Ethnic specific associations between body composition and metabolic risk and the role of sex hormones and aromatase among black and white South African women

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Table of contents

Declaration i
Acknowledgements ii
List of abbreviations iii
List of tables vi
List of figures vii
Abstract viii
Chapter 1 1
Literature Review 1
1.1 Introduction 1
1.2 Body fat distribution and risk 2
1.2.1 Ethnic-specific associations between body fat distribution and metabolic risk 4
1.2.2 Mechanisms underlying the association between body fat distribution and metabolic risk 9
1.2.2.1 FFA 9
1.2.2.2 Adipokines 11
1.2.2.3 Other 13
1.3 Determinants of body fat distribution 13
1.3.1 Gender and body composition 13
1.3.2 Menopause, obesity and body fat distribution 14
1.3.2.1 Hormonal changes and menopause 14
1.4 Oestrogen and its receptors 16
1.5 Literature conclusions 20
1.6 Aims and objectives 20
Chapter 2 22
Associations between body fat distribution, insulin resistance and dyslipidaemia in black and white South African women 22
2.1 Introduction 22
2.2 Methods and materials 23
2.2.1 Subjects: 23
2.2.2 Testing procedures: 23

2.2.2.1 Demographic and lifestyle questionnaire 23
2.2.2.2 Body composition 24
2.2.3 Blood sampling and biochemical analysis: 25
2.2.3.1 IR 25
2.2.4 Statistical analysis: 25
2.3 Results 27
2.3.1 Subject characteristics 29
2.3.2 Associations between body fatness and its distribution and cardio-metabolic risk factors. 31
2.3.3 Multivariate analysis 34
2.4 Discussion 35
Chapter 3 41
Adipose and circulating sex hormones: Associations with body composition and insulin resistance in black and white South African women 41
3.1 Introduction 41
3.2 Methods and materials 43
3.2.1 Subjects: 43
3.2.2 Testing procedures: 44
3.2.2.1 Demographic and lifestyle questionnaire 44
3.2.2.2 Body composition 44
3.2.2.3 IR 44
3.2.2.4 Fat biopsies: 45
3.2.2.5 Isolation of RNA: 45
3.2.2.6 Real-time quantitative PCR (RT-PCR): 45
3.2.3 Biochemical analysis: 46
3.2.4 Statistical analysis: 46
3.3 Results 47
3.3.1 Subject characteristics 50
3.3.2 SAT gene expression 51
3.3.3 Associations between gene expression and body fat and its distribution and IR. 55
3.3.4 Associations between circulating hormonal levels and body fat and its distribution and IR 57

3.4 Discussion 58
Chapter 4 63
Summary and conclusions 63
References 68
Appendix 91

Declaration

I, Mehreen Tootla, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is submitted for another degree in this or any other university.

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List of abbreviations

AAT – Abdominal Adipose Tissue

ApoB- Apolipoprotein B

AMPK- AMP-activated Protein Kinase

ARKO- Aromatase Knockout

BERKO- Oestrogen Receptor Beta Knockout

BMI – Body Mass Index

CHD – Coronary Heart Disease

cm - Centimetre

cm^2 – Centimetre squared

CT- Computed Tomography

CYP19A - Aromatase

CVD – Cardiovascular Disease

DAG- Diacylglycerol

DSAT – Deep Subcutaneous Adipose Tissue

DXA – Dual-energy X-ray Absorptiometry

E2 - Oestradiol

ERα – Oestrogen Receptor Alpha

ERβ – Oestrogen Receptor Beta

ERKO- Oestrogen Receptor Alpha Knockout

FFA- Free Fatty Acids

FM – Fat Mass

FMI – Fat Mass Index

FSH – Follicle Stimulating Hormone

GPAQ – Global Physical Activity Questionnaire

HC- Hip circumference

HDL-C – High-density Lipoprotein Cholesterol

HOMAIR – Homeostasis Model of Insulin Resistance

IL-6 – Interleukin 6

IMAT- Intramuscular adipose tissue

IR – Insulin Resistance

IU/L – International Units per Litre

Kg – Kilograms

Kg/m^2 – Kilograms per metre squared

LC-CoA- Long-chain Fatty Acyl-coenzyme A

LDL-C – Low-density Lipoprotein Cholesterol

LEPR- Leptin Receptor

LH – Luteinising Hormone

LPL- Lipoprotein Lipase

m - Metres

min/week – minutes per week

mmol/L – Millimoles per Litre

MVPA – Moderate to Vigorous Intensity Physical Activity

NCD- Non-communicable Diseases

ng/ml – Nanograms per Millilitre

nmol/L – Nanomoles per Litre

PI3K- Phosphoinositide 3-kinase

pg/mL – Picogram per Millilitre

PPARϒ- Peroxisome Proliferator-Activated Receptor Gamma

SA – South Africa

SANHANES – South African National Health and Nutrition Examination Survey

SAT – Subcutaneous Adipose Tissue

SCD1- Stearoyl Co-A desaturase 1

SHBG – Sex Hormone Binding Globulin

SSAT – Superficial Subcutaneous Adipose Tissue

STAT3- Signal Transducer and Activator of Transcription 3

S_I – Insulin Sensitivity Index

T2D – Type 2 Diabetes

TC – Total Cholesterol

TG - Triglycerides

TNFα – Tumour Necrosis Factor alpha

USA – United States of America

VAT – Visceral Adipose Tissue

VLDL- Very Low-density Lipoproteins

WC – Waist Circumference

WHR- Waist-hip Ratio

List of tables

Table 1: Summary of select studies that have examined differences in body fat and its distribution and the associations with cardio-metabolic risk factors in black and white women.

Table 2: Subject characteristics of black and white women.

Table 3: Body composition and body fat distribution of black and white women.

Table 4: Cardio-metabolic risk factors of black and white women.

Table 5: Correlations between body fatness and its distribution and cardio-metabolic risk factors in black and white women and the combined sample.

Table 6: Multivariate analysis for black and white women.

Table 7: Subject characteristics of normal-weight and obese black and white women.

Table 8: IR and circulating sex hormones in black and white normal-weight and obese women.

Table 9: Metabolic outcomes between contraception use in black and white women.

Table 10: Associations between physical activity and alcohol consumption and metabolic outcomes.

List of figures

Figure 1. Prevalence of overweight and obesity in South African women (SANHANES).

Figure 2: Schematic diagram of forms of testosterone and regulation by SHBG in the body.

Figure 3: Expression of genes in abdominal DSAT, SSAT and gluteal depots in normal-weight and obese black and white women.

Figure 4: Associations between ERα, ERβ, ERα/ERβ (figure 4A) and CYP19A (figure 4B) gene expression with body fat and its distribution in gluteal, SSAT and DSAT depots in black and white women.

Figure 5: Associations between ERα and ERβ gene expression with metabolic risk in gluteal and SSAT depots.

Figure 6: Associations between circulating sex hormones with body fat and IR.

Abstract

Background: Previous evidence has demonstrated ethnic differences in the relationship between body fat distribution and metabolic risk between black and white women. However, the reasons for these differences are not known and may be explained in part by differences in sex hormones. The overall aim of this thesis was therefore to i) examine ethnic-specific associations between body fat and its distribution and cardio-metabolic outcomes (study 1) and ii) examine the associations between sex hormones and subcutaneous adipose tissue (SAT) expression of oestrogen receptors (ERα and ERβ) and aromatase (CYP19A), and body fat distribution and insulin resistance (IR) among black and white women (study 2).

Methods: Study 1: In 288 black and 197 white premenopausal women, dual-energy X-ray absorptiometry (DXA) and computed tomography (CT) derived measures of body fat distribution and cardio-metabolic factors including IR (HOMAIR) and lipid levels were measured. Study 2: In a subsample consisting of 13 normal-weight and 15 obese black and 15 normal-weight and 12 obese white women, HOMAIR and S_I (frequently sampled intravenous glucose tolerance test) and ERα, ERβ and CYP19A gene expression were measured in abdominal and gluteal SAT.

Results: Study 1: Compared to white women, black women had less central and greater lower-body fat, but had similar IR and lower serum lipid concentrations. Despite these differences, the associations between body fat distribution and measures of IR, as well as TG and HDL-C concentrations were similar in black and white women. Notably, central and peripheral fat deposition was independently associated with IR in both the black and white women, and with TG in the black women. In contrast, the associations between body composition and fasting plasma glucose, TC and LDL-C concentrations differed between black and white women. Fasting glucose concentrations were associated with centralisation of body fat in black but not white women, whereas TC and LDL-C concentrations were associated with centralisation of body fat in white but not black women. In addition to body fat distribution, MVPA was associated with IR in the white women, and contraception use was associated with lipid levels in the black and white women. Study 2: CYP19A expression was positively associated with increased adiposity in black and white women in all three depots. Gluteal ERα was significantly higher and ERβ was significantly lower in the black compared to the white women, irrespective

of BMI. Gluteal ERα was negatively associated with trunk fat mass (FM) and HOMAIR in the black women only. Gluteal ERα was significantly lower in obese white compared to normal-weight white women. Additionally oestradiol (E2) levels were lower in obese compared to normal-weight white women, but did not differ by ethnicity.

Conclusion: Our results indicate that it is important in both black and white populations, to decrease centralisation of body fat. Modifiable risk factors such as MVPA and contraception use should be used as therapeutic targets to prevent and manage CVD. Additionally, oestrogen receptors may be an important determinant of body fat distribution and risk in the black women.

Chapter 1

Literature Review

1.1 Introduction

Non-communicable diseases (NCD‟s) such as Type 2 diabetes (T2D) and cardiovascular diseases (CVD) are a large global problem (1), and in 2000, were the second highest cause of death in South Africa (SA) (37%) (2). Obesity is known to be major risk factor for CVD and T2D (3-6). According to the South African National Health and Nutrition Examination Survey (SANHANES), the prevalence of overweight and obesity in SA women is high compared to SA men (24.8 and 39.2% compared to 20.1% and 10.6% for males and females, respectively) (7). Black SA women had the highest prevalence of overweight and obesity compared to the other racial groups, with 64.8% being overweight and obese and 39.9% being obese (figure 1) (7).

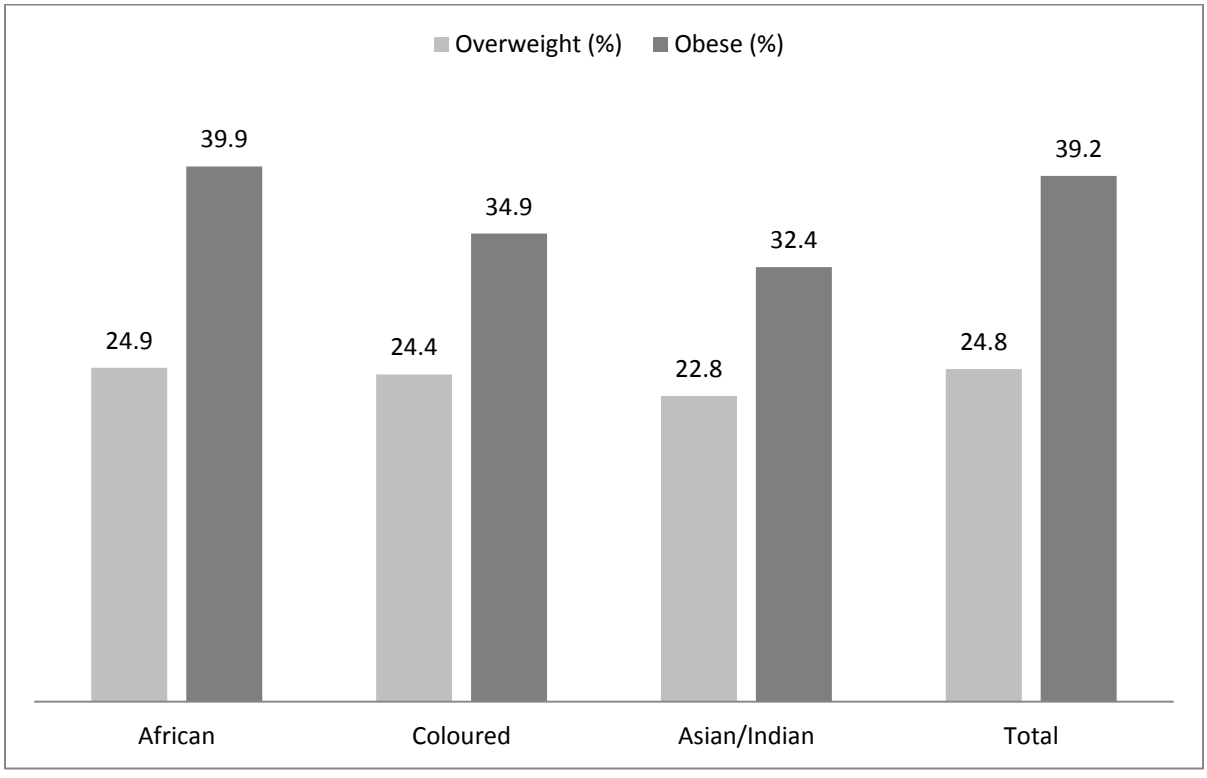


Figure 1. Prevalence of overweight and obesity in South African women (SANHANES) (7). White women were excluded due to small sample size.

The reason for the higher prevalence of obesity in African women is not known, but the aetiology of obesity is multifactorial and includes the effects of genetics. Further, environmental

and socio-economic factors may also contribute to the higher prevalence of obesity in black women (8-12). For example, in the black community an overweight body type symbolizes happiness beauty and being healthy. Another factor such as diet may contribute to higher prevalence of obesity in African women. Studies have demonstrated a general trend for increased fat intake and decreased carbohydrate intake in black SA women which may contribute to higher prevalence of obesity. Further, studies have shown that inactivity in black women from the North West was the strongest predictor of obesity and that physical activity in the highest tertile group was negatively associated with obesity. However, an in-depth discussion of these factors is beyond the scope of this thesis (8-12).

1.2 Body fat distribution and risk

Obesity is characterised by insulin resistance (IR) in association with increased insulin to an oral glucose load, and a high rate of free fatty acid (FFA) turnover/unit lean body mass (12). Further, obesity is associated with dyslipidaemia characterised by higher triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) and lower high-density lipoprotein cholesterol (HDL-C) (13 and 14).

The distribution, rather than the total amount of body fat may be a greater predictor of CVD and T2D risk factors such as IR and dyslipidaemia (15-17). There has been growing evidence indicating that not all fat stores contribute equally to CVD and T2D risk factors (18). Numerous studies have explored the relationships between central deposition of adipose tissue and CVD risk factors among women. Abdominal obesity, in particular visceral adipose tissue (VAT), is strongly associated with dyslipidaemia, IR, coronary heart disease (CHD), and T2D (19-23). Several studies using different methodologies to measure abdominal obesity have confirmed the notion that “android obesity” or male-type upper-body obesity is a high-risk form of obesity (24-26). For example, waist circumference (WC), as a measure of central fat mass (FM), has been shown to be associated with increased T2D, glucose levels, IR and a poorer lipid profile, independent of hip circumference (HC), in European and Australian men and women (27 and 28). Further, studies conducted only in sub-Saharan/SA black men and women demonstrated that an increasing WC was associated with IR and higher lipid levels (29 and 30). Additionally in rural African communities, cut-off WC points for predicting metabolic syndrome (MS) factors for women was >82cm for women (31 and 32). Using dual-energy X-ray absorptiometry (DXA)

as a measure of body fat distribution, studies conducted in European men and women have demonstrated that trunk FM independent of leg FM (33) is associated with post-load glucose and IR, as well as with higher TG and lower HDL-C concentrations (33-35). In American white women only, trunk FM as measured by DXA was associated with an unfavourable lipid profile independent of leg FM (36). Studies in the United States of America (USA) showed that within the abdominal region, obese individuals with an increase in VAT accumulation tend to have dyslipidaemia and IR. For example, in the multi-ethnic Dallas Heart Study, VAT was shown to be associated with homeostasis model of IR (HOMAIR) and dyslipidaemia, whereas subcutaneous adipose tissue (SAT) demonstrated a more benign phenotype, showing no independent association with dyslipidaemia and IR (37). Further, the Framingham Heart Study conducted in men and women demonstrated that although VAT and SAT were correlated with metabolic risk factors, VAT was a stronger correlate of metabolic risk compared to SAT. In this study it was shown that VAT had a higher odds ratio for MS compared to SAT in women and men (4.7 vs. 3.0, and 4.2 vs. 2.5, $P<0.001$ respectively) (20), suggesting that VAT is a stronger predictor of CVD risk than SAT.

In contrast to central fat accumulation, recent studies have shown that gluteo-femoral fat accumulation, described as "gynoid obesity" or lower-body obesity, which is commonly found in premenopausal women, is not a major risk for CVD and related mortality (24, 38 and 39). For example, HC, as a measure of lower-body fat was associated with lower T2D glucose levels, IR and a more favourable lipid profile independent of WC in European and Australian men and women and in Portuguese overweight and obese women, HC was associated with reduced fasting insulin independent of WC (27, 28 and 40). Using DXA derived measures, in American white women, leg FM was associated with a favourable lipid profile independent of trunk FM (36). Similarly, in a group of European men and women and postmenopausal (ethnicity not stated) women, DXA-derived measures of leg FM were independently of trunk FM (33 and 41) associated with a favourable lipid profile, reduced post load glucose concentration and IR (34 and 35). The role of arm FM on metabolic risk requires further exploration due to contradictory findings. The study conducted by Sanchez et al (2013), found no associations with arm FM (35), whereas in a group of white, pre- and postmenopausal women, a reduction in arm FM was associated with an increase in TC concentrations (36). This is in agreement with Okura et al (2004) who demonstrated that in obese and overweight women (ethnicity not stated), arm FM

was associated with reductions in LDL-C concentrations (42). Further a study conducted in Korean men and women demonstrated that appendicular fat (arm FM and leg FM) had a negative association with metabolic risk (26).

Taken together, these studies suggest that the region where fat is accumulated might have differential effects on cardio-metabolic risk. Compared to central FM, lower-body fat is more protective against MS (35).

1.2.1 Ethnic-specific associations between body fat distribution and metabolic risk

Ethnic differences in overall adiposity/body mass index (BMI) and body fat distribution and the association with cardio-metabolic risk factors in black and white women from USA/Europe and SA separately, are described in Table 1.

Table 1: Summary of select studies that have examined differences in body fat and its distribution and the associations with cardio-metabolic risk factors in black and white women.

	Reference	Subject	Age (years)	Overall obesity/BMI	VAT	SAT	Measure of central FM	Measure of leg FM
American/European	43	B vs. W	45-65 and 18-30	B>W B & W: + Insulin + Glucose + Lipids W: + TG				
	44	B vs. W	45-64	B>W B & W: + Lipids				
	45	B vs. W	005-14	B>W W: + TG				
	46 and 47	B vs. W	46 vs. 47	B>W	B=W B & W: + IR + Lipids	B>W W: + IR		
	48	B vs. W	37 vs. 41	B=W	B<W	B=W		
	56	B vs. W		B=W	B<W IR: B>W B & W: + IR	B: + IR		
	77	B vs. W	54	B>W			WHR (anthro) B=W B & W: + IR	
	78	B & W	21-46	B=W			B<W (CT) B & W: + IR + Lipids	B=W (DXA) B & W: - IR - Lipids
	79	B & W	18-65	B>W			B=W (DXA) B & W: + IR	B>W (DXA) B & W: - IR

							+ Lipids	- Lipids
	80	B vs. W	16-33	B>W			B=W (DXA) W: + Lipids	
	81	B & W	70-79	B>W				B>W (CT) B & W: - Glucose - Lipids
South African	49 and 50	B vs. W	18-45	B=W	B<W W: + IR	B>W B & W: + IR		
	51	B vs. W	45 vs. 42	B=W	B<W	B=W		
	53, 54 and 55	B vs. W		B=W	B<W IR: B>W Lipids: B<W	B=W		
	76	B vs. W		B<W			WC (anthro) B<W MS B<W	
	82	B vs. W	18-45	B=W				B>W (CT) B: + IR

B; black, W; white; +, positively associated; -, negatively associated; DXA, dual-energy X-ray absorptiometry; CT, computed tomography; and anthro, anthropometry.

In summary, for the most part, for the same or higher BMI/body fat, the association between obesity and insulin, glucose concentrations and an atherogenic lipid profile were similar in African American and white women (43 and 44). In contrast, both Folsom et al (1991) and Frierichs et al (1978) showed obesity was less strongly related to TG concentrations in the black compared to the white women (43 and 45).

Further, studies in the USA have shown that compared to Caucasian women, African American women have less VAT and more SAT, largely due to greater superficial rather than deep SAT (DSAT) (46-48). Similarly, studies in SA comparing obese black and white women have shown that when matched for BMI, black women present with lower VAT and more SAT, in particular greater superficial SAT (SSAT), than white women (49-52). Further, the studies in USA and SA showed that despite having less VAT, black women were more IR compared to their white counterparts (53-56). Moreover, these studies showed that IR was more closely associated with VAT than SAT in the white women (46, 49, 56 and 57), whereas in black women, IR was more closely associated with SAT, and more particularly DSAT, rather than with VAT (49).

Commensurate with lower VAT in black compared to white women, black women have lower TC, LDL-C and TG concentrations compared to white women (55 and 58). Studies in SA have demonstrated that black women also have the same or lower HDL-C concentrations compared to white women (51, 59 and 60), whereas studies in the USA demonstrated higher HDL-C concentrations in black compared to their white counterparts (61 and 62). Black SA women may be protected against CHD because of this more favourable serum lipid profiles which in turn may be attributed to their body fat distribution (62). However, it is not known whether their lower HDL-C concentrations may infer greater risk. Notably, in white women lipid levels, namely TG and TG/HDL-C concentrations correlate with IR, which is not the case in black women, despite their higher level of IR (59, 61 and 63-66). Rather, other studies have demonstrated that the major determinants of lipid levels in the black women were use of hormonal contraceptives, socioeconomic status, and other lifestyle factors such as diet, alcohol consumption, and smoking (59 and 67-70). It is important to note that lipid levels in the black SA population are increasing, largely due to rapid urbanisation (71).

Studies conducted in SA and USA using anthropometric and DXA-derived measures of body fat distribution demonstrated that central FM was lower in black compared to white women (72-75).

A study conducted in SA by Kalk et al (2008), demonstrated that black diabetic men had a lower prevalence of MS compared to white men, which could be explained partly by their smaller WC (76). Indeed, waist-hip ratio (WHR) was associated with increased IR in both African American and white women (77). In a random sample of premenopausal white and African American women, DXA-derived central FM was positively related to fasting insulin, IR, LDL-C, TC/HDL-C, and TG concentrations and negatively associated with HDL-C concentrations (78 and 79). In contrast, a study in USA demonstrated that the association between central FM and serum lipid levels was more strongly significant in white compared to black women (80).

In contrast to central FM, studies in the USA and Europe have shown that computed tomography (CT)- and DXA-derived measures of lower-body fat (i.e. thigh fat, relative leg fat) were higher in black compared to white women (75 and 81). Similarly, studies in SA using DXA demonstrated that black women have greater peripheral fat (arm FM and leg FM) mass compared to white women (72 and 82). Studies in the Europe demonstrated that in both black and white women, thigh fat was associated with glucose and lipid levels in black and white women independent of abdominal fat (81). In addition, in a random sample of premenopausal white and African American women, leg FM was similarly related to reduced fasting insulin, IR, LDL-C, TC/HDL-C, and TG and higher HDL-C concentrations (78 and 79). In contrast, a small SA study demonstrated that gluteal fat in the black women was associated with increased IR (82).

To date, the SA studies that have examined the ethnic-specific associations between body composition and cardio-metabolic risk among black and white women have only used anthropometric-derived measures of body composition, or specifically examined the associations between VAT and SAT, measured using CT. Studies in America/Europe have demonstrated ethnic-specific associations with body composition and metabolic risk using DXA- or CT derived measures of body composition. A major objective of this thesis was therefore to determine if differences in body fat distribution (using both DXA- and CT) between black and white women were associated with their cardio-metabolic risk profile (i.e. both IR and lipid levels), and if central vs. peripheral fat were independently associated with metabolic risk in black and white SA women. This has not been conducted in black and white South African women and is therefore novel in this study.

1.2.2 Mechanisms underlying the association between body fat distribution and metabolic risk

The mechanisms underlying the effects of body fat distribution on cardio-metabolic risk are still not known. Possible mechanisms are discussed in brief below.

1.2.2.1 FFA

It is known that FFA play an important role in the development of CVD. Whole body FFA release is increased in upper body obesity under both post-absorptive (83 and 84) and post-prandial conditions (85 and 86), compared to non-obese or lower-body obesity, due to higher rates of lipolysis in the abdominal vs. peripheral depots (83 and 85-87). The adipocytes in abdominal sites are more resistant to anti-lipolytic effects of insulin than in peripheral sites (96 and 97). The explanation for this is not clear, but some have argued that it may relate to the larger size of the abdominal or visceral adipocytes compared to the peripheral adipocytes (88-90). Indeed, reduction of fat cell size through weight loss during diet and exercise, improves insulin regulation of lipolysis (91). Notably, VAT releases FFA directly into the hepatic portal system, altering hepatic metabolism of lipids and insulin signalling. However, due to its sheer volume, SAT may contribute more FFA to the system (92). However, it has been proposed that excess FFA release is associated with ectopic fat deposition in organs such as the pancreas, liver and muscle and contribute to and worsen IR and T2D (93 and 94).

Due to the direct link of VAT to the liver through the portal vein, the delivery of FA in the portal circulation may cause reduced hepatic insulin clearance (95-97). In hepatocytes, FFA inhibits insulin binding due to a reduction in insulin receptors. This is followed by a decrease in insulin degradation and the action of insulin is also inhibited (95, 98 and 99). Increased FFA in the portal circulation may increase glucose synthesis by preventing the normal insulin mediated suppression of glucose output by the liver, and dyslipidaemia (100).

One function of very low-density lipoprotein (VLDL) is to export excess energy from the liver, in the form of TG (101). VLDL synthesis is regulated by TG which in turn is dependent on FFA levels. Portal FFA may therefore regulate the synthesis of VLDL and apolipoprotein B (ApoB) (a protein backbone for VLDL and LDL) (95 and 102). This therefore suggests that increased FFA levels results in increased synthesis and therefore increased circulating levels of VLDL,

ApoB and LDL (95, 102 and 103). An overproduction of VLDL is associated with hypertriglyceridaemia (103 and 104). Hypertriglyceridaemia is associated with increased TG, low concentrations of HDL-C and high concentrations of LDL-C (105 and 106). In addition to less VAT, studies have demonstrated that black women have less hepatic fat compared to white women (107 and 108). This might therefore explain their lower lipid levels compared to white women. Another study conducted in SA demonstrated that white women had higher late postprandial FFA levels compared to black women. This therefore suggests that higher VAT levels may be the reason for higher FFA levels and in turn dyslipidaemia demonstrated in white women (109).

Increased accumulation of fat in skeletal muscle may be due to increased availability and uptake of FA or reduction in FA oxidation (110). This process is usually demonstrated as elevated levels of FFA, TG and increase intracellular accumulation of lipid intermediates such as TG, diacylglycerols (DAG), ceramides and long-chain fatty acyl-coenzyme A (LC-CoA) (111). Compared to white women, African American women have higher post absorptive skeletal muscle lipoprotein lipase (LPL) activity and are metabolically inflexible (112). Further, *in vitro* studies have shown FA oxidation in skeletal muscle and mitochondrial function is lower in premenopausal African American compared to white women (113 and 114). Ethnic differences in muscle oxidation may potentially be related to muscle fibre composition. Studies have shown that compared to white women, African American women have less type I oxidative fibres and more type IIb glycolytic fibres (115). Increase in FA oxidation by-products, such as DAG, ceramides, and long chain acyl-CoA, rather than intramuscular adipose tissue (IMAT) alone, is associated with the development of IR (116).

Most recently it has been demonstrated that impairment of insulin secretion from chronic pancreatic exposure to FFA results in hyperglycaemia due to loss of glucose sensing by β-cells (117-119). Hyperglycaemia is associated with accumulation of TG 10-fold higher than normal in the pancreas, as hyperglycaemia inhibits fat oxidation (118 and 120). Therefore hyperglycaemia and increased FFA may act synonymously in causing damage to the B-cell due to inhibition of detoxifying lipid accumulation (121).

Compared to upper-body fat stores, the release of FFA from lower-body fat stores is reduced, and lower-body fat has been suggested to act as a metabolic sink, storing excess FFA when there

is an energy surplus (83, 85-87 and 122). Adipocytes in the gluteo-femoral region are less sensitive to lipolytic stimuli and more sensitive to anti-lipolytic stimuli than adipocytes in the abdominal region (123 and 124). LPL activity is high in the gluteo-femoral region and may therefore favour the catabolism of TG rich lipoproteins, the clearance of TG from diet and their storage in adipose tissue (93, 122, 125-128). A study conducted in a small sample of black and white women from America demonstrated that LPL activity was higher in the black population compared to their white counterparts (62). This may therefore suggest that black women may be more efficient at clearing hypertriglyceridaemia compared to white women (43). Lower-body fat may protect against ectopic fat deposition and therefore protect against IR, impaired insulin secretion and risk for CVD and T2D (81).

1.2.2.2 Adipokines

Adipose tissue can be regarded as an endocrine organ secreting factors such as adipokines (129). With obesity, adipocyte size increases which results in increase in macrophage infiltration and increase release of cytokines and decrease release of the insulin-sensitizing adipokine, adiponectin (130-136). Differences in the contribution of abdominal and gluteo-femoral fat to metabolic risk may be due to depot-specific differences in the secretion of adipokines. For example, tumour necrosis factor alpha (TNFα) is secreted by macrophages within adipocytes and may play a role as a mediator for IR in obesity (137-140). This cytokine is also present in high concentrations in abdominal obesity (141). Studies have demonstrated that TNFα interferes with insulin signalling and reduces expression of insulin-sensitive glucose transporter type 4 protein and translocation (142). This occurs by TNFα inhibiting IR tyrosine kinase in tissues responsible for insulin stimulated glucose uptake (i.e. muscle and fat) (143). Mutagenesis studies have shown that IR tyrosine kinase is very important for the biological activities of insulin (144). TNFα has been shown to increase the release of other cytokines such as interleukin 6 (IL-6) (145). IL-6 is important in whole-body energy homeostasis and inflammation regulation (140). In vitro and in vivo studies have demonstrated that IL-6 is capable of suppressing LPL activity, resulting in hypertriglyceridaemia, IR and impaired insulin secretion (140). Studies conducted in black and white women have demonstrated that TNFα expression were higher in gluteo-femoral and abdominal adipose tissue (AAT) depots of black compared to white women, and this was

particularly in the gluteo-femoral depot (146). There was no difference in the expression of IL-6 between the white and black women (146 and 147).

In contrast to AAT, gluteo-femoral adipose tissue has been shown to have a more beneficial adipokine profile (93). Population studies have demonstrated that leptin, a key regulator in energy intake and expenditure, and adiponectin levels were positively associated with gluteo-femoral fat (93). Leptin is involved in both the signal transducer and activator of transcription 3 (STAT3) pathway and insulin receptor substrate phosphoinositide 3-kinase (PI3K) pathway (148). STAT3 is important in mediating food intake and liver glucose production (148). Studies show that leptin levels are positively associated with metabolic risk (146), while obesity and IR are associated with low adiponectin levels (131, 132, 149 and 150). Adiponectin improves IR by increasing energy expenditure and FA oxidation by activation of AMP-activated protein kinase (AMPK) and by increasing the expression of peroxisome proliferator-activated receptor gamma (PPARα) target genes (151). Studies examining ethnic differences in adipokines and their association with metabolic risk are contradictory. A study conducted in black and white SA women demonstrated that adiponectin expression was lower in gluteo-femoral fat compared to abdominal fat (146), and reported no ethnic differences in the expression of adiponectin among the black and white women. In contrast, leptin gene expression was higher in abdominal fat of black compared to white women and was higher in gluteal fat compared to abdominal fat depot. In both black and white women, leptin expression was positively correlated with IR, while adiponectin expression was negatively correlated with IR irrespective of depot (146). Although black women were more IR and had a higher gluteal SAT inflammatory profile than white women, SAT inflammatory gene expression was not significantly associated with IR and only accounted for 20% of the variance. In contrast, in white women, abdominal SAT inflammatory gene expression was significantly associated with IR accounting for 56% of the variance (146). In contrast, Smith et al (2010) found no difference in abdominal expression of leptin between white and African American women matched for BMI and IR (152).

Additionally, Evans et al (2011) demonstrated that although black women had lower central obesity, serum adiponectin and leptin levels did not differ by ethnicity. However, adiponectin levels were higher and leptin levels were lower in normal-weight compared to obese women (146). In contrast, African American women had higher serum leptin concentrations compared to

white women (153). Ferris et al (2005) demonstrated that in a group of black and white SA women, serum adiponectin levels were lower in black compared to white women and correlated inversely with HOMAIR in the white women (154). Additionally, in African American and Caucasian women, serum leptin levels were found to be associated with greater central FM in Caucasian women only (155). In a group of African American men and women, serum leptin was positively correlated with VAT and HOMAIR. In contrast, serum adiponectin negatively correlated with VAT and HOMAIR (156).

This differences in findings may be due difficulty in determining the true effects of adipocytokines with traditional molecular biology methods, as adipocytokines may act in a paracrine, endocrine, and autocrine manner. We have provided evidence for the association between body fat distribution and metabolic risk and demonstrated possible mechanisms responsible for this among black and white women. However, intervention and longitudinal studies are required for a better understanding of the mechanisms underlying IR and higher prevalence of T2D in African.

1.2.2.3 Other

There are several other mechanisms that may influence body fat distribution and its association with metabolic risk. Some of these factors include behavioural factors such as physical activity, diet and demographic factors (12). Other factors include disturbances in glucocorticoid hormones as well as sex hormones.

1.3 Determinants of body fat distribution

1.3.1 Gender and body composition

Clear sexual dimorphisms exist in body fat accumulation and body fat distribution, primarily linked to reproductive function. Women generally store more fat and are more likely to be obese than males (7). In addition, females have more SAT and greater fat stores in the lower-body (gynoid) compared to males who store more fat in the upper body (android) (24).

1.3.2 Menopause, obesity and body fat distribution

The role of sex hormones in body composition is clearly evident during the menopausal transition (157). Menopause is associated with an increase in VAT and a decrease in peripheral SAT. For example a cross-sectional study conducted by Gambaccianni and colleagues measuring fat distribution using DXA in premenopausal, perimenopausal and postmenopausal women demonstrated that both perimenopausal and postmenopausal women showed increase in total and central adipose tissue compared to premenopausal women (158). These results suggest that even during the perimenopausal period, increased abdominal fat accumulation can be observed. Further, Kotani et al (1994) using CT showed that after menopause, the accumulation of VAT with age was accelerated (159). Longitudinal studies (160 and 161), over a 6 year period, showed significant increases in WHR in only the women who changed their menopause state. On average, women who became postmenopausal experienced increases in WHR two-fold higher compared to women who were premenopausal (160).

This is supported by hormone replacement therapy studies in which menopause-induced re-distribution of fat to the central depots was prevented by hormone replacement (162-164). Studies conducted by den Tonkelaar and colleagues showed that in a large sample of Dutch pre- and post-menopausal women, the use of oestrogen was associated with a lower WHR (162 and 163). A small number of oestrogen users in the longitudinal study conducted by Bjorkelund et al (1996) showed that the transition from premenopause to postmenopause resulted in an increase in WC and a decrease in HC, and the use of oestrogen replacement resulted in the postponement of this (161). However, this was not the case in older postmenopausal women, suggesting that during menopause, oestrogens may only delay the accumulation of abdominal fat (161). These results therefore suggest that hormone replacement therapy prevents or delays the increase in AAT accumulation observed at menopause and highlights the pivotal role that hormones play in determining body fat distribution.

1.3.2.1 Hormonal changes and menopause

Changes during menopause lead to progressive loss in ovarian function, leading to a reduction in sex hormones, oestrogen and progesterone (165 and 166). Oestrogen is synthesized in the ovaries. Oestradiol (E2) is synthesized by the aromatization of androstenedione to oestrone,

followed by the conversion to E2 (167). Studies have demonstrated that ovariectomy increases adipose deposition, increases the accumulation of VAT, disrupts glucose and lipid homeostasis, increases the risk for developing cardiovascular diseases and IR, and that oestrogen replacement reverses this (168-173). The extent to which the direct effect of oestrogen on adipose tissue is responsible for the prevention of fat accumulation is still under investigation. Oestrogen may exert effects on several adipokines that are produced by adipocytes (174). Oestrogen levels in premenopausal women have been shown to be closely associated with leptin levels (175 and 176). Additionally in mice, oestrogen has been shown to reduce leptin resistance (177). Oestrogen increases leptin sensitivity by controlling the expression of leptin specific receptors (176, 178 and 179). In contrast, a recent study conducted by Yeaung et al (2013) in lean and obese premenopausal women showed that greater adiposity, measured by BMI, was associated with higher E2 and lower progesterone, luteinising-hormone (LH) and follicle stimulating hormone (FSH) (180). A recent study conducted by Cassaza et al (2008) on the effect of oestrogen on insulin levels in pubertal African American and European American girls demonstrated that higher insulin levels were associated with higher E2 levels in the African American compared to European American girls (181). Further independent of E2 levels, menarche was reached at a younger age in African American girls than white girls. Menarche was associated with an accelerated fat deposition in African American but not white American girls (181). Further, some studies have demonstrated lower E2 levels in African American women than Caucasian women approaching menopause (182), while others found no significant racial difference (183).

In contrast to oestrogen, circulating levels of Sex hormone binding globulin (SHBG), may be used as a marker for androgen status and is an indirect measure of testosterone to oestrogen ratio (184), and has been shown to be negatively associated with obesity (185). SHBG is a circulating steroid binding protein, which binds reversibly and with high affinity to testosterone (184). SHBG is an important determinant of testosterone in women and is an index of both free and SHBG bound testosterone levels (figure 1) (186). Women with low SHBG have higher free testosterone that is biologically active (187). Studies conducted in premenopausal African American and Caucasian/European women demonstrated that SHBG was negatively associated with IR and obesity (187 and 188). Other studies demonstrated that SHBG was negatively correlated to BMI and IR in Caucasian and not African American women (189). As SHBG is

produced in the liver, insulin may act directly on the liver to inhibit SHBG synthesis (190). Additionally, Patel et al (2009) demonstrated that higher free testosterone was associated with IR and CHD in postmenopausal women (191). Other studies conducted in premenopausal women demonstrated that testosterone levels were positively correlated with body FM (kg and %) and lean mass in women aged 20-25 years (192). These studies therefore suggest that low SHBG, may be a phenotype that envisages CVD and its risk factors in black women. However, studies have demonstrated that although SHBG was lower and free testosterone was higher in white women with greater upper body fat compared to lower-body fat, the levels of SHBG and testosterone were not altered with extremes of body fat in the black women (193). A study conducted by Yeung et al (2010) in American women demonstrated that although SHBG levels were similar in black and white women, body fat and trunk FM as a percentage of FM was shown to be negatively associated with SHBG in the white, but not black women. However, WC and trunk FM/leg FM was negatively associated with SHBG in both the black and white women (194).

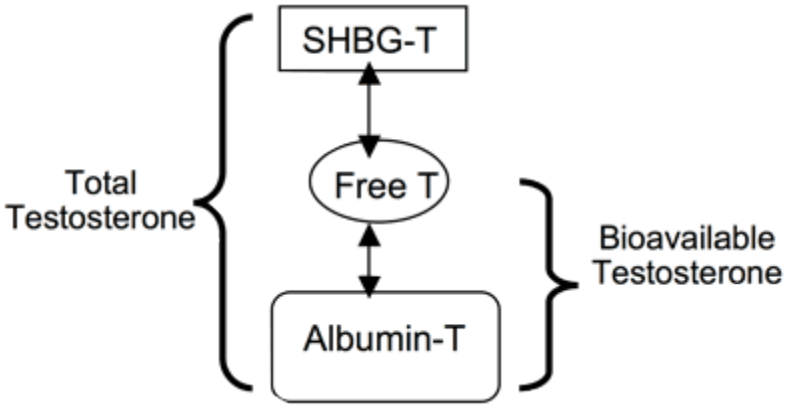


Figure 2: Schematic diagram of forms of testosterone and regulation by SHBG in the body. Free testosterone is biologically active and SHBG-bound testosterone is biologically inactive. An increase in SHBG results in an increase in SHBG bound testosterone and a decrease in bioavailable testosterone. T, testosterone; +, increases and -, decreases (186).

1.4 Oestrogen and its receptors

The known effects of oestrogen are mediated by ligand activated transcription factors known as oestrogen receptors (ER) (195). There are two types of ER's, oestrogen receptor alpha (ERα) (196) and oestrogen receptor beta (ERβ) (197). ERα is located on chromosome 6 and ERβ is

located on chromosome 14, and is homologous to ERα (195 and 198). Studies have demonstrated that ERβ inhibits the transcriptional activity of ERα depending on the concentration of oestrogens (199). Hall et al (1999) have shown that at a higher concentration of oestrogen, both ERα and ERβ had maximal transcription activity; however, the transcriptional activity of ERα was greater than ERβ. Increasing ERβ had no effect on the ERα transcriptional activity. At lower (sub-saturating) levels of oestrogen, only ERα is active. When ERβ was increased, the activity of ERα was suppressed (199). This suggests that the biological response to oestrogens is dependent on the relative ratios of ERα and ERβ levels. This suggests that different ER‟s are responsible for different functions.

Studies show that ERα plays an important role in adipose tissue deposition. Studies conducted in premenopausal women demonstrated that ERα was reduced with obesity, and increased after weight reduction (200). ERα knockout (ERKO) mice show an increase in adipose deposition, VAT, IR and impaired glucose tolerance (201-204) but also show a 8-fold increase in oestrogen levels (205), and increased signalling through ERβ could be a factor contributing to obesity of ERKO mice. Indeed, Naaz et al (2002) demonstrated that removal of oestrogen/ERβ signalling in ovariectomised ERKO mice resulted in a decrease in body weight and IR (206).

The effects of oestrogen to maintain reduced body fat appear to be mediated by ERα. Oestrogen/ERα signalling is critical in maintaining reduced IR and reduced obesity. This important role for oestrogen/ERα signalling in maintaining glucose homeostasis is supported by evidence obtained from ERβ knockout (BERKO) mice. Studies have demonstrated that BERKO mice had either no change or decreased IR and improved glucose tolerance (202, 203 and 207). Ludwig et al (2008) showed that ERβ acts through PPARϒ (207), (which is known to regulate glucose and lipid metabolism by modulating energy homeostasis in adipose tissue, liver and skeletal muscle) by inhibiting ligand mediated PPARϒ-transcriptional activity (208-210). PPARϒ was shown to enhance cellular processes involved in lipid accumulation, such as adipogenesis. The study also showed that absence of PPARϒ can lead to lipodystrophy and eventually IR (211). Studies conducted in black and white women demonstrated that in black women, PPARϒ mRNA levels were reduced with obesity (50). Goedecke et al (2011) also demonstrated that PPARϒ was down-regulated to a greater extent with obesity in black women compared to white women. Decreased expression could be an adaptive process limiting further

accumulation of adipose tissue. They also found that PPARϒ mRNA levels were negatively associated with IR in black but not white women. Consistently, low PPARϒ activity leads to cardiovascular and metabolic pathophysiological consequences such as IR, diabetes and end organ damage (208).

Another possible mechanism for ERKO mice maintaining reduced IR is demonstrated by increase in the expression of genes involved in lipid biosynthesis and a decrease in genes involved in lipid transport (202). Studies in ERKO mice have found that stearoyl-CoA desaturase (SCD1) is increased (202). SCD1 catalyses the synthesis of monounsaturated FA from saturated FA. These monounsaturated FA are used as substrates for the synthesis of TG and cholesteryl esters (212). Studies conducted in rodents have shown that SCD1 is high in conditions including obesity, diabetes and IR (213 and 214). However, a study in mice showed that a decrease in SCD1 worsens the diabetes in obese mice (215). Other studies conducted in humans have demonstrated lower adipose and hepatic SCD1 mRNA expression in obese compared to non-obese subjects and is negatively associated with IR (216 and 217).

ERKO mice also have increased plasma leptin and decreased expression of leptin receptor (LEPR) (202). Leptin is a hormone that is known to regulate adipose tissue mass by acting through LEPR (218). In other studies, increased plasma leptin levels and a reduction in LEPR expression in the hypothalamus and adipose tissue was demonstrated in ovariectomised mice. These levels were normalized by oestrogen treatment (219). Ovariectomised mice treated with E2 had significant increases in hepatic LEPR mRNA which suggests a direct role of oestrogens on the regulation of LEPR (202). It is therefore possible that the SCD1 and LEPR expression may be an important mechanism underlying IR in ERKO mice.

Further evidence supporting the role of oestrogen/ERα signalling in reducing obesity and IR was shown by a study conducted in aromatase knockout mice (ARKO) (220). ARKO mice were shown to be obese and have a phenotype that is similar to that of ERKO mice (221-223). P450 aromatase activity in adipose tissue is important in oestrogen production. Aromatase (CYP19A) converts androstenedione to oestrone followed by the conversion to E2 (167). In human and mice studies, deficiency of CYP19A results in abdominal obesity, glucose intolerance and IR, and treatment with E2 decreases IR (221, 223, 224 and 225). CYP19A transcript levels, which are highest in the gluteal region, next highest in the thigh and lowest in the abdominal SAT (226

and 227), are significantly greater in women with lower-body (gynoid) obesity than upper obesity (228). Studies conducted in Caucasian men and women (premenopausal) demonstrated that generalized obesity, characterised by BMI, was associated with an increase in CYP19A mRNA levels (229). In contrast, CYP19A mRNA levels in omental adipose tissue were inversely associated with BMI. However, this study was conducted in a smaller sample (230). The former study also suggested that an increase in ER‟s due to an increase in SAT CYP19A binding to androgen receptors may favour peripheral fat distribution. Another study demonstrated that a gain of function mutation in CYP19A was associated with female body fat distribution in a male patient (231). These studies therefore suggest that an increased CYP19A in SAT may generate more oestrogen ligands and consume more androgen ligands which may favour peripheral fat deposition in obese women.

ER gene expression levels may also be mediated by methylation in a variety of tissues. However, this has only been conducted extensively in breast tissue in which studies demonstrated that normal breast tissue and ER positive cell lines lack methylation. In contrast, ER negative breast cancer cell lines display extensive methylation due to an increase in DNA methyltransferase1 (232). Hormonal and growth factors may also modulate the level of ER‟s in order to regulate the growth of various tissues. ER transcription is important in cellular and growth potential as signals affect the growth and metabolism of oestrogen responsive tissues (232).

The studies above provide evidence for the influence of sex hormones and their receptors in changes of body fat distribution and IR. Therefore, observed differences in body fat distribution and IR between black and white women may be attributed to differences in sex hormones. There have been a few studies that have determined the association with circulating sex hormones E2 and SHBG and body fat distribution and IR in African American and European women. No studies of which we are aware have compared the difference in associations of ER‟s and body fat distribution and IR between black and white SA women. However, there has only been one other study that investigated regional differences in ERα mRNA expression (233). This study was conducted in premenopausal women but reported no difference in ERα expression between gluteal and abdominal SAT. Other studies conducted measured protein expression and found ERα was higher in AAT and ERβ was higher in gluteal adipose tissue (234).

1.5 Literature conclusions

The prevalence of overweight and obesity among black SA women is high. In addition, black women are more IR than white women, but have more peripheral fat and less VAT. This is surprising as VAT is known to be the main culprit that contributes to IR. However, the lower VAT and greater lower-body fat distribution may explain their lower prevalence of dyslipidaemia. The reasons for ethnic differences in body fat distribution are not known, however, ethnic differences in sex hormones may explain this. More specifically an increase in oestrogen and its receptors and decrease in testosterone and increase in SHBG may explain higher gluteal and lower abdominal fat in black women. We set out to determine ethnic specific associations between sex hormones and body fat distribution and IR. There have been no studies that have examined this in SA black and white women and therefore we are in a unique position to examine this in a group of women matched for BMI but in whom their body fat distribution and cardio-metabolic risk factors differs.

1.6 Aims and objectives

The overall aims of this thesis were to:

1) Examine ethnic-specific associations between body composition and cardio-metabolic risk factors;
2) Examine the association between sex hormones and body fat distribution and IR among the black and white women.

Therefore the aims will be addressed in 2 chapters

Study1: In a convenient sample of black and white premenopausal SA women, to:

1) Compare body fat distribution, IR and lipid levels;
2) Determine the associations between body fat distribution, IR and serum lipid levels;
3) Determine whether these associations differ by ethnicity.

Study 2: In a sub-sample of normal-weight and obese black and white premenopausal SA women, to:

1) Measure the gene expression of ER‟s and CYP19A in SSAT, DSAT and gluteal adipose tissue.
2) Measure circulating sex hormone concentrations
3) Examine associations between ER‟s and, CYP19A gene expression, and circulating sex hormone concentrations, body composition and IR.

Chapter 2

Associations between body fat distribution, insulin resistance and dyslipidaemia in black and white South African women

2.1 Introduction

Most recent studies show that NCD‟s account for majority of deaths globally (65.5%) (235), and that 80% of the deaths attributed to NCD‟s each year are in low-middle income countries (236). In 2000, NCD‟s such as CVD and T2D were known to be the second highest cause of death in SA (2). Obesity is known to be a major risk factor for CVD and T2D (3-6). The prevalence of obesity in SA women is high, particularly in black women (7). However, fat distribution rather than the amount of body fat has been shown to be a greater predictor of CVD and T2D risk factors such as IR and dyslipidaemia (15-17). There has been growing evidence indicating that not all fat stores contribute equally to CVD and T2D risk factors (18).

Studies in predominantly white populations have shown that central FM (measured as trunk fat on the DXA scan or WC) (27, 28, 33-35 and 36), and more specifically VAT (20 and 37), is positively associated with IR and dyslipidaemia. Conversely, lower-body SAT (gluteo-femoral) (27, 28, 33-35, 36 and 40) has been shown to be negatively associated with these cardio-metabolic risk factors. Some (27, 33 and 36), but not all of these studies have demonstrated that central and lower-body fat have independent effects on metabolic risk.

However, the relationship between fat distribution and IR appears to be altered by ethnicity. Studies in the USA and SA have shown that compared to white women, black women have less VAT and higher gluteo-femoral FM, but are more IR (46-56, 72, 75, 81 and 82). Lower VAT in the black women may however explain their lower lipid levels compared to white women (62). Notably, studies in both USA and SA have shown that despite lower VAT, black women have higher abdominal SAT than their white counterparts, which associates positively with IR (46, 49, 56 and 57). However, the SA studies have only been performed in small samples of women (n=10-15) and only focused on abdominal fat distribution. It is known that peripheral (leg and arm) FM is also an important determinant of cardio-metabolic risk. When examining whole-body fat distribution using DXA, studies in SA and the USA have shown that black women have lower central FM and higher leg FM compared to their white counterparts (72-75, 81 and 82).

To date, most studies that have explored ethnic-specific associations between whole-body fat distribution and cardio-metabolic risk have been undertaken in the USA or Europe, with no studies to our knowledge, examining the independent associations between central and peripheral fat distribution and cardio-metabolic risk in SA women.

Therefore, the aim of this study was to examine differences in whole body fat distribution between premenopausal black and white women and the ethnic-specific associations with cardio-metabolic risk. We also set out to determine if central vs. peripheral fat were independently associated with cardio-metabolic risk, and examine which body composition variable was most closely associated with cardio-metabolic risk in black and the white women, taking into account other lifestyle factors that have been shown to alter body composition, such as physical activity, contraception use, smoking and alcohol consumption.

2.2 Methods and materials

2.2.1 Subjects:

The study population consisted of 288 black and 197 white SA women who were recruited by advertisement and from local church groups, community centres and universities, as described previously (50 and 146). Inclusion criteria were: (i) aged 18-45 years; (ii) no known diseases or taking any medication for metabolic disorders; (iii) not currently pregnant, lactating or postmenopausal; and (iv) of SA ancestry. This study was approved by the Human Research Ethics Committee of the Faculty of Health Science of the University of Cape Town. Procedures and risks were explained to participants, all of whom gave written informed consent, prior to participation.

The testing procedures and biochemical analysis have been previously described, but are described in brief below (49, 50, 59 and 146).

2.2.2 Testing procedures:

2.2.2.1 Demographic and lifestyle questionnaire

A demographic questionnaire (as employed in other studies (59)), which was based on the DHS questionnaire was administered that included measures of socio-economic status, including

housing density, family history of T2D and behavioural/lifestyle factors. Contraceptive use was recorded, and women were categorized as using hormonal contraception (oral and injection) or not. Smoking was recorded and women were categorized as current smokers or not. Alcohol consumption in grams/day was also recorded using dietary recall.

Physical activity energy expenditure was characterised using the Global Physical Activity Questionnaire (GPAQ) (237 and 238). Moderate to vigorous physical activity (MVPA) was calculated as minutes of physical activity per week.

2.2.2.2 Body composition

Anthropometric measurements of participants were taken including height, weight in light clothing, WC (at the level of umbilicus) and HC (at the largest gluteal area). BMI was calculated as weight (kg) divided by the square of height (m^2). Body composition (fat and fat-free mass) was measured using DXA (Discovery-W, Software version 4.40; Hologic). Fat mass index (FMI) was calculated as total body fat (kg)/height (m^2). DXA-derived measures of body fat distribution included 1) trunk FM 2) arm FM 3) leg FM 4) android FM and 5) gynoid FM. Trunk FM included the region between the neck (line below the bottom of the jaw) and waist (line above the iliac crest) cuts with the lateral boundaries positioned to achieve separation of the upper arm and trunk at the glenoid fossa, and the inclusion of vertical lines on either side of the spine were positioned to exclude the spine. The arms included the region below the line through the glenoid fossa. Vertical lines extending downward from the waist cut were positioned to separate thigh from hands, and oblique lines were positioned to pass through the femoral neck and join the central vertical line between the legs, in order to isolate the legs (239). For android FM, the sub-region box was positioned so that that lower border was above the iliac crest and the upper border at the lower level of the ribs (excluding the bones). The lateral border was positioned so that all soft tissue was included. For the gynoid FM, the sub-region box was positioned so that the upper border passed through the most superior points of the inner pelvis. The lateral border was positioned so that all soft tissue was included (240). A CT scan (Toshiba X-press Helical Scanner; Toshiba Tokyo, Japan) at the level of the L4-L5 vertebrae was taken to determine VAT and SAT areas.

2.2.3 Blood sampling and biochemical analysis:

After an overnight fast (10-12hrs) a blood sample was drawn from the antecubital vein, for the subsequent determination of plasma glucose, and serum insulin, HDL-C, LDL-C, TC and TG levels. Plasma glucose concentrations were determined using the glucose oxidase method (YSI 2300 STAT PLUS; YSI life sciences, Yellow springs, OH). Serum insulin concentrations were determined by immunochemiluminometric assays using the ADVIA Centaur (Bayer diagnostics, Tarrytown, NJ). Blood lipids were measured using the Roche modular Autoanalyser (Roche Diagnostics GmbH, Sandhofler Strasse 116, D-68305, Mannheim, Germany). Enzymatic colourimetric assays were used to analyse TC, TG, LDL-C and HDL-C concentrations. LDL-C was calculated using the Friedewald formula (241).

2.2.3.1 IR

HOMAIR was calculated from fasting glucose and insulin levels (242).

2.2.4 Statistical analysis:

Results were analysed using Statistica version 10 (Statsoft Inc., Tulsa, Oklahoma, USA). Results are presented as median and inter-quartile range (IQR). All variables were normalized by log transformation, where required. Ethnic differences in body composition, IR, and lipid levels were determined using one-way ANCOVA, adjusting for age. Pearson chi-squared was used to determine differences in categorical variables between the black and white women. Partial correlations were used to determine the associations between the various body fat distribution variables and cardio-metabolic outcomes in the black and white women, adjusting for age and FMI. FMI was chosen as the covariate because it takes into account both the total body fat and the height of an individual (which differs significantly between black and white).

Multiple regression analysis was used to determine the independent associations between body fat distribution and IR and serum lipid levels, adjusting for age and FMI. In addition the effect of ethnicity on these relationships was tested by including ethnicity x body fat distribution interaction term in the model.

Backward stepwise regression was used to determine the model that accounted for most of the variance for each cardio-metabolic outcome. In each model, trunk FM was used as the measure

of central fat and leg FM was used as the measure of lower-body fat due to their stronger associations with cardio-metabolic outcomes (Table 5). Other variables included into the model were age, FMI, contraception use, MVPA, alcohol consumption and smoking.

2.3 Results

Table 2: Subject characteristics of black and white women

	Black	White	P-value
Age (years)	26 (22-33)	32 (24-39)	<0.01
MVPA (mins/week)	335 (90-855)	240 (120-480)	0.01
Alcohol consumption (g)	0 (0-2.8)	6.1 (0.5-14.7)	0.95
Contraception use (% (n))	32 (281)	31.1 (195)	0.74
Oral	6.3 (18)	26 (51)	<0.01
Injection	25.7 (74)	5.1 (10)	<0.01
Smoke (% (n))	10.1 (279)	17 (195)	0.04

Values presented as median and interquartile range (IQR) or %(n). MVPA, moderate-vigorous physical activity.

Table 3: Body composition and body fat distribution of black and white women.

		Black		White	P-Value
	N	Median (IQR)	N	Median (IQR)	Adjusted for age
Body composition					
Height (m)	288	1.60 (1.56-1.64)	197	1.67 (1.60-1.70)	<0.001
Weight (kg)	288	80.4 (60.9-96.2)	197	73.9 (62-94.1)	0.02
BMI (kg/m^2)	288	31.7 (23.6 -37.2)	197	26.6 (22.4-33.2)	<0.001
Fat (kg)	288	33.7 (19.4-44.3)	197	26.7 (17.1-40.3)	<0.001
Fat (%)	288	42.1 (32.7-46.9)	197	36.5 (28.9-43.9)	<0.001
FMI (kg/m^2)	288	13.3 (7.6-17)	197	9.9 (6.4-14.5)	<0.001
Body fat distribution					
WC (cm)	288	94.8 (77.3-108.6)	197	88 (78-101.5)	<0.001
Trunk FM (kg)	288	14.1 (7.4-20.6)	197	12.2 (7.4-20.3)	0.01
Trunk FM (%FM)	288	42.1 (36.7-46.7)	197	45.5 (40.7-49.7)	<0.001
Leg FM (kg)	288	13.7 (9.3-18.01)	197	10.7 (7.5-15.9)	<0.001
Leg FM (%FM)	288	44.3 (39.5-49.4)	197	41.4 (37.5-45.7)	<0.001
Trunk FM/Leg FM	288	0.95 (0.74-1.2)	197	1.1 (0.90-1.3)	<0.001
Arm FM (kg)	288	3.8 (1.9-4.9)	197	2.9 (1.8-4.6)	<0.001
Arm FM (%)	288	10.7 (9.3-12.3)	197	10.8 (9.7-11.9)	0.9
Android (kg)	288	2.5 (1.1-3.8)	197	2.02 (1.02-3.5)	0.001
Android (%FM)	288	0.07 (0.06-0.09)	197	0.07 (0.06-0.09)	0.6
Gynoid (kg)	288	6.1 (4.1-8.0)	197	5.2 (3.7-7.5)	<0.001
Gynoid (%FM)	288	0.19 (0.17-0.21)	197	0.20 (0.18-0.22)	<0.001
Android/Gynoid	288	0.38 (0.27-0.48)	197	0.38 (0.28-0.46)	0.09
VAT (cm^2)	222	71 (47-102)	153	80 (60-124)	0.04
SAT (cm^2)	220	442 (212-577)	150	297 (169-460)	<0.001
VAT/SAT	220	0.20 (0.14-0.27)	150	0.31 (0.23-0.42)	<0.001

Values presented as median and interquartile range (IQR). P values for one way ANCOVA adjusting for age. BMI, body mass index; FMI, fat mass index; WC, waist circumference; FM, fat mass; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

Table 4: Cardio-metabolic risk factors of black and white women.

	Black		White		P-value	
	N	Median (IQR)	N	Median (IQR)	Adjusted for age	Adjusted for age and FMI
Glucose (mmol/L)	280	4.5 (4.2-4.9)	196	4.7 (4.4-4.9)	0.08	<0.001
Insulin (mU/L)	287	9.8 (5.6-16.6)	197	6.9 (4.6-10.8)	<0.001	0.27
HOMAIR	279	2.1 (1.1-3.4)	196	1.5 (0.96-2.2)	<0.001	0.59
TC (mmol/L)	274	3.9 (3.3-4.5)	197	4.7 (4.1-5.3)	<0.001	<0.001
TG (mmol/L)	274	0.70 (0.50-1.0)	197	0.90 (0.60-1.2)	<0.001	<0.001
HDL-C (mmol/L)	273	1.2 (1.0-1.6)	197	1.6 (1.4-1.9)	<0.001	<0.001
LDL-C (mmol/L)	273	2.2 (1.7-2.8)	197	2.6 (2.1-3.3)	<0.001	<0.001

Values presented as median and interquartile range (IQR). P values for one way ANCOVA adjusting for age and age and FMI. HOMAIR, homeostasis model for insulin resistance; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

2.3.1 Subject characteristics

Subject characteristics are summarized in Table 2. Black women were younger than white women, and consequently, all subsequent analysis was adjusted for age. Black women had higher levels of MVPA compared to white women, and more white women smoked, whereas alcohol consumption did not differ between groups. There was no significant difference in the proportion of women who used contraceptives, but more white women used oral contraceptives, while more black women used injectable contraceptives.

Ethnic differences in body composition and fat distribution are summarized in Table 3. Black women were significantly shorter, heavier, had a higher BMI and greater FM (absolute and %) compared to white women. Black women had greater absolute measures of trunk, leg and arm FM compared to white women. However, as a percentage of total body FM, black women had less trunk FM and greater leg FM. Accordingly, trunk FM/leg FM ratio was greater in white than black women. As a percentage of total body FM, there was no significant difference in arm FM between black and white women. In addition, black women had greater absolute measures of android and gynoid FM than white women, however, as a percentage of total FM there was no significant difference between groups. Black women had less abdominal VAT and more SAT and a lower VAT/SAT ratio compared to white women.

Cardio-metabolic risk factors for black and white women are summarized in Table 4. Although there were no ethnic differences in fasting plasma glucose concentrations, black women had higher fasting insulin concentrations and HOMA-IR than white women. After adjusting for differences in age and FMI, glucose concentration were significantly lower in the black compared to the white women, but the differences in fasting serum insulin concentrations and HOMAIR were no longer significant. Black women had significantly lower TC, TG, HDL-C and LDL-C concentrations than white women, which remained significant after adjusting for age and FMI.

Table 5: Correlations between body fatness and its distribution and cardio-metabolic risk factors in black and white women and the combined sample

		Glucose (mmol/L)	Insulin (mU/L)	HOMAIR	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
FMI (kg/m^2)	B	**0.26**$^{\Delta}$	**0.53**$^{\Delta}$	**0.54**$^{\Delta}$	**0.23**$^{\Delta}$	0.035	**-0.31**$^{\Delta}$	**0.15***
	W	**0.23**$^{\Delta}$	**0.61**$^{\Delta}$	**0.61**$^{\Delta}$	**0.35**$^{\Delta}$	**0.26**$^{\Delta}$	**-0.39**$^{\Delta}$	**0.36**$^{\Delta}$
	All	**0.25**$^{\Delta}$	**0.59**$^{\Delta}$	**0.60**$^{\Delta}$	**0.30**$^{\Delta}$	**0.13**$^{\Delta\$}$	**-0.34**$^{\Delta}$	**0.25**$^{\Delta\$}$
Trunk FM (kg)	B	**0.34**$^{\Delta}$	**0.30**$^{\Delta}$	**0.34**$^{\Delta}$	**0.30**$^{\Delta}$	-0.060	**-0.23**$^{\Delta}$	-0.039
	W	0.069	**0.29**$^{\Delta}$	**0.29**$^{\Delta}$	0.099	0.043	**-0.18**$^{\Delta}$	0.12
	All	**1.04**$^{\Delta\$}$	**1.16**$^{\Delta}$	**1.23**$^{\Delta}$	**0.92**$^{\Delta}$	-0.09$^{\$}$	**-0.87**$^{\Delta}$	0.09$^{\$}$
Android (kg)	B	**0.23**$^{\Delta}$	**0.24**$^{\Delta}$	**0.26**$^{\Delta}$	**0.24**$^{\Delta}$	-0.10	**-0.20**$^{\Delta}$	-0.083
	W	0.041	**0.33**$^{\Delta}$	**0.32**$^{\Delta}$	0.14	0.072	**-0.21**$^{\Delta}$	**0.15***
	All	**0.59**$^{\Delta\$}$	**0.87**$^{\Delta}$	**0.90**$^{\Delta}$	**0.71**$^{\Delta}$	-0.13$^{\$}$	**-0.71**$^{\Delta}$	0.03$^{\$}$
VAT (cm^2)	B	0.12	**0.24**$^{\Delta}$	**0.27**$^{\Delta}$	0.078	-0.034	-0.099	0.0011
	W	-0.039	**0.21**$^{\Delta}$	**0.19***	**0.21***	0.10	**-0.25**$^{\Delta}$	**0.17***
	All	0.10$^{\$}$	**0.33**$^{\Delta}$	**0.33**$^{\Delta}$	**0.20**$^{\Delta}$	-0.04$^{\$}$	**-0.28**$^{\Delta}$	0.05$^{\$}$
SAT (cm^2)	B	0.094	-0.023	0.019	-0.11	-0.039	**0.15***	-0.071
	W	**-0.25**$^{\Delta}$	-0.0002	-0.017	-0.016	0.15	0.16	0.079
	All	0.13$^{\$}$	0.18	0.20	-0.06	-0.20$^{\$}$	-0.06	-0.19$^{\$}$
Leg FM (kg)	B	**-0.16**$^{\Delta}$	**-0.33**$^{\Delta}$	**-0.34**$^{\Delta}$	**-0.23**$^{\Delta}$	-0.011	**0.15***	-0.015
	W	-0.12	**-0.25**$^{\Delta}$	**-0.26**$^{\Delta}$	**-0.16***	-0.097	**0.19**$^{\Delta}$	**-0.15***
	All	**-0.38**$^{\Delta}$	**-0.67**$^{\Delta}$	**-0.69**$^{\Delta}$	**-0.52**$^{\Delta}$	-0.09	**0.39**$^{\Delta}$	-0.15
Gynoid (kg)	B	-0.084	**-0.28**$^{\Delta}$	**-0.27**$^{\Delta}$	**-0.19**$^{\Delta}$	-0.0003	**0.12***	-0.013
	W	-0.055	**-0.16***	**-0.16***	**-0.15***	**-0.17***	0.14	**-0.19**$^{\Delta}$
	All	-0.21	**-0.56**$^{\Delta}$	**-0.55**$^{\Delta}$	**-0.49**$^{\Delta}$	-0.17	**0.34**$^{\Delta}$	-0.23
Trunk FM/Leg FM	B	**0.30**$^{\Delta}$	**0.39**$^{\Delta}$	**0.42**$^{\Delta}$	**0.34**$^{\Delta}$	-0.027	**-0.24**$^{\Delta}$	-0.012
	W	0.12	**0.34**$^{\Delta}$	**0.34**$^{\Delta}$	**0.17***	0.092	**-0.23**$^{\Delta}$	**0.17***
	All	**0.28**$^{\Delta\$}$	**0.42**$^{\Delta}$	**0.43**$^{\Delta}$	**0.32**$^{\Delta}$	0.03$^{\$}$	**-0.28**$^{\Delta}$	0.08$^{\$}$
Android/Gynoid	B	**0.22**$^{\Delta}$	**0.34**$^{\Delta}$	**0.35**$^{\Delta}$	**0.28**$^{\Delta}$	-0.07	**-0.22**$^{\Delta}$	-0.06
	W	0.06	**0.34**$^{\Delta}$	**0.35**$^{\Delta}$	**0.20**$^{\Delta}$	**0.16***	**-0.25**$^{\Delta}$	**0.24**$^{\Delta}$
	All	**0.23**$^{\Delta\$}$	**0.45**$^{\Delta}$	**0.45**$^{\Delta}$	**0.38**$^{\Delta}$	0.03$^{\$}$	**-0.33**$^{\Delta}$	0.09$^{\$}$
VAT/SAT	B	0.043	**0.21**$^{\Delta}$	**0.21**$^{\Delta}$	**0.14***	0.0010	**-0.19**$^{\Delta}$	0.06
	W	0.13	**0.22**$^{\Delta}$	**0.23**$^{\Delta}$	**0.22**$^{\Delta}$	0.0069	**-0.37**$^{\Delta}$	0.12
	All	0.07	**0.22**$^{\Delta}$	**0.22**$^{\Delta}$	**0.20**$^{\Delta}$	0.01	**-0.27**$^{\Delta}$	0.10

Values are presented as partial correlation coefficients adjusted for age and FMI (except for FMI). $^{\Delta}$. p<0.01 and *.p<0.05. "All" values are presented as beta coefficients adjusted for age, FMI and ethnicity. $, ethnic x body composition interaction. FMI, fat mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; FM, fat mass; HOMAIR, homeostasis model of insulin resistance; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

2.3.2 Associations between body fatness and its distribution and cardio-metabolic risk factors.

Table 5 shows the partial correlations (adjusted for age and FMI) between body fatness and its distribution and cardio-metabolic risk factors for the black and white women individually and combined. In summary, when adjusted for age, greater total body fat, as defined by FMI, was positively associated with plasma glucose concentrations, measures of IR (fasting insulin and HOMA-IR), TG and LDL-C concentrations and negatively associated with HDL-C concentrations in both black and white women. FMI was positively associated with TC in white women only.

In black and white women, increased central FM and reduced lower-body fat correlated with measures of IR (fasting insulin and HOMAIR). In black women only, greater central fat, characterised by trunk FM, android FM, trunk FM/leg FM and android/gynoid, and lower leg FM were associated with increased fasting plasma glucose concentrations. Notably these associations with glucose concentrations and central FM were significantly different between black and white women. In white women only, increased abdominal SAT was associated with reduced fasting glucose concentrations, and this association differed significantly between black and white women.

In both black and white women, reduced lower-body FM characterised by leg FM and gynoid FM and increased trunk FM/leg FM and android/gynoid was associated with TG concentrations. In the black women, higher trunk FM and android FM, and in the white women higher VAT was associated with TG concentrations. In addition, in both the black and white women, central fat, characterised by trunk FM, android FM, trunk FM/leg FM and android/gynoid, was associated with reduced HDL-C concentrations. In contrast higher leg FM was associated with increased HDL-C concentrations in both the black and white women. In white women only, android/gynoid was positively associated with TC concentrations, while gynoid FM was negatively associated with TC concentrations. Notably the former relationship differed significantly between black and white women. Increased android FM, trunk FM/leg FM, android/gynoid and VAT were associated with increased LDL-C concentrations. These associations differed significantly in black and white women. In contrast, reduced lower-body fat

was associated with increased LDL-C concentrations in white, but not black women. There were no associations between arm FM and any metabolic risk factor in the black and white women.

The confounding effects of various lifestyle factors, including contraceptive use, smoking, physical activity and alcohol consumption on metabolic risk are shown in supplementary tables 9 and 10. In summary, in black women, IR was higher and HDL-C concentrations were lower in women on contraception than not. Despite few black women consuming alcohol, consumption was positively associated with serum HDL-C concentrations in black women. In white women, TC, TG and HDL-C concentrations were higher in women on contraception than not, while lower MVPA was associated with higher fasting insulin concentrations and HOMAIR.

Table 6: Multivariate analysis for black and white women

Black				**White**			
Glucose (mmol/L)							
Variable	**B**	**P**		**Variable**	**B**	**P**	
FMI (kg/m^2)	-1.11	<0.01		Age (years)	0.18	0.01	
Trunk FM (kg)	1.47	<0.01		FMI (kg/m^2)	0.24	0.00	
	R=**0.45**	R^2=**0.21**	P<**0.01**		R=**0.34**	R^2=**0.12**	P**<0.01**
Insulin (mU/L)							
Variable	**B**	**P**		**Variable**	**B**	**P**	
Age (years)	-0.33	<0.01		Age (years)	-0.25	0.00	
Trunk FM (kg)	1.09	<0.01		Trunk FM (kg)	1.02	0.00	
Leg FM (kg)	-0.53	<0.01		Leg FM (kg)	-0.37	0.00	
Contraception	0.11	<0.01					
	R=**0.64**	R^2=**0.40**	P**<0.01**		R=**0.67**	R^2=**0.45**	P<**0.01**
HOMAIR							
Variable	**B**	**P**		**Variable**	**B**	**P**	
Age (years)	-0.29	<0.01		Age (years)	-0.21	0.00	
Trunk FM (kg)	1.14	<0.01		Trunk FM (kg)	1.03	0.00	
Leg FM (kg)	-0.57	<0.01		Leg FM (kg)	-0.38	0.00	
Contraception	0.11	0.02					
	R=**0.65**	R^2=**0.42**	P**<0.01**		R=**0.67**	R^2=**0.45**	P<**0.01**
TG (mmol/L)							
Variable	**B**	**P**		**Variable**	**B**	**P**	
Age (years)	0.12	0.04		FMI (kg/m^2)	0.90	0.00	
Trunk FM (kg)	0.84	0.00		Leg FM (kg)	-0.51	0.00	
Leg FM (kg)	-0.59	0.00		Contraception	0.26	0.00	
	R=**0.48**	R^2=**0.23**	P**<0.01**		R=**0.49**	R^2=**0.24**	P**<0.01**
HDL-C (mmol/L)							
Variable	**B**	**P**		**Variable**	**B**	**P**	
Age (years)	0.20	0.07		VAT (cm^2)	-0.45	0.00	
FMI (kg/m^2)	-1.2	<0.00		Contraception	0.18	0.01	
Leg FM (kg)	0.77	0.01					
Contraception	-0.23	0.02					
Alcohol consumption	0.23	0.02					
	R=**0.51**	R$^{2=}$**0.26**	**<0.01**		R=**0.51**	R^2=**0.26**	P**<0.01**
TC (mmol/L)							
Variable	B	P		**Variable**	**B**	**P**	
Age (years)	0.22	0.0021		Age	0.22	0.00	
FMI (kg/m^2)	0.38	0.025		FMI (kg/m^2)	0.29	0.00	
SAT (cm^2)	-0.36	0.029		Contraception	0.29	0.00	
	R=**0.30**	R^2=**0.10**	P**<0.01**		R=**0.44**	R^2=**0.19**	P**<0.01**
LDL-C (mmol/L)							
Variable	**B**	**P**		**Variable**	**B**	**P**	
Age (years)	0.19	0.0071		Age	0.16	0.02	
FMI (kg/m^2)	0.46	0.0073		Trunk FM (kg)	0.38	0.00	
SAT (cm^2)	-0.33	0.049					
	R=**0.33**	R^2=**0.11**	P**<0.01**		R=**0.46**	R^2=**0.21**	P**<0.01**

2.3.3 Multivariate analysis

In separate models for black and white women, we then used backward stepwise regression to determine the factors that accounted for the greatest variance in the cardio-metabolic risk factors including the following variables in the initial model: Age, FMI, trunk FM and leg FM. Based on the results relating to the covariates described above, we also included these covariates (contraception use and alcohol) into the appropriate models. We then repeated the regression analyses including VAT and SAT in the models.

In black women, FMI and trunk FM accounted for 21% of the variance in fasting glucose concentrations, whereas in white women age and FMI contributed significantly to the model, accounting for only 12% of the variance. In both black and white women, trunk FM and leg FM were independently associated with fasting serum insulin concentrations and HOMA-IR, and together with age, and in the case of black women, contraceptive use, accounted for 40-45% of the variance in the models. The addition of VAT and SAT to the models did not contribute independently or significantly to the models for fasting plasma glucose and measures of IR in the black and white women

For the black women, trunk FM and leg FM were independently associated with TG, whereas only FMI and leg FM, as well as contraceptive use associated with TG in the white women. The addition of VAT and SAT did not contribute significantly to the model in the black and white women. In the black women, HDL-C concentrations were independently associated with age, FMI, leg FM, contraceptive use and alcohol consumption, whereas in white women, only VAT and contraceptive use contributed to the model. Notably, the associations between HDL-C concentrations and contraceptive use were opposite in the black and white women, showing a negative association in black women, and a positive association in white women. The model that explained the greatest variance in TC and LDL-C concentrations in the black women, included age, FMI and abdominal SAT, the latter being negatively associated with TC and LDL-C concentrations. This contrasts to the findings for white women, where age, FMI, contraceptive use accounted for the greatest variance in TC concentrations, and age and trunk FM contributed to the model for LDL-C concentrations.

2.4 Discussion

The main findings of this study were that compared to white women, black women had less central and greater lower-body fat, but had similar IR and lower serum lipid concentrations. Despite these differences, the associations between body fat distribution and measures of IR, as well as TG and HDL-C concentrations were similar in black and white women. The novel finding of this study was that central and peripheral fat deposition was independently associated with IR in both the black and white women, and with TG in the black women. In contrast, fasting glucose concentrations were associated with centralisation of body fat in black but not white women, whereas TC and LDL-C concentrations were associated with centralisation of body fat in white but not black women.

Black women had greater total body fat compared to their white counterparts, which is in accordance to the recent SANHANES in which the prevalence of obesity in SA black women is higher compared to other ethnic groups (7). This study also showed that, as a percentage of total body FM, black women had lower central fat and higher lower-body fat compared to the white women, which is in agreement with other SA studies that have used DXA to quantify body fat distribution (72 and 82), as well as studies from the USA and Europe (73-75 and 81). Further, within the abdominal depot, this study showed that black women had less VAT and more SAT compared to white women, which is commensurate with both SA and American studies (46-52).

Lower central, and to a lesser extent, greater peripheral FM, in the black women associated with their lower fasting glucose concentrations compared to white women, suggesting that accumulation of central FM may play a vital role in determining fasting plasma glucose concentrations, and hence the development of T2D in the black women. In contrast to the findings for fasting plasma glucose, fasting insulin levels and HOMAIR were not significantly different between black and white women, despite the differences in body fat distribution. Indeed, numerous studies have shown that compared to white women, black women have a higher prevalence of IR and T2D for the same BMI or WC (54 and 56). These results are surprising given that greater central and reduced peripheral FM were similarly associated with higher fasting insulin and HOMAIR in the black and white women, a finding supported by similar studies in the USA (77 and 79). These findings suggest that other factors, in addition to body fat distribution are important determinants of IR in black women.

Another important finding of this study was that central and peripheral FM were independently associated with fasting insulin and HOMAIR in both the black and white women. To our knowledge, this is the first study to demonstrate independent associations among black and white SA women, a finding that has been demonstrated in mostly white men and women (27, 33 and 36). Differences in the contribution of abdominal and gluteo-femoral fat to IR may be due to phenotypic differences in adipose tissue depots. Studies in predominantly white populations have shown, for example, that cytokines, such as TNFα and IL-6 are expressed in high concentrations in abdominal obesity, and have been suggested to play a role as mediators of MS in obesity (137-140 and 145). In contrast, gluteo-femoral adipose tissue has been shown to have a more beneficial adipokine profile, secreting factors such as leptin (93). Indeed, studies from our laboratory have shown that in white women, inflammatory gene expression, especially in the abdominal depot, was significantly associated with higher IR (146). However, SAT inflammatory gene expression was not significantly associated with IR in the black women, despite their being more IR and having a higher SAT inflammatory profile, especially in the gluteal depot, than white women. This finding may suggest that factors other than inflammation may contribute to higher IR in the black women.

Lower-body fat has been suggested to act as a metabolic sink, storing excess FFA when there is an energy surplus (83, 85-87 and 122), due to its lower lipolytic activity and higher LPL activity compared to upper body fat stores (93, 122 and 125-128). A study conducted in a small sample of black and white women from America, demonstrated that LPL activity was higher in the black compared to the white women, possibly explaining their greater lower-body fat deposition (62). Lower-body has been suggested to protect against ectopic fat deposition and therefore protect against risk for CVD and T2D. However, when the capacity to store excess fat in the periphery is exceeded, peripheral fat is no longer protective. A small SA study demonstrated reduced adipogenesis and lipogenesis in obese black women, and this was associated with increased IR (50). A recent study from our laboratory has shown that with increasing weight gain, black women accumulate more central relative to peripheral FM, which was associated with increased IR (Chantler et al., in review). These findings imply that the prevention of an increase in weight gain is vital for the prevention of metabolic risk in black women and it is important to determine the point at which peripheral FM is protective.

In addition to body fat distribution, other lifestyle factors also influenced IR, but differed between black and white women. In the black women only, contraceptive use, which was predominantly injectable contraceptives, was associated with increased IR. Similarly, other studies demonstrated that women using injectable contraception had significantly greater insulin levels compared to those that were on oral contraception (243). The mechanism by which injectable contraception may cause increased insulin levels has yet to be elucidated, but may reflect compensation for increased IR, which is associated with increased FA among injectable contraceptive users (244). Another mechanism may include reduced clearance, degradation, or increased return of insulin to circulation (245). In addition, physical activity was differentially associated with IR in the black and white women. There are a number of studies that demonstrated that physical activity influences metabolic risk. For example results from the Canadian Heart Health Survey showed that participants who engaged in more than 30 minutes of vigorous-intensity physical activity were less likely to have MS (246). A study conducted in black SA women using objectively measured physical activity demonstrated that light-intensity physical activity was only associated with reduced central FM and not cardiometabolic risk factors (247). In our study, despite white women having lower MVPA than black women, MVPA was associated with IR in white women only. Previous research from our laboratory has demonstrated that white women mainly perform leisure activity, typically undertaken at a higher intensity, whereas black women mainly perform physical activity for travel, typically undertaken at a lower intensity. This may suggest that the intensity of exercise is an important determinant of IR.

HDL-C and TG concentrations are often used as markers for IR (63 and 64). However, studies have demonstrated that TG/HDL-C ratio does not predict IR in African American or black SA women as it does for white women (59, 61 and 63-66). This study found that despite similar IR, HDL-C and TG concentrations were lower in the black compared to the white women, which is commensurate with some but not all studies (51, 55 and 58-62). Notably, similar to the findings for IR, higher HDL-C and lower TG concentrations were associated with reduced central and increased peripheral FM, and these associations were similar in black and white women, a finding supported by studies in the USA (79 and 248). The lower HDL-C concentrations of black women must therefore be explained by other factors. HDL-C in the black women was associated with alcohol consumption, independent of body fat distribution. Alcohol consumption may raise

HDL-C concentrations by increasing the transport rate of the major HDL apolipoprotein Apo-I and Apo-II (249). Compared with 76% of white women, 34% of black women consumed alcohol and this might therefore partly contribute to their lower HDL-C levels. Contraception use was another significant determinant of HDL-C in both the black and white women and TG in the white women. Notably contraception use was negatively associated with HDL-C in the black women and positively associated in the white women, which could be explained by the type of contraception used. Studies have demonstrated that women using injectable contraception have lower HDL-C and TG concentrations compared to those that were on oral contraception (250). This may be due to suppression of hepatic lipase by ethniyl oestradiol (present in oral contraception) which slows the transport of HDL-C and increases TG to the liver. Desogesterel, the progestin in oral contraception, is less androgenic than first and second generation progestins, so does not counteract the oestrogen effect in this contraception (250). This may therefore explain, in part, the differing HDL-C concentrations in the black and white women.

In our study we also demonstrated that independent of body fat, TC and LDL-C concentrations were lower in the black compared to the white women, which is in agreement with similar SA studies (51, 55 and 58-60). Additionally we demonstrated that increased TC and LDL-C concentrations were associated with increased central FM in the white women only. Hosain et al (2010) also demonstrated that the association between central FM and lipid levels were stronger in the white compared to the black women (80). It is important to note that increased trunk FM was significantly associated with increased LDL-C concentrations in the white women, whereas in the black women, abdominal SAT area was negatively associated with LDL-C and TC concentration, suggesting a protective effect of SAT in the black women. There have been a number of studies that have demonstrated that SAT is protective against IR and increased lipid levels, more specifically TG in women with higher BMI (251). Additionally lipodystrophic loss of SAT results in increased IR and dyslipidaemia (252). In contrast, other studies in women have demonstrated loss of abdominal SAT does not produce the same beneficial results as VAT in terms of reduced IR and dyslipidaemia (253). This is the first study of which we are aware, that has demonstrated a protective effect of abdominal SAT on cardio-metabolic risk in black women. A possible mechanism for the protective role of SAT is that it is an alternative depot for excess FFA, potentially reducing ectopic fat deposition in VAT and liver, thereby preventing lipotoxicity and reducing dyslipidaemia (254).

We found no associations between arm FM and any metabolic risk factor in the black and white women, a finding supported by a study conducted in only white men and women (35). Other studies in obese pre- and postmenopausal women found a reduction in arm FM was associated with an increase in TC and LDL-C concentrations after adjusting for total body fat and other body fat distribution variables in the study (i.e. Trunk FM, leg FM, SAT) (36 and 42). In both African American and white women, increased arm FM was associated with having 2 or more risk factors for MS (254). Although arm FM is regarded as „peripheral fat mass", this is from the upper-body and therefore may not exhibit the same protective effects as leg FM. Due to contradictory findings further exploration is required.

The strengths of this study include the state-of-the-art measures of body fat distribution, DXA and CT, and the examination of ethnic-specific associations between these measures and cardio-metabolic risk. Possible limitations of the study were the inclusion of a convenient sample of women, which is not representative of total population. Black women were more obese that white women but this maybe reflective of the population according to SANHANES. The cross-sectional design of the study limits one to derive conclusions in terms of causality. The number of women in which CT scans were conducted was low (76% of total sample) and this may have created type II error. Further, we did not measure other lifestyle factors such as diet and this has been shown to affect body fat and metabolic risk. More objective measures of physical activity using accelerometers should be used as it would give more insight into possible differences in non-exercise thermogenesis within these populations and should therefore be conducted in future studies. Future studies should also include subjects with a wider age range as metabolic risk factors differ with age.

In conclusion, this study showed that black women had lower central and greater peripheral fat compared to white women, which was associated with lower fasting glucose concentrations in the black women and higher TC and LDL-C concentrations in the white women. Increased central and reduced peripheral FM were independently associated with measures of IR in both the black and white women. In addition to body fat distribution, modifiable risk factors were identified, including MVPA, which was associated with reduced IR in the white women, and contraceptive use, which was associated with IR and lipid levels in the black and white women. Intervention studies aimed at reducing centralisation of body fat, increasing physical activity and

changing contraceptive use are required to verify these findings to provide evidence-based guidelines for the prevention and management of cardio-metabolic risk.

Chapter 3

Adipose and circulating sex hormones: Associations with body composition and insulin resistance in black and white South African women

3.1 Introduction

In chapter 2, we demonstrated that greater central body fat, characterised by increased trunk FM and android FM, was associated with increased IR, while leg FM and gynoid FM were negatively associated with IR, which is supported by various international studies (27, 28, 33-35 and 36). Despite this, this thesis also showed that although black women had less VAT and central FM, and greater lower-body fat than their white counterparts, they had similar levels of IR. Previous studies conducted in SA and the USA have demonstrated that compared to their white counterparts, black and African American women were more IR despite their lower VAT and greater lower-body fat (53-56). Further, it has been shown that VAT and SAT were similarly correlated to IR in white women, however, in black women SAT was more closely correlated with IR than VAT (46, 49, 56 and 57).

The reasons for ethnic differences in the relationship between body fat distribution and IR are not known, but could be explained in part by differences in sex hormones. Sex hormones play important roles in determining body fat and its distribution, as evidenced by sex-specific phenotypes with men displaying a preponderance towards central obesity, while women have greater peripheral obesity (7 and 24). Indeed, an increase in oestrogen levels is related to an increase in gynoid body fat (162-164). Conversely, studies have demonstrated that circulating oestrogen deficiency, experienced at the menopausal transition, is associated with an increase in central FM, IR and increased risk for developing CVD, and this is reversed with oestrogen replacement (162-164).

The effects of oestrogens are mediated by ER"s, ERα and ERβ which are expressed in human adipose tissue (195-197). It has been demonstrated that ERα and ERβ have different actions and ERβ may even oppose the actions of ERα (199). Studies conducted in ERKO mice demonstrated an increase in VAT, IR and impaired glucose tolerance (201-204). The effects of oestrogen to maintain reduced body fat appear to be mediated by ERα. Oestrogen/ERα signalling is critical in maintaining reduced IR and reduced obesity. This important role for oestrogen/ERα signalling in

maintaining glucose homeostasis is supported by evidence obtained from BERKO mice. Studies have demonstrated that BERKO mice had either no change or decreased IR and improved glucose tolerance (202, 203 and 207). Studies in humans have demonstrated that ERα expression is reduced in obese premenopausal women and expression increases after weight reduction (200). In contrast, ERβ but not ERα was significantly higher in adipose tissue of postmenopausal compared to premenopausal women (255). However, oestrogen production is also dependent on CYP19A activity in adipose tissue, which converts androstenedione to oestrone followed by the conversion to oestrogen (167). Studies in the USA have shown that CYP19A transcript levels were highest in the gluteal region and lowest in abdominal SAT (226 and 227) and were significantly greater in women with gynoid-type obesity compared to upper body obesity (228). The importance of CYP19A maintaining glucose homeostasis is supported by evidence obtained from ARKO mice, which present with glucose intolerance and IR (221-223). ARKO mice were also shown to be obese and have a phenotype similar to ERKO mice due to a lack of functional CYP19A gene, resulting in decreased oestrogen (221-223). Further, studies conducted in humans have shown that a decrease in CYP19A activity resulted in glucose intolerance and IR (223 and 224).

In postmenopausal women, increasing androgen levels (e.g. testosterone) are associated with IR (191). Other studies conducted in premenopausal women (aged 20-25 years) demonstrated that testosterone levels were positively correlated with body FM (kg and %) and lean mass (192) and were higher in women with greater central obesity (256). SHBG can be used as an indicator for androgen status and is an indirect measure of the testosterone to oestrogen ratio (184). Studies have demonstrated that women with high SHBG levels have low free testosterone levels, which were associated with reduced obesity and IR (187-189). Studies have demonstrated that although SHBG was lower and free testosterone was higher in white women with greater upper body fat compared to lower-body fat, the levels of SHBG and testosterone were not altered with extremes of body fat in the black women (193). Further, a study conducted by Yeung et al (2010) in American women demonstrated that body fat and trunk FM as a percentage of FM, were negatively associated with SHBG in white, but not black women (194).

The studies above provide evidence for differences in sex hormones and their receptors in determining body fat distribution and IR. We therefore hypothesized that difference in body fat

distribution and IR between black and white women may be associated with differences in sex hormones and their receptors. Although there have been a few studies that have examined the associations between circulating sex hormones, E2 and SHBG, and body fat distribution and IR in African American and white women, no studies of which we are aware of have compared the ethnic-specific associations of ER gene expression and body fat distribution and IR between black and white women. We are in a unique position to examine this in a group of black and white women matched for BMI, but in whom their body fat distribution and cardio-metabolic risk factors differ. Therefore in a sample of normal-weight and obese black and white SA women, the aim of this study was to examine the associations between sex hormones and body fat distribution and IR among black and white women. The objectives were therefore to:

4) Compare the ethnic differences in expression of ER‟s ERα and ERβ, and CYP19A genes in abdominal and gluteal SAT depots;
5) Compare circulating sex hormone concentrations in the black and white women;
6) Examine the associations of ERα and ERβ, and CYP19A mRNA levels within each depot and circulating sex hormones with body fat distribution and IR in black and white women.

3.2 Methods and materials

3.2.1 Subjects:

The study population, a subsample of participants described in the previous chapter, consisted of 13 normal-weight and 15 obese black and 15 normal-weight and 12 obese white SA women who were recruited by advertisement and from local church groups, community centres and universities, as described previously (24). Inclusion criteria were: (i) aged 18-45 years; (ii) no known diseases or taking any medication for metabolic disorders; (iii) not currently pregnant, lactating or postmenopausal; and (iv) of SA ancestry. This study was approved by the Human Research Ethics Committee of the Faculty of Health Science of the University of Cape Town. Procedures and risks were explained to participants, all of whom gave written informed consents, prior to participation.

3.2.2 Testing procedures:

The testing procedures including body composition, IR and fat biopsies have been previously described (49, 50, 59 and 146), but are described in brief below.

3.2.2.1 Demographic and lifestyle questionnaire

As described in chapter 2, a demographic questionnaire was administered that included measures of socio-economic status. Contraceptive use was also recorded and women were categorized as using hormonal contraception or not.

Physical activity energy expenditure was characterised using the GPAQ (237 and 238). Minutes of physical activity per week were recorded.

3.2.2.2 Body composition

Anthropometric measurements of participants, as described earlier in chapter 2 were taken including height, weight, WC and HC. Body composition (fat and fat-free mass) was measured using DXA (Discovery-W, Software version 4.40; Hologic). FMI was calculated. DXA-derived measures of body fat distribution included 1) trunk FM 2) leg FM 3) android FM and 4) gynoid FM. VAT, DSAT and SSAT areas were measured using CT scan (Toshiba X-press Helical Scanner; Toshiba Tokyo, Japan) at the level of the L4-L5 vertebrae.

3.2.2.3 IR

After an overnight fast, subjects underwent an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) to quantify IR. Cannulae were inserted into the antecibutal vein of each arm. One arm was used for glucose and insulin infusions and the other arm was used for blood sampling. Baseline samples were drawn at -15, -5 and -1 minute before the infusion of glucose (50% dextrose, 11.4 g/m^2 body surface area) over 60 seconds at time 0. At 20 minutes human insulin (0.02 U/kg; Actrapid, Novo Nordisk, Sandton, South Africa) was infused over 5 minutes at a constant rate. Plasma glucose and serum insulin concentrations were measured in 3 baseline samples and 32 samples drawn over 240 minutes after the commencement of the glucose infusion. Glucose and insulin drawn from the FSIGT were used to calculate the insulin sensitivity index (S_I) using the minimal model of glucose kinetics of Bergman et al *(1979)* (257).

HOMAIR was also calculated from fasting glucose and insulin levels (242).

3.2.2.4 Fat biopsies:

After 4h of fasting, fat biopsies were obtained from the abdominal DSAT, SSAT and gluteal areas using a mini liposuction technique. A small insertion was made directly above the umbilicus after local anaesthesia with Lignocaine hydrochloride (2%, Intramed, Port Elizabeth, South Africa). 200-300ml of normal saline with adrenaline (0.1%, Intramed) and Lignocaine (0.75%) was infused using an infiltration cannula (Lamis 14 ga × 15cm, Bryon Medical Inc., Tucson, AZ, USA). Under ultrasound guidance fat was aspirated from above (SSAT) and below (DSAT) the fascia superficialis using an aspiration cannula (Coleman. 12 ga × 15cm, Byron Medical Inc.) attached to a 10ml syringe. Gluteal samples were obtained from the upper right quadrant using the same procedure. Approximately 2ml of fat was obtained from each site and washed three times with saline until no blood was visible. Samples were placed into vials, frozen in liquid nitrogen and then stored at -80ºC (146).

3.2.2.5 Isolation of RNA:

Total RNA was isolated using the QIAGEN RNeasy system (QIAGEN Ltd., Crawley, UK) The yield and purity of the RNA was determined using a spectrophotometer (ND-1000 spectrophotometer; NanoDrop Technologies, Wilmingtom, DE, USA), and RNA integrity was analysed by 1% agarose gel electrophoresis in the presence of SYBRE gold nucleic acid stain (Invitrogen Molecular Probes^{TM}, Oregon, USA). RNA samples were stored at -80°C for subsequent analysis.

3.2.2.6 Real-time quantitative PCR (RT-PCR):

RT-PCR was performed in triplicate. Reverse transcription of 2µg RNA was performed using Taqman RT reagents (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems, Foster City, CA, USA) and RNase inhibitor (Applied Biosystems, Foster City, CA, USA)) at a final concentration of 1U/ml. mRNA levels of ERα, ERβ, and CYP19A were quantified (relative quantification standard curve method) using Taqman universal PCR master mix 2X and Taqman gene expression assays (Applied Biosystems, Foster City, CA, USA): ERα (Hs00174860_m1), ERβ (Hs00230957_m1/Hs01100353_m1), Aromatase (*CYP19A1)* (Hs00903413_m1/

Hs00240671_m1), *18S* (Hs99999901_s1), *RPLPO* (Hs99999902_m1), and *Cyclophilin (PPIA)* (Hs04194521_s1). Each sample was analysed in triplicate on a StepOnePlus real-time PCR detection system (Applied Biosystems, Foster City, CA, USA). Expression levels were calculated using a standard curve. Reference genes were evaluated by running *18S, PPIA and RPLPO* on the full study cohort and relative levels of expression genes were normalized using RPLPO as determined by NormFinder algorithm as the best housekeeping gene (258).

3.2.3 Biochemical analysis:

Plasma glucose and serum insulin concentrations were determined as described in chapter 2 (24 and 146). Circulating levels of hormones E2 (CV: 1.45%), progesterone (CV: 4.34%) and free testosterone (CV: 4.04%) were measured using competitive immunoassay (Centaur, direct chemiluminescent technology, Siemans South African Health Care Diagnostics, Johannesburg, South Africa), FSH (CV: 2.46%) and LH (CV: 2.08%) were measured using two site sandwich immunoassay (Centaur, direct chemiluminescent technology, Siemans South African Health Care Diagnostics, Johannesburg, South Africa) and SHBG (CV: 2.36%) was measured using sandwich immunoassay (Centaur, direct chemiluminescent technology, Siemans South African Health Care Diagnostics, Johannesburg, South Africa).

3.2.4 Statistical analysis:

Results were analysed using Statistica version 10 (Stat-soft Inc., Tulsa, OK). Results were presented as mean ± standard error (SE). The differences in body composition, circulating hormonal levels and mRNA levels within each depot between the ethnicity and BMI groups were analysed using two-way ANOVA with Fischer LSD post hoc analysis. Partial correlations adjusting for age, FMI and contraception were used to test the associations between circulating hormones, gene expression, body fat distribution and measures of IR in the black and white women separately. Differences in these associations between black and white women were tested using multiple regression including an interaction term (gene/hormone x ethnicity) in the model.

3.3 Results

Table 7: Subject characteristics of normal-weight and obese black and white women

	Normal-Weight				Obese				P-Value		
	N	Black	N	White	N	Black	N	White	Ethnicity	BMI	Ethnicity X BMI
Age (years)	13	23 ± 0.81$^{\#}$	15	26 ± 0.89$^{\#}$	15	28 ± 1.94$^{\#*}$	12	33 ± 1.99$^{\#*}$	0.008	<0.001	0.31
Body composition											
Height (m)	13	1.6 ± 0.02*	15	1.7 ± 0.02*	15	1.6 ± 0.01*	12	1.7 ± 0.01*	<0.001	0.60	0.50
Weight (kg)	13	56.5 ± 1.7$^{\#*}$	15	64.9 ± 1.1$^{\#*}$	15	95.7 ± 2.5$^{\#}$	12	103.7 ± 5.1$^{\#}$	0.002	<0.001	0.28
BMI (kg/m^2)	13	22.7 ± 0.37$^{\#}$	15	22.7 ± 0.39$^{\#}$	15	38.6 ± 0.74$^{\#}$	12	37.4 ± 1.5$^{\#}$	0.36	<0.001	0.41
Lean (kg)	13	36.9 ± 1.4$^{\#*}$	15	42.3 ±0.94$^{\#*}$	15	47.9 ± 1.4$^{\#*}$	12	53.04 ±2.3$^{\#*}$	0.004	<0.001	0.42
Fat (kg)	13	16.9 ±1.1$^{\#}$	15	19.2 ± 1.2$^{\#}$	15	44.6 ± 1.4$^{\#}$	12	47.3 ± 2.9$^{\#}$	0.077	<0.001	0.68
Fat (%)	13	30.3 ± 1.7$^{\#}$	15	30.03 ± 1.7$^{\#}$	15	47± 0.78$^{\#}$	12	45.9 ± 0.94$^{\#}$	0.89	<0.001	0.87
FMI (kg/m^2)	13	6.8 ± 0.45$^{\#}$	15	6.7 ± 0.42$^{\#}$	15	18.1 ± 0.54$^{\#}$	12	17.1 ± 0.98$^{\#}$	0.74	<0.001	0.87
Body fat distribution											
Waist (cm)	13	75.7 ± 1.1$^{\#}$	15	80.1 ± 1.4$^{\#}$	15	115.3 ± 2.6$^{\#}$	12	110.0 ± 4.3$^{\#}$	0.99	<0.001	0.03
Hip (cm)	13	97.9 ± 1.4$^{\#}$	15	102.3 ± 1.1$^{\#}$	15	126.6 ± 1.6$^{\#}$	12	126.2 ± 4.3$^{\#}$	0.17	<0.001	0.21
WHR	13	0.77 ± 0.01$^{\#}$	15	0.78 ± 0.01$^{\#}$	15	0.91 ± 0.02$^{\#}$	12	0.87 ± 0.01$^{\#}$	0.17	<0.001	0.08
Trunk FM (kg)	13	6.4 ± 0.53$^{\#*}$	15	8.0 ± 0.59$^{\#*}$	15	22.1 ± 0.76$^{\#}$	12	22.6 ± 1.9$^{\#}$	0.12	<0.001	0.17
Trunk FM (%FM)	13	37.9 ± 1.6$^{\#*}$	15	41.6 ± 1.6$^{\#*}$	15	49.5 ± 0.94$^{\#}$	12	47.2 ± 1.2$^{\#}$	0.89	<0.001	0.02
Leg FM (kg)	13	7.9 ± 0.59$^{\#}$	15	8.5 ± 0.53$^{\#}$	15	16.4 ± 0.77$^{\#}$	12	18.3 ± 0.86$^{\#}$	0.029	<0.001	0.47
Leg FM (%FM)	13	46.9 ± 1.8$^{\#}$	15	44.4 ± 1.3$^{\#}$	15	36.7 ± 1.07$^{\#}$	12	39.1 ± 1.2$^{\#}$	0.38	<0.001	0.04
Trunk FM/Leg FM	13	0.84 ± 0.07$^{\#}$	15	0.96 ± 0.06$^{\#}$	15	1.3 ± 0.06$^{\#}$	12	1.2 ± 0.07$^{\#}$	0.71	<0.001	0.03
Gynoid (kg)	13	3.6 ± 0.23$^{\#*}$	15	4.2 ± 0.22$^{\#*}$	15	7.6 ± 0.31$^{\#}$	12	8.5 ± 0.43$^{\#}$	0.003	<0.001	0.95
Gynoid(%FM)	13	21.5 ± 0.49$^{\#}$	15	22.2 ± 0.69$^{\#}$	15	17.2 ± 0.54$^{\#}$	12	18.2 ± 0.59$^{\#}$	0.11	<0.001	0.51
VAT (cm^2)	12	60 ± 4.8$^{\#}$	15	61 ± 6.2$^{\#}$	13	101 ± 11#*	10	163 ± 18.5$^{\#*}$	0.49	<0.001	0.05
SAT (cm^2)	12	168 ± 15.2$^{\#}$	15	182 ± 15.7$^{\#}$	13	591 ± 25.6$^{\#}$	10	520 ± 30$^{\#}$	0.77	<0.001	0.20
SSAT (cm^2)	12	99 ± 9.3$^{\#}$	15	97 ± 6.6$^{\#}$	13	321 ± 21.9$^{\#}$	10	255 ± 41.2$^{\#}$	0.28	<0.001	0.25
DSAT (cm^2)	12	68 ± 7.40$^{\#}$	15	85 ± 10.1$^{\#}$	13	269 ± 16.2$^{\#}$	10	264 ± 24.7$^{\#}$	0.59	<0.001	0.24
VAT/SAT	12	0.37 ± 0.02$^{\#}$	15	0.35 ± 0.03	13	0.2± 0.01$^{\#*}$	10	0.32 ± 0.03*	0.31	<0.001	0.001

Values are expressed as mean ± SE. P-values adjusted for age except for age. #, BMI effect; and *, ethnic effect. BMI, body mass index; FMI, fat mass index; WHR, waist-hip ratio; FM, fat mass; SSAT, superficial subcutaneous adipose tissue; DSAT, deep subcutaneous adipose tissue; VAT, visceral adipose tissue.

Table 8: IR and circulating sex hormones in black and white normal-weight and obese women.

	Normal-Weight				Obese				P-Value		
	N	**Black**	**N**	**White**	**N**	**Black**	**N**	**White**	**Ethnicity**	**BMI**	**Ethnicity X BMI**
Insulin sensitivity											
Glucose (mmol/L)	13	4.5 ± 0.11	15	4.4 ± 0.05$^{\#}$	14	4.6 ± 0.05	12	4.7 ± 0.08$^{\#}$	0.962	0.02	0.24
Insulin (mU/L)	13	7.9 ± 1.3$^{\#}$	15	5.01 ± 0.61	15	15.6 ± 1.8$^{\#*}$	12	8.1 ± 1.1*	0.003	0.001	0.40
HOMAIR	13	1.5 ± 0.28$^{\#}$	15	0.97 ± 0.12$^{\#}$	13	2.9 ± 0.39$^{\#*}$	12	1.7 ± 0.25#*	0.041	<0.001	0.66
S_I (x10^4 /min/(uU/ml))	13	2.5 ± 0.49*	14	5.7 ± 0.52$^{\#*}$	14	1.6 ± 0.38*	11	3.5 ± 0.63$^{\#*}$	<0.001	0.006	0.45
Circulating sex hormones											
E2 (pg/mL)	11	234.3 ± 36.9	14	248.9 ± 26.4$^{\#}$	13	272.3 ± 36.4	11	153.3 ± 38.9$^{\#}$	0.06	0.11	0.01
TST (nmol/L)	9	0.67 ± 0.13*	15	1.30 ± 0.16$^{\#*}$	13	0.84 ± 0.15*	11	2.46 ± 0.28$^{\#*}$	<0.001	0.04	0.16
SHBG(nmol/L)	11	63.7 ± 9.1$^{\#}$	13	77.9 ± 11.8	14	40.7 ± 6.4$^{\#}$	11	57.1 ± 19.4	0.06	0.04	0.24
PRGE (ng/mL)	11	8.3 ± 4.7	14	7.8 ± 2.9	13	2.7 ± 1.2	9	2.2 ± 0.73	0.33	0.57	0.89
LH (IU/L)	11	4.5 ± 0.75	13	6.5 ± 2.1	12	7.2 ± 2.92	11	7.4 ± 1.9	0.94	0.93	0.74
FSH (IU/L)	11	4.9 ± 0.54	14	4.9 ± 1.01	12	5.03 ± 1.2	11	6.7 ± 1.08	0.49	0.86	0.25

Values are expressed as mean ± SE. Values adjusted for age. #, BMI effect; and *, ethnic effect. S_I, sensitivity index; HOMAIR, homeostasis model for insulin resistance; E2, oestradiol; TST, testosterone; SHBG, sex hormone binding globulin; PRGE, progesterone, LH, luteinising hormone; FSH, follicle stimulating hormone

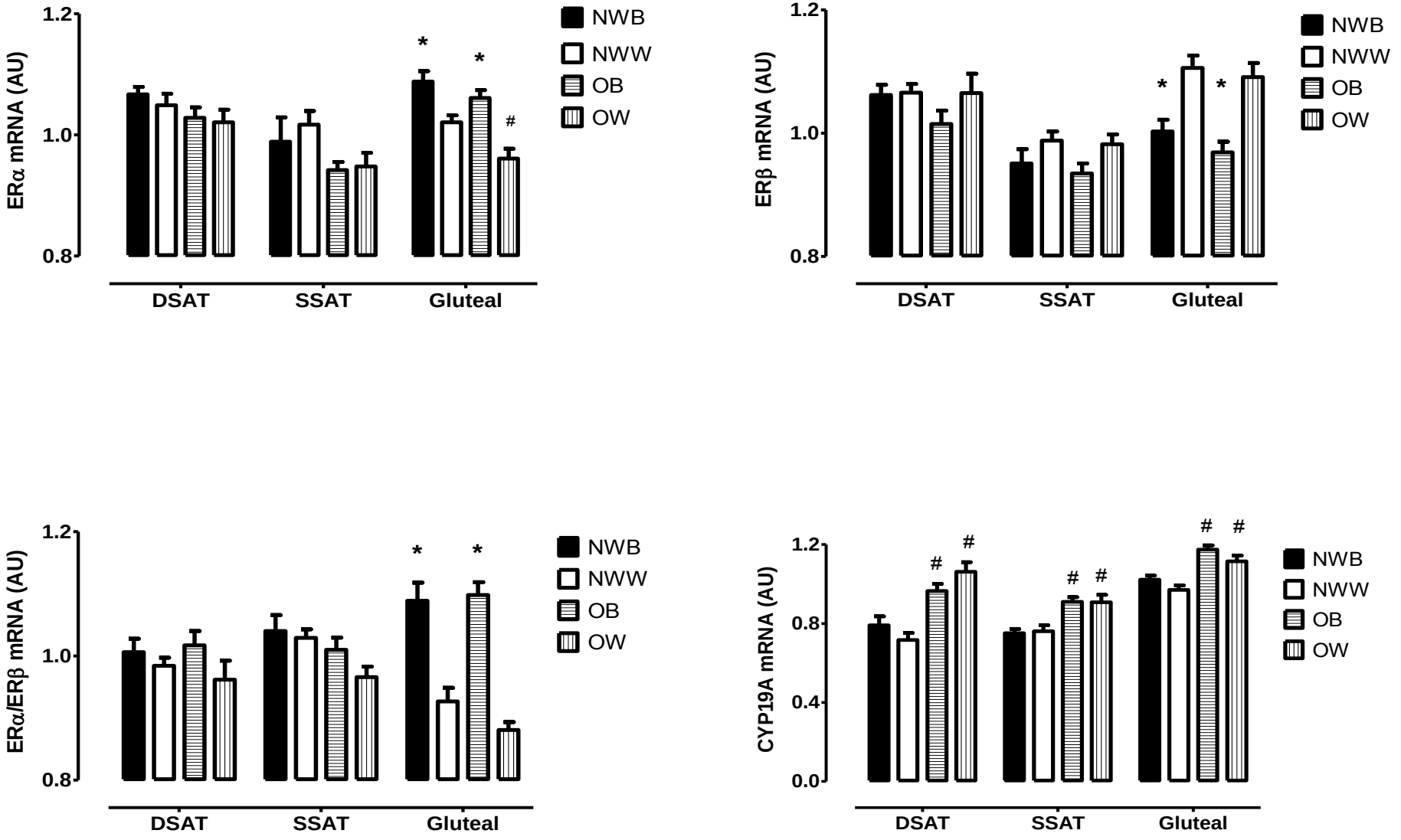


Figure 3: Expression of genes in abdominal DSAT, SSAT and gluteal depots in normal-weight and obese black and white women. Bars represent mean ± SE. *, P<0.05, Black vs. white; #, P<0.05, normal-weight vs. obese. ERα, oestrogen receptor alpha; ERβ, oestrogen receptor beta; CYP19A, aromatase, NWB; normal-weight black, NWW; normal weight white, OB; obese black and OW; obese white.

3.3.1 Subject characteristics

Subject characteristics as described previously (50 and 146) are shown in Table 7. Obese women were older than normal weight women, and obese white women were older than obese black women. There was no significant difference in hormonal contraception use between black and white women (35% vs. 26%, respectively p=0.09) however, more white women used oral contraceptives (22% vs. 0%), whereas more black women used injectable contraceptives (36% vs. 4%, $p<0.01$).

After adjusting for age, black and white women were well matched for BMI, body fat, FMI and WC and HC. When expressed relative to total body FM, normal-weight black women had less trunk FM (%) and absolute trunk FM (kg) compared to their white counterparts. With obesity, trunk FM and trunk FM/leg FM increased and leg FM decreased to a greater extent in the black compared to the white women. VAT did not differ in normal-weight women, but was significantly greater in obese white compared to obese black women. All measures of body fat were greater in obese compared to normal weight women in both groups.

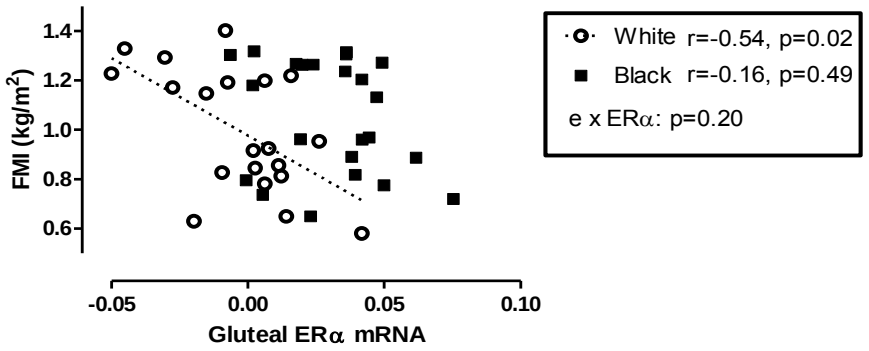
Fasting plasma glucose concentrations did not differ by ethnicity, but were significantly higher in obese white women compared to normal-weight white women. Fasting serum insulin concentrations and HOMAIR were higher in the obese black compared to obese white women. S_I was significantly lower in black women compared to white women in both BMI groups. S_I was lower, and fasting serum insulin concentrations and HOMAIR were higher in obese compared to normal-weight women in both ethnic groups.

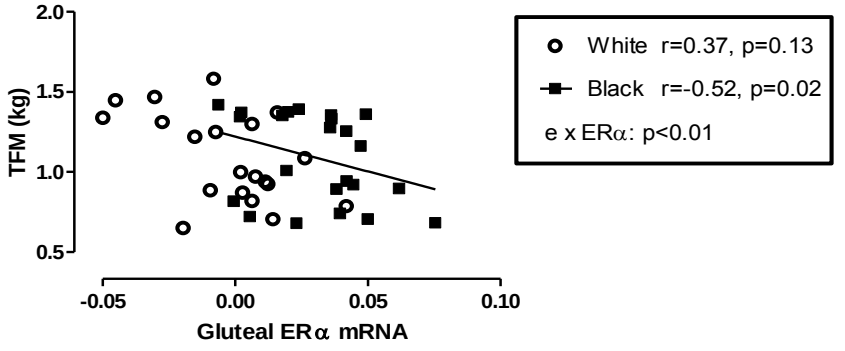
Differences in circulating sex hormones between the normal-weight and obese black and white women are presented in Table 8. E2 concentrations did not differ between normal-weight black and white women, but were lower in the obese white, but not the black women. Testosterone concentrations were significantly lower in black compared to the white women in both BMI groups. Testosterone concentrations did not differ by BMI in black women, but normal-weight white women had significantly lower testosterone concentrations compared to obese white women. SHBG concentrations did not differ by ethnicity, but normal-weight black women had significantly greater SHBG concentrations compared to obese black women. When adjusting for

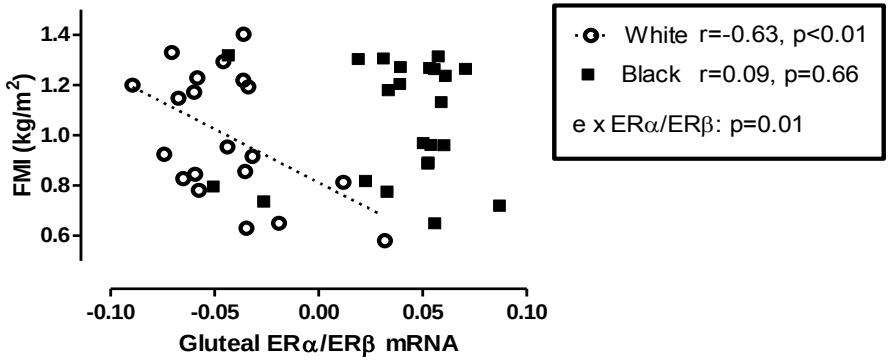
contraception use, the BMI and ethnic differences in hormone concentrations described above still remained significant (data not shown).

3.3.2 SAT gene expression

The expression of ER‟s and aromatase in DSAT, SSAT and gluteal SAT are shown in figure 3. In the gluteal depot only, ERα was significantly higher and ERβ was significantly lower in the black compared to white women in both BMI groups. Accordingly, gluteal ERα/ERβ was significantly higher in the black compared to the white women in both BMI groups. Further gluteal ERα was significantly lower in obese white compared to normal-weight white women. There were no ethnic or BMI differences in the expression of ER‟s in the SSAT or DSAT depots. CYP19A expression was higher in the obese compared to the normal-weight black and white women in all three depots. There were no ethnic differences in aromatase expression in any of the depots.


FMI (kg/m2)
1.4
1.2
1.0
0.8
0.6
-0.05
0.00
0.05
0.10
Gluteal ERα mRNA
White r=-0.54, p=0.02
Black r=-0.16, p=0.49
e x ERα: p=0.20


TFM (kg)
2.0
1.5
1.0
0.5
-0.05
0.00
0.05
0.10
Gluteal ERα mRNA
White r=0.37, p=0.13
Black r=-0.52, p=0.02
e x ERα: p<0.01


FMI (kg/m2)
1.4
1.2
1.0
0.8
0.6
-0.10
-0.05
0.00
0.05
0.10
Gluteal ERα/ERβ mRNA
White r=-0.63, p<0.01
Black r=0.09, p=0.66
e x ERα/ERβ: p=0.01

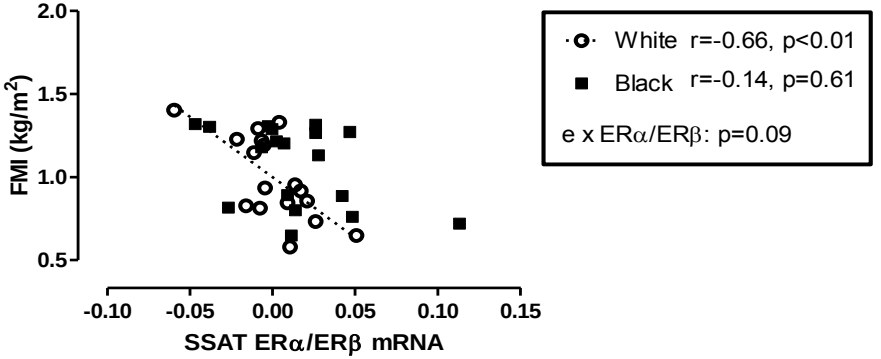

FMI (kg/m2)
2.0
1.5
1.0
0.5
-0.10
-0.05
0.00
0.05
0.10
0.15
SSAT ERα/ERβ mRNA
White r=-0.66, p<0.01
Black r=-0.14, p=0.61
e x ERα/ERβ: p=0.09

Figure 4A

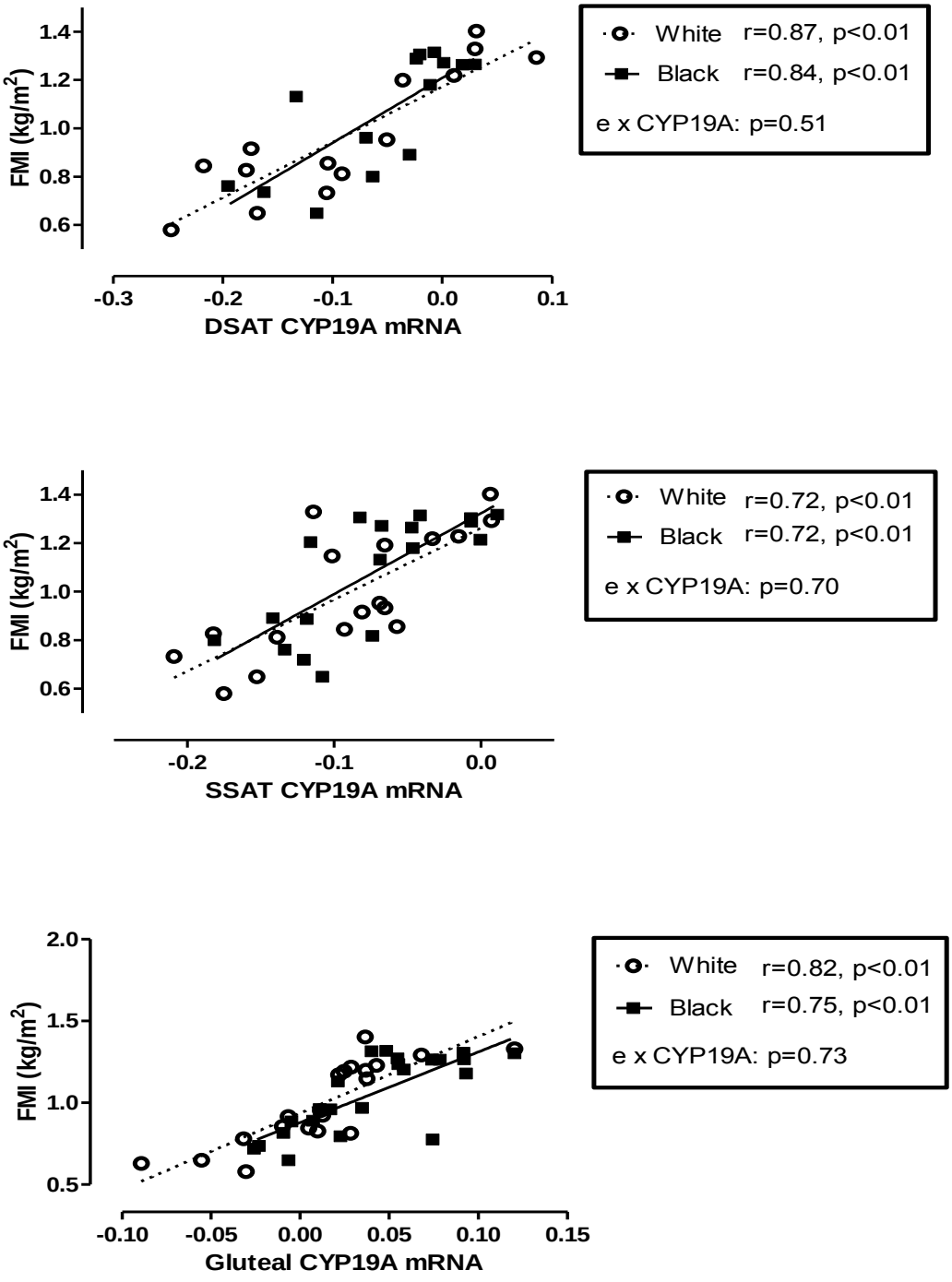


Figure 4B

Figure 4: Associations between ERα, ERβ, ERα/ERβ (figure 4A) and CYP19A (figure 4B) gene expression with body fat and its distribution in gluteal, SSAT and DSAT depots in black and white women. FMI, fat mass index; TFM, trunk fat mass; e x gene, ethnicity x gene interaction.

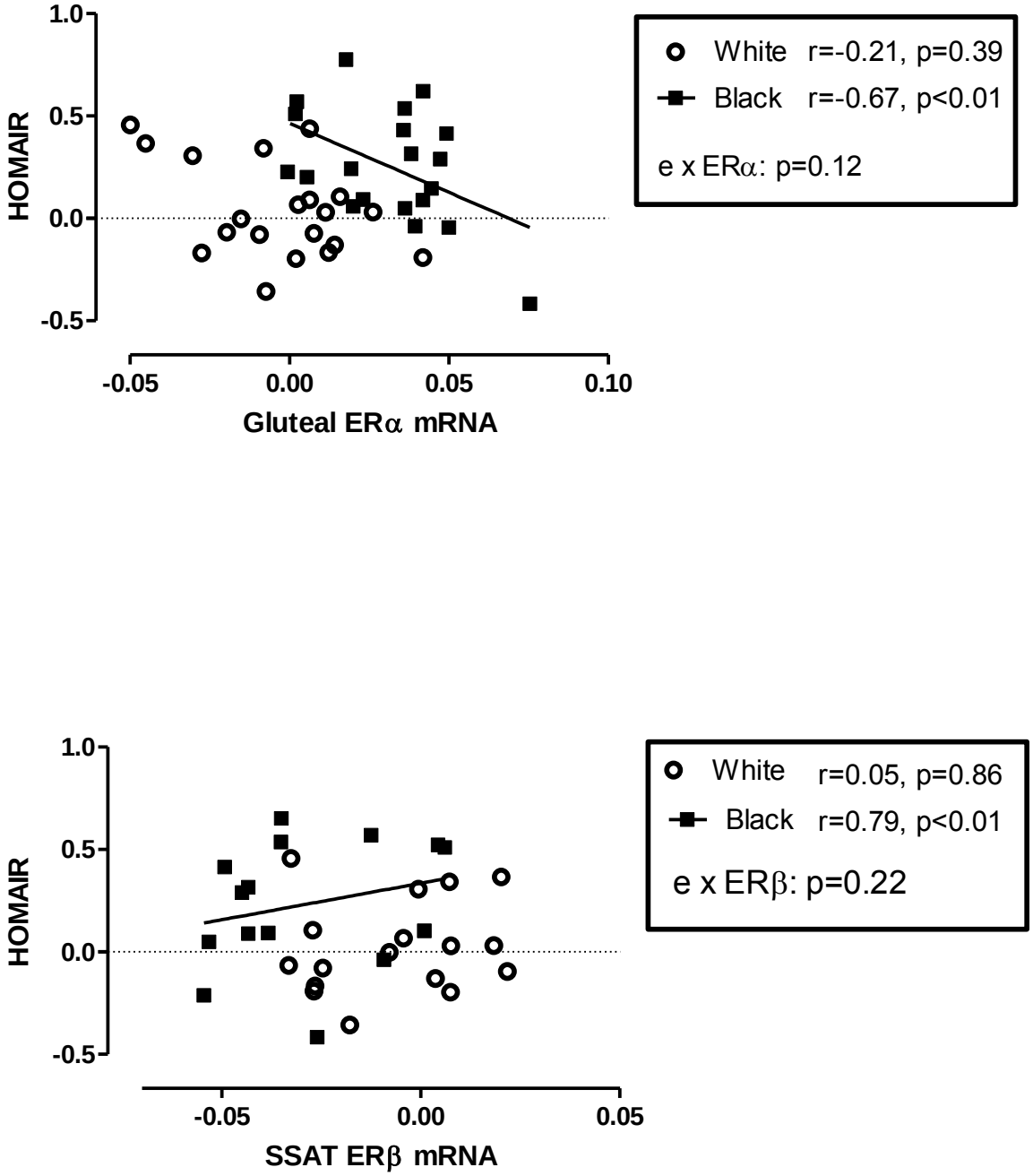


Figure 5: Associations between ERα and ERβ gene expression with metabolic risk in gluteal and SSAT depots. HOMAIR, homeostasis model of insulin resistance; e x gene, ethnicity x gene interaction.

3.3.3 Associations between gene expression and body fat and its distribution and IR.

When examining the associations between gene expression and total body fatness, lower gluteal ERα expression was associated with higher FMI in the white women only (Figure 4A). Further, there was a significant ERα/ERβ (in both the gluteal and SSAT depots) x ethnicity interaction for FMI, such that reduced ERα/ERβ expression was associated with higher FMI in the white, but not black women (Figure 4A). Higher CYP19A was associated with higher FMI in both black and white women in all 3 depots (Figure 4B). After adjusting for age and FMI, there was significant gluteal ERα x ethnicity interaction for trunk FM, such that lower ERα was associated with higher trunk FM in the black, but not white women (Figure 4A). Adipose tissue gene expression was not associated with leg FM and gynoid FM in black or white women.

After adjusting for age and FMI, increased gluteal ERα expression was associated with lower fasting insulin ($r=-0.53$, $p=0.01$) and HOMAIR in the black women only (Figure 5). Conversely, higher ERβ expression in the SSAT depot was associated with higher HOMAIR in the black women (Figure 5). The associations between gene expression and body composition and IR were not altered when further adjusting for contraception use.

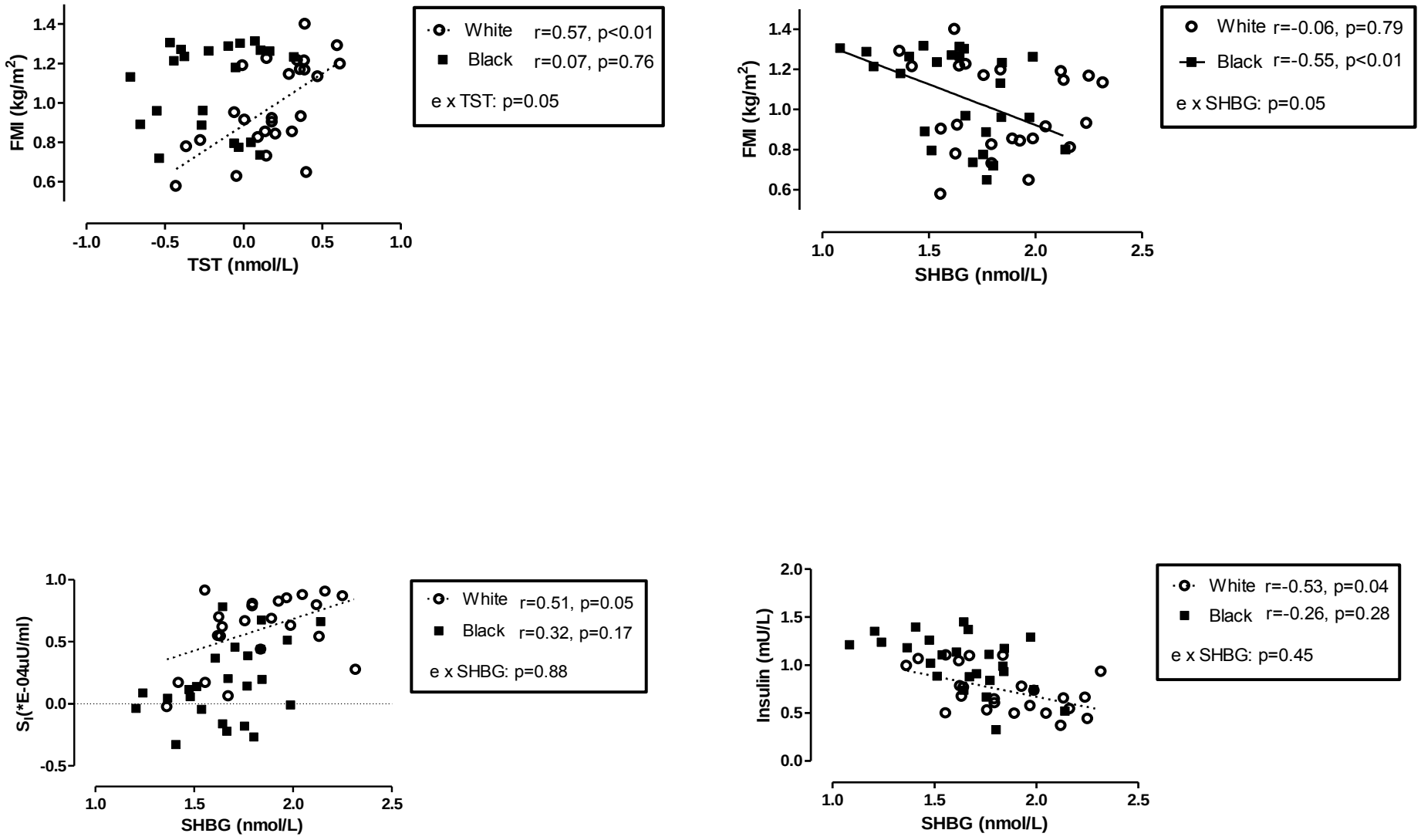


Figure 6: Associations between circulating sex hormones with body fat and IR. FMI, fat mass index, TST, testosterone; SHBG, sex hormone binding globulin; S_I, sensitivity index; e x circulating hormone, ethnicity x circulating hormone interaction.

3.3.4 Associations between circulating hormonal levels and body fat and its distribution and IR

When adjusting for age and FMI, there were significant interactions between ethnicity x hormone concentrations for FMI. Higher testosterone concentrations were associated with higher FMI in the white, but not black women. In contrast, reduced SHBG concentrations were associated with higher FMI in black, but not white women. After adjusting for age and FMI, higher SHBG was associated with lower fasting insulin concentrations in the white women only, and when also adjusting for contraception use, higher SHBG concentrations were significantly associated with higher S_I in the white women ($r=0.59$, $p=0.03$). Adjustment for contraceptive use did not alter any other finding reported above. There were no associations with FSH, LH or E2 with body fat distribution and IR.

3.4 Discussion

The main findings of this study were that gluteal expression of ERα was significantly greater and ERβ was significantly lower in the black compared to the white women. Accordingly, gluteal ERα/ERβ expression was higher in the black compared to the white women. Notably, in black women only, increased gluteal ERα expression was associated with lower central FM and lower fasting serum insulin concentrations and HOMAIR. While adipose tissue ER gene expression did not associate with body fat distribution or IR in white women, free testosterone levels were higher in white compared to black women, and correlated with adiposity in white women only. In contrast, circulating SHBG concentrations were negatively associated with adiposity in the black women, and negatively associated with fasting insulin concentrations and IR in white women after additionally adjusting for contraception use.

Compared to white women, black women had increased ERα and ERα/ERβ in the gluteal depot, but not the abdominal SAT depot. To our knowledge this is the first study to show differences in gluteal mRNA expression of ER‟s in black and white women. In white women only, lower ERα and ERα/ERβ expression in both the SSAT and gluteal depots were associated with increased adiposity. These findings are supported by studies in ERKO mice which show an obese phenotype (201-204), as well as a study conducted in European women, which demonstrated that SAT expression of ERα was lower in obese compared to normal-weight women (200).

In black women, gluteal ERα expression was associated with lower central FM. These findings are commensurate with studies that have suggested that increased ER‟s are associated with increased peripheral FM (229). Further, murine studies have demonstrated that a decrease in ERα expression results in an increase in VAT (201-204). This may therefore suggest that oestrogen receptor gene expression may be associated with the lower abdominal obesity in the black women. The reasons for these ethnic-specific associations are not known. Further studies, including longitudinal studies are required to examine this to determine possible reasons.

Increased ERα and decreased ERβ expression was associated with improved IR in the black, but not white women. Mice studies have demonstrated that an increase in ERα is associated with a decrease in IR and decrease in body fat (201-204). In BERKO mice, improvement in IR was demonstrated despite an increase in body fat (207). Studies have also shown higher SAT

expression of ERβ in postmenopausal women compared to premenopausal women (255). Despite higher ERα and lower ERβ in black compared to white women, and the association with lower IR, black women in this study were more IR than their white counterparts, as determined by fasting insulin concentrations and the FSIGT-derived measure. However, higher ERα and lower ERβ expression in the black women only associated with lower fasting measures of IR (HOMAIR and fasting insulin concentrations), which more likely reflect hepatic IR. We have recently shown that compared to white women, black women have lower hepatic, but similar peripheral IR (Goedecke et al, in review). Further, other studies have shown that the prevalence of hepatic steatosis (liver fat) is lower in the black compared to the white women (107). A study conducted in ERKO mice demonstrated by the euglycaemic hyperinsulinaemic clamp, that the normal insulin mediated suppression of glucose was blunted which revealed a pronounced hepatic IR (202). This may therefore suggest that ER‟s may be associated with lower hepatic IR in the black women.

Mechanisms that may possibly link ER expression, obesity and IR may include the expression of other genes. For example, studies have shown that ERβ inhibits PPARϒ activity (207) which regulates glucose and lipid metabolism by modulating energy homeostasis in adipose tissue and skeletal muscle (208-210). Consistently low PPARϒ activity leads to IR, diabetes and end organ damage (208). However, Goedecke et al (2011) previously demonstrated that PPARϒ was down-regulated to a greater extent with obesity in black women compared to white women, and associated negatively with IR in the black and not the white women (50). In contrast, Bower et al (2006) demonstrated no difference in mRNA expression of PPARϒ in obese black compared to obese white women (259). Due to contradictory findings, further studies examining the association between ER‟s and PPARϒ should be conducted. Further, diarylpropiolnitrile (DPN), an agonist for ERβ, was used to demonstrate that oestrogens acting via ERβ may also increase adiposity due to upregulation of 11βHSD1, which is increased in obese humans and mice models of obesity, through an ERβ agonist (255). Another possible mechanism involves the increase in the expression of genes involved in lipid biosynthesis, such as SCD1, reported in ERKO mice (202). These genes have been shown to be associated with obesity and IR (213 and 214). Studies have demonstrated that SCD1 is reduced in African American compared to Caucasian women. This may therefore be another possible explanation for the increase in ERα in the black women (152).

When comparing circulating steroid levels, lower E2 levels were observed in obese compared to normal-weight white but not black women. Although incongruous with adipose tissue ERα expression, the findings in white women are supported by studies that show that ovariectomy results in an increase in BMI, VAT and central FM (168-173). A possible mechanism through which circulating E2 causes reduced obesity is that oestrogen may exert effects on several adipokines that are produced by adipocytes. Oestrogen levels in premenopausal women have been shown to be closely associated with leptin levels (175 and 176). Additionally in mice, oestrogen has been shown to reduce leptin resistance (177), by controlling the expression of leptin specific receptors (176, 178 and 179). Studies have demonstrated that although circulating leptin levels were higher in obese black compared to obese white women, leptin concentrations were more strongly correlated with obesity indices in the white women (260) suggesting a possible reason for the association of E2 concentrations with obesity in the white and not black women.

We also found that testosterone concentrations were lower in black compared to white women, irrespective of BMI group, and were associated with greater adiposity in the white women only. Studies conducted in premenopausal women demonstrated that testosterone levels were positively correlated with body FM (kg and %) in women aged 20-25 years (192). It has been suggested that the levels of free testosterone may be elevated because of low androgen binding to SHBG, which associates with obesity (184). We found that although SHBG concentrations were not significantly different in black and white women, which is consistent with other studies (194), SHBG concentrations were negatively associated with adiposity in the black women only. This is in accordance with studies conducted in premenopausal African American women who demonstrated significant negative correlations between obesity and SHBG (187). However, this is in contrast to a study conducted in a larger sample size which demonstrated that although SHBG levels were similar in black and white women, total adiposity was associated with SHBG in the white women only (194). This is interesting as testosterone levels were lower in the black compared to the white women and it would be expected that SHBG would therefore be higher in the black women, suggesting that other factors such as contraception use may affect the levels of SHBG in the black women. Studies have demonstrated that oral contraception use increases SHBG levels (261). However, our results remained the same after taking into account contraception use of the black and white women. Other factors such as growth hormone have

also been shown to affect SHBG levels, and should therefore be measured in future studies (262). Additionally, we found that SHBG, independent of age, FMI and contraception use was negatively associated with IR in white women. Studies conducted in Caucasian women demonstrated that low SHBG levels were associated with IR (188 and 189). The reason for SHBG association with IR in the white and not black women is not known, but, could possibly be due to racial differences in sex steroid metabolism and the regulation of their cellular and physiological actions. Studies providing evidence on the specific regulation of their cellular and physiological actions should be undertaken. Therefore, the implications of this study are that higher androgenicity, due to increased testosterone may be a phenotype that is associated with obesity in white women. In contrast, SHBG may be associated with obesity in the black women and IR in the white women.

Oestrogen production is dependent on CYP19A activity in adipose tissue, which converts androstenedione to oestrone followed by the conversion to oestrogen (167). CYP19A increased with obesity and was positively associated with increased adiposity in both black and white women in all depots. This finding is supported by other studies conducted in men and premenopausal women which demonstrated that generalized obesity characterised by BMI was associated with an increase in CYP19A expression (229). It has been suggested that the mechanism by which CYP19A increases fat deposition is by consuming more androgen ligands and generating more oestrogen ligands which favours peripheral fat deposition and may cause raised plasma oestrogen levels (229). However, in our study we did not show raised plasma oestrogen levels with obesity, but rather reduced oestrogen levels with obesity in the white women. Additionally there was no association with body fat distribution as it would be expected that lower plasma oestrogen levels would be associated with reduced peripheral FM. Differences in findings could be due to differences in sample size as our study had a higher sample size compared to the study mentioned above. These findings are therefore contradictory and further studies are required to verify these results.

The strengths of this study include the precise measures of body fat distribution (i.e. DXA and CT) on a cohort of normal-weight and obese women who differed in body composition and IR. The cross-sectional design of this study limits one to derive conclusions in terms of causality, and the study is limited by a small sample size. However, no studies of which we are of aware

have compared the associations of sex hormones and body fat distribution and IR between black and white SA women. Larger samples are required to verify the relationships and determine whether these associations are ethnic-specific. We did not obtain fat biopsies or measure IR during the same menstrual cycle phase for each subject and this may affect the oestrogen receptor levels and circulating hormonal levels and the associations with body composition and IR. Further, different methods of contraception in the black and white women may have affected the results. Contraception use is known to alter body composition, IR and sex hormones, however, we found that when adjusting for contraception use the relationships did not change. This study only measured mRNA levels and measurements of protein levels are needed to verify the findings of this study.

In conclusion, these results show ethnic differences in body fat distribution were associated with regional differences in expression of ER"s. Although differences in oestrogen expression were associated with IR in the black women, further studies are required to explore other factors affecting peripheral IR in black women. In addition, CYP19A was associated with overall obesity in both the black and white women. This study also showed differences in circulating sex hormones, which associated with total body fat but not distribution. These results support the fundamental differences in the effects of sex hormones on metabolism and body composition in black and white women. Understanding the mechanisms by which sex hormones affect IR could have important preventative health implications. Based on these findings, future longitudinal studies, including a larger sample size in which menstrual cycle and contraception use are controlled, are required to verify these findings to provide further insight into strategies to prevent and manage cardio-metabolic risk.

Chapter 4

Summary and conclusions

Within SA, the prevalence of overweight and obesity is high, especially in women, with black SA women most affected (7). Obesity, and in particular central obesity, is closely associated with increased risk of developing T2D and CVD (24-26). In contrast, peripheral FM has been shown to be protective against cardio-metabolic risk (24, 38 and 39). Studies have demonstrated that black women have greater peripheral FM but lower VAT compared to their white counterparts (46-52, 72, 75, 81 and 82). Nonetheless, black SA women have a high prevalence of IR and T2D, whereas white SA women have a higher risk of dyslipidaemia and CVD (53-56 and 58). Previous research has demonstrated ethnic differences in the relationship between body fat distribution and metabolic risk in black and white women. To date, the SA studies that have examined the ethnic-specific associations between body composition and cardio-metabolic risk among black and white women have only used anthropometric-derived measures of body composition (BMI and WC) (76 and 82), or in small samples of women, specifically examined the associations between VAT and SAT, measured using CT (49-55). To our knowledge, no studies have examined the ethnic differences in whole body fat distribution using DXA, the gold standard measure of whole body composition, and explored the independent associations between central vs. peripheral fat distribution and cardio-metabolic risk in black and white SA women. Body fat distribution is intricately linked to sex hormones, a case in point being the centralisation of body fat following menopause (158-164). Differences in sex hormones and their receptors and the ethnic-specific associations with body fat and IR have not been explored in SA. Therefore, a need to explore differences in sex hormones, which could possibly explain differences in body fat distribution and thus its implications in terms of IR is important.

Therefore the aims of this thesis were to i) examine the ethnic-specific association between body fat distribution and cardio-metabolic risk; and ii) examine the associations between circulating sex hormones and the expression of their receptors in adipose tissue and body fat distribution and IR among black and white SA women.

In addressing the first aim, we found that in the first study of this thesis, black women were shorter and had greater FM compared to white women; accordingly, all analyses were adjusted for FMI. A major finding of the first study was that compared to white women, black women had lower central FM, characterised by lower trunk FM and greater lower-body FM, characterised by higher leg FM. In addition black women had lower VAT and greater abdominal SAT compared to white women. Despite lower central and greater lower-body fat, black women had similar levels of IR based on fasting insulin and HOMAIR, but had lower glucose levels compared to their white counterparts. Commensurate with previous findings, black women had lower lipid levels compared to white women. In white women only, elevated TC and LDL-C concentrations were associated with their greater central FM, whereas in black women only, lower central FM was associated with lower fasting glucose levels. This suggests that central FM may play a vital role in determining glucose concentrations and hence the risk for T2D in black women and serum lipids, and risk for CVD in white women.

In contrast to the findings for glucose and lipid levels, greater central and reduced lower-body FM were similarly associated with higher IR, as well as higher TG and lower HDL-C concentrations in the black and white women. Additionally, central and lower-body fat were independently associated with IR in both black and white women. These findings suggest that other factors, in addition to body fat are important determinants of IR and HDL-C concentrations in black women. Accordingly we showed that some of these factors include contraceptive use, physical activity and alcohol consumption. In the black women, injectable contraceptive use was associated with increased IR and reduced HDL-C, whereas in white women, oral contraception use was positively associated with HDL-C concentrations. Further, alcohol consumption in the black women was positively associated with HDL-C concentrations. In addition in white women only, MVPA was associated with reduced IR.

In the SA context, lifestyle factors such as contraception use and physical activity are influenced by socio-economic factors. Studies have shown that majority of women living in informal settlements used injectable contraception, which was a method determined by their health care provider (263 and 264). Additionally, although we showed that MVPA was higher in the black compared to white women, MVPA was only a significant correlate of IR in white women. However, previous research from our laboratory showed that black women mainly perform

physical activity for travel purposes, which are typically undertaken at a relatively low intensity, whereas white women perform physical activity for leisure, typically undertaken at a higher intensity. Based on these findings, intervention studies aimed at reducing centralisation of body fat, increasing higher intensity physical activity and changing contraceptive use are required to provide evidence-based guidelines for the prevention and management of cardio-metabolic risk.

Based on the findings of greater central and reduced lower-body fat in the white compared to the black women, we explored the associations between circulating sex hormones and their receptors in deep, superficial and gluteal adipose tissue and body fat and its distribution and IR. The major and novel finding of this study was that gluteal ERα was higher and ERβ was lower in the black compared to the white women, and that in black women only, increased gluteal ERα expression was associated with their lower central FM. This suggests that ER gene expression may be associated with lower abdominal fat demonstrated in the black women. Reasons for alterations in gene expression are unknown, but could possibly be due to genetic factors, which include genes such as SCD1, which has been shown to increase in ERKO mice (202). Further environmental factors such as, application of cosmetics containing p-hydroxybenzoic acid ester have recently been shown to be oestrogenic and has been found in human breast tissue, indicating absorption (265). The levels of oestrogen in the body may in turn affect ER expression. Thus the effects of environmental factors on ER expression should be explored in future studies. Additionally, in the black women, increased ERα and decreased ERβ were associated with lower IR. Despite higher ERα and lower ERβ in black compared to white women, and the association with lower IR, black women in this study were more IR than their white counterparts, as determined by fasting insulin concentrations and the FSIGT-derived measure, S_I. However, higher ERα and lower ERβ expression in the black women only associated with lower fasting measures of IR (HOMAIR and fasting insulin concentrations), which more likely reflect hepatic IR. This may therefore suggest that ER may be associated with lower hepatic IR in the black women.

In the white women, ERα was reduced in obese compared to normal-weight women and was associated with adiposity. Although incongruous with adipose ERα expression, lower circulating E2 levels were observed in obese compared to normal-weight women. E2 production is dependent on CYP19A activity in adipose tissue (167). We showed that CYP19A was associated with overall obesity in both the black and white women, suggesting that higher CYP19A may

play a pivotal role in determining overall obesity in the black and white women. We also found that, circulating free testosterone levels were higher in white compared to black women, and correlated with adiposity in white women only. There was no significant difference in SHBG levels between black and white women. However, in the white women only, SHBG was negatively associated with IR, independent of age, FMI and contraception use. In contrast, in the black women only, SHBG was negatively associated with adiposity. The complexity of differing associations between SHBG and adiposity and IR in the black and white women, in light of testosterone may therefore imply that other factors may affect their SHBG levels. This therefore implies that higher androgenicity as reflected by increased testosterone may be a phenotype that is associated with obesity in white women, whereas SHBG may be associated with IR in the white women, and obesity in the black women.

These results support the fundamental differences in the effects of sex hormones on metabolism and body composition in black and white women. Understanding the mechanisms by which sex hormones affect IR could have important preventative health implications. Based on these findings, longitudinal studies should be conducted in a larger sample size. Further, as menstrual cycle was not controlled for, future studies should consider this to verify these findings for further insight into strategies to prevent and manage cardio-metabolic risk.

The strengths of this study include the state-of-the-art measures of body fat distribution, DXA and CT, and the examination of ethnic-specific associations between these measures and cardio-metabolic risk. In addition, no studies of which we are of aware have compared the associations of sex hormones and their receptors in adipose tissue and body fat distribution and IR between black and white SA women.

Possible limitations of the study were the inclusion of a convenient sample of women, which is not representative of total population. Black women were more obese that white women but this maybe reflective of the population according to SANHANES (7). The cross-sectional design of the study limits one to derive conclusions in terms of causality. For study 1, the number of women in which CT scans were conducted was low (76% of total sample) and this may have created type II error. Further, we did not measure other lifestyle factors such as diet and this has been shown to affect body fat and metabolic risk. More objective measures of physical activity using accelerometers should be used as it would give more insight into possible differences in

non-exercise thermogenesis within these populations and should therefore be conducted in future studies. Future studies should also include subjects with a wider age range and metabolic risk factors could differ with age. We did not obtain fat biopsies or measure IR during the same phase of menstrual cycle for each subject, and this may affect the ER and circulating hormonal levels and consequently, the associations with body composition and IR. A major limitation of the study was that during subject recruitment data regarding the phase of menstrual cycle was not collected. We do understand that menstrual cycle phase plays an important role in sex hormone levels and is therefore a major limitation of the study, and should be considered in future studies. This study only measured mRNA levels and measurements of protein levels are needed to verify the findings. Further, different methods of contraception in the black and white women may have affected the results. Contraception use in this study is reflective of what is used at the population level (264). However, we found that when adjusting for contraception use the relationships did not change.

In conclusion, this study showed that central FM may be associated with their glucose levels in the black women and TC and LDL-C levels in the white women. The first novel finding of study 1 was that central and lower-body FM were independently associated with IR in both the black and white women. In addition, modifiable risk factors, including MVPA and contraception use were associated with cardio-metabolic risk and can be used as therapeutic targets to prevent and manage cardio-metabolic disease risk. The second novel finding of this study was that ethnic differences in body fat distribution were associated with regional differences in expression of ER‟s in gluteal depot of adipose tissue. Although we showed that differences in oestrogen expression were associated with IR in gluteal and SSAT depot in the black women, further studies are required to explore other factors affecting peripheral IR in black women. In addition, circulating sex hormones were associated with total body fat but not distribution. To our knowledge this is the first study to examine the roles of sex hormones in body fat distribution and metabolic risk among black and white SA women.

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Appendix

Table 9: Metabolic outcomes between contraception use in black and white women

	No contraception use		Contraception use		P-value
	N	Median (IQR)	N	Median (IQR)	
Black					
Glucose (mmol/L)	182	4.5 (4.2-4.9)	91	4.6 (4.2-5.0)	0.6
Insulin (mU/L)	189	9.0 (5.3-15.8)	92	11.7 (6.8-17.4)	0.03
HOMAIR	182	1.8 (1-3.2)	91	2.3 (1.3-3.7)	0.04
TC (mmol/L)	180	3.9 (3.4-4.6)	87	3.7 (3.2-4.3)	0.8
TG (mmol/L)	180	0.67 (0.5-1.0)	87	0.7 (0.5-0.9)	0.3
HDL-C (mmol/L)	180	1.3 (1.1-1.6)	86	1.1 (0.9-1.4)	<0.01
LDL-C (mmol/L)	180	2.2 (1.7-2.7)	86	2.3 (1.6-2.8)	0.7
White					
Glucose (mmol/L)	133	4.7 (4.5-4.9)	61	4.6 (4.3-4.9)	0.07
Insulin (mU/L)	134	6.4 (4.6-11.09)	61	8.2 (5.3-10.6)	0.8
HOMAIR	133	1.3 (0.95-2.3)	61	1.6 (1.04-2.1)	0.9
TC (mmol/L)	134	4.5 (4.1-5.2)	61	5 (4.5-5.5)	0.01
TG (mmol/L)	134	0.8 (0.6-1.2)	61	1 (0.7-1.4)	<0.01
HDL-C (mmol/L)	134	1.5 (1.3-1.8)	61	1.7 (1.5-2)	<0.01
LDL-C (mmol/L)	134	2.5 (2.1-3.2)	61	2.7 (2.2-3.4)	0.3

Values are presented as median and interquartile range (IQR). HOMAIR, homeostasis model of insulin resistance; TC, total cholesterol; TG, trigylcerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

Table 10: Associations between physical activity and alcohol consumption and metabolic outcomes

		Glucose (mmol/L)	Insulin (mU/L)	HOMAIR	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
MVPA mins/week	B	-0.003	-0.05	-0.08	-0.09	-0.08	-0.07	-0.03
	W	0.05	-0.19*	-0.18*	-0.02	-0.07	0.004	-0.09
Alcohol consumption (g)	B	0.12	-0.03	-0.02	0.06	-0.01	0.20*	-0.07
	W	0.05	-0.05	-0.05	0.05	-0.02	0.16	-0.13

Values are presented as correlation coefficients. *, p<0.05. MVPA, moderate-vigourous physical activity; HOMAIR, homeostasis model of insulin resistance; TG, trigylcerides; TC, total cholesterol; HDL-C high-density lipoprotein cholesterol; LDL-C low-density lipoprotein cholesterol.