R = 0.7009$; $P < 0.0001$; $R^2 = 0.4912$. Therefore, method agreement between CT-measured VAT (cm^2) and BIA-measured VAT (levels) on abdominal obesity assessment is 49.12% in the total group (60.74% in subjects without MetS and 26.74% in subjects with MetS). For results on further sub-grouping (gender), see Table 4.7.

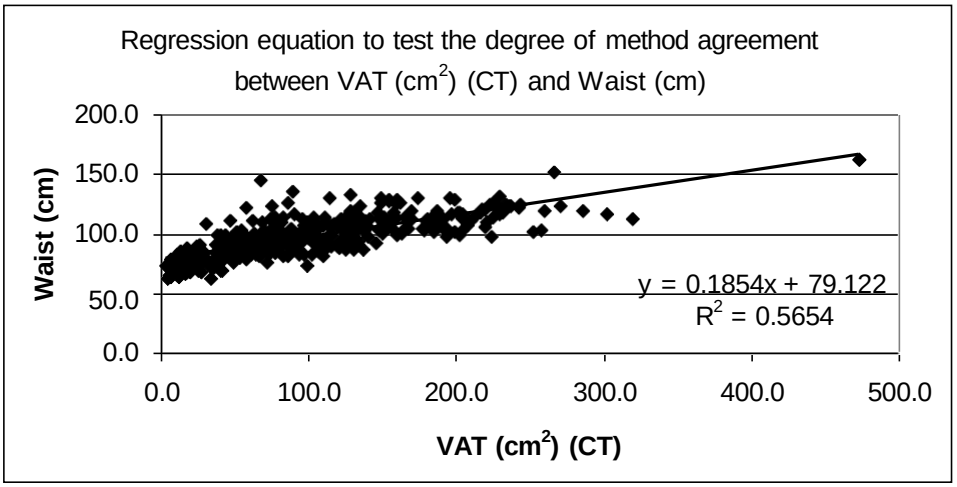


Figure 4.12. The regression (fit) equation for method agreement testing between the WC (cm) and CT-measured VAT (cm^2) for the total number of participants was y=79.1217 + 0.1854*x; R=0.7519; P<0.0001; R^2=0.5654. Therefore, method agreement between CT-measured VAT (cm^2) and the WC (cm) on abdominal obesity assessment was 56.54% in the total group.

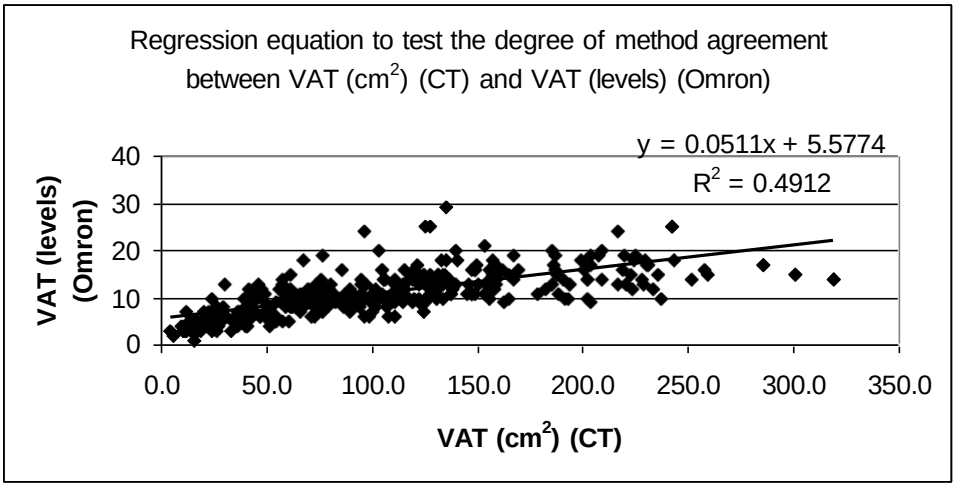


Figure 4.13. The regression (fit) equation for method agreement testing between the BIA (Omron)-measured VAT (levels) and CT-measured VAT (cm^2) for the total number of participants was y=5.5774 + 0.0511*x; R=0.7009; P<0.0001; R^2=0.4912. Therefore, method agreement between CT-measured VAT (cm^2) and BIA (Omron)-measured VAT (levels) on abdominal obesity assessment is 49.12% in the total group.

Table 4.7. Using regression equations to determine method agreement between the WC (cm) and CT-measured VAT (cm^2) as well as between BIA-measured VAT (levels) and CT-measured VAT (cm^2) in MetS sub-categories

Method agreement testing, categorized per gender and MetS categories					
Between the WC (cm) and CT-measured VAT (cm^2)					
	Regression equation	**R**	**P**	**R^2**	**% Agreement**
Total number, N401	y = 79.1217 + 0.1854*x	0.7519	< 0.0001	0.5654	56.54%
MetS No, N178	y = 74.2599 + 0.22*x	0.7648	< 0.0001	0.5849	58.49%
MetS No, Males, N46	y = 74.2888 + 0.1987*x	0.8987	< 0.0001	0.8077	80.77%
MetS No, Females, N132	y = 73.3151 + 0.2391*x	0.6974	< 0.0001	0.4864	48.64%
MetS Yes, N223	y = 88.4891 + 0.1295*x	0.6102	< 0.0001	0.3723	37.23%
MetS Yes, Males, N47	y = 86.1582 + 0.1238*x	0.6746	< 0.0001	0.4550	45.50%
MetS Yes, Females, N176	y = 88.9924 + 0.1319*x	0.5977	< 0.0001	0.3573	35.73%
Between BIA-measured VAT (levels) and CT-measured VAT (cm^2)					
	Regression equation	**R**	**P**	**R^2**	**% Agreement**
Total number, N363	y = 5.5774 + 0.0511*x	0.7009	< 0.0001	0.4912	49.12%
MetS No, N159	y = 3.5699 + 0.0711*x	0.7794	< 0.0001	0.6074	60.74%
MetS No, Males, N35	y = 2.4678 + 0.0985*x	0.8027	< 0.0001	0.6444	64.44%
MetS No, Females, N124	y = 3.942 + 0.0642*x	0.7921	< 0.0001	0.6275	62.75%
MetS Yes, N204	y = 8.3094 + 0.0341*x	0.5171	< 0.0001	0.2674	26.74%
MetS Yes, Males, N43	y = 10.5277 + 0.0355*x	0.4356	0.0035	0.1897	18.97%
MetS Yes, Females, N161	y = 7.8574 + 0.0326*x	0.6078	< 0.0001	0.3694	36.94%

4.7. Validation results for method testing

Although the Bland-Altman plot is often used to compare two assay methods, the results from the two assays need to be expressed in the same measuring units and as such these differed in the measuring modalities used in the current study, making this type of agreement testing less helpful.

4.7.1. Receiver operating characteristic (ROC) curve analysis

ROC curve analysis has been used to evaluate the diagnostic abilities of CT-measured VAT (cm^2) and BIA-measured VAT (levels), using the area under the curve (AUC) as well as % sensitivity and % specificity in the diagnostic evaluation of these measuring modalities in the classification of MetS. The WC (cm) cut-off value of 90 (cm) has been used to calculate % sensitivity and % specificity.

4.7.2. The use of ROC curve analysis to establish cut-off values for CT-measured VAT (cm^2) and BIA-measured VAT (levels) as markers for central obesity as diagnostic criteria for MetS in the mixed ancestry population has been summarized as follow:

ROC curve analysis has been used to establish cut-off values for both CT-measured VAT (cm^2) and BIA-measured VAT (levels) for optimal identification of MetS. Results are summarized in Table 4.8 and for CT-measured VAT (cm^2) also in Figures 4.14, 4.15 and 4.16 and BIA-measured VAT (levels) also in Figures 4.17, 4.18 and 4.19. Figure 4.20 demonstrates a summary of the %

sensitivity and % specificity of the derived cut-off values of CT-measured VAT (cm^2) and BIA-measured VAT (levels) to predict MetS. The % sensitivity and % specificity of the WC (cm) has been added to Table 4.8 for comparison, but the % sensitivity and % specificity have been calculated from the existing WC (cm) cut-off value; (sensitivity: % MetS cases correctly identified as positive and specificity: % controls correctly identify as negative).

4.7.2.1. Results from ROC curve analysis of CT-measured VAT (cm^2) in predicting MetS is depicted in Figure 4.14, 4.15 and 4.16 and Table 4.8: The diagnostic performance of CT-measured VAT (cm^2) as tested for by ROC curve analysis showed a cut-off value of >88.40 (cm^2) for MetS prediction in the total group of participants with sensitivity, specificity and area under the curve (AUC) values 72.2%, 70.8% and 0.81 respectively. The cut-off value in males were much lower than in the total group or in females, >72.20 (cm^2) with the performance similarly better, sensitivity, specificity and AUC values respectively 78.7%, 76.1% and 0.83. The results for females were similar as for the total group of participants.

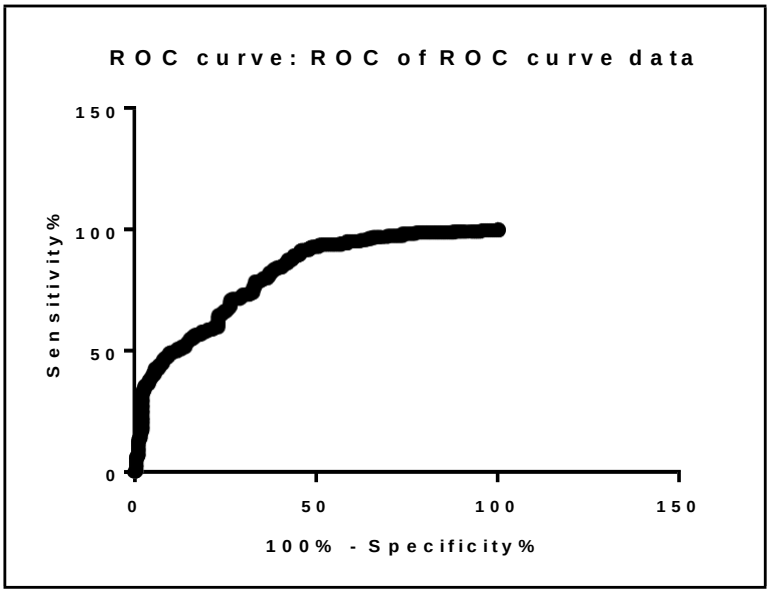


Figure 4.14. Demonstrates ROC curve analysis of CT-measured VAT (cm^2) in MetS prediction in the total group of participants, the cut-off value was >88.40 (cm^2), with sensitivity, specificity and AUC values 72.2%, 70.8% and 0.81 respectively.

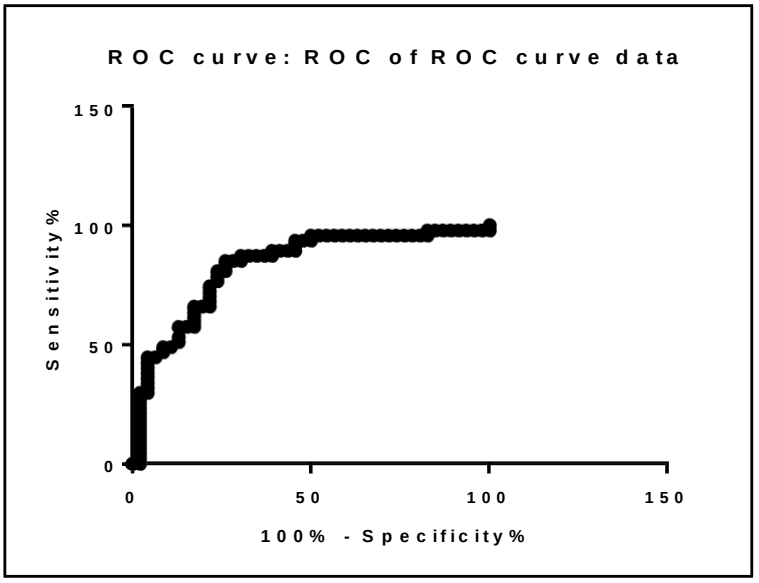


Figure 4.15. Demonstrates ROC curve analysis of CT-measured VAT (cm^2) in MetS prediction in male participants, the cut-off value was >72.20 (cm^2), with sensitivity, specificity and AUC values 78.7%, 76.1% and 0.83 respectively.

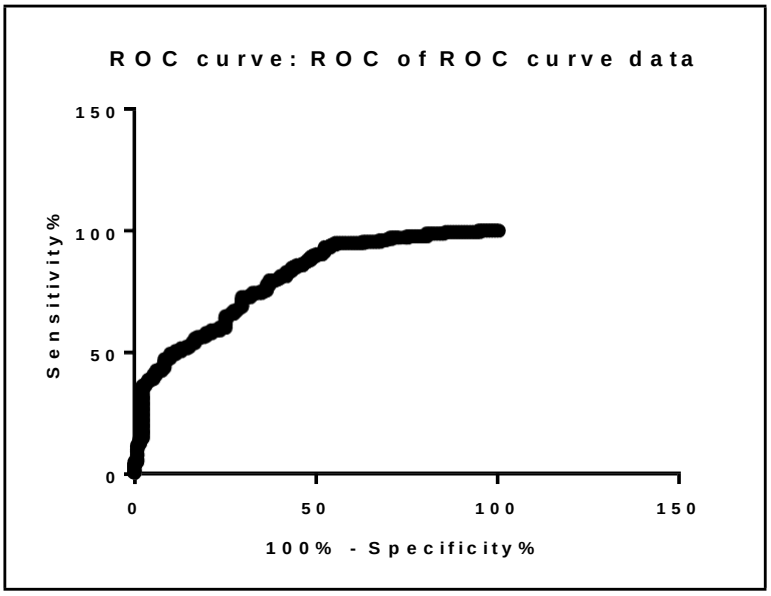


Figure 4.16. Demonstrates ROC curve analysis of CT-measured VAT (cm^2) in MetS prediction in female participants, the cut-off value was >91.40 (cm^2), with sensitivity, specificity and AUC values 72.2%, 70.5% and 0.80 respectively.

4.7.2.2. The results from ROC curve analysis of BIA-measured VAT (levels) in predicting MetS is depicted in Figure 4.17, 4.18 and 4.19 and Table 4.8: The diagnostic performance of BIA-measured VAT (levels) as tested for by ROC curve analysis was acceptable, showing a cut-off value of >10.50 (levels) for MetS prediction in the total group of participants with sensitivity, specificity AUC values 66.2%, 71.1% and 0.79 respectively, which compared well with those of

CT-measured VAT (cm^2), 72.2%, 70.8% and 0.81 respectively. The cut-off value for males and females are summarized in Table 4.8.

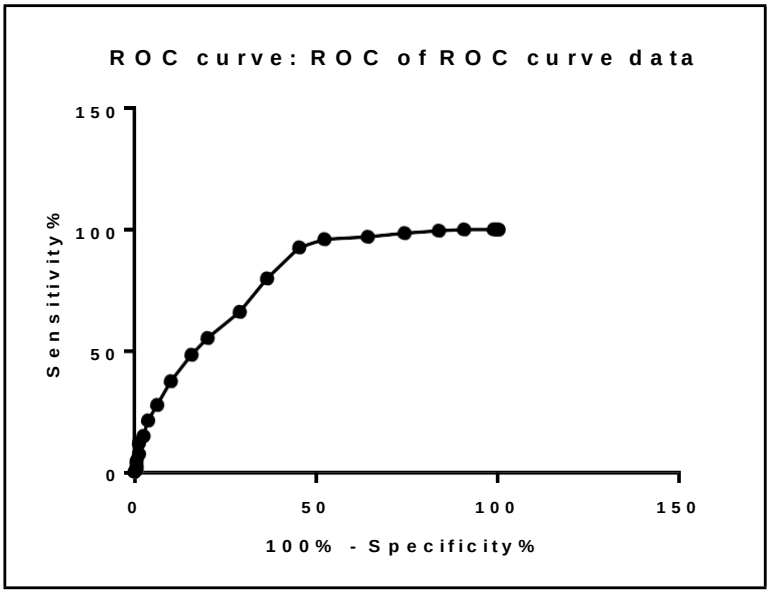


Figure 4.17. Demonstrates ROC curve analysis of BIA-measured VAT (levels) in MetS prediction in the total group of participants, the cut-off value was >10.50 (levels), with sensitivity, specificity and AUC values 66.2%, 71.1% and 0.79 respectively.

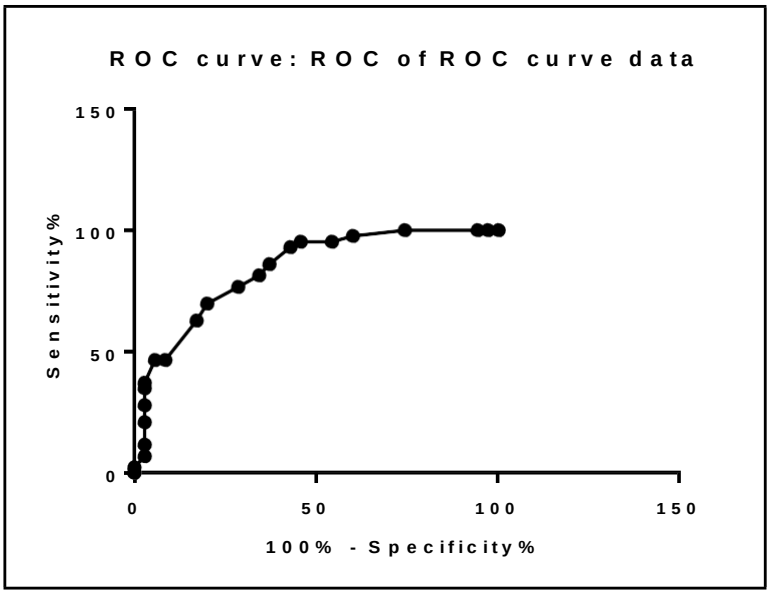


Figure 4.18. Demonstrates ROC curve analysis of BIA-measured VAT (levels) in MetS prediction in male participants, the cut-off value was >11.50 (levels), with sensitivity, specificity and AUC values 76.7%, 71.4% and 0.84 respectively.

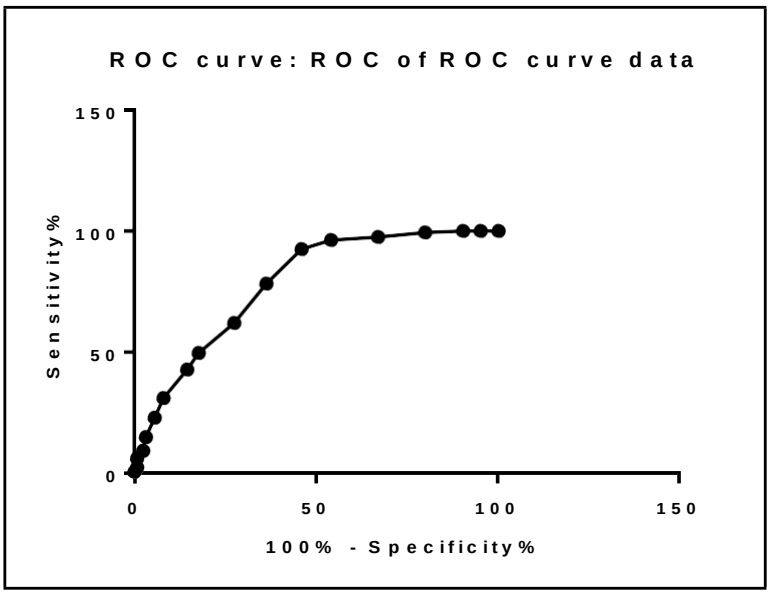


Figure 4.19. Demonstrates ROC curve analysis of BIA-measured VAT (levels) in MetS prediction in female participants, the cut-off value was >9.50 (levels), with sensitivity, specificity and AUC values 78.3%, 63.7% and 0.78 respectively.

4.7.2.3. Comparing the MetS diagnostic abilities of CT-measured VAT (cm^2), BIA-measured VAT (levels) and the WC (cm) (Table 4.8): The % sensitivity and % specificity of the WC has been calculated from the existing cut-off value of 90 (cm) and as such the AUC is not available for this measuring tool.

Using the area under the curve (AUC):

Results showed that using the AUC in the diagnostic evaluation, the BIA-measured VAT (levels) showed similar, albeit marginally lower AUC values as those of CT-measured VAT (cm^2), with both measuring tools showing the highest value in males.

Using % sensitivity and % specificity:

The % sensitivity and % specificity (as summarized in Table 4.8 and Figure 4.20) of BIA-measured VAT (levels) compared well with those of CT-measured VAT (cm^2), but with equilibrium between % sensitivity and % specificity more even in CT-measured VAT (cm^2). The % sensitivity and % specificity of the cut-off value of the WC (previously derived 90 cm) showed a higher % sensitivity as either CT-measured VAT (cm^2) or BIA-measured VAT (levels) did, but with the % specificity lower in females as compared to the those of the other two measuring modalities, especially that of the CT-measured VAT (cm^2).

Table 4.8. Summary results from ROC curve analysis; cut-off values of CT-measured VAT (cm^2), BIA-measured VAT (levels) and the WC (cm) to predict MetS

	Controls; cases (N)	AUC (SE)	95% CI	P-value	Cut-off value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Likelihood ratio
CT-measured VAT (cm^2)								
Total group, N401	178; 223	0.81 (0.02)	0.77 to 0.85	<0.0001	>88.40 (cm^2)	72.2 (65.8% to 78.0%)	70.8 (63.5% to 77.4%)	2.47
Males, N93	46; 47	0.83 (0.04)	0.74 to 0.92	<0.0001	>72.20 (cm^2)	78.7 (64.3% to 89.3%)	76.1 (61.2% to 87.4%)	3.29
Females, N308	132; 176	0.80 (0.03)	0.75 to 0.85	<0.0001	>91.40 (cm^2)	72.2 (64.9% to 78.6%)	70.5 (61.9% to 78.1%)	2.44
BIA-measured VAT (levels)								
Total group, N363	159; 204	0.79 (0.02)	0.74 to 0.84	<0.0001	>10.50 (levels)	66.2 (59.2% to 72.6%)	71.1 (63.4% to 78.0%)	2.29
Males, N78	35; 43	0.84 (0.05)	0.75 to 0.93	<0.0001	>11.50 (levels)	76.7 (61.4% to 88.2%)	71.4 (53.7% to 85.4%)	2.69
Females, N285	124; 161	0.78 (0.03)	0.73 to 0.84	<0.0001	>9.50 (levels)	78.3 (71.1% to 84.4%)	63.7 (54.6% to 72.2%)	2.16
WC (cm) (as calculated from existing cut-off value)								
Total group, N401	178; 223	NA	NA	NA	≥90.0 (cm)	91.5	63.1	NA
Males, N93	46; 47	NA	NA	NA	≥90.0 (cm)	87.2	78.3	NA
Females, N306	130; 176	NA	NA	NA	≥90.0 (cm)	92.6	57.7	NA

Key: ROC: Receiver operating characteristic; AUC (SE): Area under the curve (standard error); CI: Confidence interval.

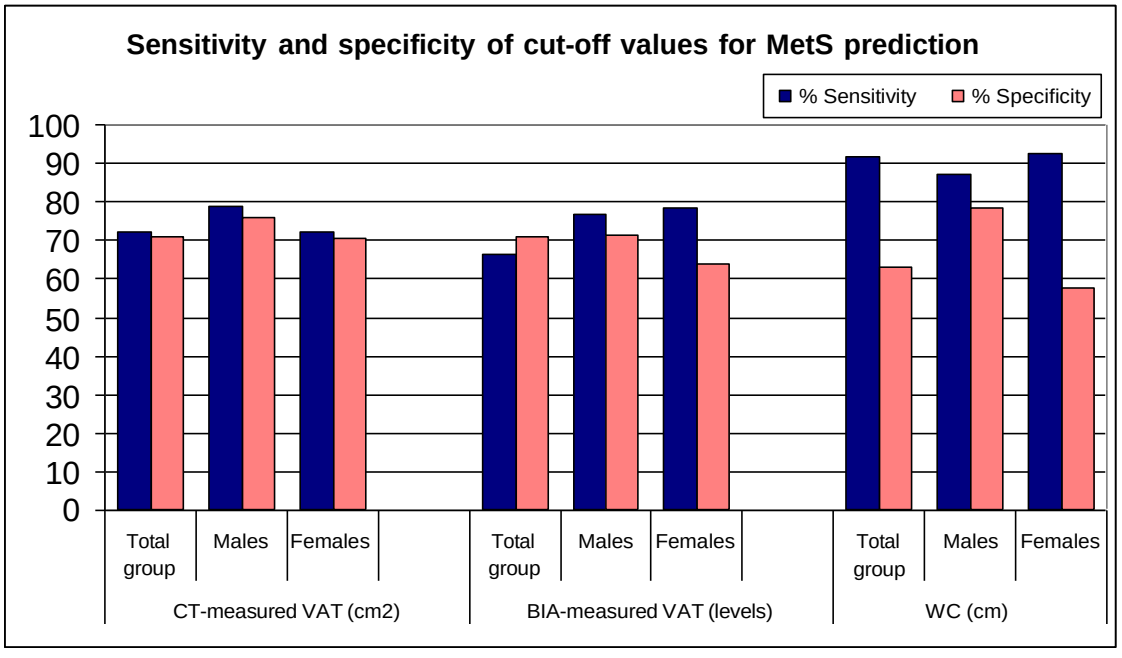


Figure 4.20. Summary of the % sensitivity and % specificity of the derived cut-off values of the three primary measuring modalities used in the current study, CT-measured VAT (cm^2), BIA-measured VAT (levels) and the WC (cm) to access abdominal obesity in MetS prediction.

CHAPTER FIVE
DISCUSSION

5.1. MetS prevalence in the current study population

MetS prevalence in the mixed ancestry population was high ((55.6%), similar to results published by Erasmus et al. (2012), with a higher prevalence rate for females (57.1%) than for males (50.5%). The prevalence rate reported for MetS ranges from 10% to 84% (Byrne & Wild, 2011), with some studies similarly reporting higher prevalence rates in females; Soares et al. (2015) reported a higher prevalence rate for MetS in females (76.2%) than in males (55.6%) in an indigenous population of Brazil. A highly significant finding was the overall high prevalence of obesity in both genders, but higher in females (59.0%) and extremely high when overweight and obesity were combined (79.8%). This prevalence is similar to the obesity prevalence reported by Goedecke, (2005) in South African women, in the mixed ancestry population (52%); amongst black women (58.5%), white women (49.2%) and Indian women (42.8%). Central obesity is a major risk factor for as well as a component of MetS diagnosis (Alberti et al., 2009; Wang, 2012) and as such highlights the importance of these findings. The prevalence of T2DM was also high in the current study population, 24.3% (known and screen detected) and 41.4% when all hyperglycaemic cases were included. Both obesity and MetS has been reported as risk factors for T2DM and CVD (Grundy et al., 2004; Stern et al., 2004; Esteghamati et al., 2008; Alberti et al., 2009) and the results from the current study confirmed the high prevalence of obesity and MetS in the South African mixed ancestry population as has been reported for other ethnicities world-wide (Grundy et al., 2004; Stern et al., 2004; Esteghamati et al., 2008; Alberti et al., 2009). The high prevalence of overweight in obesity evaluation as well as the high prevalence of intermediate hyperglyceamia in T2DM evaluation suggested a large increase in both these diseased states in the near future.

Due to the high prevalence of obesity in the current study population, one of the focus points of the study was on central obesity as measured by CT {VAT and SAT (cm^2)} as a risk factor for MetS and similarly in comparison with the other components of MetS {central obesity (other than CT measurements), glucose impairment, high blood pressure, dyslipidaemia, hyper-triglyceridaemia} that has been used in the classification of MetS (Alberti et al., 2006; Sutton & Raines, 2007;

Handelsman, 2009). Additionally, CT-measured VAT (cm^2) was used to verify the existing WC cut-off of 90 (cm) derived by Matsha et al. (2013) in MetS diagnosis.

For ease of reading the approximate course of the discussion has been outlined as follow: Measures of central tendencies and correlation coefficients have been used to discuss CT-measured SAT and VAT (cm^2) per gender and MetS classification as well as their relationship with the diagnostic components of MetS, including anthropometric measurements, glucose impairment, high blood pressure, dyslipidaemia, hyper-triglyceridaemia, and lastly additional risk factors for MetS. Regression analysis has been used to determine the inter-active combined association between the measuring modalities of central obesity and the diagnostic components of and risk factors for MetS. Method agreement testing between CT-measured VAT (cm^2) and the WC (cm) as well as BIA-measured VAT (levels) included distribution testing, comparison of measures of central tendencies and variance as well as regression equations (percentage of method agreement). ROC curve analysis has been discussed with regards to the respective abilities of CT-measured VAT (cm^2) as compared to the WC cm) and BIA-measured VAT (levels) in the diagnosis of MetS.

5.2. Assessment of the components of and risk factors for MetS

5.2.1. Assessment of central obesity

5.2.1.1. Assessment of CT-obtained SAT and VAT (cm^2), per gender

CT-measured SAT (cm^2) and VAT (cm^2) were both significantly higher in females than in males which was unaffected by age, with age similar for both genders. However, per gender results differed from the literature; CT images (Results, images 1 and 2) illustrate the difference in SAT (cm^2) and VAT (cm^2) distribution in male and female participants from the current study, but also showed that SAT (cm^2) was higher than VAT (cm^2) in both genders, with results showing similar findings in the total group of participants. This finding differed from the literature where fat distribution is said to differ in genders, with SAT higher in relation to VAT in females, but visa versa in males (Matsuzawa, 2008; Crowther & Ferris, 2010). Even so, although SAT (cm^2) was higher than VAT (cm^2) in both genders, the VAT to SAT ratio was significantly higher in males than in females, nearly doubled in males compared to the ratio in females, suggesting that VAT (cm^2) which is reported to be closer associated with MetS than SAT (cm^2) (Goodpaster et al., 2005), could still be regarded as a risk factor in males. In this regard, reports have shown that although women may be more obese than men, they are less prone to metabolic diseases as a result of the distribution of their fat (Lokuruka, 2013).

Comparison of measures of central tendency showed that the BIA-measured VAT (levels) in contrast to CT-measured SAT and VAT (cm^2) was near-significantly higher in males than in

females, suggesting that the Omron scale measurements may have been compromised in its accuracy to read VAT and that this was affected by gender, possibly by higher obesity levels in females as was shown in this study.

5.2.1.1.1. Assessment of CT-obtained SAT and VAT (cm^2) in MetS classification, per gender

Age was significantly increased in subjects with MetS in both genders, similar to reports from other groups (Ford et al., 2004; Borch-Johnsen, 2010). Due to the significant differences between the genders in most measurements in this study, results for measurements in MetS classification were done per gender group. Results showed significant increases in CT-obtained VAT and SAT (cm^2) as well as in BIA-obtained VAT (levels) in both male and female subjects with MetS, confirming reports on the association between abdominal obesity and MetS (Matsuzawa et al., 2011; Unno et al., 2012). However, the CT-obtained VAT to SAT ratio showed differences between the genders in the MetS sub-categories; in males the ratio stayed similar in subjects without and with MetS, suggesting a more constant ratio in males, while in females the VAT to SAT ratio increased in subjects with MetS, but was still not as high as the ratio was in male subjects without as well as with MetS. Even so, the prevalence of both MetS and T2DM were higher in females than in males in this study, suggesting that the significantly higher VAT (cm^2) in females may be of more importance than the higher VAT to SAT ratio in males in these obesity evaluation measurements and their association with disease.

5.2.1.1.1.1. Relationship between CT-measured SAT and VAT (cm^2) in MetS classification, per gender

Correlation coefficients confirm the dissimilarity in the relationship of CT-measured SAT and VAT (cm^2) volumes in subjects without and with MetS. CT-obtained SAT (cm^2) and VAT (cm^2) showed a significant positive correlation with each other in subjects without MetS (R=0.6128) and a much weaker, although still significant correlation in subjects with MetS (R=0.2373). The weaker correlation in subjects with MetS may have been both age and gender related. In this regard, results further showed that age in female subjects correlated in general positively with VAT (cm^2), more so that with SAT (cm^2) suggesting an increased trend in females of fat storage in VAT (cm^2) with increasing age. However, in subjects with MetS, CT-measured SAT (cm^2) showed an inverse correlation with age (years) (R=-0.2132), while CT-measured VAT (cm^2) showed a similarly strong but positive correlation with age (years) (R=0.2545), suggesting that the change to increased storage in VAT (cm^2) with aging in subjects with Mets may have been proportional to a similar decrease in SAT (cm^2) storage. These results suggested that the change in the SAT (cm^2) and VAT (cm^2) distribution profiles in subjects with MetS may be gender as well as age related and that while in males the relationship stayed relatively similar, the storage in female subjects with

MetS changed to an increased storage of abdominal fat in VAT (cm^2) volumes with increased ageing.

Comparing correlation coefficients, showed that the correlation between BIA-measured VAT (levels) was stronger with CT-measured VAT than with SAT (cm^2), R=0.7709; R=0.5803 respectively; in subjects without MetS, R=0.7959; R=0.7081 respectively and lower in subjects with MetS, R=0.6036; R=0.3138. These results showed strong correlations between CT and BIA estimations of VAT and that this was high (R=0.7959) in subjects without MetS and still relatively high in subjects with MetS (R=0.6036).

5.2.1.2. Assessment of anthropometry, per gender

Similar to CT-obtained VAT and SAT (cm^2), other measures of central obesity, including the WC (cm), a component of MetS, were similarly higher in females than in males, including the WC (cm), the hip (cm) and the BMI (kg/m^2). Confirmation of increased abdominal obesity in females as compared to males was shown by the high prevalence of obesity as classified by the BMI (kg/m^2) (WHO classification, 2004, updated 2016) which showed significantly higher obesity prevalence rates in females than in males and when overweight was combined with obesity the prevalence rates in females was extremely high (79.8%) with lower but still a high prevalence in males (57.0%). In this regard, overweight and obesity have been reported to relate to CVD mortality (Dudina et al. 2011), which strongly suggests that the high prevalence of obesity in the current study population is in need of intervention.

5.2.1.2.1. Assessment of anthropometry in MetS classification, per gender

Anthropometric measurements WC (cm), hip (cm), waist to hip ratio as well as weight (kg) and BMI (kg/m^2) were increased in both genders in subjects with MetS. This could be expected as the WC (cm) is one of the MetS components and as such included in MetS classification. The prevalence of obesity was increased in both male and female subjects with MetS, but highest in female subjects with MetS (77.3%). When overweight and obesity were combined the prevalence in females with MetS increased to 93.2%. These results confirmed reports on obesity (and possibly a sedentary lifestyle) as contributors to the high prevalence of MetS world-wide (Alberti et al., 2009; Wang, 2012).

5.2.1.2.1. Relationship between CT-measured SAT and VAT (cm^2) and anthropometry in MetS classification, per gender

The results showed that in general in the total group of participants, the anthropometric measurements showed stronger correlations with CT-measured SAT (cm^2) than with VAT (cm^2), except for the WC (cm) which showed a stronger correlation with VAT (cm^2) than with SAT (cm^2).

Per measuring tool, SAT (cm^2) showed the highest correlation strength with the BMI (kg/m^2) and least with the WC (cm), while VAT (cm^2) showed the strongest correlation strength with the WC (cm) and least with the hip (cm). Després (2006), similarly reported that VAT corresponded closer to the WC measurement and others have reported the wide use of this measurement as a rapid method to assess abdominal obesity (Atkinson & Uwaifo, 2005). In this regard, the IDF (2005) did recommend the WC as a screening tool for abdominal obesity in the diagnosis of MetS (Alberti et al., 2005) and results from the current study confirmed that of the anthropometric measurements, the WC (cm) showed the strongest correlation with CT-measured VAT (cm^2) in the mixed ancestry population as tested for in the current study population as well. The need to have a reliable, affordable substitute marker for CT-measured VAT as an abdominal measurement in MetS classification is highlighted by reports which have shown the association of elevated VAT with MetS, T2DM and CVD development (Matsuzawa et al., 2011; Unno et al., 2012).

Results from the current study have shown higher SAT (cm^2) than VAT (cm^2) values in both male and female subjects, also in MetS classification. In this regard, contributors to these increases in SAT (cm^2) as evaluated by correlation coefficients, may have been total weight as measured by the BMI (kg/m^2) (R=0.8956), weight accumulation on the hips (R=0.8936), with marginally lower accumulation of weight on the WC (cm) (R=7663) as well. The difference in correlation strengths may have been due to the general body shape of the population; that is either android (apple) with greater upper-body fat or gynoid (pear) shaped. The higher correlation of hip rather than WC with SAT (cm^2) suggested that the current study population may have been marginally more gynoid (pear) shaped. The android (apple) shape, with greater upper-body fat is associated with more metabolic complications and a greater risk for MetS as the gynoid (pear) shaped individual (Bjorntorp, 1985; Lamarche, 1998; Hansen et al., 2006; Després, 2012). However, even so the WC (cm) did show a rather strong correlation with SAT (cm^2) (R=0.7663), suggesting that the distribution of SAT (cm^2) was just marginally less in upper body fat than in lower body fat.

Similar results were shown in the MetS sub-categories, with both CT-measured SAT (cm^2) and VAT (cm^2) showing consistently stronger correlations (R) with the anthropometric measurements in subjects without MetS, than in those with MetS, which suggested that any or all of these measuring tools may over- and/or under-read in relation to the readings of the other methods, especially so in subjects with MetS. Previous studies have confirmed differences in fat measurements between CT-measured fat and anthropometric measurements (BMI levels) when it was associated with MetS (Berker et al, 2010).

5.2.2. Assessment of glucose impairment, per gender

Although FBG (mmol/L), as a component of MetS was similar in males and females, post 2HR BG (mmol/L), fasting insulin (mIU/L) and post 2HR insulin (mIU/L) measurements were all significantly increased in females as compared to males, showing changes in the levels of these parameters between the genders. However, similar to the levels of FBG (mmol/L), there was no significant difference in the prevalence of T2DM per se amongst the genders, in males (24.7%) and in females (24.2%). In this regard, T2DM classification has been done according to the WHO (2016) recommendations and as such included both glucose (mmol/L) and insulin (mIU/L) in the classification of T2DM. Increases in the glycaemic measurements in females, without a reflection of this in T2DM prevalence could possibly have indicated an increase in females with intermediate hyperglycaemia (IGT and/or IFG). In this regard, the prevalence of T2DM nearly doubled per gender when combined hyperglycaemia was evaluated; respectively in males to 38.7% and in females to 43.25% and although the difference between the genders was not significant, there was a trend of a higher hyperglycaemic prevalence in females. These results confirmed high prevalence rates for T2DM in the South African mixed ancestry population as reported by Erasmus et al. (2012) but similarly a high prevalence of intermediate hyperglycaemia.

5.2.2.1. Assessment of glucose impairment in MetS classification, per gender

FBG (mmol/L), as expected as one of the components of MetS was increased in both male and female subjects with MetS. Similarly, significant increases were found in post 2HR BG (mmol/L), HbA1c (%), fasting insulin (mIU/L) and post 2HR insulin (mIU/L) in both male and female subjects with MetS. Elevated plasma glucose, particularly in obese subjects, may be an indication of insulin resistance where more insulin is secreted to overcome hyperglycaemia (Gill et al., 2005). Insulin-resistant subjects also develop elevated plasma triglyceride levels and low HDL-C levels which are all components of the MetS profile (Sutton & Raines, 2007) and similarly results from the current study have shown increases in these measurements. Insulin resistance has been reported to be the primary mediator of MetS (Gill et al., 2005).

The prevalence of T2DM was only non-significantly increased in male subjects with MetS, but significantly in female subjects with MetS, suggesting that in females more so than in males, MetS may be associated through hyperglycaemic levels with T2DM onset. In this regard, the high prevalence of T2DM in both genders with MetS, but specifically in females (36.6%) increased substantially to 57.7% when evaluated in hyperglycaemic cases. In this regard, studies have shown that higher glucose (mmol/L) levels, even within the required reference range for normal, may pose up to a 40% higher risk of mortality than lower levels would (Bjornholt et al., 1999).

5.2.2.2. Relationship between CT-measured SAT and VAT (cm^2) and glucose impairment in MetS classification, per gender

In general, in subjects without MetS, FBG (mmol/L) showed stronger correlations with CT-measured VAT (cm^2) and insulin (mIU/L) stronger correlations with CT-measured SAT (cm^2). However, the glucose to insulin ratio showed a stronger inverse correlation with CT-measured SAT (cm^2) than with CT-measured VAT (cm^2), both in subjects without and (weaker) with MetS. The inverse correlations showed that increases in CT-measured SAT (cm^2) and to a lesser extend CT-measured VAT (cm^2) resulted in a higher increase in insulin (mIU/L) as compared to glucose (mmol/L) increases, both in subjects without and with MetS, but weaker in subjects with MetS. In addition, the glucose to insulin ratio was highly significantly decreased (median) in subjects with MetS, showing that insulin increased significantly more in relation to glucose in subjects with MetS. In this regard, both glucose (mmol/L) and insulin (mIU/L) were increased (median) in subjects with MetS and elevated plasma glucose levels, particularly in obese subjects, have been reported to be indicative of insulin resistance which would result in an increase of insulin secretion to overcome hyperglycaemia (Gill et al., 2005). Ineffectiveness of this hormone has been reported to lead to hyperglyceamia, insulin resistance and T2DM (Wang, 2012). Results from the current study therefore suggested that in subjects with MetS insulin was increased in relation to glucose and that this relationship was correlated with both VAT and SAT. Furthermore, elevated plasma glucose levels have been reported to be indicative of insulin resistance particularly in obese subjects (Gill et al., 2005) and results have shown that the percentage of obese subjects was increased in subjects with MetS in the current study. Furthermore, the weaker correlation strength in subjects with MetS suggested that the increases in glucose and insulin may have been non-linear and that contributors other than VAT and SAT were contributing to the changed profile of glucose and insulin in subjects with MetS.

5.2.3. Assessment of dyslipidaemia and hyper-triglyceridaemia, per gender

Dyslipidaemia includes low levels of HDL-chol and/or high levels of LDL-chol and triglycerides (hyper-triglyceridaemia) (Sutton & Raines, 2007; Kaur, 2014). Low levels of HDL-chol (mmol/L) is included as a component of MetS, with the cut-off value for females higher than for males, that is to be sufficient for female requirements HDL-chol should be higher in females as a similar sufficiency would be in males. Results from the current study showed significantly higher HDL-chol in females than in males, as possibly could be expected because of higher reference values for females than for males. However, there was not a significant difference in either the cholesterol (mmol/L) levels or the cholesterol to HDL-chol ratio between males and females, suggesting a similar balance between cholesterol and HDL-chol in the genders. HDL-chol is said to protect against atherosclerosis by removing cholesterol from the blood and thereby preventing build-up of

cholesterol in the blood vessels (Toth, 2005). There was also no difference between the genders in hyper-triglyceridaemia measurements, of which increased triglyceride (mmol/L) levels are also included as a component of MetS.

5.2.3.1. Assessment of dyslipidaemia as well as hyper-triglyceridaemia in MetS classification, per gender

HDL-chol (mmol/L), as expected as a component of MetS was highly significantly decreased in subjects with MetS, in both males and females. Cholesterol (mmol/L) was near-significantly increased in female subjects with MetS, but not in male subjects with MetS, but the cholesterol to HDL-chol ratio was highly significantly increased in both genders with MetS, that is, in both genders cholesterol (mmol/L) increased significantly in relation to HDL-chol (mmol/L). As expected, the triglycerides (mmol/L), similarly a component of MetS, were significantly increased in both male and female subjects with MetS. Additionally, although LDL-chol (mmol/L) is not included in the panel for MetS classification, dyslipidaemia is also characterised by increased levels of LDL-chol (Sutton & Raines, 2007; Kaur, 2014) and in the current study, LDL-chol was significantly increased in female subjects with MetS, but not in male subjects with MetS. The lipid profile of the subjects with MetS in the current study population corresponded therefore to the lipid profile of MetS subjects reported by Wierzbicki (2006) of decreased levels of HDL-chol (mmol/L), increased levels of LDL-chol (mmol/L) and increased levels of triglycerides (mmol/L), but that in the current study population these changes were more profound in females than in males.

The significant increase in the cholesterol to HDL-chol ratio in both genders with MetS suggested that the HDL-chol (mmol/L) levels may have been insufficient in relation to cholesterol (mmol/L) levels in subjects with MetS in the current study and that this may be associated with future risk in MetS subjects of atherosclerosis and associated diseases. In this regard, HDL-chol is said to protect against atherosclerosis by removing cholesterol from the blood and thereby preventing build-up of cholesterol in the blood vessels (Toth, 2005), with low levels associated as a risk factor for both DM and CVD (Wierzbicki, 2006).

5.2.3.2. Relationship between CT-measured SAT and VAT (cm^2) with dyslipidaemia and hyper-triglyceridaemia in MetS classification, per gender

None of the lipid measurements showed any correlation with either CT-measured SAT or VAT (cm^2) in subjects with MetS, except the triglycerides (mmol/L) showed a near-significant correlation with VAT (cm^2). In contrast, in subjects without MetS, CT-measured SAT (cm^2) showed significant positive correlation with LDL-chol (mmol/L) and the cholesterol to HDL-chol ratio, while VAT (cm^2) showed positive correlations with LDL-chol (mmol/L), cholesterol (mmol/L), the cholesterol to HDL-chol ratio and with the triglycerides (mmol/L) (strongest).

These results showed as expected significant correlations between the lipids and VAT and SAT (cm^2) measurements in subjects without MetS, which then suggested that the absence of significant correlations between CT-measured SAT and VAT (cm^2) with the lipids in subjects with MetS. of which the lipids were also increased (median) in subjects with MetS, suggested a non-linear increase in some or all of the lipid measurements, and that co-components and/or risk factors of MetS may have contributed to the absence of significant correlations of CT-measured fat and the blood lipid profile.

5.2.4. Assessment of the blood pressure, per gender

In the current study, the SBP (mmHg), similarly a component of MetS, was significantly higher in males than in females. There was no difference in the DBP (mmHg) between the genders. High blood pressure has been reported as a risk factor for initial stroke in non-hypertensive individuals as well as in those with hypertension (PROGRESS Collaborative Group, 2001), which suggested that male subjects from the current study showed a higher marker for this disease state than females did. Therefore, higher blood pressure values in males could be of significance because hypertension has also been shown to play an important role in the development of CVD (Kearney et al., 2005).

5.2.4.1. Assessment of the blood pressure in MetS classification, per gender

As expected, as components of MetS, the SBP (mmHg) and DBP (mmHg) were significantly increased in both male and female subjects with MetS. Hypertension has been reported to affect 85% of MetS subjects, although some authors report that the association of hypertension with MetS is still unclear, while in contrast others suggested that all MetS diagnostic criteria would contribute to hypertension (Franklin et al., 2006). In this regard, increased glucose and insulin levels have been reported associated with the development of hypertension (Reaven, 2003; Duvnjak et al., 2008).

5.2.4.2. Relationship between CT-measured SAT and VAT (cm^2) and the blood pressure in MetS classification, per gender

The correlation coefficients of both CT-measured SAT and VAT (cm^2) with the SBP and DBP (mmHg) suggested co-contributors to these correlation coefficients (results were unexpected). Regression analysis has therefore been used to evaluate blood pressure against all MetS components, adjusted for age and gender (Table 4.4.B) specifically because reports have shown that these components may all contribute to the hypertension profile in subjects with MetS (Franklin et al., 2006; Mulè et al., 2014). Results from regression analysis showed that the SBP (mmHg) was significantly positively associated with the triglycerides (mmol/L), while the DBP

(mmHg) was significantly positively associated with CT-measured SAT and VAT (cm^2). The positive association of the SBP (mmHg) with the triglycerides (mmol/L) confirmed the MetS profile in which insulin resistance has been associated with increases in plasma glucose and triglyceride levels (Sutton & Raines, 2007) contributing to the development of essential hypertension (Reaven, 2003; Duvnjak et al., 2008). These results furthermore suggested that increases in the SBP (mmHg) may be associated with high levels of fat (triglycerides) in the blood, while increases in the DBP (mmHg) appeared to be closely associated with obesity measurements, CT-measured SAT and VAT (cm^2).

5.2.5. Assessment of additional risk factors for MetS

5.2.5.1. Assessment of C-reactive protein (CRP) (mg/L) as an inflammatory marker, per gender

C-reactive protein (CRP) (mg/L) is not a component of MetS per se, but has been shown associated with obesity measurements. Furthermore, a pro-inflammatory state was one of the criteria suggested by the IDF in 2004 as a feature that should be included to confirm the presence of MetS (Alberti et al., 2006). Wang (2012) reported inflammation to be correlated with VAT, but not with SAT, while Neeland et al. (2013) reported the association of inflammatory biomarkers similar with VAT and SAT. There was no significant difference in the CRP (mg/L) between males and females in the current study, but it was significantly increased in female subjects with MetS, however not in male subjects with MetS. It is possible that the higher VAT and SAT (cm^2) in females with MetS could have contributed to the increase in the CRP (mg/L) in female subjects with MetS. The CRP is widely used as a marker of both infection and inflammation and commonly used because of its availability in routine healthcare laboratories. A disadvantage of using serum inflammatory markers is that they however don't always correlate with short-term disease progression (Giovannoni et al. 2001). In this regard, inflammation in subjects, especially in females with MetS may be chronic because of the higher prevalence of obesity in females reported in this study. Therefore, inflammation as such needs to be evaluated long-term in subjects with MetS and the effects thereof on the progression of the disease.

5.2.5.1.1. Relationship between CT-measured SAT and VAT (cm^2) and C-reactive protein (CRP) (mg/L)

The CRP (mg/L) showed significant and/or near-significant correlations with SAT and VAT (cm^2) in both subjects with and without MetS, but stronger in subjects with MetS. Of these the strongest correlation was with SAT (cm^2) in subjects with MetS. These results differed from those of Wang (2012) in that in the current study the CRP (mg/L) showed a stronger correlation with SAT, while Wang (2012) reported inflammation correlated with VAT (cm^2) only. Similarly, results from the current study differed from those of Neeland et al. (2013) who reported similar associations of

inflammatory biomarkers with VAT and SAT, while in the current study the CRP (mg/L) showed a stronger correlation with SAT than with VAT. However, both the increase in the CRP (mg/L) in female subjects with MetS, as well as the positive correlation with SAT (cm^2) and to a lesser extend with VAT (cm^2) suggested that inflammation may play a role in MetS, specifically in female subjects and that this was correlated with increases in SAT (cm^2) more so than VAT (cm^2), which indicated subcutaneous fat storage to contribute more to the inflammatory state in MetS subjects.

5.2.5.2. Assessment and relationship of Gamma GT-S (GGT) (IU/L) and cotinine (ng/mL) in MetS, per gender

Gamma GT-S (IU/L) and cotinine (ng/mL) have been included as possible risk factors for MetS, respectively as markers for alcohol consumption and smoking. Gamma GT-S (IU/L) was significantly increased in males as compared to females, which could possibly be explained by higher normal reference values for males than for females (< 55 IU/L and < 38 IU/L respectively, PathCare Reference Laboratory). However, Gamma GT-S (IU/L) was increased in subjects with MetS, in both males and females, suggesting a linked relationship with MetS. Correlation studies showed significant positive correlations between Gamma GT-S (IU/L) and CT-measured VAT (cm^2) in subjects without and with MetS, suggesting a contributing role of Gamma GT-S (IU/L) to increased values in CT-measured VAT (cm^2) in the study population.

There was no significant difference (only near-significant) in cotinine (ng/mL) between the genders and also in the MetS sub-categories, suggesting no relationship between smoking and MetS. Cotinine (ng/mL) however, showed significant inverse correlations with both CT-measured SAT and VAT (cm^2) in subjects without and with Mets, but stronger in subjects without MetS, which suggested that increases in cotinine (ng/mL) levels may be correlated with a decrease in both CT-measured SAT and VAT (cm^2) but that this was not affected by MetS status. These findings are important because they highlight the opposite effects of smoking and drinking on the central obesity profile of the mixed ancestry population.

5.3. Method agreement testing

Central obesity and more specifically VAT is said to be central to the pathogenesis of MetS and furthermore a risk factor of T2DM and CVD (Matsuzawa et al., 2011; Unno et al., 2012). As a substitute marker, the anthropometrically measured WC has been reported to be stronger associated with VAT measurements, rather than those of SAT (Després, 2006). A disadvantage of using anthropometric measurements is that it only measures VAT indirectly (Shuster et al., 2012) and the reference values of these measurements for MetS diagnosis, including that of the WC needs to be optimised per ethnic group (Alberti et al., 2009). In this regard, the International Diabetic Federation (IDF) reported that central obesity should be assessed with the use of

Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) (IDF, 2006) and validation of the WC as a substitute marker for central obesity in MetS diagnosis in the current study population needed to be validated against CT-measured VAT therefore.

Recently, the use of Bioelectrical Impedance Analysis (BIA) (scale) has become acceptable for the estimation of VAT (levels), but similar to the WC measurement, needs validation per ethnic group (Nagai et al*.,* 2010). VAT (levels) measurements using BIA (the Omron in the current study) needed therefore, similarly to the WC (cm) to be validated against CT-measured VAT (cm^2).

5.3.1. Method agreement testing, modalities used in the current study

5.3.1.1. The inherent qualities of CT imaging and anthropometric measurements

Of the imaging techniques suggested for WC (cm) verification in MetS diagnosis, CT is one of the preferred methods because of its effective evaluation, separation and quantification of various tissue types, including adipose tissue and its ability to quantify abdominal adiposity and furthermore to distinguish between and quantify VAT and SAT (Wajchenberg, 2000; Irlbeck et al., 2010; Shuster et al., 2012; Mazonakis & Damilakis, 2016) and which is of great value in clinical studies (Mazonakis & Damilakis, 2016). CT measurement has been reported superior to other measures, including surrogate markers BMI (kg/m^2) and WC (cm) to identify central obesity, with CT furthermore able to exhibit differences by gender (Brandberg, 2009; Berker et al., 2010).

However, the use of CT imaging is not always a preferential method due to radiation exposure which remains a concern (Shuster et al., 2012; Mazonakis & Damilakis, 2016). CT measurement of VAT and SAT is furthermore a time-consuming process (Roos et al. 2002). There is therefore a need to use a verified substitute measurement for central obesity, especially in the diagnosis of MetS. In the current study, the aim was to evaluate and validate the 90 cm WC cut-off value reported by Matsha et al. (2013) against CT-measured VAT (cm^2), to be used in the South African research and clinical settings. In this regard, the measurement of the WC is an inexpensive, fast and non-invasive technique, easily obtained in both clinical and research settings. Verification of its use should therefore, in addition to other factors, weigh this aspect of the measurement when contemplating especially research studies where less invasive methods of measuring central obesity may be preferred.

5.3.1.2. Bioelectrical impedance analysis (BIA) (scale)

The BIA method of VAT estimation is similar to the WC measurement simple to use but, also similar to the WC only applicable to the population it has been validated in (Kyle et al., 2004; Nagai et al*.,* 2010). The advantages of using an instrument capable of doing BIA is that it is non-invasive, relatively inexpensive, does not use ionising radiation and can be used with healthy and

chronically diseased individuals. The use of BIA as a screening tool has been validated in VAT assessment for MetS diagnosis in different ethnicities (Ozhan et al. (2012).

5.3.2. The association strength of VAT and SAT with the markers of MetS

5.3.2.1. CT-measured VAT and SAT (cm^2) and BIA-measured VAT (levels) (Table 4.5)

To determine the association of VAT (and SAT) with the diagnostic markers of MetS in the current study population, anthropometric measurements a well as markers for MetS risk factors and adjusted for age and gender have been put in a regression model. Results from regression analysis showed that it was primarily the anthropometric measurements that showed the closest associations with both VAT and SAT, followed to a lesser extend by measures of hyperglycaemia and none with the other diagnostic criteria of MetS, suggesting that obesity and to a lesser extend, hyperglycaemia may be key to the development of MetS.

5.3.3.1.1. Obesity measurements

Regression analysis showed that when adjusted for age and gender, the strongest association of the WC (cm) was with CT-measured VAT (cm^2) (b*0.4174), followed by CT-measured SAT (cm^2) (b*0.1441) and none with the BIA-measured VAT (levels). These results confirmed reports on the closer association of the WC (cm) with CT-measured VAT (cm^2) rather than with CT measured SAT (cm^2) (Després, 2006). However, there was a significant, although weaker association between the WC (cm) and CT-measured SAT (cm^2) (b*0.1441), which suggested that the WC (cm) measurements may have included SAT (cm^2) measurements to a certain extend as well.

Results also showed that when adjusted for age and gender, the strongest association of the hip (cm) was with CT-measured SAT (cm^2) (b*0.3568), inversely with CT-measured VAT (cm^2) (b*-0.2006) and none with the BIA-measured VAT (levels). The positive association of the hip (cm) with CT-measured SAT (cm^2) (b*0.3568) and in contrast an inverse association with CT-measured VAT (cm^2) (b*-0.2006) suggested that with an increase in the hip circumference, fat distribution changes, that is fat may be stored more subcutaneously and in relation less in visceral form, which would be of benefit to the subject as it is VAT that is said to be central to the pathogenesis of MetS (Matsuzawa et al., 2011; Unno et al., 2012).

The highly significant strong association of the BMI (kg/m^2) with BIA-measured VAT (levels) (b*0.7253) compared to CT-measured VAT (cm^2) (b*0.3934) suggested strongly that the BIA measurement may include fat other than VAT in its VAT measurements, hence the strong association with the BMI of which the calculation includes weight (total fat included) and height ($weight/height^2$: WHO, 2004, updated 2016). It is therefore possible that the BIA may be less reliable in obese subjects with increased BMI levels.

5.3.3.1.2. Glycaemic measurements

Regression analysis further showed that the glycaemic profile of the study subjects, when adjusted for age and gender was associated with CT-measured VAT (cm^2) rather than with SAT (cm^2) and in this regard, VAT has been reported associated with MetS as well as a risk factor for T2DM (Matsuzawa et al., 2011; Unno et al., 2012). The absence of any association between the glycaemic profile and BIA-measured VAT as compared to that of CT-measured VAT (cm^2) suggested that the BIA-measured VAT (levels) may not be optimal as compared to that of CT-measured VAT (cm^2). However, Després (2012) reported that CT scanning of the abdomen of obese individuals with excess VAT showed a positive association with some of the diagnostic criteria of MetS, but that the association with these risk factors in obese individuals with less VAT was absent (Després, 2012).

5.4. Establishing percentage method agreement between the WC (cm) and the BIA-measured VAT (levels) against that of CT-measured VAT (cm^2)

To test for percentage agreement between the measuring modalities, the distribution as well as measures of central tendencies and variance has been described, followed by a discussion of the percent agreement between the measuring modalities.

5.4.1. Distribution testing

The degree of agreement between two measuring units can be affected by a skewed distribution in one or both types of measurements. Measures of skewness, including the
Shapiro-Wilk W Test (Results; Table 4.6) showed that the distributions of the three primary tests for central obesity used in the current study CT-measured VAT (cm^2), the WC (cm) as well as BIA-measured VAT (levels) were not normally distributed, which suggested a compromised degree of method agreement between test group measurements.

5.4.2. Measures of central tendencies and variance

In addition to the skewed distributions shown in the central obesity measurements, evaluation of CT-measured VAT (cm^2), the WC (cm) and BIA-measured VAT (levels) also differ in their measuring units and as such comparing the mean (SD) or median (range) of the measurements of the three tests per se may not be a viable comparison method. However, Figure 4.11 demonstrates a summary of the different central tendency and range profiles of the three measuring modalities, CT-measured VAT (cm^2), the WC (cm) and the BIA-measured VAT (levels). Evaluation of the measures of central tendencies and ranges demonstrated the difference between the three measuring modalities in their distribution profiles. CT-measured VAT (cm^2) and the WC (cm) showed similar distribution profiles between the lower and upper quartiles, but there are however substantial differences between the minimum readings and the lower quartiles, as

well as between the upper quartiles and the maximum readings, where the WC (cm) over-reads and under-reads respectively. These differences in the distribution profiles suggested that method agreement between CT-measured VAT (cm^2) and the WC (cm) may be compromised at both lower and higher readings. It is possible that the WC measurement by tape measure may become less accurate in obese subjects, that is, the shape of the individual may make optimal measurements more difficult. However, similarly in this regard, Després (2012) reported that CT-measured VAT of obese individuals with excess VAT showed association with MetS diagnostic criteria, but that in obese individuals with less VAT the association disappears (Després, 2012). The distribution profile of BIA-measured VAT (levels) compared better with the WC (cm) distribution profile than with that of the CT-measured VAT (cm^2).

5.4.3. Regression equation to tests for percentage method agreement

5.4.3.1. CT-measured VAT (cm^2) and the WC (cm)

The regression (fit) equation for the total number of participants was $y = 79.1217 + 0.1854*x$; $R = 0.7519$; $P < 0.0001$; $R^2 = 0.5654$ (Results; Table 4.7). Therefore, method agreement between CT-measured VAT (cm^2) and the WC (cm) on abdominal obesity assessment is 56.54% in the total group. Sub-categorization of MetS showed a 58.49% in subjects without MetS (in males: 80.77%; in females: 48.64%) and a lower agreement between the two methods at 37.23% in subjects with MetS (in males: 45.50%; in females: 35.73%). These results clearly showed that the two methods agreed substantially better in subjects without MetS, than in those with MetS and that method agreement was higher in males than in females, both in subjects without and with MetS.

The highest level of agreement was in male subjects without MetS (80.77%) and the lowest level of agreement in female subjects with MetS (35.73%). It is possible that the highly significant increases in both CT-measured VAT and SAT (cm^2) in the current study population, especially in females with MetS could have contributed to the lower level of method agreement in subjects with MetS. The high obesity prevalence in female subjects with MetS (77.3% and when combined with overweight 93.2%) could possibly have affected the accuracy of the anthropometrically measured WC (cm) of obese subjects. In this regard, the specific level of WC measurement per se may be less well defined in obese subjects where the body shape may interfere with accurate location of the WC per se.

Although the WC as a measure of central tendency is a rapid method to be used in clinical practice (Atkinson & Uwaifo, 2005), this measurement has repeatedly been reported as an estimate of central obesity only and that the accuracy of this measurement may be compromised to a degree (Misra & Vikram, 2003; Unno et al., 2012). Acceptance of the level of agreement between CT-measured VAT (cm^2) and the WC (cm) as sufficient to validate the WC (cm) for use in

research and/or clinical settings in MetS diagnosis would depend on the level of accuracy required for individual need.

5.4.3.2. CT-measured VAT (cm^2) and BIA-measured VAT (levels)

The regression (fit) equation for the total number of participants was y = 5.5774 + 0.0511*x; R = 0.7009; P < 0.0001; R^2 = 0.4912 (Results; Table 4.7). Therefore, method agreement between CT-measured VAT (cm^2) and the BIA-measured VAT (levels) on abdominal obesity assessment is 49.12% in the total group, which is lower than that of CT-measured VAT (cm^2) and the WC (cm) (56.54%). Sub-categorization of MetS showed 60.74% agreement in subjects without MetS (in males: 64.44%; in females: 62.75%) and lower at 26.74% in subjects with MetS (in males: 18.97%; in females: 36.94%). These results clearly showed that the two methods agreed substantially better in subjects without MetS (equally well in males and females), than in those with MetS, where the agreement in males was very low (18.97%). In general, the BIA-measured VAT (levels) compared less well than the WC (cm) against CT-measured VAT (cm^2) as markers for obesity, and especially so in the subjects with MetS.

5.4.2. Method validation; ROC curve analysis

Regression analysis has shown a higher level of agreement between CT-measured VAT (cm^2) and the WC (cm) on abdominal obesity assessment, 56.54% in the total group of participants with a lower percentage agreement between CT-measured VAT (cm^2) and the BIA-measured VAT (levels), 49.12% in the total group, but with better agreement in subjects without MetS than in those with MetS. Results have also shown that of the anthropometric measurements, the WC (cm) has shown the strongest association with CT-measured VAT (cm^2), similar as results reported from other studies.

However, when cut-off values using ROC curve analysis where used to determine the ability of these three measuring modalities to predict MetS, results showed that BIA-measured VAT (levels) compared well of those of CT-measured VAT (cm^2), but with equilibrium between % sensitivity and % specificity more even in CT-measured VAT (cm^2). The diagnostic performance of CT-measured VAT (cm^2) as tested for by ROC curve analysis was acceptable, showing a cut-off value of >88.40 (cm^2) for MetS prediction in the total group of participants with sensitivity, specificity and area AUC values 72.2%, 70.8% and 0.81 respectively. Results from BIA-measured VAT (levels), showed a cut-off value of >10.50 (levels) for MetS prediction in the total group of participants with sensitivity, specificity and area under the curve (AUC) values 66.2 %, 71.1 % and 0.79 respectively. These results compared well with those of CT-measured VAT (levels), but with equilibrium between % sensitivity and % specificity more even in CT-measured VAT (cm^2).

However, the % sensitivity and % specificity of the cut-off value of the WC (previously derived cut-off of 90 cm) showed a very high % sensitivity as compared to the other two measuring modalities, indicating a higher level of the correct diagnosis of MetS positive cases, but with the % specificity lower in the total group as compared to that of the other two measuring modalities. The need to identify subjects at risk for MetS has been reported and similarly results from the current study has shown that both overweight and obesity were equally associated with DM prevalence (Table 4.3.B), highlighting the need to have a WC (cm) cut-off value to identify the maximum number of MetS positive cases, suggesting that the current cut-off value of 90 (cm) be accepted as a screening tool for MetS identification in the predominantly overweight/obese mixed ancestry population.

The cut-off value of CT-measured VAT (cm^2) was >88.40 (cm^2) for MetS prediction in the total group of participants which differed from published data and suggested that similar to the report from Hayashi et al. (2007) with regards to establishing cut-off values for VAT in MetS diagnosis in Japanese Americans, results from the current study showed that CT-measured VAT cut-off values for MetS diagnosis needed to be revised for the South African mixed ancestry population as well.

Evaluation of these results suggested that both the WC (cm) cut-off value of 90 cm as well as the derived cut-off value of BIA-measured VAT (levels) of >10.50 (levels) can be accepted as sufficiently comparable to that of the CT-measured VAT (cm^2) and can be suggested to be used as substitute markers for abdominal obesity in MetS diagnosis and that the derived cut-off value of >88.40 (cm^2) for CT-measured VAT (cm^2) can be used in the mixed ancestry population for MetS diagnosis.

5.5. The predictive value of obesity in MetS and T2DM diagnosis

Results from the current study (Table 4.3.B) showed that MetS prevalence increased highly significantly through normal weight to obese subjects, with very high MetS prevalence rates in the obese subjects (in obese males: 84.6%; in obese females 75.1%). These results clearly showed the association of central obesity in MetS prevalence and that the degree of obesity contributes to a higher MetS prevalence rate in both males and females. While MetS prevalence was highest in obese subjects, the results showed that T2DM prevalence was similarly increased in overweight and obese subjects in both genders; T2DM prevalence in normal weight, overweight and obese males were respectively 12.5%, 37.0% and 30.8% (P=0.0346) and in females were 8.2%, 27.0% and 28.7% (P=0.0302). These results showed that T2DM prevalence was similarly high in overweight and obese subjects in both males and females, suggesting that overweight subjects in the current study population maybe at a similar risk for T2DM onset than obese subjects were. These results strongly suggest the need for intervention and in this regard reports have shown that

weight loss in overweight subjects with or at risk for T2DM has been reported to lower the risk for T2DM (Wilding, 2014).

As elsewhere, plausible explanations for the high prevalence of obesity in the mixed ancestry population may be multiple. Obesity has been reported to be a consequence of overeating and a sedentary lifestyle which causes a chronic imbalance between energy intake and energy expenditure (Misra & Khurana, 2008; Popkin et al. 2012). Furthermore, dietary changes from natural foods to high intake of processed food have been proposed associated with obesity as well (Popkin et al. 2012).

CHAPTER SIX
CONCLUSION

The study results showed a high prevalence of MetS as classified by JIS criteria (Alberti et al., 2006) in the South African mixed ancestry population and the prevalence was significantly higher in females than in males. The increase in MetS prevalence may be in part due to a high overweight and obesity prevalence in the study population with the components of MetS similarly increased in females as compared to males. Results have also confirmed reports on the role of VAT in the MetS profile with increased values shown in subjects with MetS and also in its strong association with MetS components and risk factors, including measures of abdominal obesity, hyperglycaemia, hyperglyceridaemia, low HDL-cholesterol (dyslipidaemia) and hypertension. Similar to reports from other groups (Després, 2006) results from the current study have highlighted that of the anthropometric measurements, the WC showed the closest association with CT-measured VAT which is said to be the gold standard for abdominal obesity assessment. VAT has been reported as a major risk factor for MetS as well as a for T2DM and CVD development (Matsuzawa et al., 2011; Unno et al., 2012).

The diagnostic performance of CT-measured VAT (cm^2) as tested for by ROC curve analysis was acceptable, showing a cut-off value of >88.40 (cm^2) for MetS prediction in the total group of participants, with sensitivity, specificity and area AUC values 72.2%, 70.8% and 0.81 respectively. This cut-off value differed from published data and suggested that similar to the report from Hayashi et al. (2007) with regards to establishing cut-off values for VAT in MetS diagnosis in Japanese Americans, results from the current study showed that CT-measured VAT cut-off values for MetS diagnosis needed to be revised for the South African mixed ancestry population as well. Results from BIA-measured VAT (levels), showed a cut-off value of >10.50 (levels) with sensitivity, specificity and area under the curve (AUC) values 66.2 %, 71.1 % and 0.79 respectively, which compared well with those of CT-measured VAT (cm^2). The % sensitivity and % specificity of the cut-off value of the WC (previously derived cut-off of 90 cm) showed a very high % sensitivity as compared to the other two measuring modalities, indicating a higher level of the correct diagnosis of MetS positive cases, but with the % specificity lower in the total group as compared to that of the other two measuring modalities. Evaluation of both the WC (cm) cut-off value of 90 cm as well as the derived cut-off value of BIA-measured VAT (levels) of >10.50 (levels) suggested that these measuring modalities performed sufficiently well against the CT-measured VAT (cm^2) cut-off value in MetS diagnosis to be validated as substitute markers for abdominal obesity in MetS diagnosis. This is an important outcome as CT imaging contains a risk of radiation exposure and is also expensive, especially for use in research studies in the South African milieu. The high prevalence

of overweight and obese subjects in the current study population highlight the importance of access to simple and reliable methods for measuring body fat, which may be a challenge in specifically overweight and obese populations (Bredella et al. 2013).

STRENGHTS

The principle strength of the study was that CT scanning was done at a reputable radiology practice with reliable CT equipment. Furthermore, that the current study formed part of a greater research study, which were managed by experienced scientists in the field. All biochemical analyses were done at an accredited reference laboratory and field and site work was done by trained staff members.

LIMITATIONS

One of the limitations of the study was the difference in numbers in gender, with more females than males who participated in the study.

RECOMMENDATIONS

Results from the current study have shown association between both VAT and SAT with the components of MetS. Of the abdominal fat, VAT is said to be the metabolically active region of the abdomen with endocrine functions (Freedland, 2004; Kershaw & Flier, 2004; Després, 2006) and has been reported as central to the pathogenesis of MetS and a risk factor of T2DM and CVD (Matsuzawa et al., 2011; Unno et al., 2012). VAT is said to have a pathogenic role and therefore future studies need to investigate this aspect in research into obesity prevalence, which is a necessity in view of the associated role obesity plays in MetS, T2DM and CVD. It is possible that changes in the VAT and SAT volume as well as distribution could contribute to an imbalance in their endocrine functions and could therefore possibly contribute to the disease state of the participants.

REFERENCES

Alberti, K.G.M.M. and Zimmet, P.F., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic medicine*, *15*(7), pp.539-553.

Alberti, K.G.M.M., Zimmet, P. and Shaw, J., 2005. The metabolic syndrome—a new worldwide definition. *The Lancet*, *366*(9491), pp.1059-1062.

Alberti, K.G.M.M., Zimmet, P. and Shaw, J., 2006. Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. *Diabetic medicine*, *23*(5), pp.469-480.

Alberti, K.G.M.M., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., Fruchart, J.C., James, W.P.T., Loria, C.M. and Smith, S.C., 2009. Harmonizing the metabolic syndrome. *Circulation*, *120*(16), pp.1640-1645.

Al-Kadi, H. and Alissa, E., 2011. Prevalence of hyperlipidemia and associated risk factors among healthy young Saudi females: relationship with waist circumference and body mass index. *Endocrinol Metabol Syndrome*, p.2.

Atkinson, R.L. and Uwaifo, G., 2005. Combination Therapies for Obesity. *The Management of Eating Disorders and Obesity*, pp.277-291.

Ayvaz, G. and Rıza Çimen, A., 2011. Methods for body composition analysis in adults. *The Open Obesity Journal*, *3*(1), pp.62-69.

Balkau, B., Bertrais, S., Ducimetiere, P. and Eschwege, E., 1999. Is there a glycemic threshold for mortality risk?. *Diabetes care*, *22*(5), pp.696-699.

Berker, D., Koparal, S., Isik, S., Pasaoglu, L., Aydin, Y., Erol, K., Delibasi, T. and Güler, S., 2010. Compatibility of different methods for the measurement of visceral fat in different body mass index strata. *Diagnostic and interventional radiology*, *16*(2), p.99.

Bjørnholt, J.V., Erikssen, G., Aaser, E., Sandvik, L., Nitter-Hauge, S.I.G.U.R.D., Jervell, J., Erikssen, J. and Thaulow, E., 1999. Fasting blood glucose: an underestimated risk factor for cardiovascular death. Results from a 22-year follow-up of healthy nondiabetic men. *Diabetes care*, *22*(1), pp.45-49.

Bjorntorp, P., 1985. Regional patterns of fat distribution. *Ann Intern Med*, *103*(6), pp.994-995.

Borch-Johnsen, K. and Wareham, N., 2010. The rise and fall of the metabolic syndrome. *Diabetologia*, *53*(4), pp.597-599.

Borkan, G.A., Gerzof, S.G., Robbins, A.H., Hults, D.E., Silbert, C.K. and Silbert, J.E., 1982. Assessment of abdominal fat content by computed tomography. *The American journal of clinical nutrition*, *36*(1), pp.172-177.

Bradshaw, D., Groenewald, P., Laubscher, R., Nannan, N., Nojilana, B., Norman, R., Pieterse, D., Schneider, M., Bourne, D.E., Timæus, I.M. and Dorrington, R., 2003. Initial burden of disease estimates for South Africa, 2000. *South African Medical Journal*, *93*(9), pp.682-688.

Bradshaw, D., Pieterse, D., Norman, R. and Levitt, N.S., 2007. Estimating the burden of disease attributable to diabetes in South Africa in 2000. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, *12*(2), pp.65-71.

Brandberg, J., Lonn, L., Bergelin, E., Sjostrom, L., Forssell-Aronsson, E. and Starck, G., 2008. Accurate tissue area measurements with considerably reduced radiation dose achieved by patient-specific CT scan parameters. *The British journal of radiology*, *81*(970), pp.801-808.

Brandberg, J., 2009. *Computed Tomography and Magnetic Resonance Imaging in Determination of Human Body Composition. Methodological and Applied Studies*. Institute of Clincial Sciences. Department of Radiology, pp.1-60.

Bredella, M.A., Gill, C.M., Keating, L.K., Torriani, M., Anderson, E.J., Punyanitya, M., Wilson, K.E., Kelly, T.L. and Miller, K.K., 2013. Assessment of abdominal fat compartments using DXA in premenopausal women from anorexia nervosa to morbid obesity. *Obesity*, *21*(12), pp.2458-2464.

Bushong, S.C., 2008. Radiologic Science for Technologists, Physics, Biology & Practices.

Byrne, C.D. and Wild, S.H. eds., 2011. *The metabolic syndrome*. John Wiley & Sons, pp1-17.

Cleeman, J.I., 2001. MD. Executive Summary of the Third Report of the National Cholesterol Education (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*, *285*(19), p246.

Coppini, L.Z., Waitzberg, D.L. and Campos, A.C.L., 2005. Limitations and validation of bioelectrical impedance analysis in morbidly obese patients. *Current Opinion in Clinical Nutrition & Metabolic Care*, *8*(3), pp.329-332.

Crowther, N.J. and Ferris, W.F., 2010. The impact of insulin resistance, gender, genes, glucocorticoids and ethnicity on body fat distribution. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, *15*(3), pp.115-120.

Crowther, N.J. and Norris, S.A., 2012. The current waist circumference cut point used for the diagnosis of metabolic syndrome in sub-Saharan African women is not appropriate. *PLoS One*, *7*(11), p.e48883.

Dehghan, M. and Merchant, A.T., 2008. Is bioelectrical impedance accurate for use in large epidemiological studies? *Nutrition journal*, *7*(1), p.26.

Després, J.P., 2006. Is visceral obesity the cause of the metabolic syndrome? *Annals of medicine*, *38*(1), pp.52-63.

Després, J.P. and Lemieux, I., 2006. Abdominal obesity and metabolic syndrome. *Nature*, *444*(7121), pp.881-887.

Després, J.P., Lemieux, I., Bergeron, J., Pibarot, P., Mathieu, P., Larose, E., Rodés-Cabau, J., Bertrand, O.F. and Poirier, P., 2008. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arteriosclerosis, thrombosis, and vascular biology*, *28*(6), pp.1039-1049.

Després, J.P., 2012. Body fat distribution and risk of cardiovascular disease. *Circulation*, *126*(10), pp.1301-1313.

Dragsbæk, K., Neergaard, J.S., Laursen, J.M., Hansen, H.B., Christiansen, C., Beck-Nielsen, H., Karsdal, M.A., Brix, S. and Henriksen, K., 2016. Metabolic syndrome and subsequent risk of type 2 diabetes and cardiovascular disease in elderly women: Challenging the current definition. *Medicine*, *95*(36), pp1-8.

Dudina, A., Cooney, M.T., Bacquer, D.D., Backer, G.D., Ducimetière, P., Jousilahti, P., Keil, U., Menotti, A., Njølstad, I., Oganov, R. and Sans, S., 2011. Relationships between body mass index, cardiovascular mortality, and risk factors: a report from the SCORE investigators. *European Journal of Cardiovascular Prevention & Rehabilitation*, *18*(5), pp.731-742.

Duvnjak, L., Bulum, T. and Metelko, Z., 2008. Hypertension and the metabolic syndrome. *Diabetologia Croatica*, *37*(4), pp.83-89.

Erasmus, R.T., Soita, D.J., Hassan, M.S., Blanco-Blanco, E., Vergotine, Z., Kengne, A.P. and Matsha, T.E., 2012. High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: Baseline data of a study in Bellville, Cape Town. *SAMJ: South African Medical Journal*, *102*(11), pp.841-844.

Ervin, R.B., 2009. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States. *National health statistics reports*, *13*, pp.1-8.

Esteghamati, A., Khalilzadeh, O., Anvari, M., Ahadi, M.S., Abbasi, M. and Rashidi, A., 2008. Metabolic syndrome and insulin resistance significantly correlate with body mass index. *Archives of medical research*, *39*(8), pp.803-808.

Ford, E.S., Giles, W.H. and Mokdad, A.H., 2004. Increasing prevalence of the metabolic syndrome among US adults. *Diabetes care*, *27*(10), pp.2444-2449.

Franklin, S.S., Barboza, M.G., Pio, J.R. and Wong, N.D., 2006. Blood pressure categories, hypertensive subtypes, and the metabolic syndrome. *Journal of hypertension*, *24*(10), pp.2009-2016.

Freedland, E.S., 2004. Role of a critical visceral adipose tissue threshold (CVATT) in metabolic syndrome: implications for controlling dietary carbohydrates: a review. *Nutrition & metabolism*, *1*(12), p.1-24.

Galassi, A., Reynolds, K. and He, J., 2006. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. *The American journal of medicine*, *119*(10), pp.812-819.

Gill, H., Mugo, M., Whaley-Connell, A., Stump, C. and Sowers, J.R., 2005. The key role of insulin resistance in the cardiometabolic syndrome. *The American journal of the medical sciences*, *330*(6), pp.290-294.

Giovannoni, G., Miller, D.H., Losseff, N.A., Sailer, M., Lewellyn-Smith, N., Thompson, A.J. and Thompson, E.J., 2001. Serum inflammatory markers and clinical/MRI markers of disease progression in multiple sclerosis. *J Neurol*, 248, pp. 487-495.

Goedecke, J.H., 2005. Ethnic differences in obesity, Continuing medical education, 23(11), p.549.

Goodpaster, B.H., Krishnaswami, S., Harris, T.B., Katsiaras, A., Kritchevsky, S.B., Simonsick, E.M., Nevitt, M., Holvoet, P. and Newman, A.B., 2005. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. *Archives of internal medicine*, *165*(7), pp.777-783.

Grundy, S.M., Brewer, H.B., Cleeman, J.I., Smith, S.C. and Lenfant, C., 2004. Definition of metabolic syndrome. *Circulation*, *109*(3), pp.433-438.

Grundy, S.M., Cleeman, J.I., Daniels, S.R., Donato, K.A., Eckel, R.H., Franklin, B.A., Gordon, D.J., Krauss, R.M., Savage, P.J., Smith, S.C. and Spertus, J.A., 2005. Diagnosis and management of the metabolic syndrome. *Circulation*, *112*(17), pp.2735-2752.

Grundy, S.M., 2008. Metabolic syndrome pandemic. *Arteriosclerosis, thrombosis, and vascular biology*, *28*(4), pp.629-636.

Handelsman, Y., 2009. Metabolic syndrome pathophysiology and clinical presentation. *Toxicologic pathology*, *37*(1), pp.18-20.

Hansen, E., Hajri, T. and Abumrad, N.N., 2006. Is all fat the same? The role of fat in the pathogenesis of the metabolic syndrome and type 2 diabetes mellitus. *Surgery*, *139*(6), p.711.

Hayashi, T., Boyko, E.J., McNeely, M.J., Leonetti, D.L., Kahn, S.E. and Fujimoto, W.Y., 2007. Minimum waist and visceral fat values for identifying Japanese Americans at risk for the metabolic syndrome. *Diabetes Care*, *30*(1), pp.120-127.

Hoebel, S., Malan, L. and De Ridder, H., 2011. Differences in MetS marker prevalence between black African and Caucasian teachers from the North West Province: Sympathetic activity and ambulatory blood pressure in Africans (SABPA) Study. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, *16*(1), pp.49-56.

Hu, H.H., Nayak, K.S., Goran, M.I., 2011. Assessment of abdominal adipose tissue and organ fat content by magnetic resonance imaging. *Obesity Reviews, 12*(501), pp.e504-e515.

Ida, M., Hirata, M., Odori, S., Mori, E., Kondo, E., Fujikura, J., Kusakabe, T., Ebihara, K., Hosoda, K. and Nakao, K., 2013. Early changes of abdominal adiposity detected with weekly dual bioelectrical impedance analysis during calorie restriction. *Obesity*, *21*(9), pp.E350-E353.

International Chair on Cardiometabolic Risk www.cardiometabolic-risk.org

Irlbeck, T., Massaro, J.M., Bamberg, F., O'donnell, C.J., Hoffmann, U. and Fox, C.S., 2010. Association between single-slice measurements of visceral and abdominal subcutaneous adipose tissue with volumetric measurements: the Framingham Heart Study. *International journal of obesity*, *34*(4), pp.781-787.

Janghorbani, M. and Amini, M., 2007. Metabolic syndrome in type 2 diabetes mellitus in Isfahan, Iran: prevalence and risk factors. *Metabolic syndrome and related disorders*, *5*(3), pp.243-254.

Janssen, I., Katzmarzyk, P.T. and Ross, R., 2002. Body mass index, waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines. *Archives of internal medicine*, *162*(18), pp.2074-2079.

Janssen, I., Katzmarzyk, P.T. and Ross, R., 2004. Waist circumference and not body mass index explains obesity-related health risk. *The American journal of clinical nutrition*, *79*(3), pp.379-384.

Jebb, S.A., Cole, T.J., Doman, D., Murgatroyd, P.R. and Prentice, A.M., 2000. Evaluation of the novel Tanita body-fat analyser to measure body composition by comparison with a four-compartment model. *British Journal of Nutrition*, *83*(2), pp.115-122.

Kalk, W.J. and Joffe, B.I., 2008. The metabolic syndrome, insulin resistance, and its surrogates in African and white subjects with type 2 diabetes in South Africa. *Metabolic syndrome and related disorders*, *6*(4), pp.247-255.

Kang, J.Y., Park, I.K., Lee, J.Y., Sung, S.H., Chang, Y.K., Park, Y.K. and Choi, T.I., 2012. Use of serum homocysteine to predict cardiovascular disease in Korean men with or without metabolic syndrome. *Journal of Korean medical science*, *27*(5), pp.500-505.

Kaur, J., 2014. A comprehensive review on metabolic syndrome. *Cardiology research and practice*, *2014*, pp.1-21.

Kearney, P.M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P.K. and He, J., 2005. Global burden of hypertension: analysis of worldwide data. *The lancet*, *365*(9455), pp.217-223.

Kershaw, E.E. and Flier, J.S., 2004. Adipose tissue as an endocrine organ. *The Journal of Clinical Endocrinology & Metabolism*, *89*(6), pp.2548-2556.

Klopfenstein, B.J., Kim, M.S., Krisky, C.M., Szumowski, J., Rooney, W.D. and Purnell, J.Q., 2012. Comparison of 3 T MRI and CT for the measurement of visceral and subcutaneous adipose tissue in humans. *The British journal of radiology*, *85*(1018), pp.e826-e830.

Kuk, J.L., Katzmarzyk, P.T., Nichaman, M.Z., Church, T.S., Blair, S.N. and Ross, R., 2006.

Visceral fat is an independent predictor of all-cause mortality in men. *Obesity*, *14*(2), pp.336-341.

Kvist, H., Chowdhury, B., Grangård, U., Tylen, U. and Sjöström, L., 1988. Total and visceral adipose-tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations. *The American journal of clinical nutrition*, *48*(6), pp.1351-1361.

Kyle, U.G., Bosaeus, I., De Lorenzo, A.D., Deurenberg, P., Elia, M., Gómez, J.M., Heitmann, B.L., Kent-Smith, L., Melchior, J.C., Pirlich, M. and Scharfetter, H., 2004. Bioelectrical impedance analysis—part I: review of principles and methods. *Clinical nutrition*, *23*(5), pp.1226-1243.

Lamarche, B., Tchernof, A., Mauriège, P., Cantin, B., Dagenais, G.R., Lupien, P.J. and Després, J.P., 1998. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. *Jama*, *279*(24), pp.1955-1961.

Lear, S.A., Humphries, K.H., Kohli, S., Chockalingam, A., Frohlich, J.J. and Birmingham, C.L., 2007. Visceral adipose tissue accumulation differs according to ethnic background: results of the Multicultural Community Health Assessment Trial (M-CHAT). *The American journal of clinical nutrition*, *86*(2), pp.353-359.

Lee, D-H., Park, K.S., Ahn, S., Ku, E.J., Jung, K.Y., Kim, Y.J., Kim, K.M., Moon, J.H., Choi, S.H., Park, K.S., Jang H.C., and Lim, S., 2015. Comparison of abdominal visceral adipose tissue area measured by computed tomography with that estimated by bioelectrical impedance analysis method in Korean subjects. *Nutrients, 7*, pp.10513-10524.

Lokuruka, M.N.I., 2013. A literature review of role of obesity in adult health with reference to Africa. *African Journal of Food, Agriculture, Nutrition and Development*, *13*(1), pp.7088-7104.

Lorenzo, C., Okoloise, M., Williams, K., Stern, M.P. and Haffner, S.M., 2003. The metabolic syndrome as predictor of type 2 diabetes. *Diabetes care*, *26*(11), pp.3153-3159.

Maligie, M., Crume, T., Scherzinger, A., Stamm, E. and Dabelea, D., 2012. Adiposity, fat patterning, and the metabolic syndrome among diverse youth: the EPOCH study. *The Journal of pediatrics*, *161*(5), pp.875-880.

Matsha, T.E., Hassan, M.S., Kidd, M. and Erasmus, R.T., 2012. The 30-year cardiovascular risk profile of South Africans with diagnosed diabetes, undiagnosed diabetes, pre-diabetes or

normoglycaemia: the Bellville, South Africa pilot study: cardiovascular topics. *Cardiovascular journal of Africa*, *23*(1), pp.5-11.

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

Matsuzawa, Y., Fujioka, S., Tokunaga, K. and Tarui, S., 1987. Novel classification: visceral fat obesity and subcutaneous fat obesity. *Recent advances in obesity research*.

Matsuzawa, Y., 2008. The role of fat topology in the risk of disease. *International journal of obesity*, *32*, pp.S83-S92.

Matsuzawa, Y., Funahashi, T. and Nakamura, T., 2011. The concept of metabolic syndrome: contribution of visceral fat accumulation and its molecular mechanism. *Journal of atherosclerosis and thrombosis*, *18*(8), pp.629-639.

Mattsson, S. and Thomas, B.J., 2006. Development of methods for body composition studies. *Physics in medicine and biology*, *51*(13), pp.203.

Mazonakis, M. and Damilakis, J., 2016. Computed tomography: What and how does it measure? *European journal of radiology*, *85*(8), pp.1499-1504.

Misra, A., Wasir, J.S. and Vikram, N.K., 2005. Waist circumference criteria for the diagnosis of abdominal obesity are not applicable uniformly to all populations and ethnic groups. *Nutrition*, *21*(9), pp.969-976.

Misra, A. and Vikram, N.K., 2003. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition*, *19*(5), pp.457-466

Misra, A. and Khurana, L., 2008. Obesity and the metabolic syndrome in developing countries. *The Journal of Clinical Endocrinology & Metabolism*, *93*(11_supplement_1), pp.s9-s30.

Morricone, L., Donati, C., Hassan, T., Cioffi, P. and Caviezel, F., 2002. Relationship of visceral fat distribution to angiographically assessed coronary artery disease: results in subjects with or without diabetes or impaired glucose tolerance. *Nutrition, metabolism, and cardiovascular diseases: NMCD*, *12*(5), pp.275-283.

Motala, A.A., Mbanya, J.C. and Ramaiya, K.L., 2009. Metabolic syndrome in sub-Saharan Africa. *Ethn Dis*, *19*(2 Suppl 2), pp.S2-8.

Motala, A.A., Esterhuizen, T., Pirie, F.J. and Omar, M.A., 2011. The prevalence of metabolic syndrome and determination of the optimal waist circumference cutoff points in a rural South African community. *Diabetes care*, *34*(4), pp.1032-1037.

Mulè, G., Calcaterra, I., Nardi, E., Cerasola, G. and Cottone, S., 2014. Metabolic syndrome in hypertensive patients: An unholy alliance. *World journal of cardiology*, *6*(9), p.890.

Nagai, M., Komiya, H., Mori, Y., Ohta, T., Kasahara, Y. and Ikeda, Y., 2010. Estimating visceral fat area by multifrequency bioelectrical impedance. *Diabetes care*, *33*(5), pp.1077-1079.

Neeland, I.J., Ayers, C.R., Rohatgi, A.K., Turer, A.T., Berry, J.D., Das, S.R., Vega, G.L., Khera, A., McGuire, D.K., Grundy, S.M. and Lemos, J.A., 2013. Associations of visceral and abdominal subcutaneous adipose tissue with markers of cardiac and metabolic risk in obese adults. *Obesity*, *21*(9), pp.E439-E447.

O'Connor, M., Ryan, J. and Foley, S., 2015. Best single-slice location to measure visceral adipose tissue on paediatric CT scans and the relationship between anthropometric measurements, gender and VAT volume in children. *The British journal of radiology*, *88*(1054), pp.1-10.

Olufadi, R. and Byrne, C.D., 2008. Clinical and laboratory diagnosis of the metabolic syndrome. *Journal of clinical pathology*, *61*(6), pp.697-706.

Omron Body Composition Monitor (BF511), model HBF-511B-E / HBF-511T-E Manufacturer: Omron Healthcare Co., Ltd. 53, Kunotsubo, Terado-cho, Muko, Kyoto, 617-0002 Japan.

Ozhan, H., Alemdar, R., Caglar, O., Ordu, S., Kaya, A., Albayrak, S., Turker, Y., Bulur, S. and MELEN Investigators, 2012. Performance of bioelectrical impedance analysis in the diagnosis of metabolic syndrome. *Journal of Investigative Medicine*, *60*(3), pp.587-591.

Paniagua, L., Lohsoonthorn, V., Lertmaharit, S., Jiamjarasrangsi, W. and Williams, M.A., 2008. Comparison of waist circumference, body mass index, percent body fat and other measure of adiposity in identifying cardiovascular disease risks among Thai adults. *Obesity research & clinical practice*, *2*(3), pp.215-223.

PathCare Reference Laboratory, Cape Town, South Africa.

Piernas, C., Hernández-Morante, J.J., Canteras, M., Zamora, S. and Garaulet, M., 2009. New computed tomography-derived indices to predict cardiovascular and insulin-resistance risks in overweight/obese patients. *European journal of clinical nutrition*, *63*(7), pp.887-897.

Pietrobelli, A. and Heymsfield, S.B., 2002. Establishing body composition in obesity. *Journal of endocrinological investigation*, *25*(10), pp.884-892.

Poll, L.W., Wittsack, H.J., Koch, J.A., Willers, R., Cohnen, M., Kapitza, C., Heinemann, L. and Mödder, U., 2003. A rapid and reliable semiautomated method for measurement of total abdominal fat volumes using magnetic resonance imaging. *Magnetic resonance imaging*, *21*(6), pp.631-636.

Popkin, B.M., Adair, L.S. and Ng, S.W., 2012. Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition reviews*, *70*(1), pp.3-21.

PROGRESS Collaborative Group, 2001. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6105 individuals with previous stroke or transient ischaemic attack. *The Lancet*, *358*(9287), pp.1033-1041.

Puoane, T., Steyn, K., Bradshaw, D., Laubscher, R., Fourie, J., Lambert, V. and Mbananga, N., 2002. Obesity in South Africa: the South African demographic and health survey. *Obesity*, *10*(10), pp.1038-1048.

Qiong, Y.U., Bo, P.A.N.G., Rui, L.I.U., Wenwang, R.A.O. and Shangchao ZHANG, Y.Y., 2017. Appropriate Body Mass Index and Waist-hip Ratio Cutoff Points for Overweight and Obesity in Adults of Northeast China. *Iranian journal of public health*, *46*(8), p.1038.

Razi, T., Niknami, M. and Ghazani, F.A., 2014. Relationship between Hounsfield unit in CT scan and gray scale in CBCT. *Journal of dental research, dental clinics, dental prospects*, *8*(2), p.107.

Reaven, G.M., 2003. Insulin resistance/compensatory hyperinsulinemia, essential hypertension, and cardiovascular disease. *The Journal of Clinical Endocrinology & Metabolism*, *88*(6), pp.2399-2403.

Roberts, C.K., Hevener, A.L., Barnard, R.J., 2013. Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training. *Comprehensive Physiology*, 3(1), pp.1-58.

Roos, J.E., Desbiolles, L.M., Willmann, J.K., Weishaupt, D., Marincek, B. and Hilfiker, P.R., 2002. Multidetector-row helical CT: analysis of time management and workflow..*European radiology*, *12*(3), pp.680-685.

Ross, R., Rissanen, J. and Hudson, R., 1996. Sensitivity associated with the identification of visceral adipose tissue levels using waist circumference in men and women: effects of weight loss. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity*, *20*(6), pp.533-538.

Scharfetter, H., Riu, P., Populo, M. and Rosell, J., 2002. Sensitivity maps for low-contrast perturbations within conducting background in magnetic induction tomography. *Physiological measurement*, *23*(1), p.195.

Seeram, E., Computed Tomography, 2001. *WB Sanders Co., Philadelphia*, pp.194-195.

Seidell, J.C., Kahn, H.S., Williamson, D.F., Lissner, L. and Valdez, R., 2001. Report from a Centers for Disease Control and Prevention Workshop on use of adult anthropometry for public health and primary health care. *The American journal of clinical nutrition*, *73*(1), pp.123-126.

Shaw, J.E., Sicree, R.A. and Zimmet, P.Z., 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice*, *87*(1), pp.4-14.

Shen, W., Wang, Z., Punyanita, M., Lei, J., Sinav, A., Kral, J.G., Imielinska, C., Ross, R. and Heymsfield, S.B., 2003. Adipose tissue quantification by imaging methods: a proposed classification. *Obesity*, *11*(1), pp.5-16.

Shuster, A., Patlas, M., Pinthus, J.H. and Mourtzakis, M., 2012. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. *The British journal of radiology*, *85*(1009), pp.1-10.

Sjostrom, L., Kvist, H., Cederblad, A. and Tylen, U., 1986. Determination of total adipose tissue and body fat in women by computed tomography, 40K, and tritium. *American Journal of Physiology-Endocrinology And Metabolism*, *250*(6), pp.E736-E745.

Soares, L.P., Dal Fabbro, A.L., Silva, A.S., Sartorelli, D.S., Franco, L.F., Kuhn, P.C., Moises, R.S., Vieira-Filho, J.P.B. and Franco, L.J., 2015. Prevalence of metabolic syndrome in the Brazilian Xavante indigenous population. *Diabetology & metabolic syndrome*, *7*(1), p.105.

Sowers, J.R., Standley, P.R., Ram, J.L., Jacober, S., Simpson, L. and Rose, K., 1993. Hyperinsulinemia, insulin resistance, and hyperglycemia: contributing factors in the pathogenesis of hypertension and atherosclerosis. *American journal of hypertension*, *6*(7_Pt_2), pp.260S-270S.

Stern, L., Iqbal, N., Seshadri, P., Chicano, K.L., Daily, D.A., McGrory, J., Williams, M., Gracely, E.J. and Samaha, F.F., 2004. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Annals of internal medicine*, *140*(10), pp.778-785.

St-Onge, M.P., Janssen, I. and Heymsfield, S.B., 2004. Metabolic syndrome in normal-weight Americans. *Diabetes care*, *27*(9), pp.2222-2228.

Sutton, D.H. and Raines, D.A., 2007. Identification and Management of Metabolic Syndrome: The Role of the APN. *Topics in Advanced Practice Nursing ejournal*.

Tokunaga, K., Matsuzawa, Y., Ishikawa, K. and Tarui, S., 1983. A novel technique for the determination of body fat by computed tomography. *International journal of obesity*, *7*(5), pp.437-445.

Toth, P.P., 2005. The “good cholesterol” high-density lipoprotein. *Circulation, 111*, pp.e89-e91.

Unno, M., Furusyo, N., Mukae, H., Koga, T., Eiraku, K., Hayashi, J., 2012. The utility of visceral fat level by bioelectrical impedance analysis in the screening of Metabolic Syndrome. *Journal of atherosclerosis and thrombosis, 19*(5), pp.462-470.

Van der Kooy, K. and Seidell, J.C., 1993. Techniques for the measurement of visceral fat: a practical guide. *International journal of obesity*, *17*, pp.187-187.

Wajchenberg, B.L., 2000. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocrine reviews*, *21*(6), pp.697-738.

Wang, H.F. and Yeh, M.C., 2012. Psychological resistance to insulin therapy in adults with type 2

diabetes: mixed-method systematic review. *Journal of advanced nursing*, *68*(4), pp.743-757.

Wierzbicki, A.S., 2006. Diabetic dyslipidaemia: the triad. *European heart journal supplements, 8*(Supplement F), pp.F30-F33.

Wilding, J.P.H., 2014. The importance of weight management in type 2 diabetes mellitus. *International journal of clinical practice*, *68*(6), pp.682-691.

Won, K-B., Chang, H-J., Sung, J., Shin, S., Cho, I-J., Shim,C-Y., Hong, G-R., Kim, Y.J., Choi, B-W., Chung, N., 2014. Differential association between metabolic syndrome and coronary artery disease evaluated with cardiac computed tomography according to the presence of diabetes in a symptomatic Korean population. *BMC Cardiovascular disorders,* 14, pp.105.

World Health Organization (WHO). 1999. International Society of Hypertension Guidelines for the Management of Hypertension. Journal of Hypertension, 17: pp.151-183.

World Health Organization and International Society of Hypertension Writing Group, 2003. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *Journal of hypertension*, *21*(11), pp.1983-1992

World Health Organization (WHO) 2004. Global Database on Body Mass Index, Copyright 2006, World Health Organization, Last updated 31.08.16.

World Health Organization, 2011. Waist circumference and waist-hip ratio: Report of a WHO expert consultation, Geneva, 8-11 December 2008, pp.1-39.

World Health Organization (WHO). 2016. Global Report on Diabetes. WHO website (http://www.who.int)

Yoshizumi, T., Nakamura, T., Yamane, M., Waliul Islam, A.H.M., Menju, M., Yamasaki, K., Arai, T., Kotani, K., Funahashi, T., Yamashita, S. and Matsuzawa, Y., 1999. Abdominal fat: standardized technique for measurement at CT. *Radiology*, *211*(1), pp.283-286.

APPENDICES

Appendix 1. Ethics certificate; T.E. Matsha

Appendix 2. Ethics certificate; S. Ismail

Appendix 3. Consent form

Cape Peninsula
University of Technology
HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 Bellville 7535 South Africa Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

24 April 2017

REC Approval Reference No: CPUT/HW-REC 2015/H01 (renewal)

Faculty of Health and Wellness Sciences – Biomedical Sciences Department Dear Prof Matsha
Re: YOUR APPLICATION TO THE HW-REC FOR EXTENSION OF ETHICS APPROVAL

Approval was granted by the Health and Wellness Sciences-REC on 30 March 2017 to Prof Tandi Matsha for ethical clearance. This approval is for research activities related to staff research in the Department of Biomedical Sciences at this Institution.

TITLE: Progressive research on risk factors of type 2 diabetes and cardiovascular diseases in South Africa.

Co-investigators:
1. **Prof Rajiv Timothy Erasmums, Pathology Department – Stellenbosch University.**
2. **Prof Andre Pascal Kengne, Chronic Diseases of Lifestyle Department – MRC.**
3. **Mr Mogamat Shafick Hassan, Nursing and Radiography Department – CPUT.**

Project Manager:
1. Dr Gloudina Hon – CPUT

Comment:
Approval will not extend beyond 25 April 2018. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

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

Kind Regards

Mr. Navindhra Naidoo
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Cape Peninsula
University of Technology

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

P.O. Box 1906 • Bellville 7535 South Africa Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

4 October 2016

REC Approval Reference No: CPUT/HW-REC 2015/H18 (extension)

Faculty of Health and Wellness Sciences – Medical Imaging and Therapeutic Sciences Department Dear Ms Saaiga Ismail
Re: YOUR APPLICATION TO THE HW-REC FOR EXTENSION OF ETHICS APPROVAL

Approval was granted by the Health and Wellness Sciences-REC on 15 September 2016 to Ms Ismail for ethical clearance. This approval is for research activities related to staff research in the Department of Medical Imaging and Therapeutic Sciences at this Institution.

TITLE: Anthropometric measurements versus computed tomography for the assessment of Metabolic Syndrome in the Bellville South Community, South Africa

Supervisor: Prof T Matsha

Comment:
Approval will not extend beyond 9 October 2017. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

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

Kind Regards

Mr. Navindhra Naidoo
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM FOR RESEARCH INVOLVING GENETIC STUDIES

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

REFERENCE NUMBER:

PRINCIPAL INVESTIGATORS:	**Professor Tandi Matsha (Cape Peninsula University of Technology)** **Professor Rajiv Erasmus (Stellenbosch University)** **Professor Andre Kengne (SA Medical Research Council)**
Project manager:	**Dr Gloudina Maria Hon (Cape Peninsula University of Technology)**
ADDRESS:	**Obesity and chronic diseases of lifestyle Department of Biomedical Sciences Faculty of Health & Wellness Sciences** **Cape Peninsula University of Technology, Bellville**
CONTACT NUMBER:	**Prof T Matsha 021 959 6366 or email:matshat@cput.ac.za**
Ethics approval:	**Cape Peninsula University of Technology Ethics Reference number: CPUT/SW-REC 2015/H01** **University of Stellenbosch Ethics Reference number: N14/01/003**

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

This research study has been approved by the ethics **Faculty of Health & Wellness Sciences of the Cape Peninsula University of Technology** and it will be conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of Helsinki, and the SA Department of Health's 2004 Guidelines: *Ethics in Health Research: Principles, Structures and Processes.*

1. What is Genetic research?

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

2. What does this particular research study involve?

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

3. Why have you been invited to participate?

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

4. What procedures will be involved in this research?

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

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

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

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

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

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

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

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

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

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

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

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

5. Are there any risks involved in genetic research?

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

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

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

- Have diabetes, thereafter, you will be referred to your local health centre or general practitioner for further investigations and treatment.
- Have a condition or predisposition to developing diabetes that is treatable or avoidable
  e.g. by a lifestyle modification.
- Need genetic counselling.

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

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

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

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

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

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

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

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

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

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

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

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

You should inform your family practitioner or usual doctor that you are taking part in a research study. You can contact

Prof T Matsha at 021 959 6366 or matshat@cput.ac.za,

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

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

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

12. Declaration by participant

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

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurised to take part.
- I have received a signed duplicate copy of this consent form for my records.

13. Tick the option you choose:

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

OR

☐ I agree that my blood or tissue sample can be stored ***indefinitely,*** but I can choose to request at any time that my stored sample be destroyed. My sample will be identified

with a special study code that will remain linked to my name and contact details. I have the right to receive confirmation that my request has been carried out.

OR

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

14. Tick the option you choose:

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

OR

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

OR

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

15. Tick the option you choose: Radio Imaging

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

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

AND

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

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

AND

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

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

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

Finger print

.....

..

Signature of participant

.....

Signature of witness

16. Declaration by investigator

I *(name)* …… declare that:

- I explained the information in this document to ……..……...
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research as discussed above.
- I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*

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

.....

Signature of investigator

.....

Signature of witness

17. Declaration By Interpreter

I *(name)* …… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) …….. Using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

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

.....

Signature of interpreter

.....

Signature of witness