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University of Technology

"Body fat distribution and cardio-metabolic risk factors in South African men and women".

by

FLORENCE ELIZABETH DAVIDSON

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Supervisor: Professor TE Matsha
Co-supervisor: Professor JH Goedecke

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DECLARATION

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

Signed:  **Date: May 2020**

ABSTRACT

"Body fat distribution and cardio-metabolic risk factors in South African men and women".

Background: An analysis of pooled population-based studies conducted by the non-communicable disease (NCD) risk factor collaboration Africa working group found that estimates of adiposity and diabetes prevalence in South Africa (SA) were higher than the global average. Specifically, in the mixed-ancestry population, central obesity rates were high (87.9% and 42.2% as defined by IDF criteria) in women and men respectively. Furthermore, the mixed-ancestry population of SA present with a high prevalence of metabolic syndrome (Mets) (62%) and type-2 diabetes mellitus (28.2%), placing this population at high risk for cardio-vascular disease (CVD). Visceral adipose tissue (VAT) accumulation is a known risk factor for cardio-metabolic disease. Typically, waist circumference (WC) is the accepted proxy of VAT, however, WC and other anthropometry measurements cannot distinguish between VAT and abdominal subcutaneous adipose tissue (SAT). Imaging modalities such as computed tomography (CT) and Magnetic Resonance Imaging (MRI) are considered the gold standard for VAT and abdominal SAT differentiation, but are not readily available in the clinical setting. Whole body composition studies using dual-energy x-ray absorptiometry (DXA) have also proved to be relatively accurate in quantifying VAT and abdominal SAT. Furthermore, DXA is also able to quantify other regional body fat compartments such as gynoid fat (gluteofemoral), trunk fat, arm and leg fat, all at a substantially lower radiation dose than CT. Therefore, the overall objective of this thesis was to validate DXA as a method of quantifying central adiposity, explore the relationship between body fat distribution and cardio-metabolic risk and determine the ability of DXA compared to anthropometry to identify participants with MetS.

Methods: The study sample included a total of 46 men and 207 women, all self-described mixed-ancestry volunteers aged 20 years and older who were part of the Cape Town Vascular and Metabolic Health (VMH) parent study. Participants underwent anthropometric measurements, oral glucose tolerance test (OGTT), lipid measurements, DXA and CT scan examinations. Pearson correlation coefficients and Bland-Altman analysis were used to determine agreement between DXA and CT measurements. MetS was quantified using the Joint Interim Statement (JIS) criteria. Robust regression analyses were used to investigate associations between body fat distribution and cardio-metabolic risk factors. The area under the curve (AUC) was used to assess the performance of VAT area and anthropometry in detecting any two components of MetS (excluding WC). Optimal WC and VAT area cut-points

were derived to compare their performance for diagnosing MetS and to compare to internationally recognised cut-points

Results: The mean age in the 132 women in whom VAT and abdominal SAT were measured using single slice CT and DXA was 55 and ranged from 45 to 64 years. DXA and CT- derived measurements of abdominal VAT and SAT were significantly correlated in the overall sample ($r=0.872$ and $r=0.966$, both $p<0.001$ respectively) and within body mass index (BMI) categories. In the overall sample, the mean difference (DXA-CT estimates) was 75.3 cm^2 (95% CI: 68.8-81.8 cm^2, $p\leq0.0001$) for VAT and 54.7 cm^2 (47.1-62.3 cm^2, $p\leq0.0001$) for SAT. Within increasing BMI categories, the variance between the two modalities was fixed for VAT ($p=0.359$ for obese), whereas the variance for SAT was heteroscedastic ($p\leq0.0001$).

In the cross-sectional study, which included 207 mixed-ancestry SA women and 46 men, the men had lower body fat mass compared women (26.5 vs. 44.0%), but had more central and less peripheral fat (both $p<0.001$). Post-menopausal women had greater % fat mass, (FM), WC and VAT, and less gynoid % FM than pre-menopausal women (all $p\leq0.004$). After adjusting for age and sex, VAT accounted for greatest variance in insulin resistance ($R^2=0.27$, $p\leq0.01$), while trunk %FM and leg %FM accounted for greatest variance in triglyceride ($R^2=0.13$, $p\leq0.01$) and high-density lipoprotein cholesterol concentrations ($R^2=0.14$, $p\leq0.01$).

The highest AUC for the prediction of MetS in the 204 women was recorded for VAT, followed by waist-to height-ratio (WHtR) and WC (AUC, 0.767, 0.747 and 0.738 respectively), but these did not differ significantly (all $p\geq0.192$). In contrast, VAT was significantly better than BMI ($p=0.028$), hip ($p=0.0004$) and a body shape index (ABSI) ($p<0.0001$).

Conclusions: In conclusion, this thesis has for the first time validated DXA as a method of quantifying visceral adiposity, explored the relationship between body fat distribution and cardio-metabolic risk and determined the ability of DXA compared to anthropometry to identify mixed-ancestry participants with MetS. Although we found that DXA overestimated VAT compared to the gold standard, CT, the variance was fixed in the obese category. These findings suggest that DXA is a valid measure of VAT and abdominal SAT in the obese group which is the group most at risk for cardio-metabolic diseases. Furthermore, we have demonstrated the value of the whole body DXA scan which quantified regional fat depots and showed that central fat was the most significant correlate of cardio-metabolic risk and lower body fat was associated with reduced risk. Finally, we have demonstrated in this population that DXA-derived VAT had no advantage in discriminating MetS than WC, and therefore confirm that WC can be used as a marker of MetS provided population specific cut-offs are derived.

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DEDICATION

I dedicate this thesis to my family who have stood by me.

For

Rob, Kim, Chelsey, my mom and late dad.

PREFACE

This thesis reports an ancillary study of the larger Vascular and Metabolic Health study. The parent study was a cross-sectional study which recruited voluntary self-described mixed-ancestry men and women ≥ 20 years of age from Bellville South in the Western Cape, South Africa (SA). The SA mixed-ancestry population group has a lineage that is approximately 32-43% Khoisan, 20-36% black, 21-28% white and 2-11% Asian. The mixed-ancestry population accounts for 8.8% of the SA population and approximately 48% of the population of the Western Cape (StatsSA, 2019). This population was investigated due to the high prevalence of obesity and cardio-metabolic disease. The focus of this thesis was to characterise body fat distribution in the mixed-ancestry population using radiography imaging and anthropometry and relate it to cardio-metabolic risk factors. The thesis is comprised of six chapters, including three results chapters that have been published and are outlined below:

Chapter one is an introduction to the thesis and provides the context of the study with respect to obesity and cardiovascular disease risk in the mixed-ancestry population of SA.

Chapter two is a review of the relevant literature on adipose tissue classification, obesity and the associated cardio-metabolic risk factors from an international as well as a SA perspective. The various methods used to quantify adipose tissue, as well as the advantage and disadvantages of each are discussed. Special focus is made on the imaging methods of dual energy x-ray absorptiometry and computed tomography, as these were the methods applied in this thesis.

Chapters three, four and five are the results chapters and are represented as published research manuscripts according to the specific journal format, namely:

Chapter three: Comparison of single slice CT and DXA-derived measures of central adiposity in South African women.

Published article:
Davidson, F.E., Matsha, T.E., Erasmus, R.T., Kengne, A.P. & Goedecke, J.H. 2020. Comparison of single slice CT and DXA-derived measures of central adiposity in South African women. *European Journal of Clinical Nutrition,* published online: 22 April

Chapter four: Associations between body fat distribution and cardio-metabolic risk factors in mixed-ancestry South African women and men.

Published article:
Davidson, F.E., Matsha, T.E., Erasmus, R.T., Kengne, A.P. & Goedecke, J.H. 2019. Associations between body fat distribution and cardio-metabolic risk factors in mixed-ancestry South African women and men. *Cardiovascular journal of Africa*, 30 (September): 1–10.

Chapter five : The discriminatory power of visceral adipose tissue vs anthropometric measures as a diagnostic marker for metabolic syndrome in South African women.

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Chapter six is the summary and conclusions of the thesis.

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GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
ABSI	**A body shape index**
AUC	**Area under the curve**
BMI	**Body mass index**
CT	**Computed tomography**
CTL	**Closest top left point**
CVD	**Cardio-vascular disease**
dSAT	**Deep subcutaneous adipose tissue**
DXA	**Dual-energy x-ray absorptiometry**
FM	**Fat mass**
FFSTM	**Fat free soft tissue mass**
HbA_{1C}	**Glycated haemoglobin**
HDL-C	**High-density lipoprotein cholesterol**
HOMA-IR	**Homeostatic model assessment of insulin resistance**
hs-CRP	**High-sensitivity C-reactive protein**
IGT	**Impaired glucose tolerance**
JIS	**Joint interim statement**
LDL-C	**Low-density lipoprotein cholesterol**
MetS	**Metabolic syndrome**
MRI	**Magnetic resonance imaging**
NCDs	**Non-communicable diseases**
NGT	**Normal glucose tolerance**
OGTT	**Oral glucose tolerance test**
ROC	**Receiver operating characteristics**
ROIs	**Regions of interest**
sSAT	**Superficial subcutaneous adipose tissue**
VAT	**Visceral adipose tissue**
WC	**Waist circumference**
WHO	**World health organisation**
WHR	**Waist-to-hip ratio**
WHtR	**Waist-to-height ratio**

CHAPTER ONE: INTRODUCTION

1.0 Background and context

Globally the incidence of non-communicable diseases (NCDs) such as cardio-vascular diseases (CVDs) and type-2 diabetes mellitus is on the increase and disproportionately affects people in low-to-middle income countries (LMIC) (World Health Organisation, 2014). Obesity is associated with co-morbidities such as cardiovascular and metabolic disease including hypertension, insulin resistance, type-2 diabetes and atherogenic dyslipidaemia (Lee et al., 2013; Lemos & Neeland, 2014; O'Neill & O'Driscoll, 2015). Metabolic syndrome (MetS) is a clustering of these risk factors that when grouped together will amplify the risk for CVD and type-2 diabetes mellitus (Alberti et al., 2006; Alberti et al., 2009). Notably increased waist circumference (WC) is a major component of MetS. There has however been much debate about its definition in that initially insulin resistance was included, but the accepted harmonized criteria are the presence of any three risk factors, namley: WC ≥80cm (women), ≥94cm (men) elevated triglycerides ≥ 1.7 mmol/L (or drug treatment for elevated levels), elevated blood pressure systolic ≥ 130 and/or diastolic ≥ 85 mmHg (or antihypertensive drug treatment), elevated fasting blood glucose ≥ 5.6 mmol/L (or drug treatment of elevated glucose) and reduced HDL-C < 1.3 mmol/L (women), <1.0 mmol/L (men) (or drug treatment for reduced HDL-C) (Alberti et al., 2009). A proinflammatory state and prothrombotic state are additional components of MetS, which relate to the risk of CVD (Grundy et al., 2004). Identification of and treatment for those at risk is essential (Eckel et al., 2005; Alberti et al., 2006). Non-diabetic persons with MetS are at five times greater risk for the development of CVD and type-2 diabetes mellitus than those without MetS (Alberti et al., 2006).

In Africa, there is an impending epidemic of CVD and type-2 diabetes mellitus being observed, without population specific accurate tools for improving risk stratification and prevention (Matsha et al., 2013). In sub-Saharan Africa there has been an increasing burden of hypertension and dyslipidaemia necessitating a more aggressive treatment approach (Schutte, 2019). Furthermore, fourteen percent of ischaemic heart disease (IHD), 10% of stroke, 12% of hypertensive disease and 12% of renal disease burden in SA adults >30yrs was attributed to type-2 diabetes (Bradshaw et al., 2007). The age standardised death rates from NCDs in SA have surpassed those from HIV/AIDS and tuberculosis combined, with CVD being the leading category of NCDs (Nogilana et al., 2016). Recent research into the lifetime CVD risk in the mixed-ancestry population revealed that although high risk scores were reported in those with hyperglycaemia, the risk was still evident in younger, normal weight normo-glycaemic individuals (Matsha et al., 2012). The lineage of the SA mixed-ancestry population is approximately 32-43% Khoisan, 20-36% black, 21-28% white and 2-11% Asian (de Wit et al., 2010).

In 2019, the estimated diabetes prevalence in the adult population in Africa was approximately 3.9% (19.4 million) of which ≥9% were in SA (IDF Diabetes atlas, 2019). Furthermore, Africa was shown to have the highest proportion (59.7%, 11.6 million), of undiagnosed diabetes globally, with an estimated 500 thousand to 5 million coming from SA (IDF Diabetes atlas, 2019). Estimates of diabetes prevalence and adiposity are higher in SA than the global average (NCD-RicS-Africa Working Group, 2017). The Human Sciences Research Council (HSRC) first SA Health and Nutrition Examination Survey (SANHANES) in 2012 which included approximately 25 000 people indicated that 5% of all respondents >15yrs of age had self-reported diabetes mellitus. In the age group >55yrs, the proportion of respondents with self-reported diabetes mellitus was as high as 16% (Baleta & Mitchell, 2014). The SA mixed-ancestry population is particularly at risk for type-2 diabetes mellitus and was found to have the second-highest frequency of type-2 diabetes mellitus in South Africa after the Indian population (Charlton et al., 1997; Levitt et al., 1999; Motala et al., 2003). Recent data have shown that the prevalence of type- 2 diabetes mellitus is 28.2% in the mixed-ancestry population of Bellville, Cape Town and that the prevalence has more than doubled within a decade (Erasmus et al., 2012). Notably, a significant percentage of those with the disease (18.1%) are not aware of their condition, and are therefore not receiving appropriate interventions. Furthermore, the MetS prevalence in the mixed-ancestry population of the Western Cape was 62% (based on the JIS criteria, the highest seen in sub-Saharan populations) with significantly greater prevalence in the women most likely due to the increased central obesity in females (Erasmus et al., 2012).

Recent obesity trend analysis revealed that 603.7 million (39%) adults worldwide were overweight and obese with the prevalence in some countries more than doubling since 1980 (GBD 2015 obesity collaborators, 2017; Chooi et al., 2019). Notably, the global prevalence of obesity is generally higher in women than men and increases with age (Chooi et al., 2019). Previously, high obesity rates were more prevalent in high income countries, but an increasing trend is being observed in LMICs, in particular urban settings in sub-Saharan Africa (Biadgilign et al., 2017). For instance, in the Africa region, the prevalence of obesity has doubled from 6.2% in 1980 to 12.7% in 2015 (Chooi et al., 2019). Furthermore, the prevalence of overweight in SA increased from 49.4% in 1980 to 57.8% in 2015 (Chooi et al., 2019). Currently 68% of women and 31 % of men in South Africa are overweight or obese, with the highest prevalence occurring in women in the 45-64 years age group (National Department of Health (NDoH), 2019). There is however little difference in the prevalence of overweight or obesity by population group amongst women. However in men, the highest prevalence of overweight or obesity was among whites (75%) and lowest among Black Africans (27%)(National Department of Health (NDoH), 2019). Notably, the prevalence of overweight or obesity in both

women and men was highest in the Western Cape (73% and 44% respectively)(National Department of Health (NDoH), 2019), of which the mixed-ancestry population accounts for nearly half (48%) (STATS SA, 2019). The overall mean BMI for the mixed-ancestry female population ≥15yrs of age was 28.1kg/m^2 with the mean BMI of the 45-54-year-old population increasing to 31.7kg/m^2. Overall 34.9% of all mixed-ancestry SA women and 15.1% of all mixed-ancestry men are obese (Shisana et al., 2014). Futhermore, central obesity rates in the mixed-ancestry population were high (87.9% and 42.2% as defined by IDF criteria) in women and men respectively (Erasmus et al., 2012).

Body fat distribution appears to be a more significant discriminator of CVD and metabolic disease risk than generalised adiposity. Furthermore, the association of body fat with CVD risk differs by fat depot (Lemos & Neeland, 2014). The accumulation of central adiposity, in particular visceral adipose tissue (VAT) is associated with high risk for CVD and type-2 diabetes mellitus (Pi-sunyer, 2004; Vatanparast et al., 2009). Conversely the association between abdominal subcutaneous adipose tissue (SAT) and cardio-metabolic risk is weaker than VAT, as shown in multi-ethnic studies in men and women (McLaughlin et al., 2011; Neeland et al., 2013). The mechanisms linking VAT accumulation to metabolic complications include the higher production of proinflammatory cytokines and the higher lipolytic activity compared to SAT (Lee et al., 2013). Conversely, accumulation of lower body fat (gluteofemoral obesity) has shown opposing associations with cardio-metabolic risk (Pinnick et al., 2012; Park et al., 2014; Karpe & Pinnick, 2015), with this fat depot seen as a 'metabolic sink' which traps excess free fatty acids (Pinnick et al., 2012; Karpe & Pinnick, 2015). Body fat distribution is also sex-specific, with woman having more SAT and less VAT than men (Geer & Shen, 2009; Tchoukalova et al., 2010). Additionally, body fat distribution differs by ethnicity (Wells, 2012; Lee et al., 2013).

Typically, WC is used as a proxy for VAT in the clinical setting. Various imaging methods are able to accurately quantify regional body fat distribution, with computed tomography (CT) and magnetic resonance imaging (MRI) being the gold standard methods (Bi et al., 2015; Fosbøl & Zerahn, 2015). More recently, a more cost effective, lower radiation dose imaging modality, namely dual-energy x-ray absorptiometry (DXA), has shown to perform as well as CT in quantifying regional fat compartments in other SA ethnicities (Micklesfield et al., 2012). However, DXA has not yet been used to quantify regional adipose tissue nor been validated against CT in the mixed-ancestry population. Therefore, this thesis validated DXA as a method of quantifying central adiposity, explored the relationship between body fat distribution and cardio-metabolic risk and determined the ability of DXA compared to anthropometry to identify participants with MetS.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

Obesity is defined as a body mass index (BMI) ≥30kg/m^2 (WHO expert committee, 1995). BMI is widely used as a risk indicator for the development of several health issues (Nuttall, 2015) such as type-2 diabetes and CVDs (Shoelson et al., 2007; Vasan et al., 2018), however it is a poor indicator of body fat percentage and body fat distribution (Nuttall, 2015). Body fat distribution is a better predictor of metabolic and CVD risk (Sam & Mazzone, 2014). Body fat distribution can be accurately measured by radiological imaging, specifically CT, MRI and DXA. While CT and MRI are the criterion methods (Bi et al., 2015; Fosbøl & Zerahn, 2015), they are expensive and not readily available in the clinical setting. Furthermore patient radiation doses are considerably higher with CT than DXA. Additionally, DXA has the added advantage of being more readily available and cheaper than both CT and MRI (Vatanparast et al., 2009; Bi et al., 2015). For these reasons, DXA is more suitable for quantifying body fat distribution in large population based studies and clinical settings. Waist circumference, a measure of central adiposity, is the accepted proxy for VAT accumulation and one of the criteria of MetS (Alberti et al., 2006; Alberti et al., 2009). However due to ethnic variations in body shapes, international bodies have recommended that population derived cut-offs for WC must be developed for each ethnic group (Alberti et al., 2009).

2.1 Adipose tissue

Adipose tissue consists of two sub-types, namely white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is known to secrete a variety of substances that help to regulate metabolic processes as well as store triglycerides, while the main function of BAT is thought to be non-shivering thermogenesis (Shoelson et al., 2007). WAT is further sub divided into subcutaneous and visceral depots, with both having a distinctive role in regulating metabolic homeostasis (Shoelson et al., 2007; Gómez-Hernández et al., 2016). Adipose tissue is one of the largest body compartments and Shen et al proposed a taxonomy for its classification (Shen et al., 2003). Tables 2.1 and 2.2 below are the classifications of SAT and VAT, respectively, as proposed by Shen et al.; 2003. In this thesis, abdominal SAT and VAT are quantified using imaging. For this study, we did not differentiate between deep SAT (dSAT) and superficial SAT (sSAT).

Table 2.1 Classification of SAT (adapted from Shen et al.; 2003)

Adipose tissue compartment	Definition
SAT	The layer found between the dermis and aponeuroses and fasciae of the muscles, inclusive of mammary adipose tissue.
sSAT	The layer found between the skin and a fascial plane in the lower trunk and gluteal-thigh areas.
dSAT	The layer found between the muscle fascia and a fascial plane in the lower trunk and gluteal-thigh areas.

Table 2. 2 Classification of VAT (adapted from Shen et al.; 2003)

Adipose tissue compartment	Definition
Visceral adipose tissue (VAT)	Intraabdominal ⇒ Intraperitoneal (e.g. omental and mesenteric) ⇒ Extraperitoneal ○ Preperitoneal ○ Retroperitoneal (e.g. perirenal, pararenal, periaortic and peripancreatic)

Figures 2.1 and 2.2 below are coronal and axial sections, respectively, of a cadaver (reprinted with permission from the National Library of Medicine) demonstrating the intraabdominal VAT and abdominal SAT that was quantified in this study.

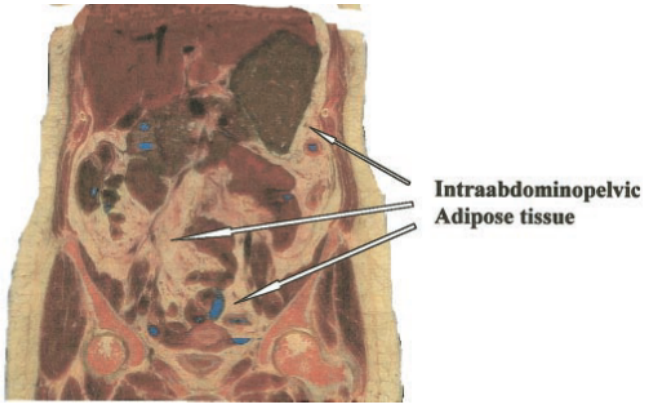

Figure 2.1 VAT compartment

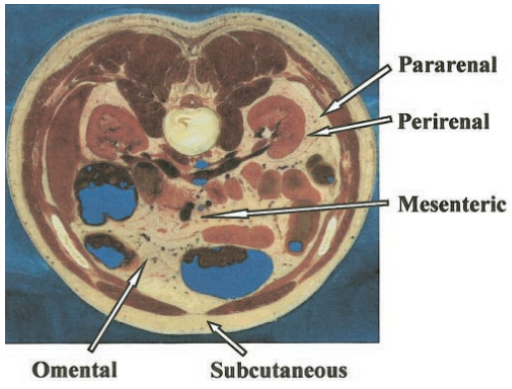

Figure 2.2 Main adipose tissue compartments

2.2 Body composition classification

Wang, et al highlights the 3 key interconnecting elements of body composition research namely: body composition levels and their organisation, measurement methods and the biological factors that impact body composition (e.g. hydration) (Wang et al., 1992).

The widely accepted five level classification by Wang, et al consists of the following levels: i) atomic, ii) molecular, iii) cellular, iv) tissue-system, and v) whole body (Wang et al., 1992). This model provides a structural framework with which to explain the relationship between the main body compartments (Fosbøl & Zerahn, 2015). The first three levels are typically quantified by direct measurements methods, e.g. neutron activation, isotope dilution and total body counting (Duren et al., 2008). The latter 2 levels, namely tissue-system and whole body are known as 'criterion' methods which typically measure the density of tissue and describes the amount of bone, muscle and adipose tissue (Duren et al., 2008). Imaging is a typical technique used for the 'criterion' method. Direct and criterion methods are collectively known as 'laboratory' methods (Duren et al., 2008). Indirect methods (also called 'field' methods), such as anthropometry and bioelectrical impedance analysis (BIA) provide estimates based on direct or criterion methods (Duren et al., 2008; Fosbøl & Zerahn, 2015).

The selection of the most appropriate method for body composition studies in clinical settings is dependent on availability of equipment, costs, safety precautions regarding radiation dose and participant cooperability to name but a few (Fosbøl & Zerahn, 2015). In order to select the most appropriate method, it is important to understand which body compartments in the classification by Wang et al., 1992 are being measured.

2.3 Body compartments and measurement methods

The methods of measuring body composition use anything from 2-compartment up to 5-compartment models. The 2-compartment model partitions the body into fat mass (FM) and fat-free mass (FFM), with the latter consisting of water, protein, carbohydrates and minerals (Fosbøl & Zerahn, 2015). Hydrostatic weighing, a 2-compartment model, uses the difference of the body weight in air and in water to calculate the body's density and then an equation is applied to estimate FM (Ball et al., 2004; Fosbøl & Zerahn, 2015; Borga et al., 2018). This method is however not well tolerated and requires expensive equipment (Duren et al., 2008). Air displacement plethysmography (ADP), another 2-compartment model, involves placing participants in an enclosed chamber and measuring the overall density and total body fat and lean tissue (Fosbøl & Zerahn, 2015; Borga et al., 2018). Both these 2-compartment models do not take into account variations in bone mineral content and hydration (Borga et al., 2018). DXA is able to differentiate between three compartments, namely FM, lean soft tissue (LST)

and bone mineral content (BMC) (Pietrobelli et al., 1996; Fosbøl & Zerahn, 2015). In addition to FM and LST, the 4-compartment model takes in account BMC and total body water (TBW)(Ball et al., 2004; Borga et al., 2018). There is no one *in vivo* method that can measure all 4-compartments (Ball et al., 2004), however DXA is able to measure the BMC as well (3-compartment), and TBW can be measured using deuterium oxide dilution (Borga et al., 2018). The 3-compartment model, which DXA is able to measure, has the added advantage over the 2-compartment models in that it allows for measurement of whole body and regional fat compartments (Borga et al., 2018). The measurement of regional fat compartments is relevant to cardio-metabolic risk research, given that the different body fat compartments (and thus distribution of fat) contribute differently to cardio-metabolic risk with central adiposity being a well-known risk factor (Lee et al., 2013; Lemos & Neeland, 2014; O'Neill & O'Driscoll, 2015).

2.3.1 Anthropometry

Indirect or field methods used for assessing body composition are anthropometry and bioelectrical impedance analysis (BIA) (Bredella et al., 2010). These measurements have proved to be predictive of cardio-metabolic outcomes for large population groups, however they are unable to differentiate between fat mass and lean mass and the amount, type and distribution of adipose tissue. In addition, anthropometry, while inexpensive and quick may be prone to high inter-rater variability (Bi et al., 2014). While WC to assess abdominal adiposity is superior to BMI, cut-off values are likely to be population-specific as previous studies have shown differences in ethnic populations in overall adiposity, abdominal obesity, VAT (Alberti et al., 2006) and SAT accumulation (Tulloch-Reid et al., 2004).

Typically WC, a measure of central adiposity, is the accepted proxy of VAT and, and is used in the clinical diagnosis of MetS (Alberti et al., 2009). The advantage of WC is that it is quick and easy to measure and does not require technical equipment. However there may be variability in tape measure placement which could affect reliability (Klein et al., 2007; Bi et al., 2015). Furthermore, a major limitation of WC, is the inability to discriminate between VAT and abdominal SAT (Wajchenberg, 2000; Bi et al., 2015). Other anthropometry measures include BMI, hip circumference (HC), waist to height ratio (WHtR), waist to hip ratio (WHR) and more recently, a body shape index (ABSI) (Amirabdollahian & Haghighatdoost, 2018). All have their benefits, as well as their weaknesses. For example, BMI, whilst being the most commonly used marker of total adiposity, fails to distinguish between muscle and fat and the location thereof (Millar et al., 2015). Unlike WC thresholds, WHtR, accounts for body size (Millar et al., 2015) and has shown less variance in sex and age (Hsieh et al., 2003). Similarly, the ABSI takes into account body size and is based on the WC adjusted for height and weight (Krakauer & Krakauer, 2012). WHR, uses the ratio of WC and HC, however the practicality of measuring circumferences may be cumbersome and prone to error (Ashwell & Gibson, 2009).

2.3.2 Imaging

Various Imaging modalities have also been used to quantify body fat compartments such as the gold standards of CT and MRI (Bi et al., 2015; Fosbøl & Zerahn, 2015). These techniques quantify total adipose tissue (TAT), SAT and VAT (Fosbøl & Zerahn, 2015) by employing additional software (Duren et al., 2008). Essentially CT provides information on tissue density as Hounsfield units (HU), while MRI uses either manual or automatic delineation of signal intensity (Fosbøl & Zerahn, 2015). Both these modalities are however costly, not easily accessible, and in the case of CT, expose participants to radiation (Duren et al., 2008; Bi et al., 2015). For these reasons, these modalities may be impractical in the clinical setting for large research studies (Bredella et al., 2010). On the other hand, DXA uses a substantially lower radiation dose (effective dose~1.5 mrem)(Neeland et al., 2016) than CT and is more readily available than the other more expensive imaging techniques (Plank, 2005).

2.3.2.1 CT scanning for abdominal obesity quantification

Participants are typically scanned in the dorsal decubitus position (Sottier et al., 2013). Segmentation of fat is either done on a single CT slice (area) or multiple slices (volume). Numerous studies have debated the optimum position, slice number and method for fat quantification. For example, studies have indicated the optimum locations for single axial slice imaging performed at the level of L4-L5 intervertebral disc space correlating well with the total VAT volume (Clasey et al., 1997; Yoshizumi et al., 1999; Piernas et al., 2009). Another study, (Irlbeck et al., 2010) found in their comparison between volumetric and specific anatomic area CT measurements of VAT and SAT, that the area at L3/4 correlated best with volume. The study by Sumner et al which examined the sex difference in VAT area in African Americans showed that single-slice CT at level L2-3 was better than at level L4-5 as the former only required adjustment for BMI and not fat content (Sumner et al., 2002). In that study, (Sumner et al., 2002), the single slice CT was compared to contiguous CT volume from the diaphragm to the iliac crest. Because of previous studies showing ethnic differences in overall adiposity, abdominal obesity, VAT (Alberti et al., 2006) and SAT accumulation (Tulloch-Reid et al., 2004), scan locations may differ depending on the specific population. More recent research which aimed at identifying a standardised anatomic space for abdominal fat quantification by CT, concluded that the site of maximum correlation is T12-L1 for SAT and L3-L4 for VAT (Tong et al., 2014). Furthermore, Schweitzer et al. (Schweitzer et al., 2015) showed that single slice MRI at the level of L3 and Shen et al (Shen et al., 2012) showed that a level 5-10 cm superior to L4-L5 to be the best compromise sites for VAT quantification. Tong et al however suggest that multiple slices provide greater accuracy for VAT than for SAT and that the optimal slices are not contiguous (Tong et al., 2014).

Prior to all scans, all standard CT quality control and equipment calibrations are performed based on the manufacturer's recommendations. Typical tube voltages of 120kv and automatic mAs and 5mm slice thickness, field of view = 40cm, image reconstruction matrix of 512x512 are used (Kim et al., 2016). Typical window settings are : width = 350 HU; Level= 50HU. The participants usually lie in a supine position on the scanning table with both arms placed in a comfortable position above the head.

The images are then transported to a CT workstation and numerous commercially available adipose tissue software (Gradmark et al., 2010; Sottier et al., 2013) can be applied which enables separation of SAT from VAT (see figure 2.3 which is a CT scan from one of our study participants).

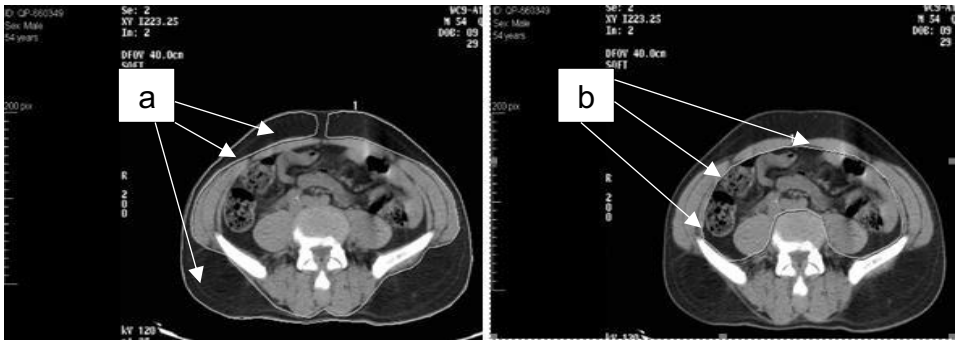

Figure 2.3 Manual delineation of a) SAT and b) VAT boundaries

The fat assessment software uses threshold based segmentation tools to identify fat (Sottier et al., 2013; Lee et al., 2015). Note that the specific threshold used may vary from one software package to another. Figure 2.4 is an example of a scan from one of the study participants indicating the VAT area in red and SAT area (deep SAT & superficial SAT together) in blue.

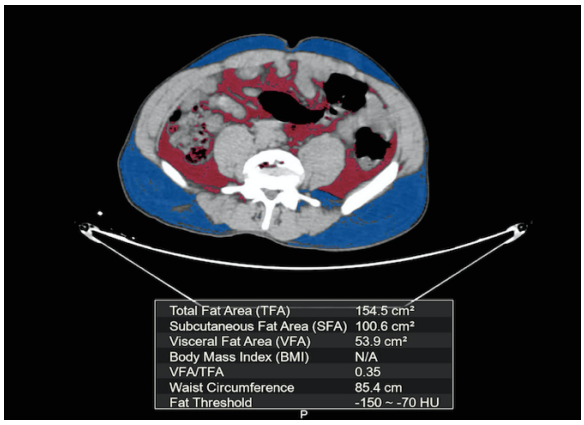

Figure 2.4 Software delineation of SAT (blue) VAT (red)

2.3.2.2 DXA for whole body and regional fat quantification

DXA is able to quantify whole body fat mass, lean mass and bone mineral mass accurately in a short time using a low radiation dose (Toombs et al., 2012). Obesity, overweight, diabetes and cirrhosis are amongst the main clinical applications of whole body DXA in adults (Albanese et al., 2003). DXA scanning, through the advancement in x-ray generation and detection technology, imaging protocols and image analysis protocols, has allowed researchers and clinicians to accurately study soft tissue composition.

The fundamental theory of DXA technology is that photon attenuation *in vivo* is an indication of tissue composition (Pietrobelli et al., 1996; Plank, 2005). This method is referred to as 'photon-absorptiometry' (Pietrobelli et al., 1996). Single photon absorptiometry was introduced in the early 1960's for quantifying appendicular bone mass, while dual photon absorptiometry (DXA) methods became clinically available ± 20 years later (Pietrobelli et al., 1996). Photon-absorptiometry systems require a photon source and a detector (Pietrobelli et al., 1996). Initial systems used a monoenergetic or dual energetic radionuclide source(s) (Pietrobelli et al., 1996). With advancements, the radionuclides were replaced by an x-ray source (Pietrobelli et al., 1996). DXA combines both a high and a low x-ray energy range to allow for maximum attenuation variances in bone and soft tissue to be captured. This is achieved by the use of energy changing structures or filters, as well as discriminating detectors (Laskey et al., 1996; Bontrager & Lampignano, 2010). The participant lies supine and is scanned in a rectilinear fashion, dividing the body into pixels with the photon attenuation measured at two different energies. The ratio of attenuation of the two energies is termed the *R* value (Plank, 2005). As mentioned previously, DXA is able to differentiate between 3 components based on the differential attenuation of fat, bone mineral and fat-free or 'lean' soft tissue. (Plank, 2005). Within the pixels, only 2 of these components can be differentially absorbed by the 2 photon energies (Plank, 2005). Thus the soft tissue (consisting mostly of water and organic compounds) and the bone differentially attenuate the photons (Plank, 2005). In the areas where bone is absent, a suitable calibration allows the distinction between fat and lean soft tissue by inference of the soft tissue over the bone (lean soft tissue) from the fat tissue (Kelly et al., 1998)(Plank, 2005). The algorithms to achieve these inferences vary between DXA manufacturers are not made public (Plank, 2005).

There has been much advancement in DXA technology with initial DXA systems using single x-ray pencil beams and detectors whilst the newer units incorporate a fan-beam assembly with a group of detectors, the latter allowing faster scanning speeds and higher image resolutions (Bontrager & Lampignano, 2010; Plank, 2005). Similarly, more recent work on DXA algorithms to estimate VAT have performed as well as a clinical CT measurement of VAT (Micklesfield et al., 2012).

Total body composition using DXA requires a suitably trained and experienced Radiographer to perform the scans. Various commercially available units can be used such Hologic, GE-Lunar and Norland (Plank, 2005). Typically, prior to all scans, all standard DXA quality control and equipment calibrations are performed based on the manufacturer's recommendations (Ball et al., 2004; Hangartner et al., 2013). No specific patient preparation is usually required. Participants undress, remove all radiopaque items and put on a cotton gown and are positioned as per the NHANES body composition manual as advocated by (Hangartner et al., 2013). Participants can be scanned using standard, thick or thin mode (appropriately chosen based on patient's body habitus). DXA-derived measures of body composition regions include six standard regions of interest (ROI), namely the whole body, the trunk defined by the lower border of the mandible and including the chest, abdomen, and pelvic triangle; the arm ROIs (right and left) are defined by a line bisecting the shoulder joint of the right and left arm; and the leg ROIs (right and left) are defined by a line bisecting the hip joint aligned with the iliac crest and pubis (Shepherd et al., 2012). Abdominal VAT cm^2 and SAT cm^2 are estimated in the android region (depicted by ‘’A" in figure 2.5, a DXA image of one of the study participants), a ROI automatically defined with a caudal limit placed on top of the iliac crests and its height set to 20% of the distance from the top of the iliac crest to the base of the skull as the cephalic limit (Kaul et al., 2012; Bosch et al., 2014), coinciding approximately with the 4^{th} lumbar vertebrae as indicated in Figure 2.5. The direct lateral SAT measured is used to estimate (using appropriate algorithms) the SAT in the anterior posterior dimension (due to the limited 2-directional plane of DXA) and then this is subtracted from the total abdominal tissue (TAT) to give the VAT estimate (Micklesfield et al., 2012; Schousboe et al., 2018). The gynoid region (denoted by "G" in figure 2.5) has its upper limit below the top of the iliac crests at a distance of 1.5 times the android height with the total height of the gynoid region being twice the height of the android ROI and the lateral borders being the arm cut lines (Stults-Kolehmainen et al., 2012).

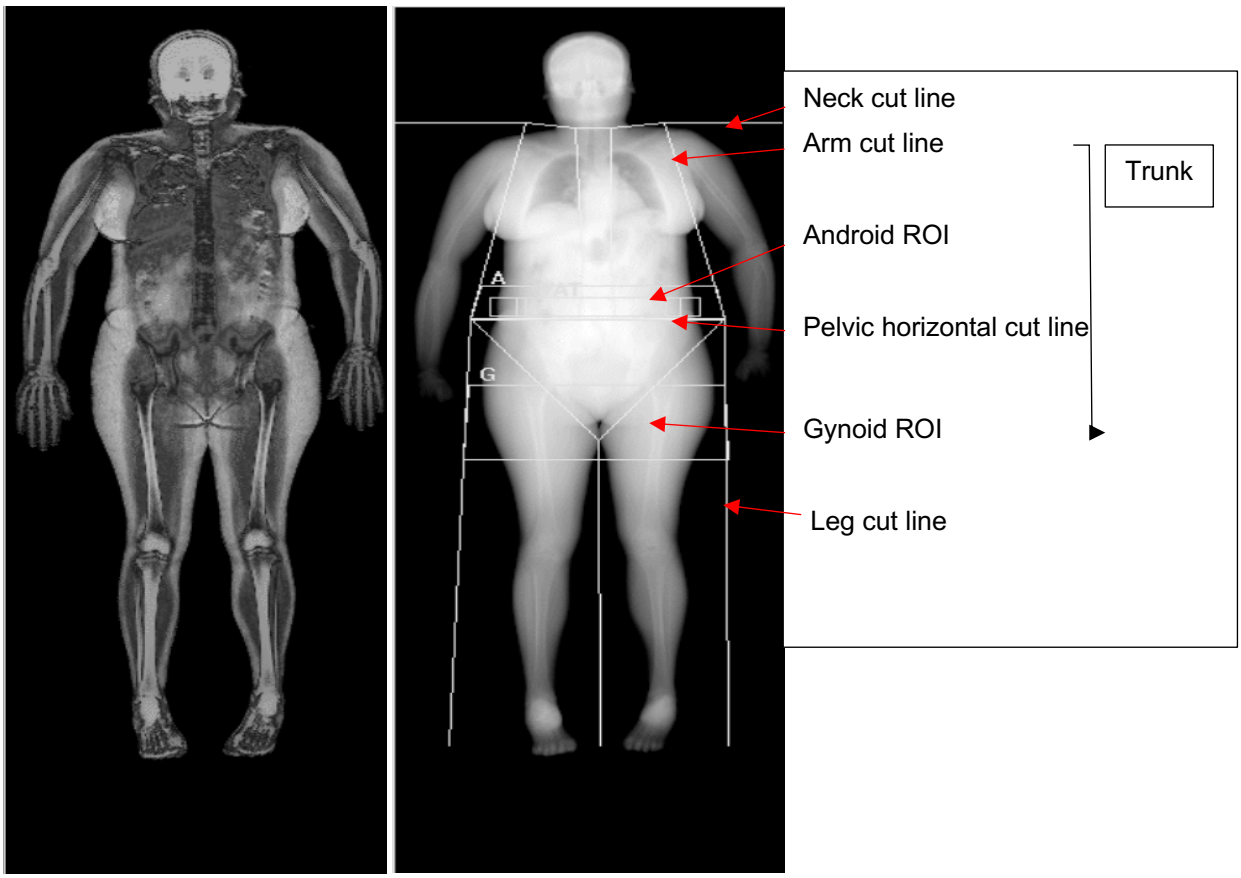

Figure 2.5 DXA scan with ROIs

The parameters indicated in Table 2.3 are calculated automatically by the software. The computer of the DXA unit then typically transports this data into a Microsoft access database. The data is then accessed in Microsoft excel and also transported into the statistical package. Table 2.3 below represents an example of the whole body data set produced by the DXA unit as per the NHANES data sets (Kelly et al., 2009).

Table 2.3 Typical body composition parameters automatically calculated by the DXA software (example of data from study participant)

Region	Fat mass (g)	Lean+BMC* (g)	Total (g)	% Fat
L Arm	1871	1926	3797	49.3
R Arm	1836	2111	3948	46.5
Trunk	16414	19023	35437	46.3
L Leg	4464	5475	9939	44.9
R Leg	4629	5744	10373	44.6
Subtotal	29214	34279	63493	46.0
Head	872	3830	4702	18.6
Total	30086	38108	68195	44.1
Android (A)	3049	3090	6140	49.7
Gynoid (G)	4897	5654	10551	46.4

**BMC Bone mineral content; L left; R right; %fat calculated as (fat mass divided by total mass)x100; FM fat mass)*

The measurement of VAT and SAT is taken in a 5cm ROI in the android region as indicated in figure 2.5. Table 2.4 below explains the quantification of VAT and SAT from the DXA whole body scan.

Table 2.4 DXA quantification of VAT and SAT

Tissue	Definition[#]	DXA report *
VAT	Fat that is deep inside the abdominal wall only (not extremities) and usually surrounds the organs. Includes the visceral adipose area just at the inner edge of the abdominal wall	VAT area: cross sectional area (cm^2) of fat inside the abdominal cavity
SAT	Superficial subcutaneous adipose tissue (the layer found between the skin and a fascial plane in the lower trunk and gluteal-thigh area) Deep subcutaneous adipose tissue (the layer found between the muscle fascia and a fascial plane in the lower trunk and gluteal-thigh areas)	SAT area: cross sectional area (cm^2) of fat outside the abdominal cavity Note DXA is unable to differentiate between sSAT and dSAT.

[#](Shen et al., 2003), *(Hologic, 2012)

2.4 Comparisons between anthropometry and regional depot body composition studies for CVD risk

Central adiposity, in particular VAT accumulation, poses the greatest risk for cardio-metabolic diseases. Typically WC is the accepted proxy of VAT and central adiposity and is used in the clinical diagnosis of MetS (Alberti et al., 2009). However, a major limitation of WC and other anthropometric indices such as BMI, hip circumference, WHtR and ABSI is the inability to distinguish between VAT and abdominal SAT (Wajchenberg, 2000; Bi et al., 2015). Although CT and MRI are the criterion imaging methods for adipose tissue quantification (Bi et al., 2015; Fosbøl & Zerahn, 2015), DXA algorithms have proved to be just as reliable (Kaul et al., 2012; Micklesfield et al., 2012). However, in LIMCs, access to expensive imaging modalities such and DXA and less so, CT and MRI, is limited and not viable for large scale screening. It is thus important to determine if these imaging modalities perform better than cheaper anthropometry measures in detecting participants at risk of cardio-metabolic diseases. Numerous studies have compared the discriminatory power of anthropometry and body composition studies using imaging modalities such as DXA, MRI and CT for detecting CVD risk. For example, the cross-sectional Netherlands epidemiology of obesity study involving mostly obese white middle-aged women indicated MRI-derived VAT was most closely associated with cardio-metabolic risk followed by WC and WHR (Snijder et al., 2005). In Japanese women, CT-derived VAT performed better than WC in predicting MetS (Hayashi et al., 2007). Furthermore, in a large cross-sectional study (Vasan et al., 2018) of nearly 5000 men and women from the Oxford biobank, conventional anthropometry underestimated the associations of regional adiposity with type-2 diabetes and CVD risk markers compared to

DXA. Within the SA context the discriminatory power of WC, WHtR compared to CT-derived measures of VAT in predicting MetS were similar in pre-menopausal black African and white women (Evans et al., 2011). While a WC≥80cm is one of the five clinical criteria for MetS diagnosis and an accepted proxy for VAT, various studies (Nicklas et al., 2003; Hayashi et al., 2007; Evans et al., 2011) have noted that cut points for diagnosing MetS other than WC, vary with age and ethnicity.

On the other hand, measures of total adiposity such a BMI and a body shape index (ABSI) appear not to be as closely related to cardio-metabolic risk (Amirabdollahian & Haghighatdoost, 2018). For example, several studies have shown weak correlations for ABSI in predicting MetS (Haghighatdoost et al., 2014; Behboudi-Gandevani et al., 2016). Likewise in a large cross sectional study in an Iranian population, BMI had the lowest AUC for predicting MetS when compared to other measures of central adiposity (Bener et al., 2013).

While comparisons between the discriminatory ability of anthropometry and DXA-derived body composition to identify individuals with cardio-metabolic risk has been done in other populations, no study has investigated the mixed-ancestry population of SA who present with high prevalence of obesity, MetS and type-2 diabetes. It is not known whether DXA, a more readily available imaging modality in a LMIC like SA, is able to provide more precise estimates of cardiovascular risk in relation to total and regional adiposity in mixed-ancestry SA's with high central adiposity rates.

2.5 Comparison between DXA and other gold standard imaging modalities for body composition

Limited studies have assessed the accuracy of DXA against criterion methods such as CT and MRI. For example in Asian Chinese men and women representing wide age and BMI ranges, DXA overestimated VAT volume compared to CT scanning by 263cm^3 (95% limits of agreement of -232-755 cm^3) (Lin et al., 2013). The study by Kaul et al., which compared the automated DXA method of VAT to contiguous CT in white American men and women over a range of BMIs and VAT volumes showed a combined bias of 56cm^3, however they suggest that the VAT algorithm may not be applicable to all ethnic groups and should be further investigated (Kaul et al., 2012). Similarly in overweight and obese middle-aged Kuwaiti men and women, DXA overestimated VAT volume compared to volumetric MRI scanning covering the same android region scanned by DXA by 79.7cm^3 and 48.6cm^3 in men and women respectively (±7% overall) (Mohammad et al., 2017). Notably in both these studies (Lin et al., 2013; Mohammad et al., 2017), the imprecision of DXA increased with increasing VAT levels. Similarly in the multi-ethnic Dallas heart study (mean age of cohort 44 year) where 75% of the participants were overweight or people with obesity, DXA overestimated VAT when compared

to single slice MRI by 0.09kg in men and 0.01kg in women (Neeland et al., 2016). Furthermore in a comparative study between single slice CT and DXA-derived VAT and SAT, in pre-menopausal American women over a range of BMI categories, DXA overestimated both VAT and SAT compared to CT (Bredella et al., 2013). Conversely, another study in white and black pre-menopausal women SA across a range of BMIs, a newly derived DXA algorithm performed as well as CT in measuring VAT (Micklesfield et al., 2012). Various factors could be responsible for these biases, such as inherent participant characteristics (e.g. amount of VAT present, age, BMI category and ethnicity) and methodological issues. For instance, Bredella et al., 2013 showed that measurement bias between DXA and CT was influenced by BMI category such that the largest percentage bias for VAT was seen in the anorexia category (46.8%) and the smallest in the overweight/obese category (23.5%) (Bredella et al., 2013). Similarly for SAT, DXA overestimated SAT by 60.9% in the anorexia category and 17.4% overweight/obese category (Bredella et al., 2013). Given the differential variances across BMI categories seen by Bredella et al, the authors suggest that DXA VAT measurements may be more useful in quantifying abdominal fat depots in overweight and obese individuals, however they recognise that there are sex and age related differences in DXA measurements which need further exploration (Bredella et al., 2013). Furthermore, the above studies (Lin et al., 2013; Mohammad et al., 2017) have shown that there was increasing bias between imaging modalities with increasing VAT amounts and thus the accuracy of DXA for example in post-menopausal women in different BMI categories (who are known to have increased VAT accumulation) (Krishnan et al., 2018) would warrant further investigation.

Methodological differences between imaging modalities are also thought to affect VAT and SAT bias. For example variations in anatomical site of measurement between modalities (Clasey et al., 1997; Micklesfield et al., 2012; Bredella et al., 2013), assumptions of VAT distribution (Thomas & Bell, 2003) being equal for all, variations in compartment sizes used for measurement (Mohammad et al., 2017) as well as the use of multi-slice scanning techniques instead of single slice techniques (Thomas & Bell, 2003) are proposed to influence bias.

Furthermore, ethnic variations in VAT and abdominal SAT are known. For example, for similar waist circumferences, ethnic differences in the amount of VAT and abdominal SAT have been observed (Goedecke et al., 2009; Katzmarzyk et al., 2010; Micklesfield et al., 2010; Keswell et al., 2016). Newly derived DXA algorithms have proved to be relatively accurate compared to CT in fat quantification in European American men and women (Kaul et al., 2012) and women of European and black African ancestry (Micklesfield et al., 2012), however these algorithms have not been tested on other African populations, specifically the mixed-ancestry population of South Africa.

2.6 The influence of sex and ethnicity on body fat distribution and cardio-metabolic risk

Obesity is a well-known risk factor for CVD and metabolic diseases (Lee et al., 2013; Lemos & Neeland, 2014; O'Neill & O'Driscoll, 2015), but the distribution of body fat may be a more important risk factor for cardio-metabolic diseases than generalised adiposity. For example, the meta-analysis of 40 observational studies on the associations of different adipose tissue depots with insulin resistance revealed the strongest correlate of insulin resistance to be VAT (Zhang et al., 2015). Furthermore, in the large cross-sectional study (Vasan et al., 2018) of nearly 5000 men and women from the Oxford Biobank, which compared DXA regional fat measurements and conventional anthropometry and their association with type-2 diabetes and CVD risk, DXA measured fat masses were strongly associated with type-2 diabetes and CVD risk factors. In that study, in terms of central adiposity, DXA-derived VAT showed an overall higher risk, while abdominal SAT showed a protective role except for insulin resistance in women**.** Similar findings were seen between the relationship between abdominal SAT and cardio-metabolic risk which was weaker than that of VAT as shown in multi-ethnic studies in men and women (McLaughlin et al., 2011; Neeland et al., 2013). Furthermore, greater central adiposity, in particular, VAT, in men confers higher insulin resistance (Geer & Shen, 2009), type-2 diabetes mellitus (Nordström et al., 2016) and adverse cardio-metabolic risk profiles probably because, in women, oestrogen regulates insulin sensitivity and female adipocytes are more insulin sensitive compared to those of males (Shi & Kumar, 2012).

On the other hand, the accumulation of lower body SAT (gluteofemoral obesity) was associated with lower cardio-metabolic risk (Pinnick et al., 2012; Karpe & Pinnick, 2015; Park et al., 2014). An example of this protective effect was also observed in a large sample of Asian men and women in that those with MetS had less lower body peripheral fat than those without MetS (Park et al., 2014). Furthermore, in both sexes, an increase in leg or gynoid fat mass was associated with a substantially lower risk of hypertriglyceridemia in participants from the large Oxford Biobank study (Vasan et al., 2018), however other studies have shown this protective effect of lower body SAT to favour women over men (Schorr et al., 2018). Arm fat has also be shown to have similar risk associations to central adiposity (Jensen, 2008), but in the Oxford Biobank study (Vasan et al., 2018) arm fat was protective in the men only.

Body fat distribution is sex and ethnic specific. For example women have more SAT and less VAT than men (Geer & Shen, 2009; Tchoukalova et al., 2010). Furthermore changes in body composition and body typology are evident in women transitioning into menopause (Sowers et al., 2007). Examples of these changes include an increase in fat mass and decrease in skeletal muscle mass and an increase in WC (Sowers et al., 2007). In women, after menopause particularly (Gurka et al., 2016), weight gain and increased central adiposity are

common (Karvonen-Gutierrez & Kim, 2016), translating to increased cardio-metabolic risk (Peer et al., 2014). Changes in body composition and fat distribution at menopause are primarily due to ovarian aging (Karvonen-Gutierrez & Kim, 2016) and oestrogen deficiency with resultant acceleration of CVD in woman after menopause (Carr, 2003). The prevalence of MetS is known to increase with menopause, possibly explained by the emergence of features such as increased central adiposity, a more atherogenic lipid profile and increased glucose and insulin levels (Carr, 2003).

Ethnic influences on body fat distribution have been reported in international studies such that Asian Indians have more total and central fat mass than their European and Black counterparts (Rush et al., 2007; Nazare et al., 2012; Eastwood et al., 2015). Black Africans however have less VAT but more abdominal SAT (Goedecke et al., 2009; Katzmarzyk et al., 2010; Micklesfield et al., 2010; Keswell et al., 2016), and greater gluteofemoral fat mass compared to European women (Keswell et al., 2016). The effects of ethnicity and associated cardio-metabolic risk are seen with African American women having a weaker association between VAT and blood pressure, triglyceride concentrations, high-density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) than European women, while African American men displayed a stronger association between VAT, triglycerides, low HDL-C and metabolic syndrome than their European counterparts (Liu et al., 2013).

Even though differences in body fat distribution, and associations with cardio-metabolic risk between black African, European and Asian women have been described in SA (Ali & Crowther, 2005; Goedecke et al., 2009; Crowther & Norris, 2012), there is a paucity of literature examining the mixed-ancestry population of SA, who present with a high prevalence of metabolic syndrome (62%) and type-2 diabetes mellitus (28.2%), placing this population at high risk for CVD (Erasmus et al., 2012).

2.7 Obesity and cardio-metabolic complications

Obesity is associated with adverse alterations in adipose tissue that predispose to metabolic dysregulation (Greenberg & Obin, 2006; Sam & Mazzone, 2014). In obese individuals, there is an accumulation of lipids in adipocytes which triggers cellular stress resulting in the recruitment of macrophages, production of proinflammatory molecules and reactive oxygen species (Gómez-Hernández et al., 2016). In addition, the lipid overflow theory has been proposed (Figure 2.6, adapted from Tchernof & Després, 2013) which starts with, a) a positive energy balance, b) saturation of expansion capacity or the inability of subcutaneous adipose tissue to expand resulting in c) a lipid overflow, d) deposition of lipids in around the viscera (VAT), the liver epi/pericardial and myocardial, muscle, renal sinus and pancreas, which is then linked to greater risk for insulin resistance and CVD. The mechanisms linking VAT

accumulation to metabolic complications involve the greater production of proinflammatory cytokines and the greater lipolytic action compared to SAT, with the resultant increase in cytokines and free fatty acid transfer to the hepatic portal system impacting on insulin sensitivity (Lee et al., 2013). In contrast, the lower body fat depot is seen as a 'metabolic sink', which traps excess free fatty acids (FFA) due to its increased lipoprotein lipase activity and lower lipolytic activity compared to the abdominal fat depot, thus protecting other tissues from lipid overflow and insulin resistance associated with ectopic lipotoxicity (Dulloo et al., 2010; Pinnick et al., 2012; Karpe & Pinnick, 2015).

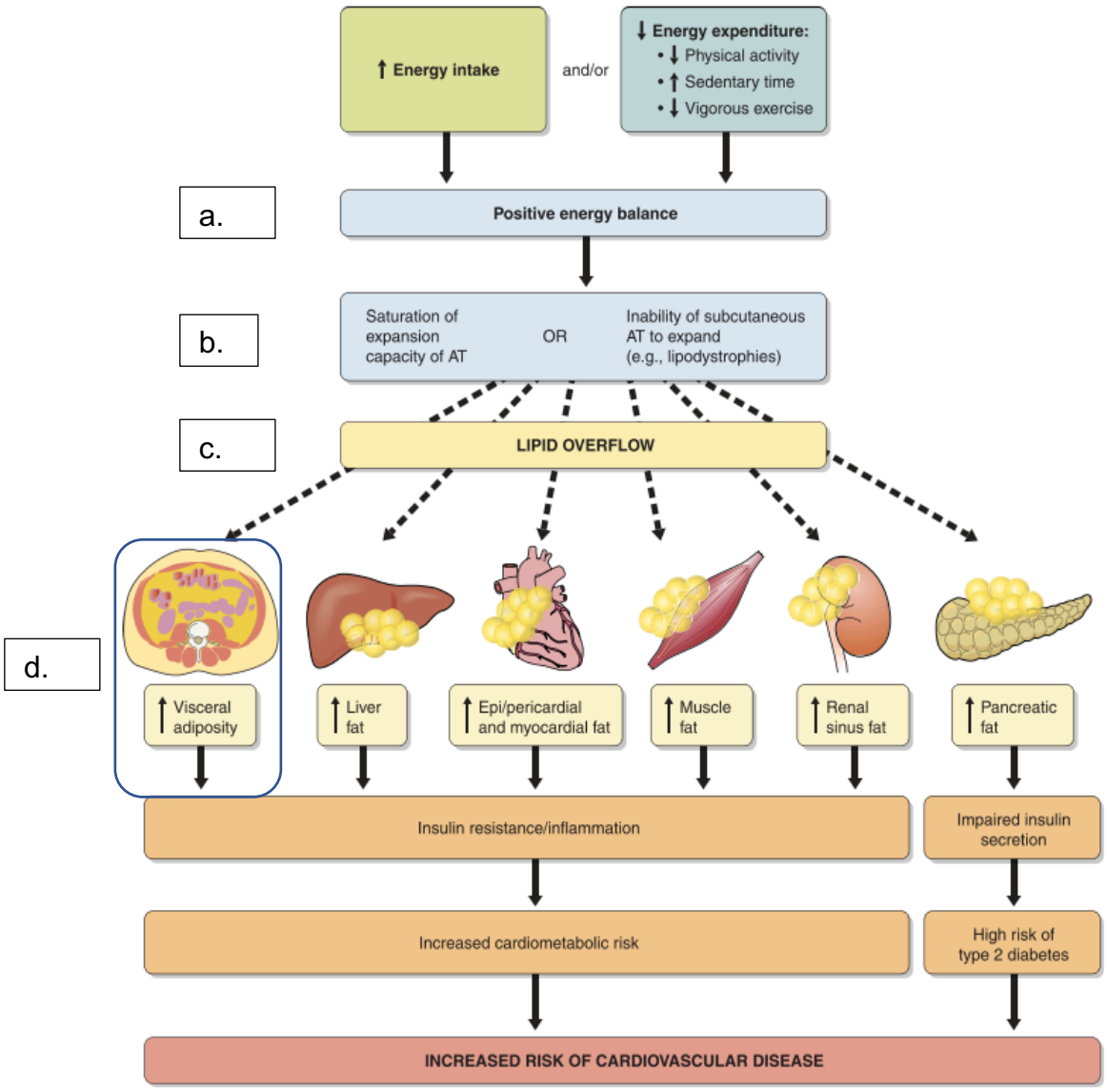

Figure 2.6 Working model of the relationship of excess VAT and cardio-metabolic risk
Adapted from (Tchernof & Després, 2013)

2.8 Significance of the research

Obesity is a well-known risk factor for CVD and metabolic diseases, however the distribution of body fat is a more important risk factor than generalised adiposity, with VAT accumulation

posing the highest risk irrespective of sex and age. Sex, age and ethnic differences however exist for VAT accumulation, as well as the relationship between intra-abdominal fat and cardio-metabolic risk. CT and MRI are the gold-standard imaging methods for VAT quantification, however in clinical practice anthropometry is used as a proxy for VAT. DXA has proven its accuracy for body composition in numerous population-based studies, at a substantially lower radiation dose than CT and is more readily available. However, in a resource poor setting such as SA, it is not known whether DXA is able to provide more precise estimates of CVD risk in relation to total and regional adiposity in mixed-ancestry SA's with high central adiposity rates.

2.9 Aims, objectives and hypotheses

The overall objective of this thesis was to validate DXA as a method of quantifying central adiposity, explore the relationship between body fat distribution and cardio-metabolic risk and determine the ability of DXA compared to anthropometry to identify participants with MetS. This overall objective was met by the following three aims and associated hypotheses:

1. To compare the agreement between DXA and the gold standard, CT, for abdominal fat quantification in a cross-sectional sample of 132 mixed-ancestry SA women across the three World Health Organisation BMI categories to determine its accuracy.
   H_0 DXA is able to accurately quantify abdominal fat compared to the gold-standard, CT, irrespective of BMI category.
   H_1 DXA is not able to accurately quantify abdominal fat compartments across BMI categories.

2. To investigate the relationship between body fat compartments and cardio-metabolic risk factors in mixed-ancestry SA women and a small sample of men, and explore the effects of menopausal status on these relationships in the women.

a) **H_0** Body compartments do not differentially affect cardio-metabolic risk.
   H_1 Cardio-metabolic risk is differentially influenced by body compartments.
b) **H_0** Menopause does not differentially affect cardio-metabolic risk.
   H_1 Menopause influences cardio-metabolic risk.

3. To compare the ability of DXA-derived VAT area and anthropometric measures of adiposity for diagnosing any 2 components of MetS (other than WC) in 204 women.
   H_0 There is no difference in the discriminatory power of DXA-derived VAT area and waist circumference in detecting any 2 components of MetS.
   H_1 DXA-derived VAT area is better at discriminating participants with any 2 components of MetS (other than waist circumference)

References

Albanese, C. V, Diessel, E. & Genant, H.K. 2003. Clinical applications of body composition measurements using DXA. *Journal of Clinical Densitometry*, 6(2): 75–85.

Alberti, K., Zimmet, P. & Shaw, J. 2006. Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. *Diabetic Medicine*, 23: 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. & Smith, S.C. 2009. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force. *Circulation*, 120: 1640–1645.

Ali, A.T. & Crowther, N.J. 2005. Body fat distribution and insulin resistance. *SAMJ South African medical journal*, 95(11): 878–880.

Amirabdollahian, F. & Haghighatdoost, F. 2018. Anthropometric Indicators of Adiposity Related to Body Weight and Body Shape as Cardiometabolic Risk Predictors in British Young Adults : Superiority of Waist-to-Height Ratio. *Journal of Obesity*: 1–15.

Ashwell, M. & Gibson, S. 2009. Waist to height ratio is a simple and effective obesity screening tool for cardiovascular risk factors: Analysis of data from the British national diet and nutrition survey of adults aged 19-64 years. *Obesity Facts*, 2(2): 97–103.

Baleta, A. & Mitchell, F. 2014. Country in Focus: diabetes and obesity in South Africa. *The Lancet Diabetes & Endocrinology*, 2(9): 687–688.

Ball, S.D., Altena, T.S. & Swan, P.D. 2004. Comparison of anthropometry to DXA: a new prediction equation for men. *European journal of clinical nutrition*, 58: 1525–1531.

Behboudi-Gandevani, S., Ramezani Tehrani, F., Cheraghi, L. & Azizi, F. 2016. Could “a body shape index” and “waist to height ratio” predict insulin resistance and metabolic syndrome in polycystic ovary syndrome? *European Journal of Obstetrics Gynecology and Reproductive Biology*, 205: 110–114.

Bener, A., Yousafzai, M.T., Darwish, S., Al-Hamaq, A.O.A.A., Nasralla, E.A. & Abdul-Ghani, M. 2013. Obesity index that better predict metabolic syndrome: Body mass index, waist circumference, waist hip ratio, or waist height ratio. *Journal of Obesity, Hindawi Publishing*, 2013: 1–9.

Bi, X., Seabolt, L., Shibao, C., Buchowski, M., Kang, H., Keil, C.D., Tyree, R. & Silver, H.J. 2015. DXA-measured visceral adipose tissue predicts impaired glucose tolerance and metabolic syndrome in obese Caucasian and African-American women. *European journal of clinical nutrition*, 69(3): 329–336.

Biadgilign, S., Mgutshini, T., Haile, D., Gebremichael, B., Moges, Y. & Tilahun, K. 2017. Epidemiology of obesity and overweight in sub-Saharan Africa: A protocol for a systematic review and meta-analysis. *BMJ Open*, 7(11): 7–10.

Bontrager, K; Lampignano, J. 2010. *Textbook of radiographic positioning and related anatomy.* 7th ed. Missouri: Mosby.

Borga, M., West, J., Bell, J.D., Harvey, N.C., Romu, T., Heymsfield, S.B. & Leinhard, O.D. 2018. Advanced body composition assessment: From body mass index to body composition profiling. *Journal of Investigative Medicine*, 66(5): 887–895.

Bosch, T.A., Dengel, D.R., Kelly, A.S., Sinaiko, A.R., Moran, A. & Steinberger, J. 2014. Visceral adipose tissue measured by DXA correlates with measurement by CT and is associated with cardiometabolic risk factors in children. *Pediatric obesity*, 10(21): 172–179.

Bradshaw, D., Norman, R., Pieterse, D. & Levitt, N.S. 2007. Estimating the burden of disease attributable to diabetes in South Africa in 2000. *SAMJ South African medical journal*, 97(7): 700–706.

Bredella, M.A., Ghomi, R.H., Thomas, B.J., Torriani, M., Brick, D.J., Gerweck, A. V, Misra, M., Klibanski, A. & Miller, K.K. 2010. Comparison of DXA and CT in the assessment of body composition in premenopausal women with obesity and anorexia nervosa. *Obesity (Silver Spring, Md.)*, 18(11): 2227–2233.

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

Carr, M.C. 2003. The emergence of the metabolic syndrome with menopause. *Journal of Clinical Endocrinology and Metabolism*, 88(6): 2404–2411.
Charlton, K.E., Levitt, N.S. & Lombard, C.J. 1997. The prevalence of diabetes mellitus and associated risk factors in elderly coloured South Africans. *SAMJ South African medical journal*, 87(3 Suppl): 364–367.
Chooi, Y.C., Ding, C. & Magkos, F. 2019. The epidemiology of obesity. *Metabolism Clinical and Experimental*, 92: 6–10.
Clasey, J.L., Bouchard, C., Wideman, L., Kanaley, J., Teates, C.D., Thorner, M.O., Hartman, M.L. & Weltman, A. 1997. The influence of anatomical boundaries, age, and sex on the assessment of abdominal visceral fat. *Obesity research*, 5(5): 395–401.
Crowther, N.J. & 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).
Dulloo, A.G., Jacquet, J., Solinas, G., Montani, J.P. & Schutz, Y. 2010. Body composition phenotypes in pathways to obesity and the metabolic syndrome. *International Journal of Obesity*, 34(S2): S4–S17.
Duren, D.L., Ph, D., Sherwood, R.J., Ph, D., Czerwinski, S.A., Ph, D., Lee, M., Ph, D., Choh, A.C., Ph, D., Siervogel, R.M., Ph, D., Chumlea, W.C. & Ph, D. 2008. Body Composition Methods : Comparisons and Interpretation. *Journal of Diabetes Science and Technology*, 2(6): 1139–1146.
Eastwood, S. V., Tillin, T., Dehbi, H.M., Wright, A., Forouhi, N.G., Godsland, I., Whincup, P., Sattar, N., Hughes, A.D. & Chaturvedi, N. 2015. Ethnic differences in associations between fat deposition and incident diabetes and underlying mechanisms: The SABRE study. *Obesity*, 23(3): 699–706.
Eckel, R.H., Alberti, K.G.M.M., Grundy, S.M. & Zimmet, P.Z. 2005. The metabolic syndrome. *Lancet*, 365: 1415–1428.
Erasmus, R.T., Soita, D.J., Hassan, M.S., Blanco-Blanco, E., Vergotine, Z., Kegne, A.P. & Matsha, T.E. 2012. High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: baseline data of a study in Bellville, Cape Town. *South African medical journal*, 102(11): 841–844.
Evans, J., Micklesfield, L., Jennings, C., Levitt, N.S., Lambert, E. V., Olsson, T. & Goedecke, J.H. 2011. Diagnostic Ability of Obesity Measures to Identify Metabolic Risk Factors in South African Women. *Metabolic Syndrome and Related Disorders*, 9(5): 353–360.
Fosbøl, M.O. & Zerahn, B. 2015. Contemporary methods of body composition measurement. *Clinical Physiology and Functional Imaging*, 35(2): 81–97.
GBD 2015 obesity collaborators. 2017. Health effects of overweight and obesity in 195 countries over 25 years. *New England Journal of Medicine*, 377(1): 13–27.
Geer, E.B. & Shen, W. 2009. Gender differences in insulin resistance, body composition, and energy balance. *Gender medicine*, 6(Suppl 1): 60–75.
Goedecke, J.H., Dave, J.A., Faulenbach, M. V, Utzschneider, K.M., Lambert, E. V, West, S., Collins, M., Olsson, T., Walker, B.R., Seckl, J.R., Kahn, S.E. & Levitt, N.S. 2009. Insulin response in relation to insulin sensitivity: an appropriate beta-cell response in black South African women. *Diabetes Care*, 32(5): 860–865.
Gómez-Hernández, A., Beneit, N., Díaz-Castroverde, S. & Escribano, Ó. 2016. Differential Role of Adipose Tissues in Obesity and Related Metabolic and Vascular Complications. *International Journal of Endocrinology, Hindawi Publishing*, 2016: 1–15.
Gradmark, A.M.I., Rydh, A., Renström, F., De Lucia-Rolfe, E., Sleigh, A., Nordström, P., Brage, S. & Franks, P.W. 2010. Computed tomography-based validation of abdominal adiposity measurements from ultrasonography, dual-energy X-ray absorptiometry and anthropometry. *British Journal of Nutrition*, 104(4): 582–588.
Greenberg, A.S. & Obin, M.S. 2006. Obesity and the role of adipose tissue in inflammation and metabolism. *American Journal of Clinical Nutrition*, 83(2): 461–465.
Grundy, S.M., Brewer, H.B., Cleeman, J.I., Smith, S.C. & Lenfant, C. 2004. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation*, 109(3): 433–438.
Gurka, M.J., Vishnu, A., Santen, R.J. & DeBoer, M.D. 2016. Progression of Metabolic

Syndrome Severity During the Menopausal Transition. *Journal of the American Heart Association*, 5(8): e003609.
Haghighatdoost, F., Sarrafzadegan, N., Mohammadifard, N., Asgary, S., Boshtam, M. & Azadbakht, L. 2014. Assessing body shape index as a risk predictor for cardiovascular diseases and metabolic syndrome among Iranian adults. *Nutrition*, 30(6): 636–644.
Hangartner, T.N., Warner, S., Braillon, P., Jankowski, L. & Shepherd, J. 2013. The Official Positions of the International Society for Clinical Densitometry: Acquisition of Dual-Energy X-Ray Absorptiometry Body Composition and Considerations Regarding Analysis and Repeatability of Measures. *Journal of Clinical Densitometry*, 16(4): 520–536.
Hayashi, T., Boyko, E.J., McNeely, M.J., Leonetti, D.L., Kahn, S.E. & Fujimoto, W.Y. 2007. Minimum waist and visceral fat values for identifying Japanese Americans at risk for the metabolic syndrome. *Diabetes Care*, 30(1): 120–127.
Hologic. 2012. *Hologic APEX 3.4/4.0 Data Dictionary*. Bedford, USA: Hologic Inc.
Hsieh, S.D., Yoshinaga, H. & Muto, T. 2003. Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. *International Journal of Obesity*, 27(5): 610–616.
IDF Diabetes atlas. 2019. IDF DIABETES ATLAS, 9th edition. In International Diabetes Federation: 1–164.
Irlbeck, T., Massaro, J.M., Bamberg, F., Donnell, C.J.O., Hoffmann, U. & 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): 781–787.
Jensen, M.D. 2008. Role of Body Fat Distribution and the Metabolic Complications of Obesity. *The Journal of Clinical Endocrinology & Metabolism*, 93(11): S57–S63.
Karpe, F. & Pinnick, K.E. 2015. Biology of upper-body and lower-body adipose tissue—link to whole-body phenotypes. *Nature Reviews Endocrinology*, 11(2): 90–100.
Karvonen-Gutierrez, C. & Kim, C. 2016. Association of Mid-Life Changes in Body Size, Body Composition and Obesity Status with the Menopausal Transition. *Healthcare*, 4(42): 1–16.
Katzmarzyk, P.T., Bray, G. a, Greenway, F.L., Johnson, W.D., Jr, R.L.N., Ravussin, E., Ryan, D.H., Smith, S.R. & Bouchard, C. 2010. Racial differences in abdominal depot – specific adiposity in white and African American adults. *American journal of clinical nutrition*, 91: 7–15.
Kaul, S., Rothney, M.P., Peters, D.M., Wacker, W.K., Davis, C.E., Shapiro, M.D. & Ergun, D.L. 2012. Dual-energy X-ray absorptiometry for quantification of visceral fat. *Obesity*, 20(6): 1313–8.
Kelly, T.L., Berger, N. & Richardson, T.L. 1998. DXA body composition: theory and practice. *Applied Radiation and Isotopes*, 49(5–6): 511–513.
Kelly, T.L., Wilson, K.E. & Heymsfield, S.B. 2009. Dual energy X-ray absorptiometry body composition reference values from NHANES. *PLoS ONE*, 4(9, September): e7038.
Keswell, D., Tootla, M. & Goedecke, J.H. 2016. Associations between body fat distribution, insulin resistance and dyslipidaemia in black and white South African women. *Cardiovascular Journal of Africa*, 27(May): 1–7.
Kim, Y.J., Park, J.W., Kim, J.W., Park, C.-S., Gonzalez, J.P.S., Lee, S.H., Kim, K.G. & Oh, J.H. 2016. Computerized Automated Quantification of Subcutaneous and Visceral Adipose Tissue From Computed Tomography Scans: Development and Validation Study. *JMIR medical informatics*, 4(1): e2, 1–10.
Klein, S., Allison, D., Heymsfield, S. & Kelley, D. 2007. Waist circumference and cardiometabolic risk: A consensus statement from shaping America's health: Association for weight management and obesity prevention; NAASO, the Obesity society; the American society for nutrition; and the American diabetes associat. *Obesity*, 15(5 May): 1061–1067.
Krakauer, N.Y. & Krakauer, J.C. 2012. A New Body Shape Index Predicts Mortality Hazard Independently of Body Mass Index. *PLoS ONE*, 7(7): e39504.
Krishnan, K.C., Mehrabian, M. & Lusis, A.J. 2018. Sex differences in metabolism and cardiometabolic disorders. *Curr Opin Lipidol*, 29(5): 404–410.

Laskey, M.A.N.N., Phil, D. & Dunn, M.R.C. 1996. Dual-energy X-Ray absorptiometry and body composition. *Nutrition*, 12: 45–51.
Lee, D.H., Park, Kyeong Seon, Ahn, S., Ku, E.J., Jung, K.Y., Kim, Y.J., Kim, K.M., Moon, J.H., Choi, S.H., Park, Kyong Soo, Jang, H.C. & 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(12): 10513–10524.
Lee, M.J., Wu, Y. & Fried, S.K. 2013. Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications. *Molecular Aspects of Medicine*, 34(1): 1–11.
Lemos, J.A. De & Neeland, I.J. 2014. Separating the VAT from the FAT New Insights Into the Cardiometabolic Risks of Obesity. *JACC: Cardiovascular Imaging*, 7(12): 1236–1238.
Levitt, N.S., Steyn, K., Lambert, E. V., Reagon, G., Lombard, C.J., Fourie, J.M., Rossouw, K. & Hoffman, M. 1999. Modifiable risk factors for Type 2 diabetes mellitus in a peri-urban community in South Africa. *Diabetic Medicine*, 16(11): 946–950.
Lin, H., Yan, H., Rao, S., Xia, M., Zhou, Q., Xu, H., Rothney, M.P., Xia, Y., Wacker, W.K., Ergun, D.L., Zeng, M. & Gao, X. 2013. Quantification of visceral adipose tissue using lunar dual-energy X-ray absorptiometry in Asian Chinese. *Obesity*, 21(10): 2112–2117.
Liu, J., Hickson, D.A., Musani, S.K., Talegawkar, S.A., Carithers, T.C., Tucker, K.L., Fox, C.S. & Taylor, H.A. 2013. Dietary Patterns, Abdominal Visceral Adipose Tissue, and Cardiometabolic Risk Factors in African Americans: The Jackson Heart Study. *Obesity*, 21(3, March): 644–651.
Matsha, T.E., Hassan, M.S., Hon, G.M., Soita, D.J., Kengne, A.P. & Erasmus, R.T. 2013. Derivation and validation of a waist circumference optimal cutoff for diagnosing metabolic syndrome in a South African mixed ancestry population. *International Journal of Cardiology*, 168(3): 2954–2955.
Matsha, T.E., Hassan, M.S., Kidd, M. & Erasmus, R.T. 2012. The 30-year cardiovascular risk profile of South Africans with diagnosed diabetes, undiagnosed diabetes, pre-diabetes or normoglycaemia: the Bellville, South Africa pilot study. *Cardiovascular journal of Africa*, 23(1): 5–11.
McLaughlin, T., Lamendola, C., Liu, A. & Abbasi, F. 2011. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. *Journal of Clinical Endocrinology and Metabolism*, 96(11): 1756–1760.
Micklesfield, L.K., Evans, J., Norris, S.A., Lambert, E. V, Jennings, C., Joffe, Y., Levitt, N.S. & Goedecke, J.H. 2010. Dual-energy X-ray absorptiometry and anthropometric estimates of visceral fat in Black and White South African Women. *Obesity (Silver Spring, Md.)*, 18(3): 619–624.
Micklesfield, L.K., Goedecke, J.H., Punyanitya, M., Wilson, K.E. & Kelly, T.L. 2012. Dual-Energy X-Ray Performs as Well as Clinical Computed Tomography for the Measurement of Visceral Fat. *Obesity*, 20(5, May): 1109–1114.
Millar, S.R., Perry, I.J. & Phillips, C.M. 2015. Assessing cardiometabolic risk in middle-aged adults using body mass index and waist-height ratio: Are two indices better than one? A cross-sectional study. *Diabetology and Metabolic Syndrome*, 7(1): 1–11.
Mohammad, A., Rolfe, E.D.L., Sleigh, A., Kivisild, T., Behbehani, K., Wareham, N.J., Brage, S. & Mohammad, T. 2017. Validity of visceral adiposity estimates from DXA against MRI in Kuwaiti men and women. *Nature Publishing Group*, 7(1): e238-5.
Motala, A.A., Pirie, F.J., Gouws, E., Amod, A. & Omar, M.A.K. 2003. High incidence of Type 2 diabetes mellitus in South African Indians: A 10-year follow-up study. *Diabetic Medicine*, 20(1): 23–30.
National Department of Health (NDoH). 2019. *National Department of Health (NDoH), Statistics South Africa, South African Medical Research Council (SAMRC), and ICF. 2019.* Pretoria, South Africa.
Nazare, J.A., Smith, J.D., Borel, A.L., Haffner, S.M., Balkau, B., Ross, R., Massien, C., Alméras, N. & Després, J.P. 2012. Ethnic influences on the relations between abdominal subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: The international study of prediction of intra-abdominal adiposity and its relationship with cardiometabolic risk/intra-. *American Journal of Clinical Nutrition*, 96(4):

714–726.
NCD-RicS-Africa Working Group. 2017. Trends in obesity and diabetes across Africa from 1980 to 2014 : an analysis of pooled population-based studies. *International Journal of Epidemiology*, (October): 1–12.
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. & De 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): 439–447.
Neeland, I.J., Grundy, S.M., Li, X., Adams-Huet, B. & Vega, G.L. 2016. Comparison of visceral fat mass measurement by dual-X-ray absorptiometry and magnetic resonance imaging in a multiethnic cohort: the Dallas Heart Study. *Nutrition & Diabetes*, 6(7): e221.
Nicklas, B.J., Penninx, B.W.J.H., Ryan, A.S., Berman, D.M., Lynch, N.A. & Dennis, K.E. 2003. Visceral Adipose Tissue Cutoffs Associated With Metabolic Risk Factors for Coronary Heart Disease in Women. *Diabetes Care*, 26(5): 1413–1420.
Nogilana, B., Bradshaw, D., Pillay-van Wyk, V., Msemburi, W., Somdyala, N., Joubert, J. & Groenewald, P. 2016. Persistent burden from non-communicable diseases in South Africa needs strong action. *SAMJ South African medical journal*, 106(5): 436–437.
Nordström, A., Hadrévi, J., Olsson, T., Franks, P.W. & Nordström, P. 2016. Higher prevalence of type 2 diabetes in men than in women is associated with differences in visceral fat mass. *Journal of Clinical Endocrinology and Metabolism*, 101(10): 3740–3746.
Nuttall, F.Q. 2015. Body mass index: Obesity, BMI, and health: A critical review. *Nutrition Today*, 50(3): 117–128.
O'Neill, S. & O'Driscoll, L. 2015. Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. *Obesity Reviews*, 16(1): 1–12.
Park, S.Y., Kwon, K.Y., Kim, J.H., Choi, H.H., Han, K.H. & Han, J.H. 2014. Association between appendicular fat mass and metabolic risk factors. *Korean Journal of Family Medicine*, 35(4): 182–189.
Peer, N., Kengne, A.-P., Motala, A.A. & Mbanya, J.C. 2014. IDF Diabetes Atlas. Diabetes in the Africa region: An update. *Diabetes Research and Clinical Practice*, 103: 197–205.
Pi-sunyer, F.X. 2004. The Epidemiology of Central Fat Distribution in Relation to Disease. *NutriRev*, 62(7): S120–S126.
Piernas, C., Hernández-Morante, J.J., Canteras, M., Zamora, S. & 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): 887–897.
Pietrobelli, A., Formica, C., Wang, Z. & Heymsfield, S.B. 1996. Dual-energy X-ray absorptiometry body composition model: Review of physical concepts. *American Journal of Physiology - Endocrinology and Metabolism*, 271(6 34-6): 941–951.
Pinnick, K.E., Neville, M.J., Fielding, B.A., Frayn, K.N., Karpe, F. & Hodson, L. 2012. Gluteofemoral adipose tissue plays a major role in production of the lipokine palmitoleate in humans. *Diabetes*, 61(6): 1399–1403.
Plank, L.D. 2005. Dual-energy X-ray absorptiometry and body composition. *Current opinion in clinical nutrition and metabolic care*, 8(3): 305–9.
Rush, E.C., Goedecke, J.H., Jennings, C., Micklesfield, L., Dugas, L., Lambert, E. V & Plank, L.D. 2007. BMI , fat and muscle differences in urban women of five ethnicities from two countries. *International Journal of Obesity*, 31: 1232–1239.
Sam, S. & Mazzone, T. 2014. Adipose tissue changes in obesity and the impact on metabolic function. *Translational Research*, 164(4): 284–292.
Schorr, M., Dichtel, L.E., Gerweck, A. V., Valera, R.D., Torriani, M., Miller, K.K. & Bredella, M.A. 2018. Sex differences in body composition and association with cardiometabolic risk. *Biology of Sex Differences*, 9(1): 1–10.
Schousboe, J.T., Kats, A.M., Langsetmo, L., Vo, T.N., Taylor, B.C., Schwartz, A. V., Cawthon, P.M., Lewis, C.E., Barrett-Connor, E., Hoffman, A.R., Orwoll, E.S. & Ensrud, K.E. 2018. Central Obesity and Visceral Adipose Tissue Are Not Associated With Incident Atherosclerotic Cardiovascular Disease Events in Older Men. *Journal of the American Heart Association*, 7(16).

Schutte, A.E. 2019. Urgency for South Africa to prioritise cardiovascular disease management. *The Lancet Global Health*, 7(2): e177–e178.
Schweitzer, L., Geisler, C., Pourhassan, M., Braun, W., Glüer, C.C., Bosy-Westphal, A. & Müller, M.J. 2015. What is the best reference site for a single MRI slice to assess whole body skeletal muscle and adipose tissue volumes in healthy adults? *American Journal of Clinical Nutrition*, 102(1): 58–65.
Shen, W., Chen, J., Gantz, M., Velasquez, G., Punyanitya, M. & Heymsfield, S.B. 2012. A single MRI slice does not accurately predict visceral and subcutaneous adipose tissue changes during weight loss. *Obesity*, 20(12): 2458–2463.
Shen, W., Wang, Z., Punyanita, M., Lei, J., Sinav, A., Kral, J.G., Imielinska, C., Ross, R. & Heymsfield, S.B. 2003. Adipose tissue quantification by imaging methods: a proposed classification. *Obesity research*, 11(1): 5–16.
Shepherd, J.A., Fan, B., Lu, Y., Wu, X.P., Wacker, W.K., Ergun, D.L. & Levine, M.A. 2012. A multinational study to develop universal standardization of whole-body bone density and composition using GE Healthcare Lunar and Hologic DXA systems. *Journal of Bone and Mineral Research*, 27(10): 2208–2216.
Shi, H. & Kumar, S. 2012. Sex Differences in Obesity-Related Glucose Intolerance and Insulin Resistance. In *Glucose Tolerance*. : http://www.intechopen.com/books: Intech open access book publisher: 37–66.
Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Reddy, P., Parker, W., Hoosain, E., Naidoo, P., Hongoro, C., Mchiza, Z., Steyn, N., Dwane, N., Makoae, M., Maluleke, T., Ramlagan, S., Zungu, N., Evans, M., Jacobs, L. & Faber, M. 2014. *The South African National Health and Nutrition Examination Survey, 2012 SANHANES-1*. Cape Town: HSRC Press, Cape Town.
Shoelson, S., Herrero, L. & Naaz, A. 2007. Obesity, inflammation, and insulin resitance. *Gastroenterology*, 132(6): 2169–2180.
Snijder, M.B., Visser, M., Dekker, J.M., Goodpaster, B.H., Harris, T.B., Kritchevsky, S.B., De Rekeneire, N., Kanaya, A.M., Newman, A.B., Tylavsky, F.A. & Seidell, J.C. 2005. Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study. *Diabetologia*, 48(2): 301–308.
Sottier, D., Petit, J.M., Guiu, S., Hamza, S., Benhamiche, H., Hillon, P., Cercueil, J.P., Krausé, D. & Guiu, B. 2013. Quantification of the visceral and subcutaneous fat by computed tomography: Interobserver correlation of a single slice technique. *Diagnostic and Interventional Imaging*, 94(9).
Sowers, M.F., Zheng, H., Tomey, K., Karvonen-Gutierrez, C., Jannausch, M., Li, X., Yosef, M. & Symons, J. 2007. Changes in body composition in women over six years at midlife: Ovarian and chronological aging. *Journal of Clinical Endocrinology and Metabolism*, 92(3): 895–901.
StatsSA. 2019. Midyear Population Estimate 2019. *Population Estimates*, (July).
Stults-Kolehmainen, M. a., Stanforth, P.R. & Bartholomew, J.B. 2012. Fat in Android, Trunk, and Peripheral Regions Varies by Ethnicity and Race in College Aged Women. *Obesity*, 20(3): 660–665.
Sumner, A.E., Farmer, N.M., Tulloch-Reid, M.K., Sebring, N.G., Yanovski, J.A., Reynolds, J.C., Boston, R.C. & Premkumar, A. 2002. Sex differences in visceral adipose tissue volume among African Americans. *American Journal of Clinical Nutrition*, 76: 975–979.
Tchernof, A. & Després, J.-P. 2013. Pathophysiology of human visceral obesity: an update. *Physiological reviews*, 93(1): 359–404.
Tchoukalova, Y.D., Koutsari, C., Votruba, S.B., Tchkonia, T., Giorgadze, N., Thomou, T., Kirkland, J.L. & Jensen, M.D. 2010. Sex- and depot-dependent differences in adipogenesis in normal-weight humans. *Obesity (Silver Spring, Md.)*, 18(10): 1875–1880.
Thomas, E.L. & Bell, J.D. 2003. Influence of undersampling on magnetic resonance imaging measurements of intra-abdominal adipose tissue. *International Journal of Obesity*, 27(2): 211–218.
Tong, Y., Udupa, J.K. & Torigian, D. a. 2014. Optimization of abdominal fat quantification on CT imaging through use of standardized anatomic space: A novel approach. *Medical*

physics, 41(6): 063501.
Toombs, R.J., Ducher, G., Shepherd, J.A. & Souza, M.J. De. 2012. The Impact of Recent Technological Advances on the Trueness and Precision of DXA to Assess Body Composition. *Obesity*, 20(1).
Tulloch-Reid, M.K., Hanson, R.L., Sebring, N.G., Reynolds, J.C., Premkumar, A., Genovese, D.J. & Sumner, A.E. 2004. Both subcutaneous and visceral adipose tissue correlate highly with insulin resistance in african americans. *Obesity research*, 12(8): 1352–1359.
Vasan, S.K., Osmond, C., Canoy, D., Christodoulides, C., Neville, M.J., Di Gravio, C., Fall, C.H.D. & Karpe, F. 2018. Comparison of regional fat measurements by dual-energy X-ray absorptiometry and conventional anthropometry and their association with markers of diabetes and cardiovascular disease risk. *International Journal of Obesity*, 42(4): 850–857.
Vatanparast, H., Chilibeck, P.D., Cornish, S.M., Little, J.P., Paus-Jenssen, L.S., Case, A.M. & Biem, H.J. 2009. DXA-derived abdominal fat mass, waist circumference, and blood lipids in postmenopausal women. *Obesity (Silver Spring, Md.)*, 17(8): 1635–1640.
Wajchenberg, B.L. 2000. Subcutaneous and Visceral Adipose Tissue : Their relation to the metabolic syndrome. *Endocrine Reviews*, 21(6): 697–738.
Wang, Z.M., Pierson, R.N. & Heymsfield, S.B. 1992. The five-level model: A new approach to organizing body-composition research. *American Journal of Clinical Nutrition*, 56(1): 19–28.
Wells, J.C.K. 2012. Ethnic variability in adiposity, thrifty phenotypes and cardiometabolic risk: Addressing the full range of ethnicity, including those of mixed ethnicity. *Obesity Reviews*, 13(Suppl. 2): 14–29.
WHO expert committee. 1995. *WHO Physical status: the use and interpretation of anthropometry*.
de Wit, E., Delport, W., Rugamika, C.E., Meintjes, A., Möller, M., Van Helden, P.D., Seoighe, C. & Hoal, E.G. 2010. Genome-wide analysis of the structure of the South African Coloured Population in the Western Cape. *Human Genetics*, 128(2): 145–153.
World Health Organisation. 2014. *Global status report on noncommunicable diseases*.
Yoshizumi, T., Nakamura, T., Yamane, M., Islam, a H., Menju, M., Yamasaki, K., Arai, T., Kotani, K., Funahashi, T., Yamashita, S. & Matsuzawa, Y. 1999. Abdominal fat: standardized technique for measurement at CT. *Radiology*, 211(1): 283–286.
Zhang, M., Hu, T., Zhang, S. & Zhou, L. 2015. Associations of Different Adipose Tissue Depots with Insulin Resistance: A Systematic Review and Meta-analysis of Observational Studies. *Scientific reports*, 5(18495): 1–6.

CHAPTER THREE: COMPARISON OF SINGLE SLICE CT AND DXA-DERIVED MEASURES OF CENTRAL ADIPOSITY IN SOUTH AFRICAN WOMEN.

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Introduction

Accumulation of central adiposity, in particular visceral adipose tissue (VAT) is associated with a high risk of cardiovascular disease (CVD) and type-2 diabetes mellitus (1,2). At similar waist circumferences, ethnic differences in VAT and subcutaneous adipose tissue (SAT) have been reported amongst different ethnic groups (3). Accordingly, it is imperative that accurate, safe and cost-effective methods are used for the quantification of body fat distribution. Computerised tomography (CT) and magnetic resonance imaging (MRI) are considered the gold standard for quantifying and comparing regional fat distribution in research settings, however, both these modalities are costly, not easily accessible, and in the case of CT, expose participants to radiation (2,4).

In contrast, dual-energy x-ray absorptiometry (DXA) is able to measure total and regional body fat at a substantially lower radiation dose than CT (5). The latest DXA technology uses algorithms to segment abdominal fat (AF) into visceral fat (VAT) and subcutaneous fat (SAT) (6). This DXA-derived technology proved to be better at predicting CT-derived VAT than previous DXA-derived android measures of body fat (7). A limited number of studies have reported the performance of this new DXA technology against gold standard imaging. For example, DXA was shown to overestimate VAT in overweight middle-aged Kuwaiti and Asian women when compared to MRI- and CT- derived VAT, respectively (8,9). In the multi-ethnic Dallas heart study where 75% of the participants were overweight or obese, at higher levels of VAT, DXA overestimated VAT when compared to MRI (10). Based on these studies, it appears that the precision of DXA to quantify VAT and SAT may be influenced by BMI and the amount of VAT and SAT present in an individual. There is therefore the need to further evaluate the accuracy of DXA-VAT, especially in high risk populations, such as the mixed-ancestry population of South Africa who present with a high prevalence of obesity, metabolic syndrome and type-2 diabetes (11). Thus, the main aim of this study was to assess the validity of DXA-derived VAT against the gold standard, CT across a range of BMI categories in mixed ancestry women.

Methods and procedures

Participants

The study sample included 132 self-described mixed ancestry women who volunteered for CT and DXA scans as part of the Cape Town Vascular and Metabolic Health (VMH) study described previously (12). Inclusion criteria were adults aged 20 years and older, self-described ethnicity being mixed ancestry. Participants were excluded if they were pregnant or acutely ill. Participants provided written consent and the study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). Ethical approval

was obtained from the Human Research Ethics Committees of the Cape Peninsula University of Technology and Stellenbosch University (respectively, NHREC:REC-230 408-014, CPUT/HWS-REC 2015/H03 and N14/01/003). The CT and DXA scans took place within the same week.

Measurements

Anthropometric measurements included body weight, height and BMI which are described in detail previously (12). Participants were grouped according to the World Health Organisation (WHO) BMI categories, namely normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥30 kg/m^2).

DXA protocol

Total body composition was acquired by one suitably trained and experienced Radiographer at the approved research site using a Hologic Discovery W DXA whole body scanner configured with software version 12.1 (Hologic, Bedford, MA). Prior to all scans, standard DXA quality control and equipment calibrations were performed based on the manufacturer's recommendations (13,14). Undressed participants with no radiopaque items, put on a cotton gown and were positioned as per the NHANES body composition manual according to the official positions of the international society for clinical densitometry (14). Participants were scanned using standard, thick or thin mode (appropriately chosen based on participants' body habitus). Abdominal VAT and SAT areas were measured in the android region, a region of interest (ROI) automatically defined with a caudal limit placed on top of the iliac crests and its height set to 20% of the distance from the top of the iliac crest to the base of the skull as the cephalic limit (6,15), coinciding approximately with the 4^{th} lumbar vertebrae. The whole body DXA datasets from the National Health and Nutrition Examination Survey (NHANES 1999-2004) (16–18) was used for the development of the algorithm to estimate the VAT and SAT areas. The algorithm is proprietary to the DXA manufacturer (19) and was previously validated against single slice CT-derived VAT at the level of L4-5 in a sample of normal-weight and obese white and black SA women (7). The algorithm has not been validated in predominantly older, obese mixed- ancestry women who present with high prevalence of central obesity (11).

CT protocol

A suitably trained and experienced radiographer performed the CT scans. Prior to all scans, all standard CT quality control and equipment calibrations were performed based on the manufacturer's recommendations. A scout image was taken from the lower border of the xiphisternum to the symphysis pubis and a single axial scan was performed at the level of L4-L5 intervertebral disc space, as this level correlated well with the total VAT volume in previous studies (20–22). A GE 16 slice Lightspeed CT scanner was used, with sequential scanning

mode as previously described (23). A tube voltage of 120kv and automatic mAs and 5mm slice thickness, field of view = 40cm, image reconstruction matrix of 512x512 was used. The window settings were: width = 350 HU; Level= 50HU.

The images were transported to a CT workstation and the adipose tissue software, Vitrea Core (Version 6.4.4083.268, Minnetonka, MN, USA) was applied. SAT was separated from VAT by a manually drawn line using a cursor which demarcated the abdominal muscular wall. SAT was defined as the layer between the skin and the muscles of the abdominal wall, while the VAT area was defined by tracing around the inner margin of the abdominal musculature (21). This was done by the two dedicated CT radiographers and then checked by the radiologist. The fat assessment software uses threshold based segmentation tools, using an adipose threshold of -150 to -70 HU.

Statistical analysis

Data were analysed using SPSS® version 25 (Armonk, NY: IBM Corp.), STATA® version 14.2 (STATA corporation, Texas, USA) and Microsoft excel, version 2013. Categorical variables are presented as frequencies and percentages, while continuous variables are presented as mean ± standard deviation (s.d.) for normally distributed variables and median and 25^{th}-75^{th} percentiles for skewed variables. Data was tested for normality using the Shapiro-Wilk test. The sample was split into three groups based on the WHO BMI categories, namely normal weight, overweight and obese. Group comparisons were made using one-way ANOVA for normally distributed data and Kruskal-Wallis for non-normally distributed data. Pearson correlation coefficients were used to estimate the correlation between DXA- and CT- derived VAT and SAT areas. Regression analysis was performed to test if the relationship between DXA- and CT-derived VAT and SAT areas were influenced by BMI category (DXA * BMI category). The paired-sample t-test was used to assess differences between the means of DXA and CT VAT and SAT areas. Bland-Altman analysis was used to assess the agreement between the two methods of VAT and SAT measurement. α was set at 0.05.

Results

Body composition and body fat distribution of the participants according to BMI categories

The body composition of the participants, according to BMI categories are shown in table 1. The median age of the participants was 55 (45-64) years, while the median BMI was 32.5 (28.7-37.8) kg/m^2. Of the 132 participants, 92 (69.7%) were classified as obese, 20 (15.2%) as overweight and 20 (15.2%) as normal weight. The normal-weight participants were younger than the overweight and obese (p=0.048). The CT and DXR derived VAT and SAT

measurements differed between the normal-weight, overweight and obese groups (all ≤0.0001).

Correlations between DXA and CT-derived body composition measurements

DXA and CT- derived measurements of abdominal VAT and SAT were significantly correlated in the overall sample (r=0.872, p<0.001 and r=0.966, p<0.001 respectively) and within the three BMI categories (Supplementary file, Figure S1). We explored the effect of BMI category on the relationship between DXA- and CT-derived VAT and SAT measures (DXA * BMI category interaction), and showed that BMI category did not modify this relationship (p ≥ 0.266 and p ≥ 0.210 for VAT and SAT, respectively).

Agreement between DXA and CT abdominal VAT and SAT measurements

Table 2 represents the mean differences (cm^2 and %) and confidence intervals between DXA- and CT-derived measures of VAT and SAT. DXA consistently acquired higher values for both VAT and SAT in the overall sample and within the BMI categories, with the mean difference of both narrowing in the overweight and obese BMI categories.

The Bland-Altman analysis in the overall sample indicated significant heteroscedasticity and proportional bias such that the mean of the differences between the modalities increased with increasing VAT (r=0.457, p≤0.0001) and decreased with increasing SAT (r= -0.440, p≤0.0001) (Figure 1). In the normal weight group (Supplementary file, Figure S2), the mean difference increased with increasing VAT (r=0.605, p=0.005), while remaining constant with increasing SAT (r=0.056, p=0.816). In the overweight and obese BMI categories, the Bland-Altman plots for VAT in overweight category (Supplementary file, Figure S3) and obese category (Figure 2) were homoscedastic, indicating a fixed bias, whereas there was significant heteroscedasticity for SAT (p=0.02 and p≤0.0001 respectively) (Supplementary file, Figure S3 & Figure 2), such that the difference between DXA and CT narrowed with increasing SAT area.

Discussion

Although there was a significant positive correlation between DXA- and CT-derived VAT and SAT measures, DXA overestimated VAT and to a lesser extent, abdominal SAT, in predominantly obese middle-aged mixed ancestry women. Notably, the agreement between the two modalities varied between the adipose tissue depots and BMI categories. With increasing VAT area, the difference between the two modalities increased, whereas with increasing abdominal SAT area, the difference between the two modalities decreased. Further, the differential bias between depots was also dependent on the BMI category, such that within

increasing BMI categories, the variance between the two modalities was constant for VAT, whereas the bias for SAT decreased. Although DXA overestimated VAT by nearly 90% in both the overweight and obese categories, the bias was homoscedastic in the obese category, implying that this technique may be applicable in body composition research in obese people who are particularly at risk of cardio-metabolic complications from VAT.

We showed that DXA overestimated VAT and SAT compared to CT-derived measures, a trend found in similar studies in overweight and obese premenopausal women (24) and overweight middle-aged Asian Chinese women (9). The extent of the bias for VAT was influenced by the amount of VAT present such that as the VAT increased, so did the bias, a trend seen in similar studies (8–10,24). BMI category also influenced the bias such that although DXA measured higher amounts of VAT and SAT in all the BMI groups, the largest mean difference was observed in the normal-weight BMI category (105% for VAT) and the smallest in the obese category (12% for SAT). Although the greater bias in the normal-weight and overweight BMI categories compared to the obese BMI category could have been explained by the small sample in these categories, similar trends have been observed in other studies (24). The study of Bredella et al (24) showed a 33.5% and 23.5% bias for VAT, while the bias for SAT was 37.9 % and 17.4 % for the normal-weight and overweight/obese categories, respectively. A possible explanation for the greater bias observed in our study compared to that of Bredella et al (24) may be due to this population having a propensity for central obesity (11) with 90% of this sample having a WC >80cm. Additionally the majority of our sample were post-menopausal women in whom VAT accumulation is known to be high (25).

Another plausible reason for DXA overestimating both VAT and SAT in our study could be that the anatomical level at which the two imaging modalities measure VAT and SAT are not exactly the same, a limitation noted previously (7)(24). In this study, the single slice CT scan was performed at the level of the L4-L5 intervertebral disc space, while the inferior border of the 5cm wide DXA ROI is placed at L4, being limited by bone pixels from the iliac crests (7,26). Additionally, the size of the compartment and whether the measurement is a cross-sectional area or volume may influence results. For example, the study by Mohammad et al (8) compared MRI VAT volumes from the identical android defined region used by DXA and found that DXA overestimated VAT in both men and women by only 7%. Although single slice imaging methods are frequently used for body composition assessment in research studies, these method are likely be imprecise for abdominal VAT quantification due to inaccuracies in slice position and the assumption that VAT distribution is the same in all individuals (27). The authors posit that only multi-slice CT or MRI will give precise results (27), however recent studies have shown that single slice MRI at the level of L3 (28) and 5-10 cm superior to L4-L5 (29) to be the best compromise sites for VAT quantification.

We postulate that the overestimation of VAT by DXA in our study may also be explained by the amount and position of VAT and SAT present. Whereas VAT can be directly measured by CT, the same is not true for the limited two-directional projection of DXA-derived VAT as SAT lies above and below the VAT. The SAT measured with DXA is modelled from the SAT measured laterally and this is then subtracted from the total abdominal tissue to give the VAT estimate which may be prone to error (7,30), particularly at high SAT areas. The majority of our sample were obese, having large amounts of abdominal SAT (median: 458 cm^2 measured by single slice CT), which could therefore have affected the bias. Indeed, we showed an inverse relationship between the bias related to VAT and SAT (Figure 2A&B). Further, abdominal SAT is more prone to movement than VAT, especially with change in posture. It is plausible that the SAT moves laterally as the subject lies supine for the scan, resulting in a larger lateral SAT area and thus directly affecting the modelled anterior to posterior VAT amount. This lateral movement of SAT would explain the reason for the decrease in measurement bias of SAT as the amount of SAT increased in our study. Mohammed et al.(8) cites a possible reason for the substantial overestimation of DXA VAT, particularly at higher VAT levels in their study being due to the diminished ability of tissue differentiation in larger individuals due to increased attenuation of the two x-ray wavelengths, which we suggest could be relevant to our findings. Finally, the overestimation of VAT observed in our study could be explained by respiratory state during scan acquisition. For example, the study by Lin et al. (9) in overweight Asian Chinese women of similar age to our sample found that DXA moderately overestimated CT VAT volume. The authors attributed this bias to VAT movement during breath-holding for the CT scan acquisition. This variation in measurement may explain the DXA bias in our study as the DXA scan was acquired during gentle breathing while the single slice CT scan was acquired during arrested inspiration.

To our knowledge, this is the first study to investigate the accuracy of DXA-derived VAT in mixed-ancestry women of South Africa who are known to have high central adiposity (11). Areas for future study would be to use a larger more representative sample and include men who are known to have higher VAT/SAT ratios. A limitation of the study was the small sample size in the normal-weight and overweight BMI categories, making meaningful comparisons between these groups difficult. Only one DXA and one single slice CT scan was performed and future research should include repeat measurements on the same participants to determine precision of the measurements (31–33). Estimates of body composition have been shown to vary by scanner make, model and software; therefore these results may not be generalisable to all DXA scanners (34). Additionally, ensuring that compartment size is the same when comparing modalities may improve precision and reduce the measurement bias. In terms of agreement between the two methods, setting of a *priori* clinical criteria as to what

the acceptable limits of agreement would be and then testing these would establish whether one method can replace the other (33,35).

In conclusion, DXA overestimated VAT and to a lesser extent abdominal SAT in a sample of women with a high prevalence of central obesity. With an increase in VAT area, the difference between the two modalities increased, whereas when abdominal SAT area increased, the difference between the two modalities decreased. Within increasing BMI categories, the variance between the two modalities was constant for VAT, whereas the bias for SAT decreased. Notably although the bias for VAT was large, it was homoscedastic in the obese BMI category, an indication that DXA may be valid for body composition studies in obese people. Further validation studies are needed in a more representative sample, as well as in males.

Supplementary information is available at EJCN's website.

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Conflict of interest

The authors state that they have no conflict of interest.

Contribution statement

FED: data analysis and interpretation, preparation of the first draft and approval of final draft
TEM: conception and design, acquisition and interpretation of data, revision for important intellectual content and approval of final draft
RTE: conception and design, revision for important intellectual content and approval of final draft
SI: revision for important intellectual content and approval of final draft
APK: conception and design, data analysis and interpretation of data, revision for important intellectual content and approval of final draft
JHG: data analysis and interpretation, revision for important intellectual content and approval of final draft

Data access, responsibility and analysis

FED, APK, TEM and JHG, had access to the data in the study and TEM, APK and JHG take responsibility for the integrity of the data and accuracy of the data analysis.

Availability of data and material:

The datasets used and/or analysed during the current study are available from the PI (TEM), Vascular and Metabolic Health Study on reasonable request.

Disclaimers

None

Acknowledgments

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Table 1:Body composition and abdominal fat parameters of study participants

	All	Normal-weight (18.5-24.9 kg/m^2)	Overweight (25-29.9 kg/m^2)	Obesity group (≥30 kg/m^2)	*p*-value
N (%)	132	20 (15.2%)	20 (15.2%)	92 (69.6%)	
Age (years)	55 (45-64)	45 (30-61)^{c}	54.5 (48-64)	57 (45-65)^{c}	0.048
Height (cm)	1.56 (6.1)	1.58 (5.8)	1.56 (5.3)	1.56 (6.3)	0.401
Weight (kg)	80.2 (68-93)	56 (53-59)^{C}	68 (65-72.3)^{C}	86.1 (78.1-101.1)^{AB}	≤0.001
Waist circumference (cm)	101 (15.1)	77.6 (7.7)^{ABC}	91.6 (5.7)^{ABC}	108 (11)^{ABC}	≤0.001
BMI (kg/m^2)	32.5 (28.7-37.8)	23.2 (21.3-23.9)^{AC}	28.2 (26.9-29.5)^{BC}	36.4 (32.2-39.7)^{ABC}	≤0.001
Fat free mass (kg)	41.3 (6.9)	33.7 (3.7)^{B}	37.6 (4.6)^{C}	43.8 (6.2)^{AB}	≤0.001
Body fat (%)	43.4 (7.2)	33.1 (6)^{BC}	39.1 (3.6)^{AC}	46.5 (5.1)^{AB}	≤0.001
CT VAT (cm^2)	99.3 (71.4-148.4)	37.9 (16.6-63.8)^{aA}	87.6 (60-112.1)^{bB}	119 (85.1-159.1)^{Cc}	≤0.001
DXA VAT (cm^2)	185.9 (76.5)	74.4 (39.8)^{ABC}	147.0 (48.6)^{ABC}	218.6 (58.8)^{ABC}	≤0.001
CT SAT (cm^2)	399 (160.1)	182 (69)^{AC}	282.4 (72.3)^{aC}	458 (378.3-558.2)^{ABC}	≤0.001
DXA SAT (cm^2)	453.7 (138.6)	246.2 (66.6)^{ABC}	365.6 (50.3)^{ABC}	517.9 (104.9)^{ABC}	≤0.001

Values are mean (standard deviation) or median (25th-75th percentile).

BMI (WHO classification) body mass index; CT VAT Computerized Tomography derived Visceral Adipose Tissue; DXA VAT Dual x-ray absorptiometry derived Visceral Adipose Tissue; CT SAT Computerized Tomography derived Subcutaneous Adipose Tissue; DXA SAT Dual x-ray absorptiometry derived Subcutaneous Adipose Tissue.

p<0.05 a- Normal weight; b- overweight; c- obese, p≤0.001 A-Normal weight; B-overweight; C-obese

Group comparisons by one-way analysis of variance using Bonferroni post hoc test or Kruskal-Wallis test with pairwise comparison.

Table 2: Mean differences of DXA and CT derived abdominal VAT and SAT

	All (*n*=132)	**Normal-weight (18.5-24.9 kg/m^2) (*n*=20)**	**Overweight (25-29.9 kg/m^2) (*n*=20)**	**Obesity group (≥30 kg/m^2) (*n*=92)**
VAT	75.33** (68.84-81.82) cm^2 90.9 (79.1-103.0) %	33.16** (24.15-42.16) cm^2 105.3 (73.5-137.1) %	56.44**(46.42-66.45) cm^2 85 (56.7-114.2) %	88.6** (81.4-95.9) cm^2 89.1 (74.4-103.7)%
SAT	54.66** (47.06-62.26) cm^2 20.7 (16.7-24.7) %	64.66**(52.74-76.57) cm^2 44.5 (30.6-58.3) %	83.19**(65.11-101.28) cm^2 35.9 (21.6-50.2) %	46.3** (36.8-55.8) cm^2 12.2 (9.6-14.8) %

*Using paired-sample t-test; values expressed in absolute values (cm^2) mean difference (95% confidence interval; % mean difference (95% confidence interval) and **p=≤0.0001.*

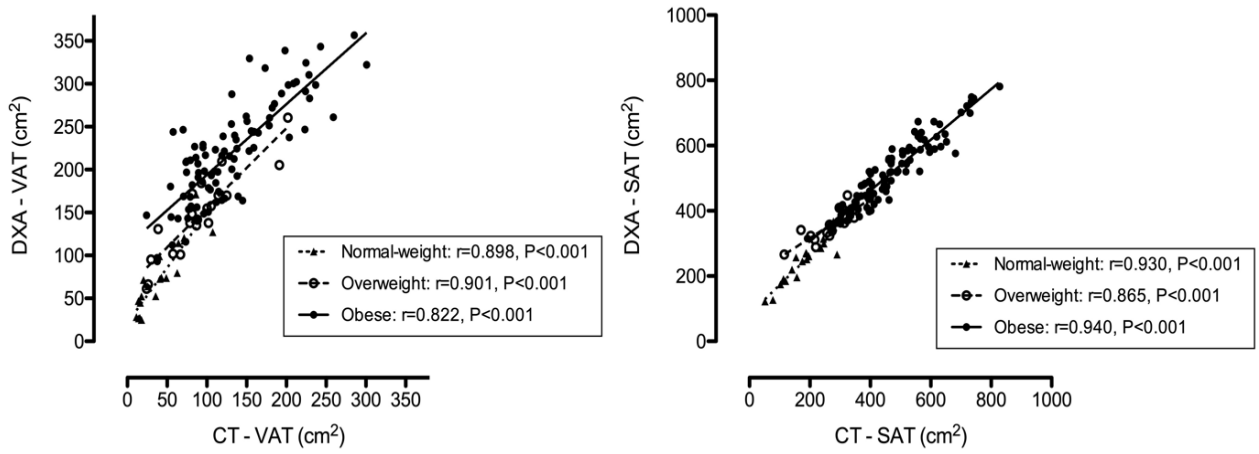


Figure S1 Correlations analysis between DXA and CT derived measurements of abdominal (A) Visceral adipose tissue (VAT); B) Subcutaneous adipose tissue (SAT) for normal weight, overweight and Participants with obesity.

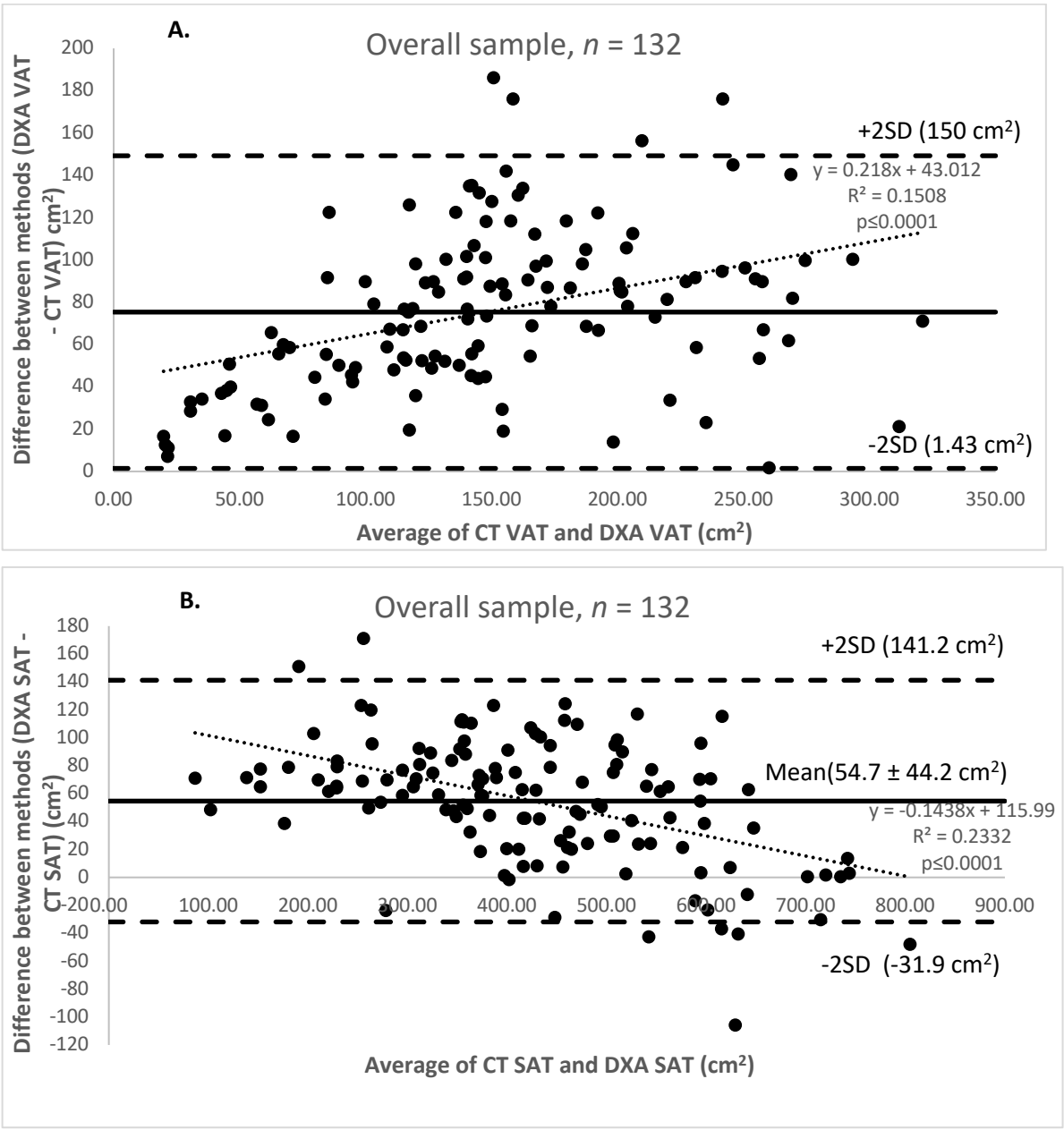


Figure 2 Bland-Altman analysis overall sample: CT and DXA VAT (A); CT and DXA SAT (B)

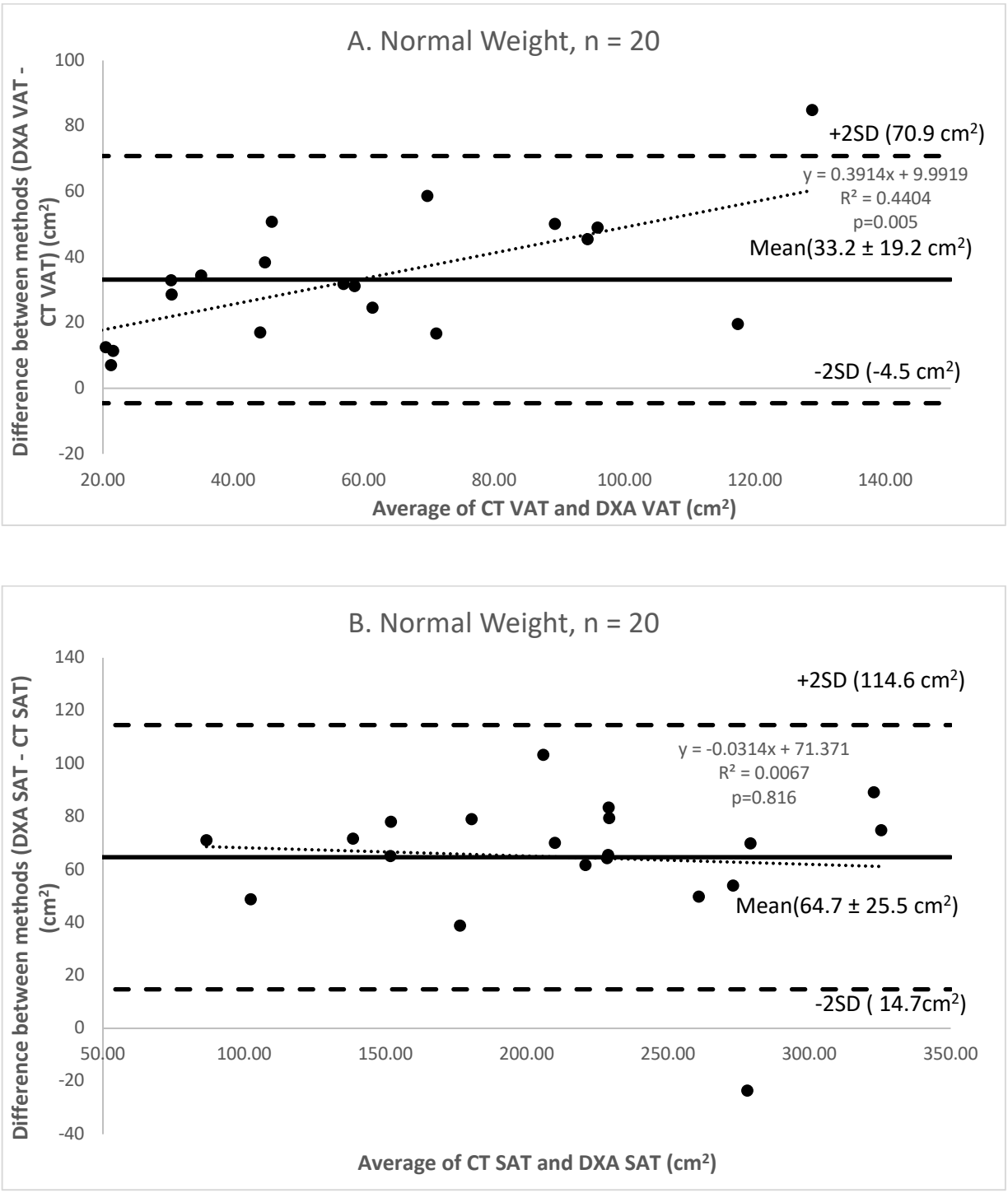
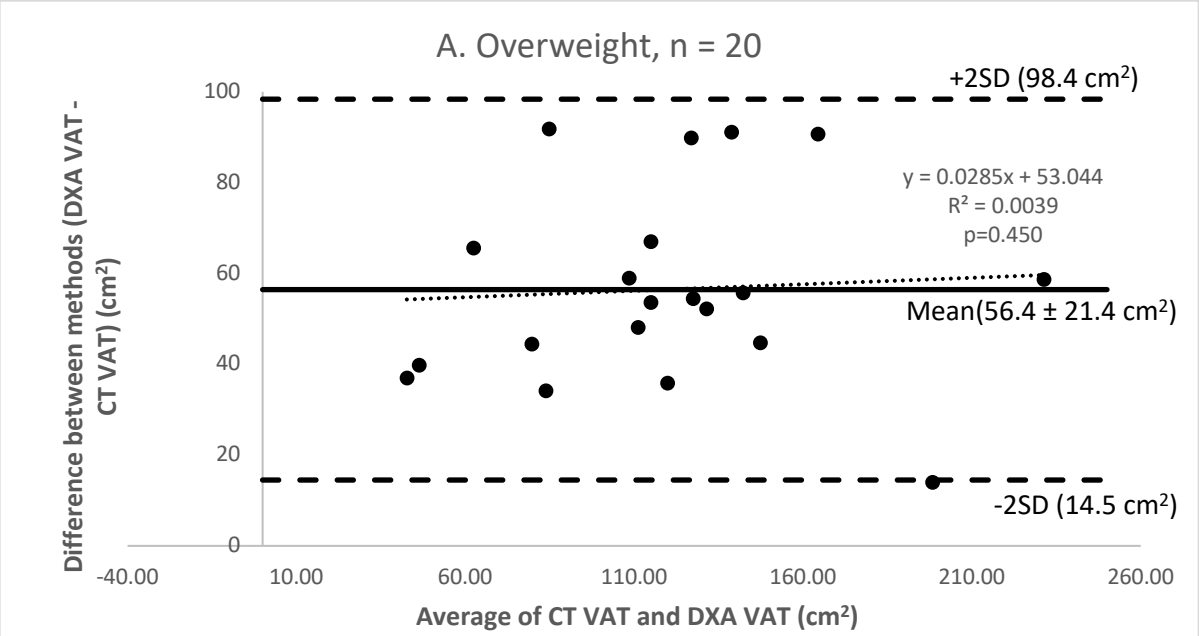


Figure 3 Bland-Altman analysis normal weight group: CT and DXA VAT (A); CT and DXA SAT (B)



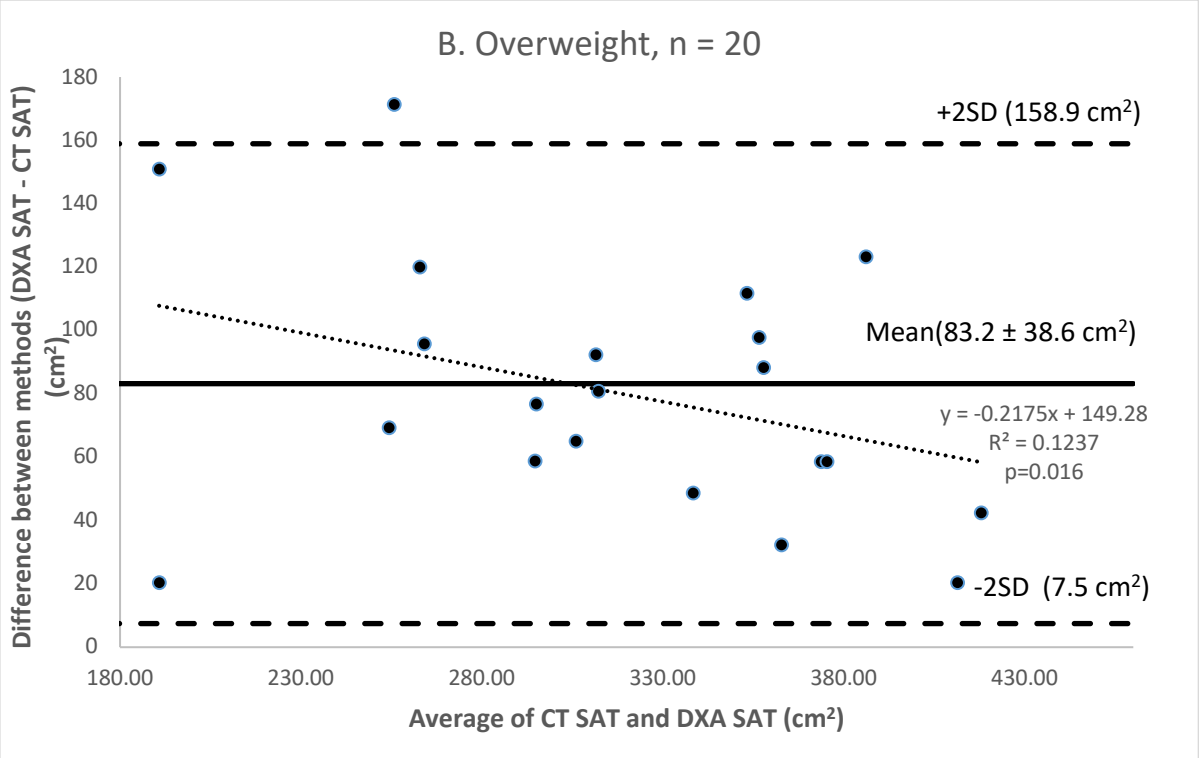
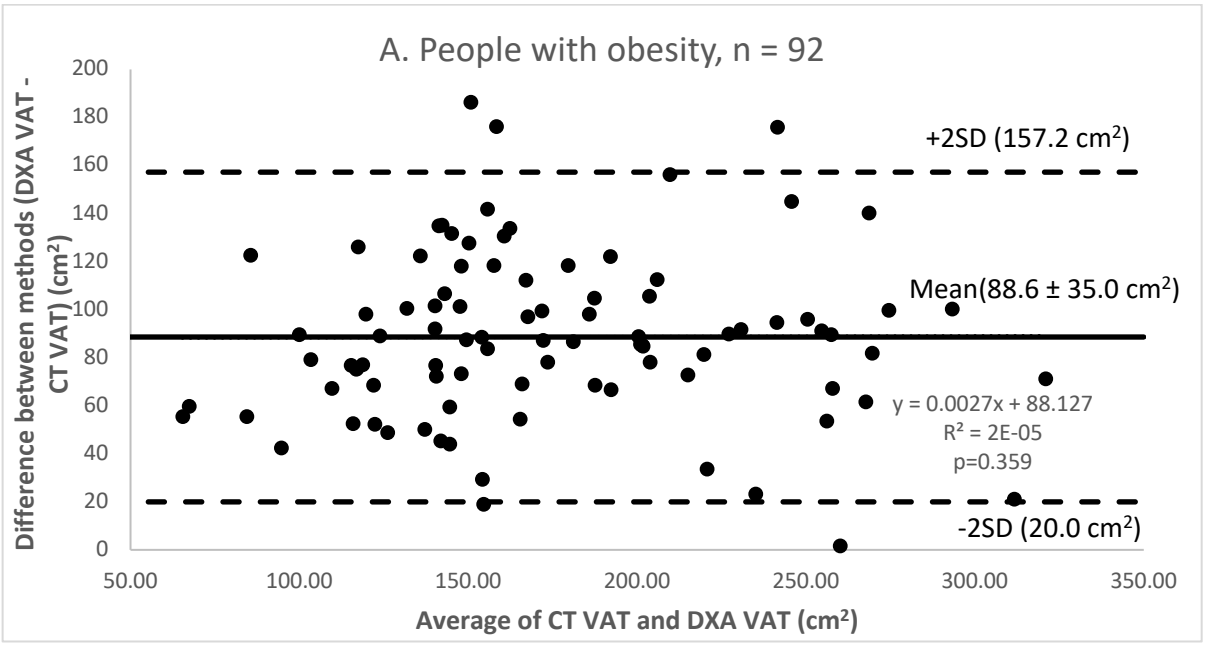


Figure 4 Bland -Altman analysis overweight group: CT and DXA VAT(A); CT and DXA SAT (B)



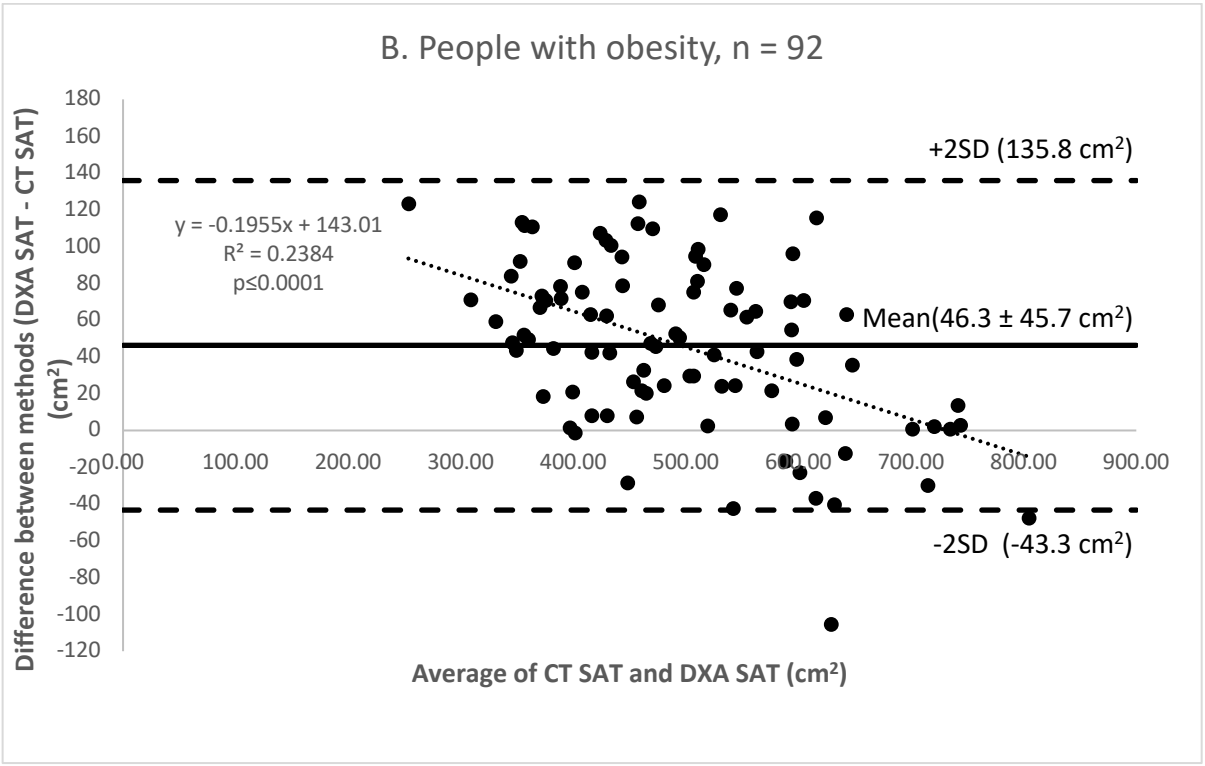
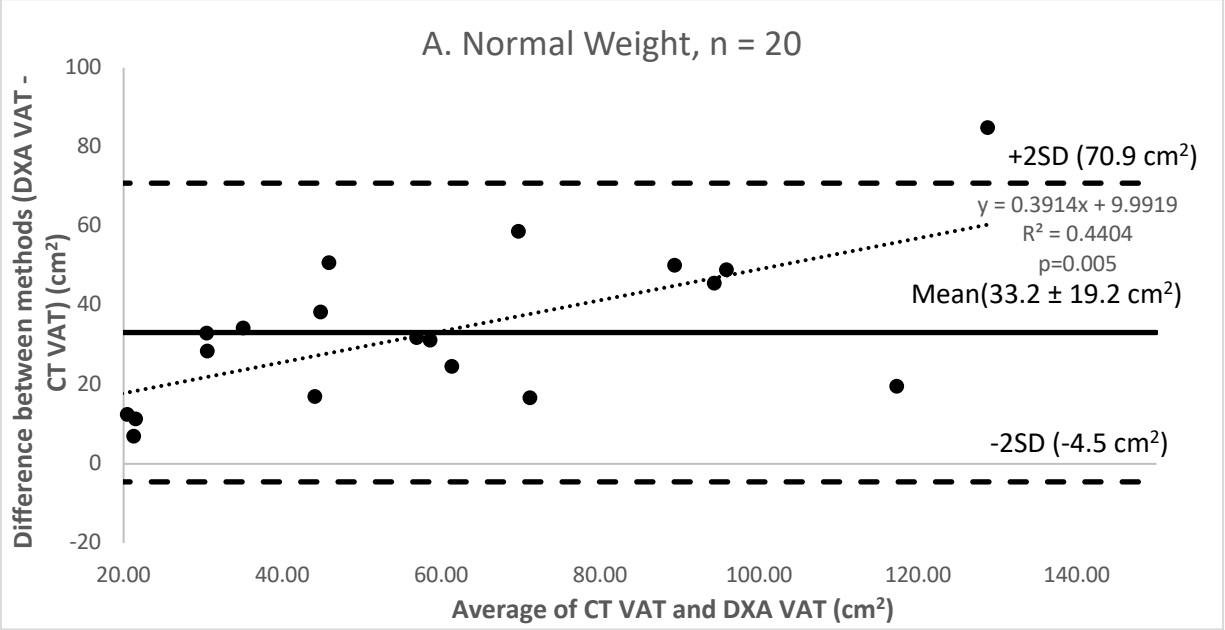


Figure 5 Bland-Altman analysis of people with obesity: CT and DXA VAT(A); CT and DXA SAT (B)



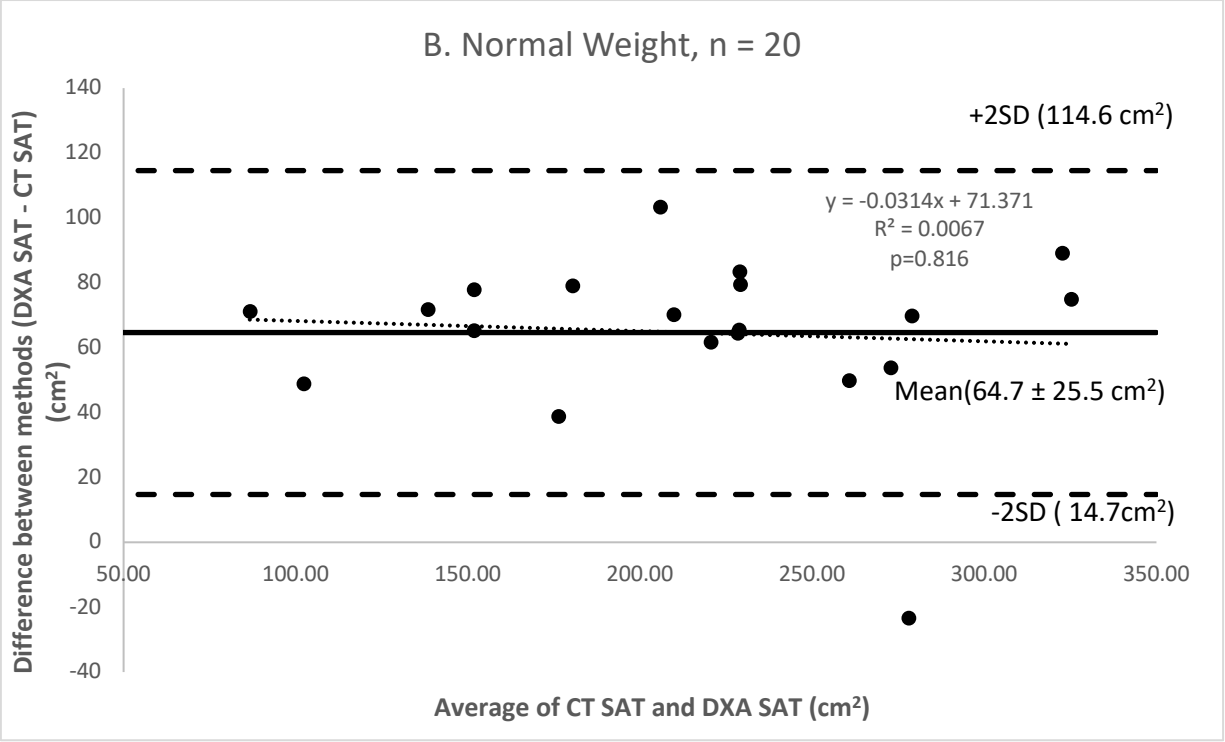
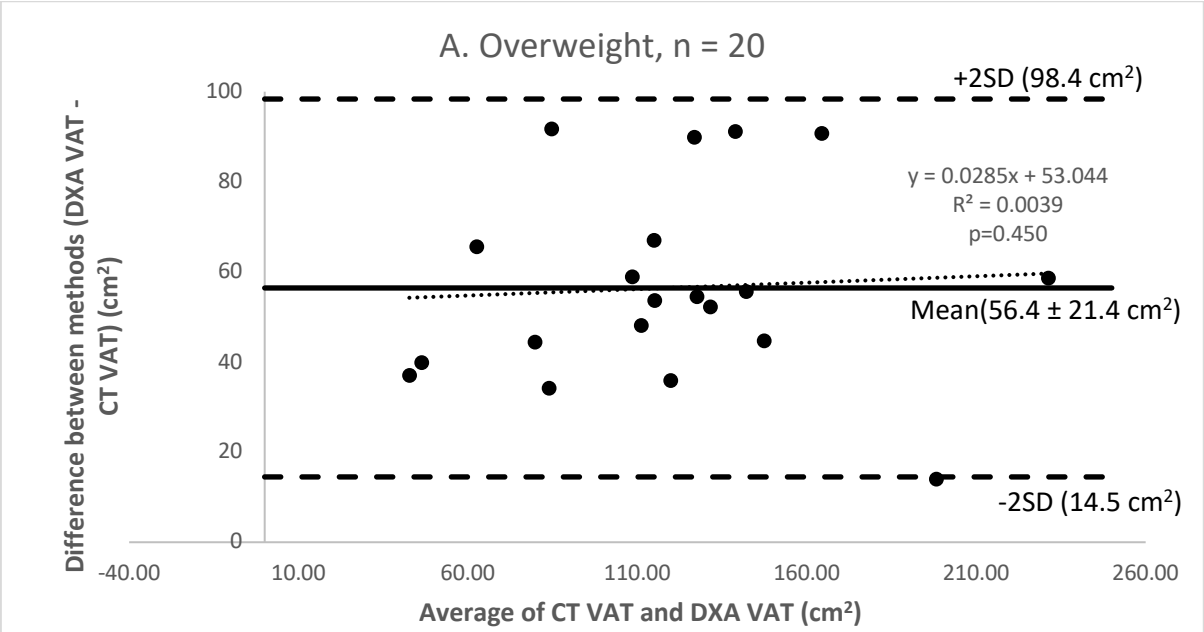


Figure S2 Bland-Altman analysis normal weight group: CT and DXA VAT (A); CT and DXA SAT (B)



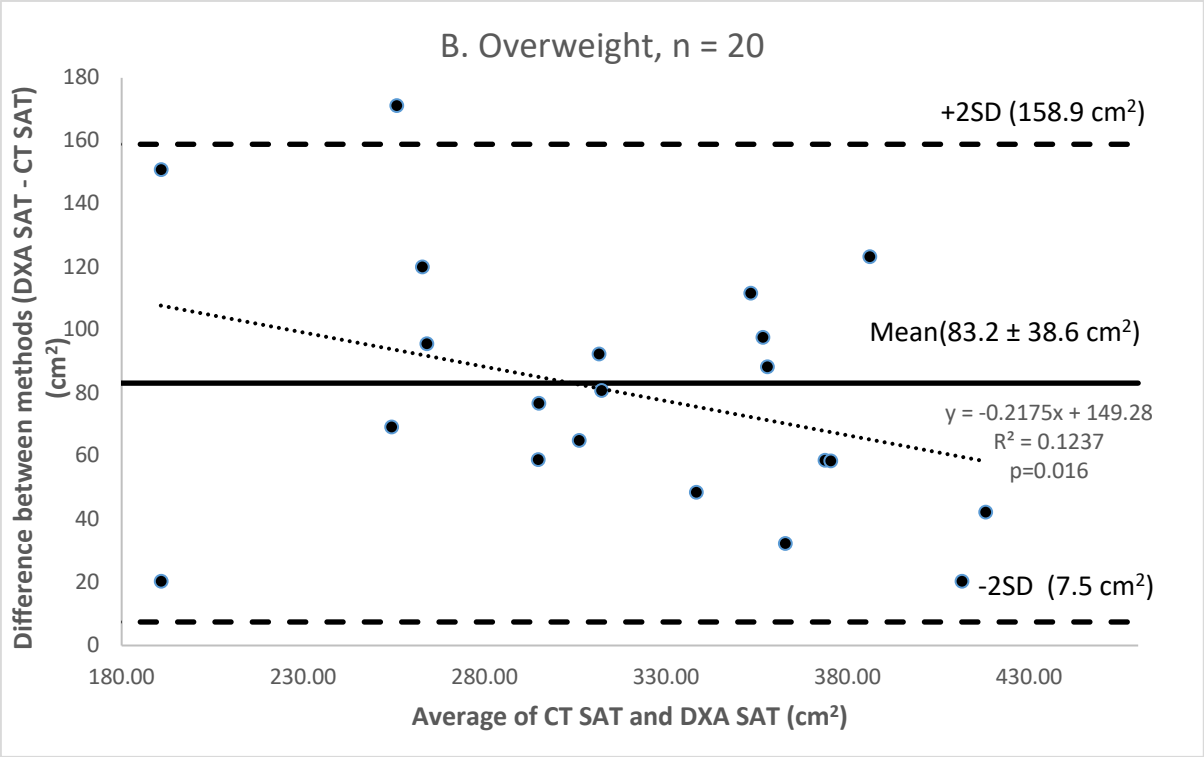


Figure S3 Bland -Altman analysis overweight group: CT and DXA VAT(A); CT and DXA SAT (B)

References

1. Pi-sunyer FX. The Epidemiology of Central Fat Distribution in Relation to Disease. NutriRev. 2004;62(7):S120–6.
2. Vatanparast H, Chilibeck PD, Cornish SM, Little JP, Paus-Jenssen LS, Case AM, et al. DXA-derived abdominal fat mass, waist circumference, and blood lipids in postmenopausal women. Obesity. 2009;17(8):1635–40.
3. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med. 2013;34(1):1–11.
4. Bi X, Seabolt L, Shibao C, Buchowski M, Kang H, Keil CD, et al. DXA-measured visceral adipose tissue predicts impaired glucose tolerance and metabolic syndrome in obese Caucasian and African-American women. Eur J Clin Nutr. 2014;1–8.
5. Plank LD. Dual-energy X-ray absorptiometry and body composition. Curr Opin Clin Nutr Metab Care. 2005 May;8(3):305–9.
6. Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, et al. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity. 2012;20(6):1313–8.
7. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-Energy X-Ray Performs as Well as Clinical Computed Tomography for the Measurement of Visceral Fat. Obesity. 2012;20(5, May):1109–14.
8. Mohammad A, Rolfe EDL, Sleigh A, Kivisild T, Behbehani K, Wareham NJ, et al. Validity of visceral adiposity estimates from DXA against MRI in Kuwaiti men and women. Nat Publ Gr. 2017;7(1):e238-5.
9. Lin H, Yan H, Rao S, Xia M, Zhou Q, Xu H, et al. Quantification of visceral adipose tissue using lunar dual-energy X-ray absorptiometry in Asian Chinese. Obesity. 2013;21(10):2112–7.
10. Neeland IJ, Grundy SM, Li X, Adams-Huet B, Vega GL. Comparison of visceral fat mass measurement by dual-X-ray absorptiometry and magnetic resonance imaging in a multiethnic cohort: the Dallas Heart Study. Nutr Diabetes. 2016;6(7):e221.
11. Erasmus RT, Soita DJ, Hassan MS, Blanco-Blanco E, Vergotine Z, Kegne AP, et al. High prevalence of diabetes mellitus and metabolic syndrome in a SA coloured population: baseline data of a study in Bellville, Cape Town. SA Med J. 2012 Nov;102(11):841–4.
12. Kengne AP, Erasmus RT, Levitt NS, Matsha TE. Alternative indices of glucose homeostasis as biochemical diagnostic tests for abnormal glucose tolerance in an African setting. Prim Care Diabetes II Eur. 2017;11(2):119–31.
13. Ball SD, Altena TS, Swan PD. Comparison of anthropometry to DXA: a new prediction equation for men. Eur J Clin Nutr. 2004;58:1525–31.
14. Hangartner TN, Warner S, Braillon P, Jankowski L, Shepherd J. The Official Positions of the International Society for Clinical Densitometry: Acquisition of Dual-Energy X-Ray Absorptiometry Body Composition and Considerations Regarding Analysis and Repeatability of Measures. J Clin Densitom. 2013;16(4):520–36.
15. Bosch TA, Dengel DR, Kelly AS, Sinaiko AR, Moran A, Steinberger J. Visceral adipose tissue measured by DXA correlates with measurement by CT and is associated with cardiometabolic risk factors in children. Pediatr Obes. 2014;10(21):172–9.
16. Hangartner TN, Warner S, Braillon P, Jankowski L, Shepherd J. The Official Positions of the International Society for Clinical Densitometry: Acquisition of Dual-Energy X-Ray Absorptiometry Body Composition and Considerations Regarding Analysis and Repeatability of Measures. J Clin Densitom. 2013;16(4):520–36.
17. Kelly TL, Wilson KE, Heymsfield SB. Dual energy X-ray absorptiometry body composition reference values from NHANES. PLoS One. 2009;4(9):e7038.
18. Petak S, Barbu CG, Yu EW, Fielding R, Mulligan K, Sabowitz B, et al. The Official Positions of the International Society for Clinical Densitometry: Body Composition Analysis Reporting. J Clin Densitom. 2013;16(4):508–19.

19. Kelly T, Wilson KE, Ruth C. Estimating visceral fat by dual-energy x-ray absorbtiometry. United States; US7,725,153 B2, 2010.
20. Clasey JL, Bouchard C, Wideman L, Kanaley J, Teates CD, Thorner MO, et al. The influence of anatomical boundaries, age, and sex on the assessment of abdominal visceral fat. Obes Res. 1997;5(5):395–401.
21. Yoshizumi T, Nakamura T, Yamane M, Islam a H, Menju M, Yamasaki K, et al. Abdominal fat: standardized technique for measurement at CT. Radiology. 1999;211(1):283–6.
22. Piernas C, Hernández-Morante JJ, Canteras M, Zamora S, Garaulet M. New computed tomography-derived indices to predict cardiovascular and insulin-resistance risks in overweight/obese patients. Eur J Clin Nutr. 2009;63(7):887–97.
23. Matsha TE, Ismail S, Speelman A, Hon GM, Davids S, Erasmus RT, et al. Visceral and subcutaneous adipose tissue association with metabolic syndrome and its components in a SA population. Clin Nutr ESPEN. 2019;32(August):76–81.
24. Bredella MA, Gill CM, Keating LK, Torriani M, Anderson EJ, Punyanitya M, et al. Assessment of abdominal fat compartments using DXA in premenopausal women from anorexia nervosa to morbid obesity. Obesity. 2013;21(12):2458–64.
25. Krishnan KC, Mehrabian M, Lusis AJ. Sex differences in metabolism and cardiometabolic disorders. Curr Opin Lipidol. 2018;29(5):404–10.
26. Fourman LT, Kileel EM, Hubbard J, Holmes T, Anderson EJ, Looby SE, et al. Comparison of visceral fat measurement by dual-energy X-ray absorptiometry to computed tomography in HIV and non-HIV. Nutr Diabetes. 2019;9(1):1–10.
27. Thomas EL, Bell JD. Influence of undersampling on magnetic resonance imaging measurements of intra-abdominal adipose tissue. Int J Obes. 2003;27(2):211–8.
28. Schweitzer L, Geisler C, Pourhassan M, Braun W, Glüer CC, Bosy-Westphal A, et al. What is the best reference site for a single MRI slice to assess whole body skeletal muscle and adipose tissue volumes in healthy adults? Am J Clin Nutr. 2015;102(1):58–65.
29. Shen W, Chen J, Gantz M, Velasquez G, Punyanitya M, Heymsfield SB. A single mri slice does not accurately predict visceral and subcutaneous adipose tissue changes during weight loss. Obesity. 2012;20(12):2458–63.
30. Schousboe JT, Kats AM, Langsetmo L, Vo TN, Taylor BC, Schwartz A V., et al. Central Obesity and Visceral Adipose Tissue Are Not Associated With Incident Atherosclerotic Cardiovascular Disease Events in Older Men. J Am Heart Assoc. 2018;7(16).
31. Earthman CP. Body Composition Tools for Assessment of Adult Malnutrition at the Bedside. J Parenter Enter Nutr. 2015;39(7):787–822.
32. Altman DG, Bland JM. Measuring agreement in method comparison studies. Stat Methods Med Res. 1999;8(99):135–60.
33. Chhapola V, Kanwal SK, Brar R. Reporting standards for Bland–Altman agreement analysis in laboratory research: a cross-sectional survey of current practice. Ann Clin Biochem. 2015;52(3):382–6.
34. Taylor AE, Kuper H, Varma RD, Wells JC, Bell JD, Radhakrishna K V., et al. Validation of Dual Energy X-Ray Absorptiometry Measures of Abdominal Fat by Comparison with Magnetic Resonance Imaging in an Indian Population. PLoS One. 2012;7(12).
35. Giavarina D. Understanding Bland Altman analysis Lessons in biostatistics. Biochem Medica. 2015;25(2):141–51.

CHAPTER FOUR: ASSOCIATIONS BETWEEN BODY FAT DISTRIBUTION AND CARDIO-METABOLIC RISK FACTORS IN MIXED-ANCESTRY SOUTH AFRICAN WOMEN AND MEN

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Introduction

Globally chronic non-communicable diseases (NCDs) are responsible for more deaths than any other cause, with people from the low-and middle income countries being disproportionately affected (1). In 2012, cardiovascular diseases (CVDs) and diabetes accounted for 46.2% and 4% of NCDs-related deaths, respectively (1). The SA (SA) cause of death profile for 2012 shows similar trends (2). An analysis of pooled population-based studies conducted by the NCD risk factor collaboration Africa working group found that that estimates of adiposity and diabetes prevalence in SA were higher than the global average (3). NCDs deaths are attributable to the high prevalence of major risk factors, including obesity, which is driven by lifestyle factors such as poor dietary intake and physical inactivity (4).

Obesity is a well-known risk factor for CVD and metabolic diseases (5–7), but body fat distribution appears to be a more significant discriminator of risk than generalized adiposity. The association of body fat with CVD risk differs by fat depot. A meta-analysis of 40 observational studies on the associations of different adipose tissue depots with insulin resistance revealed the strongest correlate of insulin resistance to be visceral adipose tissue (VAT) (8). In contrast, the relationship between abdominal subcutaneous adipose tissue (SAT) and cardio-metabolic risk is weaker than VAT, as shown in multiethnic studies in men and women (9,10). In contrast, the accumulation of lower body SAT (gluteofemoral obesity) has shown opposing associations with cardio-metabolic risk (11–13).

Body fat distribution is also sex-specific, with women having more SAT and less VAT than men (14,15). The greater central adiposity, in particular, VAT, in men translates to higher insulin resistance (14), type 2 diabetes (16) and adverse cardio-metabolic risk profile in general. The risk of cardio-metabolic disease increases with age (17) and in women, after menopause (18), when weight gain and increased central adiposity are common (19).

Differences also exist in body fat distribution amongst different ethnic groups (5,20). International studies have shown that Asian Indians have more total and central fat mass than their Caucasian and Black counterparts (21–23). Black Africans on the other hand have less VAT but more abdominal SAT than Caucasians (24–27), and greater gluteofemoral fat mass compared to Caucasian women (27). In addition to differences in body fat distribution, the association with cardio-metabolic risk also differs according to sex, age and ethnicity. For example, African American women were shown to have a weaker association between VAT and blood pressure, triglyceride concentrations, high-density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) than Caucasian women, while African American men displayed a stronger association between VAT, triglycerides, low HDL-C and metabolic syndrome than their Caucasian counterparts (28). While differences in body fat distribution, and associations

with cardio-metabolic risk between black, Caucasian and Asian women have been described in SA (24,29,30), no studies have examined the mixed-ancestry population of SA, who present with a high prevalence of metabolic syndrome (62%) and type 2 diabetes (28.2%), placing this population at high risk for CVD (31).

The composition of the mixed-ancestry (collectively referred to as "Coloured") population of SA is Khoesan (32–43%), Bantu-speaking Africans (20–36%), European (21–28%) and a smaller Asian contribution (9–11%)(32). This population accounts for 8.9% of the SA population and 48.8% of the population of the Western Cape Province (33). The aims of the study were therefore, for the first time, to investigate the relationship between whole-body fat distribution and cardio-metabolic risk factors in mixed-ancestry SA men and women, and to explore the effect of menopausal status on these relationships in women.

Methods

Participants

The study sample included all self-described mixed-ancestry volunteers who completed a whole body dual x-ray absorptiometry (DXA) scan as part of the Cape Town Vascular and Metabolic Health (VMH) study described previously (34). Inclusion criteria were adults aged 20 years and older. Participants were excluded if they were pregnant or acutely ill. Ethical approval was obtained from the Ethics Committees of the Cape Peninsula University of Technology and Stellenbosch University (respectively, NHREC:REC-230 408-014, CPUT/HWS-REC 2015/H03 and N14/01/003). All participants signed written informed consent and the study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). A total of 46 men and 207 women volunteered for the study.

Body composition

Anthropometric measurements were taken and included body weight, height and body mass index (BMI) as described in detail previously (34). Body composition (fat mass and fat-free mass) was acquired by a suitably trained and experienced radiographer using a Hologic Discovery W DXA whole body scanner configured with software version 13.4.1 (Hologic, Bedford, MA). Participants were positioned as per the NHANES body composition manual as advocated by Hangartner (35). DXA-derived measures of body composition regions included six standard regions of interest (ROI), namely the whole body, the trunk defined by the lower border of the mandible and including the chest, abdomen, and pelvic triangle; the arm ROIs (right and left) were defined by a line bisecting the shoulder joint of the right and left arm; and the leg ROIs (right and left) were defined by a line bisecting the hip joint aligned with the iliac crest and pubis (27). For the android fat measurement, the ROI is automatically defined with a caudal limit placed on top of the iliac crests and its height set to 20% of the distance from

the top of the iliac crest to the base of the skull as the cephalic limit (36). The height of the gynoid ROI is double that of the android ROI with the separation between the two regions equating to 1.5 times the height of the android ROI. VAT and SAT were estimated within this android region. DXA has proved to be as accurate as a clinical computed tomography scan in the quantification of VAT and SAT in adults (36). Subtotal body fat % and kg, which excludes the head, were used in the analysis. The head was excluded to reduce possibilities of any artefacts in the head region and total adipose body tissue classification excludes the head. Regional fat distribution (arms, legs, trunk, android and gynoid) were expressed as a percentage relative to sub-total fat mass (%FM).

Cardio-metabolic risk factors

Blood pressure was measured according to World Health Organisation (WHO) (37) guidelines using a semi-automatic digital blood pressure monitor (Omron M6 comfort-preformed cuff BP Monitor) on the right arm, in sitting position and at rest for 10-minutes. The lowest of three consecutive readings were taken in the analyses (34).

After an overnight fast (8-14 hrs) blood samples were taken to measure glycated haemoglobin (HbA_{1c}), glucose, insulin, lipid profile and biochemical marker for inflammation, high-sensitivity C-reactive protein (hs-CRP). After collection of the fasting blood sample, the participants without previously diagnosed diabetes underwent an oral glucose tolerance test (OGTT) as per the WHO criteria (38) . Participants drank 75g of anhydrous glucose in 250-300ml of water over the course of five minutes (39), following which blood samples were collected after the 2-h test load.

Blood samples were transported daily in an icebox for processing using standard pathology practices.

Biochemical parameters were analysed at an ISO 15189 accredited Pathology practice (Pathcare, Reference Laboratory , Cape Town, South Africa) as described previously elsewhere (34). Plasma glucose was measured by the enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa). HbA_{1c} was assessed by high performance liquid chromatography (Biorad Variant Turbo, BioRad, South Africa). Insulin was measured by paramagnetic particle chemiluminescence assay (Beckman DXI, Beckman Coulter, South Africa). High-density lipoprotein cholesterol (HDL-C) was by enzymatic immunoinhibition, and triglycerides by glycerol phosphate oxidase-peroxidase and Low-density lipoprotein cholesterol (LDL-C) by enzymatic selective protection – End Point (Beckman AU, Beckman Coulter, South Africa). hs-CRP analysis was performed on the BNA nephelometer (Dade Behring) by particle-enhanced immunonephelometry with a detection limit of 0.18 mg/L and a measuring range of 0.18–1150 mg/L.

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated from fasting glucose and insulin levels (40). The metabolic syndrome were quantified using the Joint Interim Statement (JIS) criteria (39) and the WHO glucose tolerance categories were used.

Statistical analysis

Data were analysed using SPSS® version 24 (Armonk, NY: IBM Corp.) and STATA® version 14.2 (STATA corporation, Texas, USA). The participants in the study were a convenient sub-sample of the larger study. The 253 available volunteers who participated in the study provided an 80% power at a 5% significance level to detect a coefficient of determination (R2) of 0.042 or greater from the linear regression model comprising three predictors. Categorical variables are presented as frequencies and percentages, while continuous variables are presented as mean ± standard deviation (s.d.) for normally distributed variables and median and 25^{th}-75^{th} percentiles for skewed variables. Data was tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk statistic. Women were also split into two groups based on menopausal age, which is estimated to be 50 years in this population (41). Group comparisons were made using the Mann- Whitney U test or chi-square test. Robust regression analyses were used to investigate the associations between body fat distribution and cardio-metabolic risk factors (insulin resistance, lipid levels, blood pressure and inflammatory markers), adjusting for age and sex. In addition, we explored the interactions between sex and body composition on cardio-metabolic risk factors, adjusting for age, and in women, between menopausal age and body composition. To investigate whether the one body compartment was more closely associated with the risk factor than the other, coefficients of determination (R^2) were used from robust regressions. We calculated the R^2 for the model with covariates only (age and sex), then the R^2 for models containing covariates and each of the adiposity measures.

Results

Body composition

In all, 253 participants (18% men and 82% women) were included. The average age of the participants was 55 years, and was similar between men and women (p=0.630). Differences in body composition and body fat distribution between women and men, as well as between pre- and post-menopausal women are presented in Table 1. On average, women were obese (mean BMI=32.6±7.2 kg/m^2), whereas men were overweight (mean BMI=27.4±6.1 kg/m^2) ($p<0.001$).

Men had higher fat free soft tissue mass (FFSTM) compared to women, ($p<0.001$), but body fat mass (kg and %) was significantly higher in women than men ($p<0.001$). As a percentage of total fat mass, women had significantly less central fat mass ($p<0.001$) and greater

peripheral fat mass (arm, leg and gynoid fat %, $p \leq 0.003$ for all) than men. VAT area was not different between men and women (p=0.474), but SAT area was higher in women than men ($p<0.001$).

When examining differences in body composition between the pre- and post-menopausal women, we found that although there were no differences in BMI, more post-menopausal women than pre-menopausal women were obese (68.9% vs. 57.3%) and post-menopausal women had greater fat mass (p=0.026) and %FM (p<0.001) than pre-menopausal women. Although trunk fat mass (%) and android fat mass (%) did not differ between pre- and post-menopausal women (both p≥0.415), post-menopausal women had greater waist circumference and VAT (both p≤0.004), and less gynoid %FM (p=0.001) than pre-menopausal women.

Cardio-metabolic risk

Differences in cardio-metabolic risk factors between mixed-ancestry men and women, and between pre- and post-menopausal women are described in Table 2. While blood pressure, fasting glucose, insulin and lipid levels were not different between men and women (all p≥0.085), 2-hour post prandial glucose ($p<0.05$) and HDL-C ($p<0.001$) concentrations, were higher in women than men. The majority of the sample had normal glucose tolerance (NGT) (men 63.1% and women 57.3%). The prevalence of diabetes (30.4% and 26.7% respectively), and screen-detected diabetes (8.7% and 9.2%) was similar in men and women, respectively (p=0.249).

Post-menopausal women had higher systolic blood pressure (SBP), fasting glucose (p<0.001), 2-hour glucose (p=0.01), triglyceride (p<0.01), TC (p<0.05) and HDL-C (p<0.001) concentrations than pre-menopausal women. The prevalence of impaired glucose tolerance (IGT)/ impaired fasting glucose (IFG) and type 2 diabetes was similar in the pre-and post-menopausal women (p=0.166).

Associations between body fat and its distribution and cardio-metabolic risk factors

Table 3 shows the associations between body fat variables and cardio-metabolic risk factors in the whole sample, adjusting for sex and age. In terms of total body fat (kg and %), positive associations were observed for diastolic blood pressure ($p<0.05$), 2-hour glucose, fasting insulin, HOMA-IR (all p<0.01), triglyceride concentrations ($p<0.05$) as well as hs-CRP, and in the case of body fat %, TC ($p<0.01$) and LDL-C ($p<0.05$).

When examining associations between central fat mass (trunk fat %FM, android %FM, VAT and SAT area) and cardio-metabolic risk profile, we found positive associations with diastolic

blood pressure (DBP), fasting glucose, 2-hour glucose, fasting insulin and HOMA-IR, triglyceride concentrations and hs-CRP (p<0.01 for all), and negative association with HDL-C levels (p<0.01 for all). When examining the relationships of peripheral fat mass, we found that arm fat mass was positively associated with SBP (p<0.05), DBP (p<0.01), fasting insulin (p<0.05), and HOMA-IR (p<0.01), and negatively associated with HDL-C (p<0.01). In contrast, lower body peripheral fat mass (gynoid % FM and leg % FM) was negatively associated with all CVD risk markers, except for HDL-C, which was positively associated with gynoid and leg % FM (p<0.01).

We then compared the proportion of the variance that age, sex and the different body composition measures explained for each cardio-metabolic risk factor. Together with age and sex, VAT area accounted for the greatest variance in fasting insulin (29%) and HOMA-IR (27%), while SAT area accounted for the greatest variance in hs-CRP (15%). Trunk %FM and leg FM% contributed equally to the greatest variance in triglyceride concentrations (13%). Additionally, leg FM% also accounted for the greatest variance in HDL-C (14%). Body composition did not add to variance in TC and LDL-C concentrations above that of age and sex.

There were sex-specific differences in the associations between body fat distribution and measures of cardio-metabolic risk. While fat mass (kg) and abdominal SAT were associated with 2-hour glucose (figure 1A and 1C), HOMA-IR (figure 1B and 1D) and fasting insulin in both men and women, the association was stronger in men compared to women (all interaction p≤0.027). Conversely, the associations between serum triglycerides and the distribution of body fat was significant in women, but not men. Specifically, central adiposity measures (trunk %FM and android %FM) were positively associated with serum triglyceride concentrations (figure 2A & B, all interaction p≤0.014), and peripheral fat mass (leg %FM and gynoid %FM) were negatively associated with serum triglyceride levels (figure 2D & E, both interaction p≤0.022) in women, but not men. The association between VAT and triglyceride concentrations was stronger in women than men (figure 2C, interaction p=0.012).

The associations between body composition and cardio-metabolic risk factors in pre-and post-menopausal women are shown in Table 4. For the most part, the association between body fat distribution and cardio-metabolic risk did not differ between the pre- and post-menopausal women. Significant interactions were however seen between FM (kg), central fat distribution (FM%, trunk %FM, VAT and SAT area) and SBP (all interaction p≤0.042) and VAT and DBP (interaction p=0.030), such that these were significant in pre-menopausal women and not post-menopausal women. Similarly, FM (kg) FM (%), VAT and SAT were associated with TC (all interactions p≤0.002) and LDL-C (all interactions p≤0.007) in the pre-menopausal women only.

FM (kg) was associated with fasting insulin in both pre- and post-menopausal women, but the association was stronger in pre- than post- menopausal women (interaction p=0.019), while FM (kg) was associated with triglycerides concentrations in the pre-menopausal women only (interaction p=0.016). Peripheral fat (arm %FM and gluteofemoral %FM) was associated with LDL-C (both interaction p≤0.032) and hs-CRP (both interaction p≤0.012) in the pre-menopausal women and not post -menopausal women.

Discussion

This is the first study to investigate the relationship between body composition and cardio-metabolic risk profile in the mixed-ancestry SAs. The main findings of the study are that body fat and, in particular central adiposity, were associated with unfavourable cardio-metabolic risk profile, while lower body peripheral fat was associated with favourable risk profile. However, the associations between body fat distribution and cardiovascular risk profile differed by sex and menopausal status, such that the associations were stronger in men and pre-menopausal women.

Although the women in our sample had nearly twice as much body fat mass, and had higher obesity rates than men, the prevalence of cardio-metabolic risk factors was similar between sex (apart from 2-hour glucose and HDL-C concentrations being higher in women). This may be explained by the fact that despite marked differences in total body fat, VAT area was similar in men and women. Indeed, VAT was the most consistent and significant correlate of cardio-metabolic risk (insulin resistance, glucose tolerance, triglyceride concentrations and HDL-C) in this sample. Further, the association between VAT and cardio-metabolic risk did not differ by sex. Similarly, a recent study amongst Korean men and women showed that DXA-derived VAT was the best correlate of diabetes and pre-diabetes (42). Likewise, the meta-analysis by Zhang and co-workers (8) supports VAT as the strongest correlate of insulin resistance, followed by total fat mass. The mechanisms linking VAT accumulation to metabolic complications include the higher production of proinflammatory cytokines and the higher lipolytic activity compared to SAT, with the consequent increase in cytokines and free fatty acids delivery to the hepatic portal system impacting on insulin sensitivity (5). VAT is also proposed to be a marker of insulin resistance as a consequence of lipotoxicity, in particular an increase in fat deposition in the liver (5)

In contrast to VAT, women had more abdominal SAT than men (14,15). Notably, the relationship between both total adiposity and abdominal SAT and insulin resistance was stronger in men than women. These differences may relate to the fact that oestrogens regulate insulin sensitivity and that female adipocytes are more insulin sensitive compared with male adipocytes (43). Alternatively, the sex-specific relationship between abdominal SAT and

insulin resistance could, in part, be explained by the fact that men have greater deep subcutaneous adipose tissue (dSAT) and less superficial SAT (sSAT) than women (44). Nazare et al., showed that of the two SAT layers, dSAT had a higher association with inflammation and oxidative stress, suggesting that dSAT is an important determinant of metabolic syndrome (MetS)(22). Accordingly, abdominal SAT should be considered as two functionally distinct compartments rather than a single entity (22) . A suggestion for further investigation in this population would be to explore the role of sSAT and dSAT using alternative imaging methods such as computerised tomography or magnetic resonance imaging, as DXA is unable to differentiate between sSAT and dSAT.

Unlike associations with insulin sensitivity, the associations between measures of body fat distribution (VAT, android, gynoid and leg % FM) and triglyceride concentrations were more pronounced in women than men. This finding is supported by the results of the Framingham Heart Study (45) where the relationship between VAT in particular, and triglyceride concentrations was stronger in women than men, likely explained by the higher rates of lipolysis of VAT in women compared to men (46).

Greater lower body peripheral fat mass was associated with a lower cardio-metabolic risk, commensurate with findings from previous studies in African American and Caucasian men and women (47). Similarly, the protective effect of lower body peripheral fat was observed in a large sample of Asian men and women, showing that those with MetS had less lower body peripheral fat that those without MetS (13). Notably, the study by Shorr *et al* (48) which examined the differences between sex, body composition and cardiometabolic risk, showed the protective effect of lower body fat to be stronger in women than men, which supports our study results. The lower body fat depot is seen as a 'metabolic sink' which traps excess free fatty acids (FFA) due to the increased lipoprotein lipase activity and lower lipolytic activity in this depot compared to the abdominal fat depot, thus protecting other tissues from lipid overflow and insulin resistance associated with ectopic lipotoxicity (11,12,44). The protective effect of lower body peripheral fat on triglyceride concentrations was however not observed in the sample of men, who had significantly less lower body peripheral fat than women.

We found positive associations between arm fat and cardio-metabolic risk, in particular insulin resistance, similar to those found with central adiposity. A possible explanation for this may be that upper body adiposity is more sensitive to lipolysis and secretes a greater number of inflammatory cytokines (49). Accordingly, not all peripheral fat may be regarded as protective and these differences should be further investigated.

Contrary to the findings for triglyceride concentrations and HDL-C, TC and LDL-C concentrations were not associated with body fat in either men or women. This is at variance with findings from similar studies in other ethnic groups (50), but similar to those shown in black SA women (27). These findings suggest that factors other than body fat and its distribution, including genetics, dietary intake, physical activity and smoking influence HDL-C, TC and LDL-C concentrations.

Commensurate with the decline in oestrogen following menopause, the post-menopausal women had greater VAT and lower gynoid %FM compared to the pre-menopausal women, corresponding to their greater cardio-metabolic risk, as previously demonstrated (18,19). However, the association between body fat distribution and cardio-metabolic risk was weaker in the post- compared to pre-menopausal women. A possible explanation for this is that as oestrogen declines and levels of bioavailable testosterone increase at menopause, this results in a shift in body weight and body fat distribution and disruptions in glucose regulation (43). Interestingly studies have shown that aging and lack of physical activity rather than menopause are the main reasons for weight gain and obesity in midlife women (19). This study adds to the literature the associations between body composition and cardio-metabolic risk factors in the mixed ancestry population which previously had not been researched. In particular, the women in our study had higher VAT than the men which is in contrast to other studies and ethnicities (48). This is possibly due to the vast difference in total body fat between men and women which may be unique in this sample. Additionally, post-menopausal women had increased VAT compared to the pre-menopausal women which is commensurate with recent literature (51). In clinical practice the importance of preventing weight gain and centralization of body fat prior to menopause should be highlighted. Even though the women in our study had substantially more abdominal SAT, the relationship between abdominal SAT and insulin resistance was stronger in the men, a finding similar to that of the Netherlands epidemiology of obesity study (52).

The strengths of the study include the proven accuracy of DXA to measure body composition, and the use of robust analytic approaches to carefully explore the targeted associations. Although there were multiple comparisons, the relationships were consistent, which suggests that false positive results were unlikely. Possible limitations were the cross-sectional nature of the study and the inclusion of a convenient sample of women and only a small sample of men. However, this is typical of a SA population survey in which more women are included than men (31). Further, the sex disparities in obesity prevalence shown in this study are similar to those reported in the national prevalence data (53). We did not have an objective measure of menopause age. These findings could thus reflect an age effect and warrants further investigation. We lacked information on important potential confounders such as socio-

economic status, diet, physical activity and smoking which are known to affect body fat and cardio-metabolic risk. In addition, we did not adjust for medication use, but the participants were instructed not to take any medications prior to testing.

In summary, central fat mass was associated with increased cardio-metabolic risk and lower body peripheral fat mass was associated with reduced risk. However, these associations were influenced by sex and menopausal status. Notably, VAT was the most consistent and significant correlate of insulin resistance. Future studies should focus on the mechanisms underlying the sex-specific associations between SAT (in particular dSAT and sSAT) and cardio-metabolic risk. Additionally, the relationship between DXA-derived VAT and SAT and simpler anthropometry measurements to predict cardio-metabolic risk should be investigated. Specific VAT cut-points for cardio-metabolic risk in the mixed ancestry populations should be derived in an effort to identify high risk individuals.

Contribution statement

FED: data analysis and interpretation, preparation of the first draft and approval of final draft
TEM: conception and design, acquisition and interpretation of data, revision for important intellectual content and approval of final draft
RTE: conception and design, revision for important intellectual content and approval of final draft
APK: conception and design, data analysis and interpretation of data, revision for important intellectual content and approval of final draft
JHG: data analysis and interpretation, revision for important intellectual content and approval of final draft

Data access, responsibility and analysis

FED, APK, TEM and JHG, had access to the data in the study and TEM, APK and JHG take responsibility for the integrity of the data and accuracy of the data analysis.

Conflict of interest

The authors state that they have no conflict of interest.

Disclaimers

None

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Table 1 Comparison of body composition and body fat distribution between mixed-ancestry men and women, and pre- and post-menopausal women

	Men		Women		Men vs. women	Women				Pre- vs. post-menopausal women
	n	Total sample	n	Total sample	*p*-value	n	20-49 years	n	≥50 years	*p*-value
Age (years)	46	53.5(44.8-65.3)	207	55.0 (45.0-63.0)	0.630	75	39.0(31.0-45.0)	131	61.5(56.0-67.0)	<0.001
Anthropometry										
Height (cm)	46	168.0(163.3-173.6)	206	156.0(151.5-160.5)	<0.001	75	157.5(153.0-160.5)	131	155.0(151.0-160.5)	0.043
Weight (kg)	46	75.6(63.6-89.1)	206	78.2(66.3-90.4)	0.443	75	74.5(62.1-92.2)	131	79.8(67.6-90.2)	0.196
BMI (kg/m^2)	46	27.4±6.1	206	32.6±7.2	<0.001	75	31.4±7.7	131	33.4±6.9	0.083
Waist (cm)	46	96.2±17.5	206	99.3±15.0	0.284	75	95.0±17.1	131	101.9±13.2	0.004
BMI Category	46	%	207	**% of total sample**	Pearson Chi-Square	75	%	132	%	Pearson Chi-Square
Underweight	2	4.3	3	1.4	<0.001	2	2.7	1	0.8%	0.010
Normal	14	30.4	29	14.0		18	24	11	8.3	
Overweight	16	34.8	41	19.8		12	16	29	22	
Obese	14	30.5	134	64.7		43	57.3	91	68.9	
DXA-derived body composition and body fat distribution										
Fat free soft tissue mass (kg)	46	50.4(43.8-56.8)	207	38.9(35.6-44.7)	<0.001	75	38.9(34.3-44.7)	132	37.3(34.2-42.2)	0.243
Body fat (kg)	46	16.4(12.7-27.8)	207	31.2(24.4-40.0)	<0.001	75	30.0±12.5	132	34.1±11.9	0.026
Body fat (%)	46	26.5(19.9-32.5)	207	44.0(39.8-48.6)	<0.001	75	41.5(35.3-46.8)	132	44.9(41.4-49.6)	<0.001
Trunk fat (% FM)	46	57.1±5.2	207	50.7±6.01	<0.001	75	50.1±6.9	132	51.0±5.4	0.415
Arm Fat (% FM)	46	10.7±1.5	207	12.5±1.97	<0.001	75	12.1±1.8	132	12.7±2.0	0.059
Leg Fat (% FM)	46	31.7(29.0-34.9)	207	36.1(31.9-40.5)	<0.001	75	37.5(32.0-41.5)	132	35.8(31.5-40.2)	0.180
Android (% FM)	46	10.8±2.0	207	8.9±1.57	<0.001	75	8.8±1.8	132	9.1±1.4	0.445
Gynoid (% FM)	46	15.6(14.6-17.2)	207	17.2(15.4-19.1)	0.003	75	18.1(16.0-20.7)	132	16.9(14.9-18.5)	0.001
VAT (cm^2)	46	167.0(101.2-260.7)	207	180(135-236)	0.474	75	154.5(93.2-211.0)	132	197.2(149.4-244.1)	<0.001
SAT (cm^2)	46	263.8±143.7	207	451±142	<0.001	75	432.6±160.6	132	461.0±129.5	0.220

Values presented as means ± standard deviations (SD), median and 25th-75th percentiles, or %

BMI(WHO classification), body mass index; WC. Waist circumference; FM, fat mass expressed as a percentage relative to sub-total fat mass; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

Table 2 Comparison of cardio-metabolic risk factors between mixed-ancestry men and women, and pre- and post-menopausal women

	Men		Women		Men vs. women	Women				Pre- vs. post-menopausal women
	n	Total sample	n	Total sample	*p*-value	n	20-49 years	n	≥ 50 years	*p*-value
SBP (mmHg)	46	128.5±22.5	207	127.2±20.6	0.894	75	116.6±19.0	132	133.3±19.1	<0.001
DBP (mmHg)	46	81.9±12.9	207	82.6±11.6	0.472	75	81.3±12.0	132	83.4±11.4	0.228
Fasting glucose (mmol/l)	46	5.1(4.6-5.8)	205	5.1(4.7-6.0)	0.550	74	4.8(4.5-5.5)	131	5.3(4.8-6.3)	0.001
2hr Glucose (mmol/l)	35	5.8(4.5-7.5)	170	6.6(5.5-8.0)	0.017	63	6.3(5.0-7.1)	107	7.1(5.7-8.2)	0.010
Fasting insulin (mIU/L)	46	6.7(3.8-13.2)	205	8.4(5.6-12.6)	0.085	74	8.5(5.9-14.6)	131	8.4(5.3-12.1)	0.362
HOMA-IR	46	1.6(0.9-3.3)	204	2.1(1.2-3.6)	0.085	73	2.0(1.3-4.2)	131	2.1(1.2-3.5)	0.940
TG (mmol/l)	46	1.5(1.0-1.9)	205	1.4 (0.1-2.0)	0.665	74	1.2(0.8-1.8)	131	1.5(1.1-2.1)	0.008
TC (mmol/l)	46	5.1±1.3	205	5.4±1.2	0.208	74	5.2±1.1	131	5.5±1.1	0.017
LDL-C (mmol/l)	42	3.1(2.3-3.9)	201	3.2(2.7-4.1)	0.385	72	3.3(2.6-4.0)	129	3.2(2.7-4.1)	0.527
HDL-C (mmol/l)	46	1.1(0.9-1.3)	205	1.3 (1.1-1.5)	<0.001	74	1.2(1.0-1.3)	131	1.3(1.1-1.5)	<0.001
CRP (mg/L)	39	2.8(1.6-5.5)	152	3.3(1.7-5.7)	0.467	58	2.8(1.3-6.6)	94	3.3(1.8-5.7)	0.508
Glucose tolerance categories										
NGT, n/%	29	63.1	118	57.3%	0.249	50	66.7	68	51.9	0.166
IGT/IFG, n/%	3	6.5	33	16.0%		9	12.0	24	18.3	
Diabetes, n/%	14	30.4	55	26.7%		16	21.3	39	29.8	

Values presented as means ± standard deviations (SD), median and 25th-75th percentiles or %. SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model for insulin resistance; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein.

Table 3 Associations between body composition and cardio-metabolic risk factors in the whole sample,♦ adjusted for sex and age.

		SBP	DBP	Fasting glucose	2 hr glucose	Fasting insulin	HOMA IR	TG	TC	LDL-C	HDL-C	hsCRP
Age and sex	**R^2**	0.23	0.03	0.07	0.08	0.01	0.02	0.03	0.03	0.01	0.06	0.01
FM (kg)	**β (95%CI)**	0.053 (-0.14 to 0.24)	0.142* (0.02 to 0.27)	0.01** (0.00 to 0.01)	0.03**# (0.01 to 0.05)	0.17**# (0.13 to 0.22)	0.04**# (0.03 to 0.05)	0.01** (0.00 to 0.02)	0.01(-0.00 to 0.02)	0.01(-0.00 to 0.02)	-0.01**(-0.01to 0.00)	0.09**(0.06 to 0.12)
	R^2	0.23	0.04	0.09	0.11	0.20	0.20	0.04	0.04	0.01	0.08	0.13
FM (%)	**β (95%CI)**	0.02(-0.29 to 0.33)	0.26*(0.05 to 0.46)	0.15*(0.00 to 0.02)	0.05**(0.02 to 0.09	0.27**(0.20 to 0.34)	0.06**(0.05 to 0.08)	0.01*(0.00 to 0.03)	0.03**(0.01 to 0.05)	0.02*(0.00 to 0.04)	-0.00(-0.00 to 0.00	0.16**(0.09 to 0.24)
	R^2	0.23	0.05	0.10	0.13	0.21	0.21	0.04	0.05	0.03	0.10	0.13
Trunk fat (%FM)	**β (95%CI)**	0.38(-0.00 to 0.77)	0.54**(0.30 to 0.79)	0.01**(0.00 to 0.03)	0.08**(0.04 to 0.12)	0.26**(0.15 to 0.37)	0.06**(0.03 to 0.08)	0.04**◊ (0.30 to 0.06)	0.02(-0.00 to 0.48)	0.01(-0.01 to 0.03)	-0.02**(-0.02 to -0.01)	0.11**(0.04 to 0.18)
	R^2	0.24	0.10	0.09	0.13	0.10	0.10	0.13	0.04	0.01	0.12	0.06
Arm fat (%FM)	**β (95%CI)**	1.19*(0.01 to 2.36)	1.12**(0.34 to 1.90)	0.01(-0.03 to 0.05)	0.04 (-0.10 to 0.18)	0.40*(0.03 to 0.76)	0.09**#(0.01 to 0.18)	0.04(-0.10 to 0.09)	-0.00(-0.08 to 0.08)	0.01(-0.06 to 0.08)	-0.03**(-0.05 to -0.01)	0.19(-0.02 to 0.41)
	R^2	0.24	0.05	0.07	0.08	0.03	0.03	0.03	0.03	0.01	0.08	0.02
Leg fat (%FM)	**β (95%CI)**	-0.43*(-0.79 to -0.08)	-0.56**(-0.79 to -0.34)	-0.01**(-0.03 to-0.00)	-0.06**(-0.10 to -0.03)	-0.25**(-0.36 to -0.15)	-0.06**(-0.08 to -0.03)	-0.04**◊ (-0.05 to -0.03)	-0.02(-0.04 to 0.00)	-0.01(-0.03 to 0.01)	0.02**(0.01 to 0.02)	-0.11**(-0.17 to -0.05)
	R^2	0.24	0.12	0.09	0.13	0.10	0.10	0.13	0.03	0.01	0.14	0.07
Android fat (%FM)	**β (95%CI)**	0.46(-0.91 to 1.82)	1.74**(0.87 to 2.62)	0.08**(0.04 to 0.13)	0.29**(0.14 to 0.42)	1.01**(0.65 to 1.39)	0.24**(0.15 to 0.32)	0.13**◊ (0.08 to 0.18)	0.10*(0.01 to 0.19)	0.06(-0.02 to 1.43)	-0.05**(-0.07 to -0.03)	0.59**(0.35 to 0.82)
	R^2	0.23	0.08	0.13	0.14	0.12	0.13	0.10	0.04	0.01	0.11	0.11
Gynoid fat (%FM)	**β (95%CI)**	-1.12**(-1.93 to -0.31)	-1.13**-(1.66 to -0.60)	-0.03**(-0.06 to -0.00)	-0.15**(-0.23 to -0.06)	-0.70**(-0.91 to -0.47)	-0.17**(-0.22 to -0.11)	-0.10**◊ (-0.13 to -0.07)	-0.04(-0.09 to 0.02)	-0.01(-0.06 to 0.04)	0.03(**0.02 to 0.05)	-0.25**(-0.40 to -0.10)
	R^2	0.25	0.09	0.09	0.12	0.15	0.15	0.12	0.03	0.01	0.11	0.06
VAT(cm^2)	**β (95%CI)**	0.02(-0.01 to 0.05)	0.03**(0.01 to 0.05)	0.00**(0.00 to 0.00)	0.01**(0.00 to 0.01)	0.04**(0.03 to 0.05)	0.01**(0.01 to 0.01)	0.00**◊ (0.00 to 0.00)	0.00(-0.00 to 0.00)	0.00(-0.00 to 0.00)	-0.00**(-0.00 to -0.00)	0.01**(0.01 to 0.02)
	R^2	0.23	0.07	0.12	0.16	0.29	0.27	0.09	0.03	0.01	0.12	0.14
SAT(cm^2)	**β (95%CI)**	0.01(-0.01 to 0.02)	0.02**(0.01 to 0.03)	0.00**(0.00 to 0.00)	0.00**#(0.00 to 0.00)	0.01**#(0.01 to 0.02)	0.00**#(0.00 to 0.00)	0.00**(0.00 to 0.00)	0.00*(0.00 to 0.00)	0.00(-0.00 to 0.00)	-0.00**(-0.00 to -0.00)	0.01**(0.01 to -0.01)
	R^2	0.23	0.06	0.11	0.12	0.19	0.20	0.06	0.05	0.02	0.09	0.15

♦Diabetic participants excluded from insulin sensitivity measurements. Beta coefficients, confidence intervals, total R^2 for: %FM, expressed as a percentage of sub-total fat mass (FM); HOMA-IR, homeostasis model for insulin resistance; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue. *p<0.05 **P<0.01. # Significant in men, ◊ Significant in women.

Table 4 Associations between body composition and cardio-metabolic risk factors in the pre- and post-menopausal women

		SBP	DBP	Fasting insulin	TG	TC	LDL-C	hsCRP
FM (kg)	Pre-meno Post-meno	0.264[a] -0.092 #	0.292[a] 0.028	0.629[A] 0.380[B] #	0.502[A] 0.081 #	0.459[A] -0.120 #	0.394[A] -0.079 #	0.592[A] 0.198
FM (%)	Pre-meno Post-meno	0.198 -0.123 #	0.246[a] 0.006	0.509[A] 0.318[B]	0.357[A] 0.001	0.462[A] -0.073 #	0.451[A] -0.053 #	0.528[A] 0.226[b]
Trunk fat (%FM)	Pre-meno Post-meno	0.443[A] -0.002 #	0.507[A] 0.118	0.562[A] 0.358[B]	0.585[A] 0.525[B]	0.199 0.126	0.128 0.004	0.504[A] 0.067 #
Arm fat (%FM)	Pre-meno Post-meno	0.209 0.131	0.283[a] 0.158	0.156 0.215[b]	0.203 0.076	0.190 -0.098	0.260[a] -0.076 #	0.400[A] -0.023 #
Gynoid (%FM)	Pre-meno Post-meno	-0.449[A] -0.158	-0.531[A] -0.107	-0.615[A] -0.388[B]	-0.591[A] -0.450[B]	-0.242[a] 0.023	-0.164 0.137 #	-0.679[A] 0.074 #
VAT $(cm)^2$	Pre-meno Post-meno	0.415[A] -0.047 #	0.436[A] 0.037 #	0.737[A] 0.519[B]	0.635[A] 0.336[B]	0.411[A] -0.086 #	0.339[A] -0.098 #	0.597[A] 0.243[b]
SAT $(cm)^2$	Pre-meno Post-meno	0.274[a] -0.117 #	0.334[A] 0.063	0.601[A] 0.387[B]	0.533[A] 0.132	0.430[A] -0.059 #	0.369[A] -0.040 #	0.605[A] 0.231[b]

Values are Spearman's correlation coefficients. %FM, expressed as percentage of sub-total fat mass (FM), SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; hsCRP, C-reactive protein.
[a]p<0.05 and [A]P<0.01 for pre-menopausal women; [b]p<0.05 and [B]P<0.01 for post-menopausal women.
#p<0.05 for age x body composition interaction

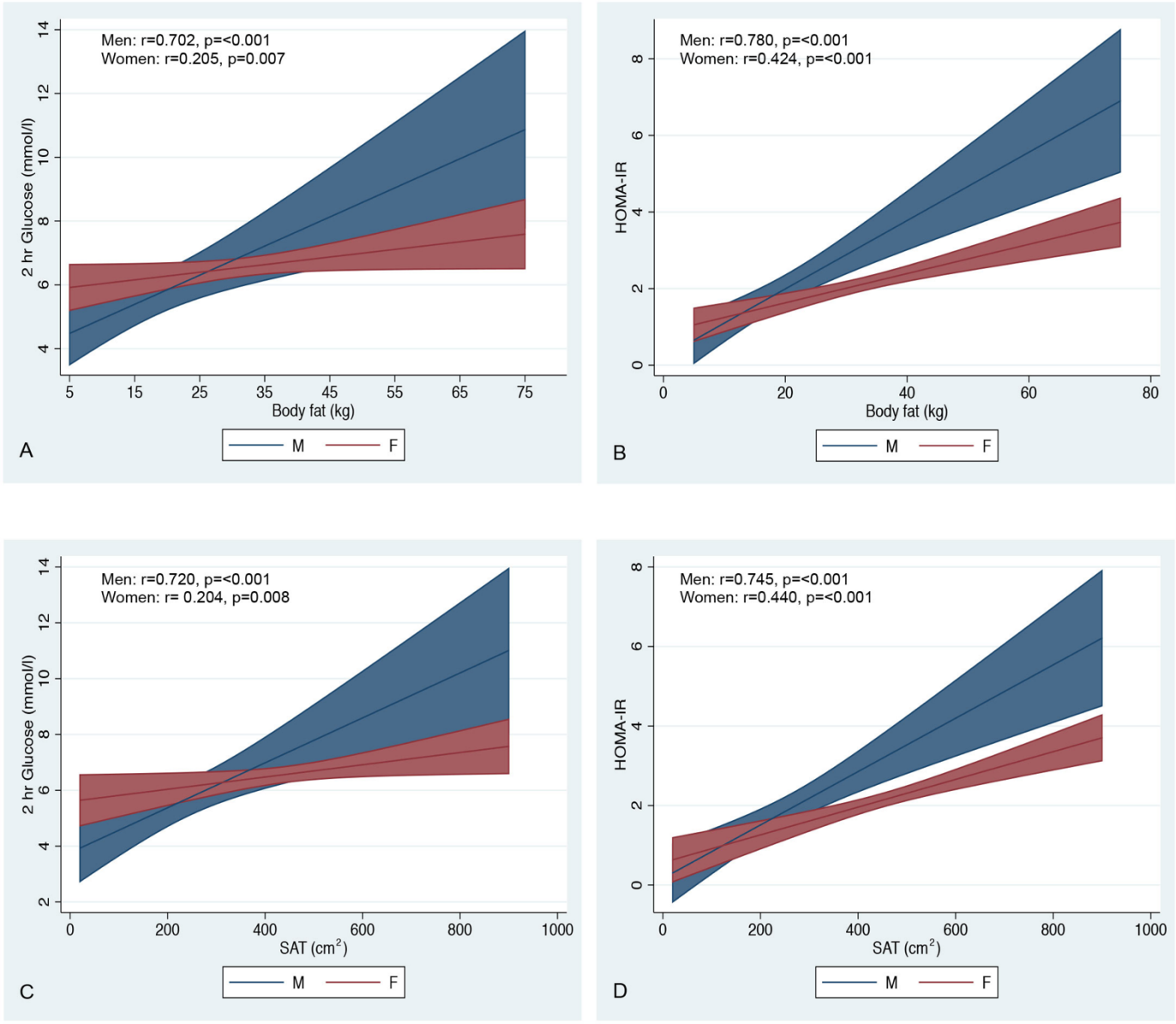

Fig 1- Sex-specific associations between total body fat and abdominal subcutaneous adipose tissue (SAT) and 2-hour glucose (A&C) and insulin resistance, estimated using HOMA-IR (B&D), respectively.

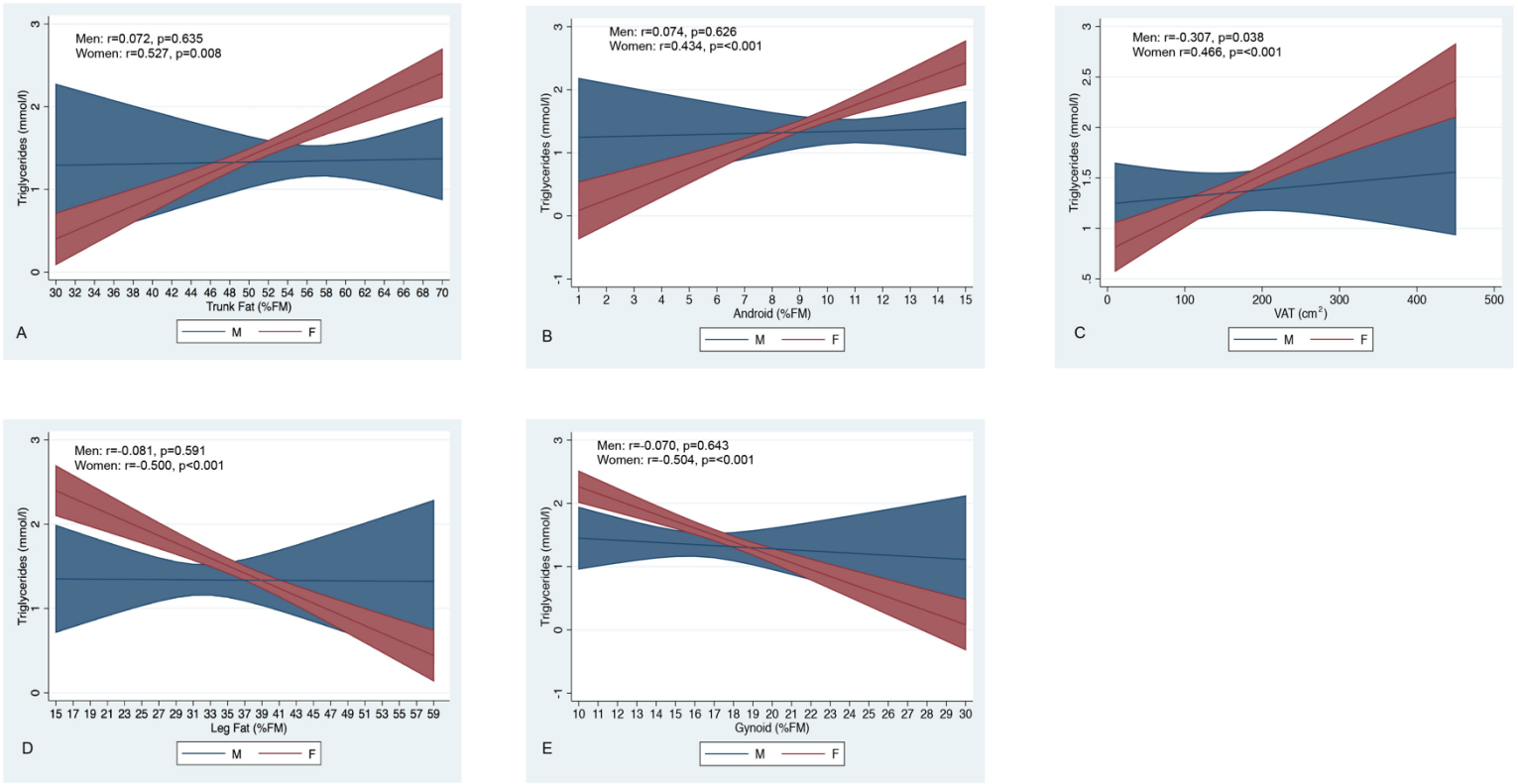

Fig 2 – Sex-specific associations between serum triglyceride concentrations and trunk % fat mass (%FM) (A) android %FM(B), visceral adipose tissue area (VAT)(C), leg %FM (D) and gynoid %FM (E)

References

1. WHO. Global status report on noncommunicable diseases. 2014.
2. Msemburi W, Pillay-van Wyk V, Dorrington RE, Neethling I, Nannan N, Groenewald P, Laubscher R, Joubert J, Matzopoulos R, Nicol E, Nojilana B, Prinsloo M SN, Somdyala N, Bradshaw D. Second national burden of disease study for South Africa: Cause of death profile for South Africa, 1997-2012. Cape Town, South Africa; 2016.
3. NCD-RicS-Africa Working Group. Trends in obesity and diabetes across Africa from 1980 to 2014 : an analysis of pooled population-based studies. Int J Epidemiol. 2017;(October):1–12.
4. Wandai M, Day C. Trends in risk factors for non-communicable diseases in South Africa. Durban, South Africa; 2015.
5. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med. 2013;34(1):1–11.
6. Lemos JA De, Neeland IJ. New Insights Into the Cardiometabolic Risks of Obesity. JACC Cardiovasc Imaging. 2014;7(12):1236–8.
7. O'Neill S, O'Driscoll L. Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. Obes Rev. 2015;16(1):1–12.
8. Zhang M, Hu T, Zhang S, Zhou L. Associations of Different Adipose Tissue Depots with Insulin Resistance: A Systematic Review and Meta-analysis of Observational Studies. Sci Rep. 2015;5:18495.
9. McLaughlin T, Lamendola C, Liu A, Abbasi F. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. J Clin Endocrinol Metab. 2011;96(11):1756–60.
10. Neeland IJ, Ayers CR, Rohatgi AK, Turer AT, Berry JD, Das SR, et al. Associations of visceral and abdominal subcutaneous adipose tissue with markers of cardiac and metabolic risk in obese adults. Obesity. 2013;21(9):439–47.
11. Pinnick KE, Neville MJ, Fielding BA, Frayn KN, Karpe F, Hodson L. Gluteofemoral adipose tissue plays a major role in production of the lipokine palmitoleate in humans. Diabetes. 2012;61(6):1399–403.
12. Karpe F, Pinnick KE. Biology of upper-body and lower-body adipose tissue—link to whole-body phenotypes. Nat Rev Endocrinol. 2014;11(2):90–100.
13. Park SY, Kwon KY, Kim JH, Choi HH, Han KH, Han JH. Association between appendicular fat mass and metabolic risk factors. Korean J Fam Med. 2014;35(4):182–9.
14. Geer EB, Shen W. Gender differences in insulin resistance, body composition, and energy balance. Gend Med. 2009;6(Suppl 1):60–75.
15. Tchoukalova YD, Koutsari C, Votruba SB, Tchkonia T, Giorgadze N, Thomou T, et al. Sex- and depot-dependent differences in adipogenesis in normal-weight humans. Obesity (Silver Spring). 2010;18(10):1875–80.
16. Nordström A, Hadrévi J, Olsson T, Franks PW, Nordström P. The Higher Prevalence of Type 2 Diabetes in Men. 2016;(August):1–8.
17. Peer N, Kengne A-P, Motala AA, Mbanya JC. IDF Diabetes Atlas Diabetes in the Africa region: An update. Diabetes Res Clin Pract. 2014;103:197–205.
18. Gurka MJ, Vishnu A, Santen RJ, DeBoer MD. Progression of Metabolic Syndrome Severity During the Menopausal Transition. J Am Heart Assoc. 2016;5(8):e003609.
19. Karvonen-Gutierrez C, Kim C. Association of Mid-Life Changes in Body Size, Body Composition and Obesity Status with the Menopausal Transition. Healthcare. 2016;4(3):42.
20. Wells JCK. Ethnic variability in adiposity, thrifty phenotypes and cardiometabolic risk: Addressing the full range of ethnicity, including those of mixed ethnicity. Obes Rev. 2012;13(Suppl. 2):14–29.
21. Rush EC, Goedecke JH, Jennings C, Micklesfield L, Dugas L, Lambert E V, et al. BMI , fat and muscle differences in urban women of five ethnicities from two countries. Int J Obes. 2007;31:1232–9.
22. Nazare JA, Smith JD, Borel AL, Haffner SM, Balkau B, Ross R, et al. Ethnic influences

on the relations between abdominal subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: The international study of prediction of intra-abdominal adiposity and its relationship with cardiometabolic risk/intra-. Am J Clin Nutr. 2012;96(4):714–26.
23. Eastwood S V., Tillin T, Dehbi HM, Wright A, Forouhi NG, Godsland I, et al. Ethnic differences in associations between fat deposition and incident diabetes and underlying mechanisms: The SABRE study. Obesity. 2015;23(3):699–706.
24. Goedecke JH, Dave JA, Faulenbach M V, Utzschneider KM, Lambert E V, West S, et al. Insulin response in relation to insulin sensitivity: an appropriate beta-cell response in black SA women. Diabetes Care. 2009;32(5):860–5.
25. Katzmarzyk PT, Bray, George A, Greenway FL, Johnson WD, Newton RL, Ravussin E, et al. Racial differences in abdominal depot – specific adiposity in white and African American adults 1 – 3. Am J Clin Nutr. 2010;91:7–15.
26. Micklesfield LK, Evans J, Norris SA, Lambert E V, Jennings C, Joffe Y, et al. Dual-energy X-ray absorptiometry and anthropometric estimates of visceral fat in Black and White SA Women. Obesity (Silver Spring). 2010;18(3):619–24.
27. Keswell D, Tootla M, Goedecke JH. Associations between body fat distribution, insulin resistance and dyslipidaemia in black and white SA women. Cardiovasc J Afr. 2016;27(May):1–7.
28. Liu J, Hickson DA, Musani SK, Talegawkar SA, Carithers TC, Tucker KL, et al. Dietary Patterns, Abdominal Visceral Adipose Tissue, and Cardiometabolic Risk Factors in African Americans: The Jackson Heart Study. Obesity. 2013;21(3, March):644–51.
29. Ali AT, Crowther NJ. Body fat distribution and insulin resistance. S Afr Med J. 2005;95(11):878–80.
30. Crowther NJ, Norris SA. The Current Waist Circumference Cut Point Used for the Diagnosis of Metabolic Syndrome in Sub-Saharan African Women Is Not Appropriate. PLoS One. 2012;7(11).
31. Erasmus RT, Soita DJ, Hassan MS, Blanco-Blanco E, Vergotine Z, Kegne AP, et al. High prevalence of diabetes mellitus and metabolic syndrome in a SA coloured population: baseline data of a study in Bellville, Cape Town. SA Med J. 2012 Nov;102(11):841–4.
32. De Wit E, Delport W, Rugamika CE, Meintjes A, Möller M, Van Helden PD, et al. Genome-wide analysis of the structure of the SA Coloured Population in the Western Cape. Hum Genet. 2010;128(2):145–53.
33. Stats SA. Statistical release (Revised) Census 2011. Pretoria, South Africa; 2012.
34. Kengne AP, Erasmus RT, Levitt NS, Matsha TE. Alternative indices of glucose homeostasis as biochemical diagnostic tests for abnormal glucose tolerance in an African setting. Prim Care Diabetes II Eur. 2017;11(2):119–31.
35. Hangartner TN, Warner S, Braillon P, Jankowski L, Shepherd J. The Official Positions of the International Society for Clinical Densitometry: Acquisition of Dual-Energy X-Ray Absorptiometry Body Composition and Considerations Regarding Analysis and Repeatability of Measures. J Clin Densitom. 2013;16(4):520–36.
36. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-Energy X-Ray Performs as Well as Clinical Computed Tomography for the Measurement of Visceral Fat. Obesity. 2012;20(5, May):1109–14.
37. Chalmers J, MacMahon S, Mancia G, Whitworth J, Beilin L, Hansson L, et al. 1999 World Health Organization International Society of Hypertension guidelines for the management of hypertension. J Hypertens. 1999;17(2):151–83.
38. WHO. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia. World Health. 2006.
39. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force. Circulation. 2009;120(16):1640–5.
40. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
41. Gold EB. The Timing of the Age at Which Natural Menopause Occurs. Obstet Gynecol

Clin North Am. 2011;38(3):425–40.
42. Jung SH, Ha KH, Kim DJ. Visceral Fat Mass Has Stronger Associations with Diabetes and Prediabetes than Other Anthropometric Obesity Indicators among Korean Adults. Yonsei Med J. 2016;57(3):674–80.
43. Shi H, Kumar S. Sex Differences in Obesity-Related Glucose Intolerance and Insulin Resistance. In: Glucose Tolerance. Intech open access book publisher; 2012. p. 37–66.
44. Dulloo AG, Jacquet J, Solinas G, Montani J-P, Schutz Y. Body composition phenotypes in pathways to obesity and the metabolic syndrome. Int J Obes (Lond). 2010;34 Suppl 2(S2):S4-17.
45. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, et al. Abdominal visceral and subcutaneous adipose tissue compartments: Association with metabolic risk factors in the framingham heart study. Circulation. 2007;116(1):39–48.
46. Nielsen S, Guo Z, Johnson CM, Hensrud DD, Jensen MD. Splanchnic lipolysis in human obesity. J Clin Invest. 2004;113(11):1582–8.
47. Snijder MB, Visser M, Dekker JM, Goodpaster BH, Harris TB, Kritchevsky SB, et al. Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study. Diabetologia. 2005;48(2):301–8.
48. Schorr M, Dichtel LE, Gerweck A V., Valera RD, Torriani M, Miller KK, et al. Sex differences in body composition and association with cardiometabolic risk. Biol Sex Differ. 2018;9(1):1–10.
49. Jensen MD. Role of Body Fat Distribution and the Metabolic Complications of Obesity. J Clin Endocrinol Metab. 2008;93(11):S57–63.
50. Luo Y, Ma X, Shen Y, Hao Y, Hu Y, Xiao Y, et al. Positive relationship between serum low-density lipoprotein cholesterol levels and visceral fat in a Chinese nondiabetic population. PLoS One. 2014;9(11):1–7.
51. Krishnan KC, Mehrabian M, Lusis AJ. Sex differences in metabolism and cardiometabolic disorders. Curr Opin Lipidol. 2018;29(5):404–10.
52. de Mutsert R, Gast K, Widya R, de Koning E, Jazet I, Lamb H, et al. Associations of Abdominal Subcutaneous and Visceral Fat with Insulin Resistance and Secretion Differ Between Men and Women: The Netherlands Epidemiology of Obesity Study. Metab Syndr Relat Disord. 2018;16(1):54–63.
53. Shisana O, Labadarios D, Rehle T, Simbayi L, Zuma K, Dhansay A, Reddy P, Parker W, Hoosain E, Naidoo P, Hongoro C, Mchiza Z, Steyn NP, Dwane N, Makoae M, Maluleke T, Ramlagan S, Zungu N, Evans MG, Jacobs L FM. The SA National Health and Nutrition Examination Survey, 2012 SANHANES-1. Cape Town: HSRC Press, Cape Town; 2014. 1–398 p.

CHAPTER FIVE: THE DISCRIMINATORY POWER OF VISCERAL ADIPOSE TISSUE AREA VS ANTHROPOMETRIC MEASURES AS A DIAGNOSTIC MARKER FOR METABOLIC SYNDROME IN SOUTH AFRICAN WOMEN.

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Introduction

The International Diabetes Federation estimates the global prevalence of MetS to be around 25% (1,2). Previous research in South Africa showed that the mixed ancestry population were a high risk group for MetS (3). It is therefore essential in a high risk population like this that one is able to identify those at risk and introduce lifestyle modifications.

Typically WC is the accepted proxy of visceral adipose tissue (VAT) and measure of central adiposity, and is used in the clinical diagnosis of MetS (4). The advantage of WC is that it is quick and easy to measure and does not require technical equipment (5,6). Other anthropometric measures of total adiposity and body fat distribution such as BMI, WC, hip circumference (HC), waist to height ratio (WHtR), waist to hip ratio (WHR) and more recently, a body shape index (ABSI) have also been used as indicators of cardiometabolic risk (7). All have their benefits, as well as their weaknesses. For example, while BMI is most commonly used as an indicator of total adiposity, it does not differentiate between muscle and fat or the location of fat (8). WHtR, unlike WC thresholds, takes into account body size (8) and has shown closer agreement of values between men and women at all ages (9) . Similarly, the ABSI takes into account body size and is based on the WC adjusted for height and weight (10). In contrast, WHR, uses the ratio of WC and HC, however the practicality of measuring 2 circumferences may be cumbersome and prone to error (11).

While metabolic abnormalities can be due to differential distribution of adipose tissue and or adipose dysfunction (12), a major limitation of WC, and other anthropometric measurements, is the inability to discriminate between VAT and subcutaneous adipose tissue (SAT) (6,13). Findings of the Framingham heart study revealed that both SAT and VAT correlated with metabolic risk factors, but that VAT was more powerfully associated with an unfavourable metabolic risk profile even after accounting for easily measured anthropometric indexes such as BMI and WC (14). The mechanisms linking VAT accumulation to metabolic complications involve the greater production of proinflammatory cytokines and the greater lipolytic action compared to SAT, with the resultant increase in cytokines and free fatty acid transfer to the hepatic portal system impacting on insulin sensitivity (15).

In the clinical setting, VAT is however difficult to measure as it requires expensive technical equipment for imaging (13)(16)(6). Although computed tomography (CT) and magnetic resonance imaging (MRI) are considered the gold standard imaging methods for quantifying body fat and its distribution, reliable algorithms have been developed using dual x-ray absorptiometry (DXA) software. These have been validated against CT in women with varying body mass index (BMI) (17,18). Numerous studies in various populations have identified associations between DXA-derived VAT area and cardio-metabolic risk factors (19–22).

Although waist circumferences may be similar, ethnic variations in VAT and subcutaneous (SAT) have been documented amongst different ethnic groups (15). Few studies have attempted to measure VAT in African populations and more specifically in the mixed ancestry women of South Africa where the prevalence of MetS is high.

The main aim of the study was to compare the ability of DXA-derived VAT area and anthropometry measures of total and central adiposity for diagnosing MetS in this high-risk sample of SA women.

Materials and Methods

Study setting and population

Participants

The current study data was collected from participants from the Cape Town Vascular and Metabolic Health (VMH) study, an extension of the Cape Town Bellville South study described previously (23,24). A mixed race was chosen as this population accounts for 8.9% of the SA population, 48.8% of the population of the Western Cape Province and 76% of the geographical area surveyed. This population has a high prevalence of MetS and type-2 diabetes (3), and therefore risk detection is key to early prevention and management.

Self-described mixed-ancestry female volunteers who took part in the above mentioned cross-sectional study were invited to complete a whole body DXA scan. The DXA scans were performed from April 2015 to June 2016. Being 20 years or older was an inclusion criterion. Participants were excluded if they were pregnant or acutely ill. Ethical approval was obtained from the Ethics Committees of the Cape Peninsula University of Technology and Stellenbosch University (NHREC:REC-230 408-014, CPUT/HWS-REC 2015/H03 and N14/01/003). Participants provided written consent to participate in the study. A total of 204 women volunteered for the study.

Body composition

Anthropometric measurements were taken, as described in detail previously (3,24). Body weight was measured (to the nearest 0.1 kg) with the participants in light clothing and without shoes. Height was measured to the nearest centimetre using a stadiometer. BMI was calculated as weight divided by height in square meters (kg/m^2). Waist circumference was measured using a non-elastic tape at the level of the narrowest part of the torso as seen in an antero-posterior projection and hip circumference around the widest part of the buttocks. The WHtR is calculated as the waist measurement divided by the height, while WHR is calculated as waist measurement divided by the hip measurement. ABSI was based on WC adjusted for weight and height, and was calculated using the formula,

ABSI = $\frac{WC}{BMI^{2/3}\, height^{1/2}}$ (10). All anthropometric measurements were performed three times, and the average measurements were used for analysis.

Body composition (fat mass and fat-free mass) was acquired by a trained and experienced radiographer using a Hologic Discovery W DXA whole body scanner configured with software version 13.4.1 (Hologic, Bedford, MA). Participants were positioned as per the NHANES body composition manual as advocated by Hangartner (25). VAT and SAT area were estimated within the android region, which is automatically defined with a caudal limit placed on top of the iliac crests and its height set to 20% of the distance from the top of the iliac crest to the base of the skull as the cephalic limit (18). DXA has proved to be as accurate as a clinical CT scan in the quantification of VAT and SAT in adults (18). Subtotal body fat % and kg, which excludes the head, were used in the analysis. The head was excluded to reduce the possibility of any artefacts in the head region. Total adipose body tissue classification excludes the head.

Blood sample collection and analysis

After an overnight fast, blood samples were taken to measure glycated haemoglobin (HbA_{1c}), glucose, insulin and lipid profile. Blood samples were transported daily on ice for processing using standard pathology practices. Biochemical parameters were analysed at an ISO 15189 accredited Pathology practice (Pathcare, Reference Laboratory , Cape Town, South Africa) as described elsewhere (24). Plasma glucose was measured by the enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa). Insulin was measured by paramagnetic particle chemiluminescence assay (Beckman DXI, Beckman Coulter, South Africa). High-density lipoprotein cholesterol (HDL-C) was by enzymatic immunoinhibition, and triglycerides by glycerol phosphate oxidase-peroxidase and Low density lipoprotein cholesterol (LDL-C) by enzymatic selective protection – End Point (Beckman AU, Beckman Coulter, South Africa).

Metabolic syndrome was quantified using the Joint Interim Statement (JIS) criteria (4), namely the presence of any 3 risk factors: WC ≥80 cm, elevated triglycerides ≥ 1.7 mmol/L (or drug treatment for elevated levels), elevated blood pressure systolic ≥ 130 and/or diastolic ≥ 85 mmHg (or antihypertensive drug treatment), elevated fasting blood glucose ≥ 5.6 mmol/L (or drug treatment of elevated glucose) and reduced HDL-C < 1.3 mmol/L (or drug treatment for reduced HDL-C). In our analysis we excluded WC in the discrimination of MetS as WC is part of the JIS MetS criteria, but used DXA-VAT as an independent variable to discriminate those with MetS.

Statistical methods

General characteristics of the study groups are summarized as count and percentage for categorical variables, mean and standard deviation (SD) or median and 25^{th}-75^{th} percentiles for quantitative variables. The *pROC* package (26) of the R statistical software version 3.4.3 [30-11-2017], (The R Foundation for Statistical Computing, Vienna, Austria) was used for receiver operating characteristics (ROC) analyses. The area under the curve (AUC) was then used to assess and compare the ability of VAT area, WC, BMI, WHR, WHtR and ABSI to predict the presence of any two components of metabolic syndrome, excluding WC, with AUC comparisons through non-parametric methods (27). The optimal WC and VAT area was determined by applying both the Youden's index approach (28) and the closest top left point approach (29). For comparison purposes, the optimal VAT and WC thresholds derived for this sample were tested alongside cut points commonly advocated in African and other populations (4,30,31).

Results

Participant characteristics

The characteristics of the participants are presented in Table 1. The mean age of the participants was 53.1 (± 13.7) years. The mean BMI was 32.6 (± 7.2), with 19.8% of the participants being overweight, and the majority (64.7%) obese. Twenty seven percent were classified as having diabetes and 57.1% had MetS, with 88.7% presenting with a WC ≥80cm. The median VAT area was 181 cm^2. The most prevalent MetS components after the high WC, was high blood pressure (74%), low HDL (48%) with triglycerides (34.3%) and high fasting glucose (33.5%) being the least frequent component.

Discriminatory power of anthropometric variables and VAT for the prediction of Metabolic syndrome

The discriminatory power of all anthropometric variables and VAT area for the prediction of any two JIS-defined components of MetS (excluding WC, as this is part of the MetS criteria) is shown in Table 2. The highest point estimate of AUC for the prediction of MetS was recorded for VAT, followed by WHtR and WC (AUC, 0.767, 0.747 and 0.738 respectively), but these did not differ significantly (all $p \geq 0.083$). In contrast, VAT had significantly greater discriminatory power than BMI ($p=0.028$), and VAT, WHtR and WC had greater discriminatory power than hip ($p<0.0004$) and ABSI ($p<0.0001$).

Optimal waist circumference and VAT threshold values

Given that WC is commonly used as a proxy for VAT in the clinical setting, we examined the thresholds for WC and VAT area to diagnose MetS (excluding WC) in this sample. The receiver

operating characteristic curves (ROC) for the prediction of the presence of at least two components of MetS using WC or VAT area are presented in Figure 1.The Youden's index method and the CTL approach used to derive the optimal WC threshold values in this sample identified the same threshold of 94.4 cm (Table 3). The optimal VAT area threshold was 174 cm^2 based on the Youden's index method and 175.50 cm^2 based on the CTL approach.

The WC cut-point of 94.4 cm in this sample yielded a sensitivity of 61.4% and specificity of 80.8% . The sensitivity of this cut-point was higher than the recommended JIS ≥80cm WC (20.5%) cut-point, and the 90cm WC (48.2%) cut-point recommended by Matsha and co-workers for both genders in the larger study of this population (31). Conversely, the specificities of the WC derived for this sample (80.8%) was lower than that for the JIS criteria (95.8%) and the larger study in this population (89.2%) (31). The sensitivity and specificity of the 174 cm^2 VAT thresholds obtained from the Youden index were 72.3% and 70% respectively.
When comparing the sensitivity and specificity of WC and VAT cut-points for predicting any two components of MetS in this population, the sensitivity of the VAT area cut-point was greater than WC (72.3% v 61%), however the specificity of VAT area was less than WC (70% v 80.8%). When comparing the accuracy of VAT and WC for predicting any two components of MetS, they were very similar (70.9%-72.3%, for Youden index and CTL). Further, the positive predictive values (PPV) were similar for VAT area and WC in diagnosing any two components of MetS (63% V 69%) with similar 95% CI's. The likelihood of a positive test (LH^+) for VAT area and WC were also similar (2.1-2.45 v 3.21 respectively).

Discussion

In this study we set out to compare the discriminatory power of DXA-derived VAT area and other anthropometric measurements to diagnose any two components of MetS in a sample of high-risk mixed ancestry women from South Africa. The main finding was that VAT area, WC and WHtR performed similarly, lending support to the current recommendation of using the WC measurement for the diagnosis of MetS.

VAT accumulation is an important predictor of MetS (32) and is more closely associated with MetS risk than SAT due to its greater lipolytic activity and higher inflammatory profile (15). WC is an internationally recognized surrogate for VAT and one of the five JIS criteria for the diagnosis of MetS (4). As WC and other anthropometric measurements are however unable to discriminate between VAT and SAT, we set out to determine whether VAT would perform better than these measures and could therefore be used in risk prediction. Notably, we found that DXA- derived VAT did not perform better that WC and some of the other measures of central adiposity in diagnosing MetS in this sample. A possible explanation for this is that abdominal SAT is heterogenous, is of greater volume than VAT and has similar metabolic

activity to VAT, thus also impacting on the development of insulin resistance and MetS (12). The larger volume of SAT vs. VAT is particularly true for women compared to men, with abdominal SAT measured at L4-L5 being roughly five fold greater than VAT in the same location (12). Moreover, abdominal SAT can be divided at the level of the fascia superficialis into deep (dSAT) and superficial SAT (sSAT), with the dSAT having higher metabolic activity and inflammatory profile than sSAT, and intermediate to VAT (33). This suggests that the metabolic effects of accumulation of both VAT and abdominal SAT on MetS risk may be additive. Other studies have shown that VAT area performs better than WC, WHtR and WHR in determining MetS risk. For example, the results of the cross-sectional Netherlands epidemiology of Obesity study involving mostly white middle-aged obese women indicated that MRI-derived VAT was most strongly associated with cardiometabolic risk factors followed by WC and WHR (34). Likewise in a study in Japanese women, CT-derived VAT performed better than WC in predicting MetS (35). In contrast, and similar to our findings, Evans et al., (2011) found that WC, WHtR and a CT-derived measure of VAT performed similarly in predicting MetS in pre-menopausal black and white SA women. These findings may be explained by ethnic-specific associations between adipose tissue distribution and insulin sensitivity. Indeed, studies have shown that VAT was the most significant correlate of insulin sensitivity in white women, whereas in black women, SAT performed similarly or better to VAT (Goedecke et al., (2013). Another possible explanation for VAT not performing significantly better than WC and WHtR in diagnosing MetS may lie in the methodological limitation of imaging to precisely distinguish the various anatomical adipose tissue compartments (38). Although DXA-derived VAT and SAT have been validated against the gold standard imaging methods such as CT and MRI in other ethnic groups (17,18), they have not yet been validated in a mixed ancestry population. Additionally, DXA is unable to differentiate between dSAT and sSAT.

When comparing the performance of the VAT area and WC thresholds for detecting any two components of MetS in this sample, we found that the sensitivity of VAT area was higher than WC (73% vs 61%), but the specificity was lower (70% vs. 80.8%). The implication of this is that VAT may be more sensitive than WC to detect MetS, but may also over diagnose MetS in screening. Furthermore, the accuracy and PPV of the WC and VAT area, as well as the DOR were very similar, reiterating the view that there may not be any advantage of using the more costly measurement of VAT in the clinical setting.

In contrast to WC and WHtR, we found that VAT area performed better than BMI, HC, WHR and ABSI in diagnosing any two components of MetS. A possible reason for this is that the latter anthropometry measures are essentially measures of total adiposity, which is not as closely associated with MetS as central adiposity (7). For example, several studies have shown weak correlations for ABSI in predicting MetS (39,40). Similarly in a large cross-sectional study

in an Iranian population, BMI had the lowest AUC in women for predicting Mets (41). WC only requires one measurement unlike BMI and ABSI which requires height to be measured which can be challenging in the clinical setting (42).

The optimal VAT area for predicting any 2 components of MetS (other than WC) in this sample was 174 cm^2 (CI: 137.7-181.5). This is higher than the ≥163cm^2 found in peri and post-menopausal African and Caucasian American women (19). Our VAT thresholds were also considerably higher than those used to predict metabolic risk variables in black (>48 cm) and white (>107 cm) pre-menopausal SA women (36). Notably, recommended VAT cut points for diagnosing any two components of MetS (other than WC) differ by age and ethnicity (19,35,36). Indeed, studies have shown that for the same BMI or WC, black women have less VAT than white women (43). Further, it is well known that VAT accumulation occurs at menopause (44), and that VAT and total fat mass are independent with regards to metabolic risk (45), supporting the notion that WC corresponding to critical levels of VAT area may be age specific (19,35). This is supported by results of the study by Evans et al., (2011) which identified considerably lower CT-derived VAT thresholds (>88 cm^2) for MetS risk factors in younger pre-menopausal white SA women than those recommended for peri- and post-menopausal women from our study (174 cm^2) and others (≥163cm^2) (19).

The strengths of the study are that we derived WC and VAT area cut-points specific to this sample, which enabled us to directly compare the performance of these cut-points to diagnose any 2 components of MetS. Additionally, the two statistical approaches used to derive the cut-points yielded very similar results, an indication that the relationships are consistent. Furthermore, this is the first study to measure DXA-derived VAT area in mixed ancestry SA women. Although CT and MRI are considered the gold-standard for measuring VAT area , DXA has been proven to be an accurate imaging tool for deriving VAT (18). The limitations of this study are that the sample included mostly post-menopausal women and thus the results cannot be generalised to younger women or men given that there are specific age and sex thresholds for WC that correspond to critical levels of VAT cm^2 (19,35). Additionally, the sample size was relatively small, yielding unstable estimates. A larger representative study is needed to determine whether VAT performs better than WC for diagnosing MetS and if shown to be better, ethnic, sex and age specific cut-points developed.

Conclusion

Our study showed that there is no advantage of measuring VAT over WC in the diagnosis of MetS as VAT area, WC and WHtR performed similarly in predicting two components of MetS

in this sample of mixed ancestry SA women. WC is easier to measure in the clinical setting than other anthropometric measures and an universally recognised proxy for central adiposity.

Declarations:

Ethics approval and consent to participate:

Ethical approval was obtained from the Ethics Committees of the Cape Peninsula University of Technology and Stellenbosch University (NHREC:REC-230 408-014, CPUT/HWS-REC 2015/H03 and N14/01/003). Participants provided written consent to participate in the study.

Consent for publication:

Not applicable

Availability of data and material:

The datasets used and/or analysed during the current study are available from the PI (TEM) Vascular and Metabolic Health Study on reasonable request.

Competing interests

The authors state that they have no competing interests.

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Authors' contributions:

FED: data analysis and interpretation, preparation of the first draft and approval of final draft
TEM: conception and design, acquisition and interpretation of data, revision for important intellectual content and approval of final draft
RTE: conception and design, revision for important intellectual content and approval of final draft
APK: conception and design, data analysis and interpretation of data, revision for important intellectual content and approval of final draft
JHG: data analysis and interpretation, revision for important intellectual content and approval of final draft

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Table 1: Participant characteristics

Variable	Overall
n	204
Age (years)	53.1 (13.7)
Body composition	
Height (m)	1.56 (0.06)
Weight (kg)	79.3 (18.3)
BMI (kg/m^2)	32.6 (7.2)
WC (cm)	99 (15.0)
Hip (cm)	113 (14.0)
WHR	0.88 (0.07)
WHtR	0.64 (0.1)
ABSI	0.078 [0.075-0.082]
Body fat (%)	44.0 (39.8-48.6)
Body fat (kg)	31.2 (24.4-40.0)
Cardiometabolic risk factors	
Fasting glucose (mmol/L)	6.3 (3.3)
Fasting serum insulin (µU/mL)	8.4 (5.7-12.7)
Triglycerides (mmol/L)	1.6 (1.0)
LDL-cholesterol (mmol/L)	3.3 (1.0)
HDL-cholesterol (mmol/L)	1.3 (0.3)
Total cholesterol (mmol/L)	5.4 (1.2)
Systolic blood pressure (mmHg)	127 (21)
Diastolic blood pressure (mmHg)	82 (12)
Metabolic syndrome (JIS)	
High waist circumference (n(%))	181 (88.7)
High blood pressure (n(%))	151 (74.0)
High fasting blood glucose (n(%))	68 (33.5)
High triglycerides (n(%))	70 (34.3)
Low HDL (n(%))	98 (48.0)
3 components or more (n(%))	116 (57.1)

Values are mean (standard deviation) or median (interquartile range).

BMI (WHO classification) body mass index; WC Waist circumference; WHR Waist- to- hip ratio; WHtR Waist- to -height- ratio; ABSI A body shape index

Table 2 – Comparison of the performance of anthropometric variables and VAT area in the discrimination of any two components of metabolic syndrome (not including the WC criteria in the analysis)

Variables	AUC (95% CI)	p-value for differences in AUC						
		Vs. BMI	Vs. WC	Vs. Hip	Vs. WHR	Vs. WHtR	Vs. ABSI	Vs. VAT area
BMI	0.716 (0.643-0.788)	-	0.187	0.002	0.703	0.109	0.014	0.028
WC	0.738 (0.667-0.810)	0.187	-	0.0005	0.288	0.413	0.0004	0.192
Hip	0.664 (0.587-0.741)	0.002	0.0005	-	0.528	0.001	0.132	0.0004
WHR	0.698 (0.624-0.771)	0.703	0.288	0.528	-	0.181	<0.0001	0.083
WHtR	0.747 (0.675-0.819)	0.109	0.413	0.001	0.181	-	0.0001	0.397
ABSI	0.575 (0.495-0.655)	0.014	0.0004	0.132	<0.0001	0.0001	-	<0.0001
VAT area	0.767 (0.700-0.834)	0.028	0.192	0.0004	0.083	0.397	<0.0001	-

ABSI, A Body Shape Index; AUC, area under the receiver-operating characteristic curve; BMI, body mass index; Hip, hip circumference; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; 95% CI, 95% confidence interval

Table 3- Performance of different VAT and waist optimal thresholds to detect two components of the metabolic syndrome (excluding waist circumference)

Thresholds*	Methods	App prev	True prev	Sensitivity	Specificity	Accuracy	DOR	NND	Youden index	PPV	NPV	LR+	LR-
VAT area													
174.0 (137.7-181.5)	Youden index	47.3 (40.3-54.4)	40.9 (34.0-48.0)	0.723 (0.614-0.815)	0.700 (0.610-0.780)	0.709 (0.642-0.770)	6.02 (3.28-11.31)	2.36 (1.68-4.48)	0.423 (0.223-0.596)	0.625 (0.520-0.722)	0.785 (0.695-0.859)	2.41 (1.78-3.27)	0.40 (0.27-0.57)
175.50 (154.6-180.1)	CTL	47.8 (40.7-54.9)	40.9 (34.0-48.0)	0.735 (0.627-0.826)	0.700 (0.610-0.780)	0.714 (0.647-0.775)	6.47 (3.46-12.08)	2.30 (1.65-4.23)	0.435 (0.236-0.606)	0.629 (0.525-0.725)	0.792 (0.703-0.865)	2.45 (1.81-3.31)	0.38 (0.26-0.55)
WC													
94.40 (89.7-102.3)	Youden index	36.4 (29.8-43.5)	40.9 (34.0-48.0)	0.614 (0.501-0.719)	0.808 (0.726-0.874)	0.729 (0.662-0.789)	6.72 (3.56-12.67)	2.36 (1.68-4.39)	0.423 (0.228-0.594)	0.689 (0.571-0.792)	0.752 (0.668-0.824)	3.21 (2.14-4.81)	0.48 (0.36-0.63)
94.40 (93.3-101.2)	CTL	36.4 (29.8-43.5)	40.9 (34.0-48.0)	0.614 (0.501-0.719)	0.808 (0.726-0.874)	0.729 (0.662-0.789)	6.72 (3.56-12.67)	2.36 (1.68-4.39)	0.423 (0.228-0.594)	0.689 (0.571-0.792)	0.752 (0.668-0.824)	3.21 (2.14-4.81)	0.48 (0.36-0.63)
80[#]		10.8 (6.9-15.9)	40.9 (34.0-48.0)	0.205 (0.124-0.307)	0.958 (0.905-0.986)	0.650 (0.580-0.716)	5.92 (2.09-16.79)	6.13 (3.40-33.87)	0.163 (0.029-0.294)	0.773 (0.546-0.922)	0.635 (0.561-0.705)	4.92 (1.89-12.80)	0.83 (0.74-0.93)
90[#]		26.1 (20.2-32.7)	40.9 (34.0-48.0)	0.482 (0.371-0.594)	0.892 (0.822-0.941)	0.724 (0.657-0.784)	7.66 (3.73-15.71)	2.68 (1.87-5.19)	0.374 (0.193-0.535)	0.755 (0.617-0.862)	0.713 (0.634-0.784)	4.45 (2.54-7.78)	0.58 (0.47-0.72)

*CI = P2.5 and P97.5 from bootstrap, WC 80cm[#](JIS), WC 90cm[#](31)
App prev, apparent prevalence; DOR, diagnostic odd ratio; LR-, likelihood of a negative test; LR+, likelihood of a positive test; NND, number needed to diagnose; NPV, negative predictive value; PPV, positive predictive value; thresh, threshold; True prev, true prevalence

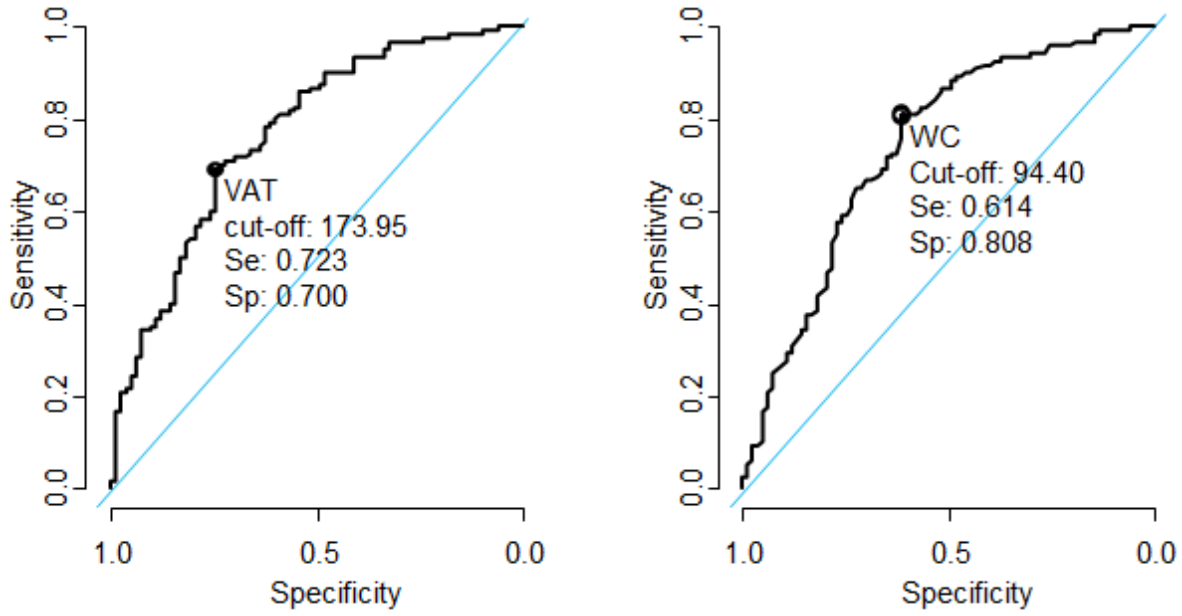

Figure 1 Receive operating characteristic curves (ROC) using visceral adipose tissue area (VAT) and waist circumference (WC) for the prediction of the presence of at least two components of the metabolic syndrome. Se, sensitivity; Sp, specificity (using the Youden Index).

References

1. O'Neill S, O'Driscoll L. Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. Obes Rev. 2015;16(1).
2. Nolan PB, Carrick-Ranson G, Stinear JW, Reading SA, Dalleck LC. Prevalence of metabolic syndrome and metabolic syndrome components in young adults: A pooled analysis. Prev Med Reports. 2017;7:211–5.
3. Erasmus RT, Soita DJ, Hassan MS, Blanco-Blanco E, Vergotine Z, Kegne AP, et al. High prevalence of diabetes mellitus and metabolic syndrome in a SA coloured population: baseline data of a study in Bellville, Cape Town. SA Med J. 2012 Nov;102(11):841–4.
4. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force. Circulation. 2009;120(16):1640–5.
5. Klein S, Allison D, Heymsfield S, Kelley D. Waist circumference and cardiometabolic risk: A consensus statement from shaping America's health: Association for weight management and obesity prevention; NAASO, the Obesity society; the American society for nutrition; and the American diabetes associat. Obesity. 2007;15(5 May):1061–7.
6. Bi X, Seabolt L, Shibao C, Buchowski M, Kang H, Keil CD, et al. DXA-measured visceral adipose tissue predicts impaired glucose tolerance and metabolic syndrome in obese Caucasian and African-American women. Eur J Clin Nutr. 2014;1–8.
7. Amirabdollahian F, Haghighatdoost F. Anthropometric Indicators of Adiposity Related to Body Weight and Body Shape as Cardiometabolic Risk Predictors in British Young Adults : Superiority of Waist-to-Height Ratio. J Obes. 2018;1–15.
8. Millar SR, Perry IJ, Phillips CM. Assessing cardiometabolic risk in middle-aged adults using body mass index and waist-height ratio: Are two indices better than one? A cross-sectional study. Diabetol Metab Syndr. 2015;7(1):1–11.
9. Hsieh SD, Yoshinaga H, Muto T. Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. Int J Obes. 2003;27(5):610–6.
10. Krakauer NY, Krakauer JC. A New Body Shape Index Predicts Mortality Hazard Independently of Body Mass Index. PLoS One. 2012;7(7):e39504.
11. Ashwell M, Gibson S. Waist to height ratio is a simple and effective obesity screening tool for cardiovascular risk factors: Analysis of data from the British national diet and nutrition survey of adults aged 19-64 years. Obes Facts. 2009;2(2):97–103.
12. Patel P, Abate N. Role of subcutaneous adipose tissue in the pathogenesis of insulin resistance. J Obes. 2013;2013.
13. Wajchenberg BL. Subcutaneous and Visceral Adipose Tissue : Endocr Rev. 2000;21(6):697–738.
14. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, et al. Abdominal visceral and subcutaneous adipose tissue compartments: Association with metabolic risk factors in the framingham heart study. Circulation. 2007;116(1):39–48.
15. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med. 2013;34(1):1–11.
16. Bouchard C. BMI, fat mass, abdominal adiposity and visceral fat: Where is the 'beef'? Int J Obes. 2007;31(10):1552–3.
17. Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, et al. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity. 2012;20(6):1313–8.
18. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-Energy X-Ray Performs as Well as Clinical Computed Tomography for the Measurement of Visceral Fat. Obesity. 2012;20(5, May):1109–14.
19. Nicklas BJ, Penninx BWJH, Ryan AS, Berman DM, Lynch NA, Dennis KE. Visceral Adipose Tissue Cutoffs Associated With Metabolic Risk Factors for Coronary Heart Disease in Women. Diabetes Care. 2003;26(5):1413–20.

20. Katzmarzyk PT, Heymsfield SB, Bouchard C. Clinical utility of visceral adipose tissue for the identification of cardiometabolic risk in white and African American adults. Am J Clin Nutr. 2013;97:480–6.
21. Rothney MP, Catapano AL, Xia J, Wacker WK, Tidone C, Grigore L, et al. Abdominal visceral fat measurement using dual-energy X-ray: Association with cardiometabolic risk factors. Obesity. 2013;21(9):1798–802.
22. Miazgowski T, Krzyżanowska-Świniarska B, Dziwura-Ogonowska J, Widecka K. The associations between cardiometabolic risk factors and visceral fat measured by a new dual-energy X-ray absorptiometry-derived method in lean healthy Caucasian women. Endocrine. 2014;47(2):500–5.
23. Masconi K, Matsha TE, Erasmus RT, Kengne AP. Independent external validation and comparison of prevalent diabetes risk prediction models in a mixed-ancestry population of South Africa. Diabetol Metab Syndr. 2015;7(1):42.
24. Kengne AP, Erasmus RT, Levitt NS, Matsha TE. Alternative indices of glucose homeostasis as biochemical diagnostic tests for abnormal glucose tolerance in an African setting. Prim Care Diabetes II Eur. 2017;11(2):119–31.
25. Hangartner TN, Warner S, Braillon P, Jankowski L, Shepherd J. The Official Positions of the International Society for Clinical Densitometry: Acquisition of Dual-Energy X-Ray Absorptiometry Body Composition and Considerations Regarding Analysis and Repeatability of Measures. J Clin Densitom. 2013;16(4):520–36.
26. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: An open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12.
27. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves : A Nonparametric Approach. Biometrics. 1988;44(3):837–45.
28. Youden WJ. Index for rating diagnostic tests. Cancer. 1950;3(1):32–5.
29. Perkins NJ, Schisterman EF. The inconsistency of ‘optimal’ cutpoints obtained using two criteria based on the receiver operating characteristic curve. Am J Epidemiol. 2006;163(7):670–5.
30. Motala AA, Esterhuizen T, Pirie FJ, Omar MA. The prevalence of metabolic syndrome and determination of the optimal waist circumference cutoff points in a rural SA community. Diabetes Care. 2011;34(4 April):1032–7.
31. Matsha TE, Hassan MS, Hon GM, Soita DJ, Kengne A, Erasmus RT. Derivation and validation of a waist circumference optimal cutoff for diagnosing metabolic syndrome in a SA mixed ancestry population. Int J Cardiol. 2013 Oct;168(3):2954–5.
32. Arsenault BJ, Després JP, Boekholdt SM. Hypertriglyceridemic waist: Missing piece of the global cardiovascular risk assessment puzzle? Clin Lipidol. 2011;6(6):639–51.
33. Alvehus M, Burén J, Sjöström M, Goedecke J, Olsson T. The human visceral fat depot has a unique inflammatory profile. Obesity. 2010;18(5, May):879–83.
34. Elffers TW, Mutsert D, Lamb HJ, Roos A De, Willems van Dijk K, Rosendaal FR, et al. Body fat distribution , in particular visceral fat , is associated with cardiometabolic risk factors in obese women. PLoS One. 2017;12(9):e0185403.
35. Hayashi T, Boyko EJ, McNeely MJ, Leonetti DL, Kahn SE, Fujimoto WY. Minimum waist and visceral fat values for identifying Japanese Americans at risk for the metabolic syndrome. Diabetes Care. 2007;30(1):120–7.
36. Evans J, Micklesfield L, Jennings C, Levitt NS, Lambert E V., Olsson T, et al. Diagnostic Ability of Obesity Measures to Identify Metabolic Risk Factors in SA Women. Metab Syndr Relat Disord. 2011;9(5):353–60.
37. Goedecke JH, Levitt NS, Evans J, Ellman N, Hume DJ, Kotze L, et al. The role of adipose tissue in insulin resistance in women of African ancestry. J Obes. 2013;2013.
38. Tchernof A, Després J-P. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93(1):359–404.
39. Haghighatdoost F, Sarrafzadegan N, Mohammadifard N, Asgary S, Boshtam M, Azadbakht L. Assessing body shape index as a risk predictor for cardiovascular diseases and metabolic syndrome among Iranian adults. Nutrition. 2014;30(6):636–44.

40. Behboudi-Gandevani S, Ramezani Tehrani F, Cheraghi L, Azizi F. Could “a body shape index” and “waist to height ratio” predict insulin resistance and metabolic syndrome in polycystic ovary syndrome? Eur J Obstet Gynecol Reprod Biol. 2016;205:110–4.
41. Bener A, Yousafzai MT, Darwish S, Al-Hamaq AOAA, Nasralla EA, Abdul-Ghani M. Obesity index that better predict metabolic syndrome: Body mass index, waist circumference, waist hip ratio, or waist height ratio. J Obes. 2013;2013.
42. Frid H, Adolfsson ET, Rosenblad A, Nydahl M. Agreement between different methods of measuring height in elderly patients. J Hum Nutr Diet. 2013;26:504–11.
43. Sumner AE, Micklesfield LK, Ricks M, Tambay A V, Avila NA, Thomas F, et al. Waist circumference, BMI, and visceral adipose tissue in white women and women of African descent. Obesity (Silver Spring). 2011;19(3):671–4.
44. Toth MJ, Tchernof A, Sites CK, Poehlman ET. Effect of menopausal status on body composition and abdominal fat distribution. Int J Obes. 2000;24(2):226–31.
45. Bosch TA, Steinberger J, Sinaiko AR, Moran A, Jacobs DR, Kelly AS, et al. Identification of sex-specific thresholds for accumulation of visceral adipose tissue in adults. Obesity. 2015;23(2):375–82.

CHAPTER SIX: SUMMARY AND CONCLUSION

Obesity, in particular central adiposity, is a well-known risk factor for cardiovascular and metabolic diseases (Lee et al., 2013; Lemos & Neeland, 2014; O'Neill & O'Driscoll, 2015). Within SA, the prevalence of overweight or obesity in both women and men was highest in the Western Cape (73% and 44% respectively)(National Department of Health (NDoH), 2019), of which the mixed-ancestry population accounts for nearly half (48%) (StatsSA, 2019). Central obesity rates in the mixed-ancestry population are high in women and men (87.9% and 42.2% as defined by IDF criteria), respectively, with MetS being significantly more prevalent in the women (Erasmus et al., 2012). Having a better understanding of the role of body fat distribution in CVD and type-2 diabetes mellitus risk in different ethnic groups will assist with risk stratification and disease prevention. Cost effective methods are thus necessary to accurately quantify body fat. Therefore, the main objective of this thesis was to validate DXA as a method to objectively quantify central adiposity, explore the relationship between body fat distribution and cardio-metabolic risk and determine the ability of DXA compared to anthropometry to identify participants with MetS. The main findings of this thesis are discussed below.

Anthropometry is commonly used in the clinical setting as an indicator of obesity, however it fails to differentiate between different adipose tissue compartments, namely abdominal VAT and SAT. Differences in VAT and SAT accumulation for similar waist circumferences have been reported amongst different ethnic groups (Lee et al., 2013). CT and MRI are the gold standard imaging modalities used to accurately quantify adipose tissue compartments. These modalities are however expensive and in the case of CT, expose the participants to high radiation doses. DXA on the other hand, is cheaper, more readily available in SA and is able to quantify central and regional adipose tissue compartments at a substantially lower radiation dose than CT, making it more practical to use for screening in high risk populations. Therefore, the first study of the thesis compared the validity of DXA-derived VAT and SAT against the criterion measure, CT, across a range of BMI categories in 132 mixed-ancestry women. The main finding was that DXA overestimated VAT and to a lesser extent SAT in all BMI categories, commensurate with similar studies (Bredella et al., 2013; Lin et al., 2013; Neeland et al., 2016; Mohammad et al., 2017). In the overall sample the bias between the two modalities increased as the amount of VAT increased. Notably, the extent of the bias was influenced by BMI category such that within increasing BMI categories, the variance was constant for VAT. Although DXA overestimated VAT by nearly 90% in both the overweight and obese categories, the bias was homoscedastic in the obese category, which comprises the majority of the sample (70%). These findings imply that DXA-derived measures of VAT may be applicable in body composition research in obese women who are at particularly high risk for cardio-metabolic complications associated with VAT. Unfortunately, the sample size in the normal weight and

overweight categories was too small to make meaningful comparisons about the bias. This is the first study in the mixed-ancestry population which has quantified both whole body, central and regional body composition using DXA and then validated it against the criterion method, CT.

Obesity, as well as being an independent risk factor for CVD and type-2 diabetes mellitus, is also associated with other risk factors for MetS such as insulin resistance, dyslipidaemia and elevated blood pressure (Lee et al., 2013; Lemos & Neeland, 2014; O'Neill & O'Driscoll, 2015). However, this association between risk differs by fat depot with some depots for example conferring a protective effect. Furthermore, ethnic, sex and age have been shown to affect these relationships. As DXA was able to quantify regional body fat compartments, in addition to whole body and central adiposity, particularly in obese individuals, the second study of the thesis went on to explore the associations between the DXA- derived whole body and regional fat distribution and cardio-metabolic risk in predominantly obese pre and post-menopausal women and a small sample of overweight men. The study additionally explored whether menopause status and sex affected these associations. The main finding was that central fat mass (VAT in particular) was associated with increased cardio-metabolic risk and lower body peripheral fat mass was associated with reduced risk. Indeed, VAT was the most consistent and significant correlate of insulin resistance even after accounting for age and sex. Cardio-metabolic risk factors such as blood pressure, fasting glucose, insulin and lipid levels were not different between men and women despite the women having twice as much body fat and higher obesity rates than the men. Notably the women had similar amounts of VAT to the men which is in contrast to other studies and ethnicities (Geer & Shen, 2009; Tchoukalova et al., 2010). The similar amounts of VAT could however possibly be explained by the higher obesity rates in women compared to men (BMI 32.6±7.2 vs. 27.4 ±6.1kg/m^2 in women and men, respectively). It may be worthwhile to investigate this further in a more representative sample of men and women and to adjust for total body fat and BMI to get a more accurate view of the sex differences in VAT (Sumner et al., 2002).

When comparing women based on estimated menopausal status, post-menopausal women had higher systolic blood pressure, fasting glucose, 2-hour glucose, triglyceride, TC and HDL-C concentrations than pre-menopausal women. In contrast, the prevalence of impaired glucose tolerance (IGT)/ impaired fasting glucose (IFG) and type 2 diabetes was similar in the pre-and post-menopausal women. For the most part, the association between body fat distribution and cardio-metabolic risk did not differ between the pre- and post-menopausal women. The post-menopausal women did however have greater VAT compared to the pre-menopausal women, and thus in clinical practice, the importance of preventing weight gain

and centralization of body fat prior to menopause should be highlighted. We did not however have an objective measure of menopause and thus the results may reflect an age effect.

In terms of sex differences, even though the women in this study had substantially more abdominal SAT than men, the relationship between abdominal SAT and insulin resistance was stronger in men than women. These findings contrast to similar international studies (Geer & Shen, 2009; Vasan et al., 2018), but are similar to those reported in black African and mixed-ancestry populations in South Africa (Goedecke et al., 2016; Matsha et al., 2019). This may be due to men having more dSAT and less sSAT compared to women (Dulloo et al., 2010), with concomitant higher inflammation and oxidative stress associated with dSAT (Nazare et al., 2012). The differential role of dSAT and sSAT in cardio-metabolic risk between sexes is worth investigating further in this population with a more representative sample of women and men. Greater lower body peripheral fat mass was associated with a lower cardio-metabolic risk, commensurate with findings from previous studies in African American and Caucasian men and women (Snijder et al., 2005) and in a large sample of Asian men and women (Park et al., 2014). Notably, although this protective effect was stronger in the women in this study, which is similar to that found by Schorr (Schorr et al., 2018), this study was limited by the small sample of men.

The first study in this thesis validated DXA-derived VAT and SAT in this sample of predominantly obese women and the second study showed that VAT was the most consistent and significant correlate of insulin resistance after accounting for age and sex. However, DXA is not commonly available in clinical practise. Rather, several indices of central obesity, such as waist circumference and WHtR are used in clinical practice for the early detection of individuals at risk for CVD, type-2 diabetes mellitus and MetS. In order to see if DXA-derived VAT is worth incorporating into risk screening for early detection of cardio-metabolic disease, I hypothesised that DXA-derived VAT would have greater discriminatory power over simpler anthropometric measures. Therefore the third and final study in this thesis compared the discriminatory power of DXA-derived VAT and simpler anthropometry to identify mixed-ancestry women with MetS. The main finding was that DXA-VAT performed similarly to WC in diagnosing MetS, which is contrary to various international studies (Hayashi et al., 2007; Elffers et al., 2017) but similar to that for Black SA women (Evans et al., 2011). The implication of this is that WC, which is easier and cheaper than imaging examinations, can continue to be used as a risk marker for screening purposes, particularly in low resource settings, such as in SA. Although the WC cut-point (94.4cm) derived for this study was purely for comparative purposes, it is similar (90cm) to that derived by Matsha and co-workers for both sexes in a larger study of this population (Matsha et al., 2013). This result further strengthens the notion that at similar WC, there are ethnic variations in the amount of VAT and SAT accumulation,

thereby necessitating the need for population specific WC cut-points. Further longitudinal studies are however needed to define these cut-points. To my knowledge, this was the first study to compare the discriminatory power of DXA-VAT and WC to identify mixed-ancestry participants with MetS.

The thesis has a number of limitations that should be acknowledged. This thesis was limited by the cross-sectional nature and the inclusion of a convenient sample of women and only a small sample of men, however, this is typical of SA population surveys in which more women tend to voluntarily participate (Erasmus et al., 2012). The validity of DXA-VAT compared to the criterion method of CT in chapter 3 included women only a very small sample of normal-weight and overweight participants, and thus these results may not be reliable for those categories or be generalized to men. Further studies should thus be conducted on a more representative sample. Furthermore, only one DXA and CT scan was performed, and future research should include repeat measurements to determine precision of the measurements. Importantly, estimates of body composition have been shown to vary by scanner make, model and software; therefore these results may not be generalisable to all DXA scanners. The investigation into the associations between cardio-metabolic risk and DXA-VAT in chapter 4 consisted of predominantly obese peri- and post-menopausal women. We did not have an objective measure of menopause status and these findings could therefore reflect an age effect. Future studies in which menopausal status is objectively measured are required to confirm the findings of this chapter. In chapter 5, a larger representative sample of women and men is needed to determine whether VAT performs better than WC for diagnosing MetS, and if shown to be better, ethnic, sex and age specific cut-points should be developed.

Despite these limitations, this thesis has some important novel findings regarding the validity of DXA-VAT and SAT and associated cardio-metabolic risks in the high risk mixed-ancestry population. The thesis has shown that cardio-metabolic risk factors such as blood pressure, fasting glucose, insulin and lipid levels were similar between men and women, despite women having twice as much body fat and higher obesity rates than the men. DXA was shown to be valid for VAT quantification particularly in the obese participants (70% of the participants sampled). Indeed, DXA-derived VAT accounted for the greatest variance in insulin resistance irrespective of age and sex. VAT amounts were similar in the men and women which is in contrast to other studies and ethnicities, but may be explained by the greater total body fatness in women compared to men. The practical implication of these findings is that, in high risk populations, with very high levels of total and central adiposity, DXA could be used to understand the pathogenesis of type-2 diabetes and CVD. Furthermore the DXA-derived regional body fat associations with obesity co-morbidities can be better understood. Post-menopausal women had more VAT than pre-menopausal women and thus an important

clinical application would be to highlight the importance of the prevention of weight gain and centralisation of body fat prior to menopause. DXA-VAT was no better than WC in predicting MetS in this sample and thus the clinical implication of this is that WC should continue to be used in the clinical setting, particularly in a LMIC country like SA.

In conclusion, DXA proved to be a valid measure of VAT and SAT, particularly in the obese peri and post-menopausal women. I therefore accept the null hypothesis that DXA is able to accurately quantify abdominal fat compared to the gold -standard, CT, particularly in the obese BMI category. VAT was the most consistent and significant correlate of cardio-metabolic risk irrespective of age and sex, assisting with our understanding of cardio-metabolic risk in a high-risk mixed-ancestry population. I therefore accept the alternative hypothesis as cardio-metabolic risk was differentially influenced by body compartments. However, VAT did not perform significantly better than WC for identifying participants with MetS, and thus in LMICs, WC should still be used as a proxy for VAT for screening for type-2 diabetes mellitus and CVD risk. The null hypothesis is thus accepted as there was no statistically significant difference between VAT area and WC in MetS diagnosis.

Recommendations for clinical applications based on the findings of this study

- DXA may be useful for body composition studies in obese mixed-ancestry women who are particularly at risk for cardiovascular disease.
- The importance of preventing weight gain and centralization of body fat must be emphasised in the high risk mixed-ancestry group.
- Waist circumference should continue to be used as one of the criteria for MetS identification in the post-menopausal mixed-ancestry group until such time as a larger more representative study proves otherwise.

References

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

Dulloo, A.G., Jacquet, J., Solinas, G., Montani, J.P. & Schutz, Y. 2010. Body composition phenotypes in pathways to obesity and the metabolic syndrome. *International Journal of Obesity*, 34(S2): S4–S17.

Elffers, T.W., Mutsert, D., Lamb, H.J., Roos, A. De, Willems van Dijk, K., Rosendaal, F.R., Jukema, W.J. & Trompet, S. 2017. Body fat distribution , in particular visceral fat , is associated with cardiometabolic risk factors in obese women. *PLoS ONE*, 12(9): e0185403.

Erasmus, R.T., Soita, D.J., Hassan, M.S., Blanco-Blanco, E., Vergotine, Z., Kegne, A.P. & Matsha, T.E. 2012. High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: baseline data of a study in Bellville, Cape Town. *South African medical journal*, 102(11): 841–844.

Evans, J., Micklesfield, L., Jennings, C., Levitt, N.S., Lambert, E. V., Olsson, T. & Goedecke, J.H. 2011. Diagnostic Ability of Obesity Measures to Identify Metabolic Risk Factors in South African Women. *Metabolic Syndrome and Related Disorders*, 9(5): 353–360.

Geer, E.B. & Shen, W. 2009. Gender differences in insulin resistance, body composition, and energy balance. *Gender medicine*, 6(Suppl 1): 60–75.

Goedecke, J.H., George, C., Veras, K., Peer, N., Lombard, C., Victor, H., Steyn, K. & Levitt, N.S. 2016. Sex differences in insulin sensitivity and insulin response with increasing age in black South African men and women. *Diabetes Research and Clinical Practice*, 122: 207–214.

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

Lee, M.J., Wu, Y. & Fried, S.K. 2013. Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications. *Molecular Aspects of Medicine*, 34(1): 1–11.

Lemos, J.A. De & Neeland, I.J. 2014. Separating the VAT from the FAT New Insights Into the Cardiometabolic Risks of Obesity. *JACC: Cardiovascular Imaging*, 7(12): 1236–1238.

Lin, H., Yan, H., Rao, S., Xia, M., Zhou, Q., Xu, H., Rothney, M.P., Xia, Y., Wacker, W.K., Ergun, D.L., Zeng, M. & Gao, X. 2013. Quantification of visceral adipose tissue using lunar dual-energy X-ray absorptiometry in Asian Chinese. *Obesity*, 21(10): 2112–2117.

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

Matsha, T.E., Ismail, S., Speelman, A., Hon, G.M., Davids, S., Erasmus, R.T. & Kengne, A.P. 2019. Visceral and subcutaneous adipose tissue association with metabolic syndrome and its components in a South African population. *Clinical Nutrition ESPEN*, 32(August): 76–81.

Mohammad, A., Rolfe, E.D.L., Sleigh, A., Kivisild, T., Behbehani, K., Wareham, N.J., Brage, S. & Mohammad, T. 2017. Validity of visceral adiposity estimates from DXA against MRI in Kuwaiti men and women. *Nature Publishing Group*, 7(1): e238-5.

National Department of Health (NDoH). 2019. *National Department of Health (NDoH), Statistics South Africa, South African Medical Research Council (SAMRC), and ICF. 2019.* Pretoria, South Africa.

Nazare, J.A., Smith, J.D., Borel, A.L., Haffner, S.M., Balkau, B., Ross, R., Massien, C., Alméras, N. & Després, J.P. 2012. Ethnic influences on the relations between abdominal subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: The international study of prediction of intra-abdominal adiposity and its relationship with cardiometabolic risk/intra-. *American Journal of Clinical Nutrition*, 96(4): 714–726.

Neeland, I.J., Grundy, S.M., Li, X., Adams-Huet, B. & Vega, G.L. 2016. Comparison of

visceral fat mass measurement by dual-X-ray absorptiometry and magnetic resonance imaging in a multiethnic cohort: the Dallas Heart Study. *Nutrition & Diabetes*, 6(7): e221.
O'Neill, S. & O'Driscoll, L. 2015. Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. *Obesity Reviews*, 16(1): 1–12.
Park, S.Y., Kwon, K.Y., Kim, J.H., Choi, H.H., Han, K.H. & Han, J.H. 2014. Association between appendicular fat mass and metabolic risk factors. *Korean Journal of Family Medicine*, 35(4): 182–189.
Schorr, M., Dichtel, L.E., Gerweck, A. V., Valera, R.D., Torriani, M., Miller, K.K. & Bredella, M.A. 2018. Sex differences in body composition and association with cardiometabolic risk. *Biology of Sex Differences*, 9(1): 1–10.
Snijder, M.B., Visser, M., Dekker, J.M., Goodpaster, B.H., Harris, T.B., Kritchevsky, S.B., De Rekeneire, N., Kanaya, A.M., Newman, A.B., Tylavsky, F.A. & Seidell, J.C. 2005. Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study. *Diabetologia*, 48(2): 301–308.
StatsSA. 2019. Midyear Population Estimate 2019. *Population Estimates*, (July).
Sumner, A.E., Farmer, N.M., Tulloch-Reid, M.K., Sebring, N.G., Yanovski, J.A., Reynolds, J.C., Boston, R.C. & Premkumar, A. 2002. Sex differences in visceral adipose tissue volume among African Americans. *American Journal of Clinical Nutrition*, 76: 975–979.
Tchoukalova, Y.D., Koutsari, C., Votruba, S.B., Tchkonia, T., Giorgadze, N., Thomou, T., Kirkland, J.L. & Jensen, M.D. 2010. Sex- and depot-dependent differences in adipogenesis in normal-weight humans. *Obesity (Silver Spring, Md.)*, 18(10): 1875–1880.
Vasan, S.K., Osmond, C., Canoy, D., Christodoulides, C., Neville, M.J., Di Gravio, C., Fall, C.H.D. & Karpe, F. 2018. Comparison of regional fat measurements by dual-energy X-ray absorptiometry and conventional anthropometry and their association with markers of diabetes and cardiovascular disease risk. *International Journal of Obesity*, 42(4): 850–857.