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THE IMPACT OF OBESITY AND INFLAMMATION ON METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASE AND TYPE TWO DIABETES IN BLACK AND WHITE SOUTH AFRICAN WOMEN

By

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RELATIVE CONTRIBUTION TO THIS THESIS

My role and contribution to each chapter of this thesis is presented below. My supervisors Dr Julia Goedecke and Professor Tommy Olsson helped me define my research questions and construct the study designs.

Chapter 2.

I drafted the research hypothesis and collected all the data on the white women. I analysed and interpreted the data and drafted the main body of the chapter.

Chapter 3.

I drafted the research hypothesis and collected all the data on the white women. I analysed and interpreted all the data and drafted the whole chapter.

Chapter 4.

I drafted the research hypothesis and performed the genotyping of the \textit{IL-18} -137G/C polymorphism on the whole cohort under the guidance of Dr Malcolm Collins. I was assisted with the statistical analyses and interpretation of the data. I drafted the whole chapter.

Chapter 5.

I drafted the research hypothesis and performed the adipose tissue protein quantification and determination and cell size analysis. I performed all univariate statistical analyses, and was assisted with interpretation of the data. I drafted the main body of the chapter.
DECLARATION

I, Juliet Evans, do hereby declare that the experiments presented in this thesis were conceived and executed by myself except where otherwise indicated.

Neither the substance nor any part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in the university or any other university.

This thesis is presented in fulfilment of the requirements for the degree of PhD.

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Signed:

Date:
PUBLICATIONS

Peer reviewed publications related to this thesis


abdominal adipose tissue distribution on insulin sensitivity in black and white South African women. 2009 Aug. Obesity (Silver Spring); 17(8):1506-12.

**International conference presentations related to this thesis**


**Local conference presentations related to this thesis**

**Evans J, Micklesfield LK, Jennings C, Levitt NS, Lambert EV, Olsson T, Goedecke JH.** Association between central obesity measures and metabolic risk variables: a study of black and white South African women. SEMDSA, January 2010 (Oral presentation)

ABBREVIATIONS

AT  Adipose tissue
ARIC  Atherosclerosis Risk in Communities
AUC  Area Under the Curve
BP  Blood pressure
BMI  Body mass index
CCL2  Chemokine C-C motif ligand 2
CCR2  Chemokine C-C motif receptor 2
CSF-1  Colony stimulating factor
CT  Computer tomography
CVD  Cardiovascular disease
DSAT  Deep subcutaneous adipose tissue
DXA  Dual x-ray absorptiometry
FFA  Free fatty acids
FSIGT  Frequently sampled intravenous glucose tolerance test
HDL-C  High density lipoprotein cholesterol
HOMA-IR  Homeostasis model of insulin resistance
hsCRP  High sensitivity C-reactive protein
HSE  Health Survey for England
IDF  International Diabetes Federation
IFNγ  Interferon gamma
IRAS  Insulin Resistance Atherosclerosis Study
GENOA  Genetic Epidemiology Network of Arteriopathy
IL-  Interleukin-
IR  Insulin resistance
LDL-C  Low density lipoprotein cholesterol
MIF  Macrophage inhibitory factor
MRI  Magnetic resonance imaging
NCEP ATPIII National Cholesterol Education Programme Adult Treatment Panel 3
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHANES</td>
<td>National Health and Nutritional Examination Survey</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PA</td>
<td>Physical activity</td>
</tr>
<tr>
<td>POWIRS</td>
<td>Profiles of Obese Women with Insulin Resistance Syndrome</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operator characteristic</td>
</tr>
<tr>
<td>SADHS</td>
<td>South African Demographic Health Survey</td>
</tr>
<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>S_I</td>
<td>Insulin Sensitivity</td>
</tr>
<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
</tr>
<tr>
<td>SSAT</td>
<td>Superficial subcutaneous adipose tissue</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SVF</td>
<td>Stromal vascular fraction</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
</tr>
<tr>
<td>TC</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>THUSA</td>
<td>Transition and Health during Urbanisation of South Africa</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TNFR</td>
<td>Tumor necrosis factor receptor</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral adipose tissue</td>
</tr>
<tr>
<td>WC</td>
<td>Waist circumference</td>
</tr>
<tr>
<td>WHtR</td>
<td>Waist-height ratio</td>
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<td>WHR</td>
<td>Waist-hip ratio</td>
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GLOSSARY OF TERMS

Obesity

Obesity is viewed as excess accumulation of body fat, and was defined based on the following BMI thresholds: Normal weight=BMI<25kg/m$^2$; Overweight=BMI:25kg/m$^2$-30kg/m$^2$; Obese=BMI>30kg/m$^2$

Dyslipidemia

Dyslipidemia is a disorder of lipoprotein metabolism, and for the purpose of my thesis classified as elevated triglycerides (TG), elevated total cholesterol (TC), and/or reduced high density lipoprotein cholesterol (HDL-C).

Insulin sensitivity (SI)/Insulin resistance (IR)

Insulin sensitivity refers to the ability of endogenous insulin to promote glucose clearance and inhibit hepatic glucose output. Insulin resistance is defined as decreased insulin sensitivity.

Adipose tissue secretory products

Macrophage Markers CD68, CD14, CD163

Pro-inflammatory cytokines IL-18, IL-6, TNF-α, CSF-1, MIF

Anti-inflammatory cytokine IL-10

Pro-inflammatory chemokines CCL2 and its receptor CCR2

Pro-inflammatory adipokine Leptin

Anti-inflammatory adipokine Adiponectin
ABSTRACT

Abdominal obesity, particularly accumulation of visceral adipose tissue (VAT), is characterised by a chronic low-grade inflammatory response and is associated with increased risk of insulin resistance (IR), type two diabetes mellitus (T2DM) and cardiovascular disease (CVD). Importantly, body fat distribution, inflammation (at both a genetic and circulating level), metabolic risk factors for CVD and T2DM, as well as socioeconomic status (SES) and behavioural/lifestyle factors, have been shown to differ across ethnic groups. However, the ethnic-specific association between body fat distribution, inflammation and risk for CVD and T2DM, and the interaction with SES and behavioural/lifestyle factors, has not been comprehensively explored in a South African population and is required to identify potential mechanisms underlying these diseases and highlight specific areas for intervention.

There is currently limited data on the effectiveness of central obesity measures, including VAT, and its anthropometric proxies (waist circumference (WC), waist- to hip ratio (WHR) and waist-to-height ratio (WHtR)), for early detection of CVD risk factors in different ethnic groups in South Africa. Furthermore, several studies have examined circulating inflammatory markers, in particular the high sensitivity measure of the acute phase protein C-reactive protein (hsCRP), as an inflammatory biomarker of increased CVD risk. There is less consistent information available on other inflammatory biomarkers. Interleukin (IL)-18, a proinflammatory cytokine known to be associated with T2DM and CVD risk, has been suggested as a novel biomarker of risk. However, this has not been examined in a multi-ethnic population, such as South Africa, nor is it known how ethnic differences in SES, behavioural/lifestyle and genetic factors influence circulating IL-18 concentrations. Further, given the differences in body fat distribution between black and white women it remains to be explored if there are ethnic differences in the depot-specific expression of inflammatory genes within different adipose tissue (AT) depots, namely abdominal and gluteal depots, and
if a depot-specific AT inflammation could explain ethnic differences in metabolic risk factors for CVD and T2DM in black and white women.

Therefore, the overall aim of this thesis was to investigate the ethnic-specific role of inflammation in obesity and related metabolic risk factors associated with T2DM and CVD in apparently healthy black and white premenopausal South African women. More specifically, the objectives were to; i) compare the discriminative ability of central obesity measures to identify black and white South African women with or without known metabolic risk factors for CVD and T2DM, including elevated blood pressure (BP), dyslipidaemia (defined by elevated triglyceride (TG), high total cholesterol (TC)/high density lipoprotein cholesterol (HDL-C) ratio, and/or reduced HDL-C concentrations), and IR (estimated using the homeostasis model of insulin resistance (HOMA-IR) and defined by the upper tertile for the whole cohort); ii) investigate the association between circulating inflammatory markers, namely IL-18 and hsCRP with metabolic risk factors for CVD in black and white South African women, taking into account socioeconomic and behavioural/lifestyle factors known to influence both inflammation and CVD risk; iii) investigate whether ethnic variation in the distribution of the \(-137\ G/C\) polymorphism within the \(IL-18\) gene is associated with differences in serum IL-18 concentrations and metabolic risk factors for CVD in black and white South African women; iv) determine the ethnic- and depot-specific associations between AT inflammatory gene and protein expression with insulin sensitivity (S\(_i\)), a major risk factor for T2DM, in black and white South African women.

In summary, this thesis has important novel findings regarding the ethnic specific role of obesity and inflammation (at a circulating, genetic and AT-specific level) with metabolic risk factors for CVD and T2DM in apparently healthy black and white South African women. The data show that central obesity measures were less robust indicators of metabolic risk
variables in black compared to white women. Furthermore, basic, cost-effective anthropometric measures of obesity (WC and WHtR) were able to identify an equivalent amount of risk compared to CT-derived VAT. The data also show that increased circulating concentrations of the inflammatory markers hsCRP and IL-18 were associated with obesity, low SES, smoking, contraceptive use and metabolic risk factors for CVD in both black and white women. The relationship between circulating IL-18 and hsCRP with risk was largely due to the effects of obesity and not SES, supporting the role of AT-derived inflammation in linking obesity to increased CVD and T2DM risk. However, differences in circulating IL-18 concentrations between black and white women could not be explained by ethnic differences in the distribution of the \textit{IL-18} -137G/C gene polymorphism. Further investigation is therefore needed into other factors influencing circulating concentrations of IL-18. The data also showed that although black women have higher inflammatory gene expression within the abdominal SAT and gluteal depots, this was not able to explain their lower $S_I$. In conclusion, despite an up-regulation of inflammation at a circulating, genetic and AT-specific level in black women, this could not explain ethnic differences in metabolic risk factors between black and white South African women. Future research should investigate the contribution of inflammation from other tissues which may affect metabolic risk factors in black populations. Compared to other factors explored in this thesis, central obesity explained the greatest proportion of variance in metabolic risk factors. However, a large proportion of risk still remained unexplained. Therefore future studies should focus on other factors involved, in particular exploring more objective measures of lifestyle, such as physical activity and dietary intake. Furthermore, prospective studies are also required to establish whether these relationships are similar in individuals who develop T2DM and CVD.
CHAPTER ONE

LITERATURE REVIEW

Data from this chapter have been published in part in: Evans J, Goedecke JH. Inflammation in relation cardiovascular disease risk: A comparison of black and white women from the United States, United Kingdom and South Africa. 2011 March Current Cardiovascular Risk Reports (In press).

1.1. INTRODUCTION

Obesity (defined by a body mass index (BMI)>30kg/m²) is highly prevalent in South African women [1] and is associated with increased risk of non-communicable diseases, including cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [2;3]. In addition to being an independent risk factor for CVD and T2DM, obesity also increases the risk of other metabolic risk factors, namely insulin resistance (IR), hypertension and dyslipidaemia [4]. The metabolic effects of excess adipose tissue (AT) are exerted partly via increased release of free fatty acids (FFA) and the production of adipose-derived proteins, including adipocyte hormones, inflammatory cytokines, chemokines and acute phase proteins, which have both metabolic and inflammatory functions [5]. The distribution of AT influences its metabolism and thereby disease risk, independently of the size of the AT stores. Accumulation of fat in the abdominal area, particularly in the visceral fat compartment (VAT), is associated with increased risk of IR, T2DM, hypertension, dyslipidaemias and atherosclerosis [6], whereas fat stored in the lower body is associated with a more favourable metabolic profile [7]. It is hypothesised that the differential effects of body fat distribution on metabolic risk outcome are, in part, mediated via inflammatory pathways, with a putative origin in AT [8].
Studies in developed countries have highlighted the importance of ethnicity in the relationship between obesity and disease risk [9,10]. Subsequently, apparent ethnic differences in body composition, fat distribution, inflammation, and metabolic risk factors for CVD and T2DM have been shown in South African women [11-13] (Figure 1.1). Furthermore, it must also be recognised that developmental influences (such foetal nutrition) have lifelong effects on cardiovascular and metabolic function, and that this may be especially relevant in a developing country such as South Africa undergoing epidemiological transition (as reviewed by Hanson and Gluckman [14]). To add to the complexity of the association between obesity, inflammation and metabolic risk, are inherent ethnic differences in socioeconomic status (SES) and environmental/lifestyle factors, which may influence disease risk and outcome [15]. Although these differences are mainly a result of the socio-political history of the country, genetic variability and/or epigenetic differences between ethnicities may also be involved [16-18]. Of particular relevance to this thesis are ethnic differences in inflammation, which have been shown at both a genetic and circulating level.

A better understanding of the relevant predisposing factors associated with metabolic disease risk in South African women is important for early disease detection and prevention. Notably, ethnic differences in the aetiology of cardiovascular disease and type two diabetes are multifactorial, included in which are interactions of both AT endocrine and immune abnormalities with non-AT organs (as reviewed by Lee et al. [19]). However, for the purpose of this thesis, the following review focuses on studies investigating the associations between obesity, inflammation at a circulating, genetic level and AT-specific level, and metabolic risk factors for CVD and T2DM, with particular focus on differences in black and white premenopausal South African women.
Figure 1.1. Impact of ethnicity on the relationship between obesity, adipose tissue distribution, inflammation and metabolic risk factors for cardiovascular disease and type two diabetes mellitus. VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; SES, socioeconomic status.

1.2. ETHNIC DIFFERENCES IN CARDIOVASCULAR DISEASE AND TYPE TWO DIABETES

It is estimated that the burden of disease attributable to non-communicable diseases, including CVD and T2DM, constituted the second most important cause of death in South African adults in 2000 [20]. Earlier population based surveys in South Africa show a high prevalence of hypertension (14-33%) and T2DM (4.8-6%) [21;22], with variation in prevalence found across ethnic groups. Specifically, black South African women were twice
as likely to present with T2DM compared to white women (7.0% vs. 3.6%) [23;24] and white South African women displayed a higher prevalence of dyslipidaemia [25] and ischemic heart disease [21;26;27], compared to black women.

Similar black-white differences in the prevalence of CVD and T2DM have been found in America and the United Kingdom (UK). According to American national prevalence data published in 2007, the prevalence of T2DM was 6.6% in white versus 11.8% in black women [28], and the prevalence of coronary heart disease was 6.6% in white compared to 5.2% in black women [29]. Data from the Health Survey for England (HSE) in 2006 showed the prevalence for T2DM and CVD was 7.6% and 9.2% in black Afro-Caribbean, 2% and 5.5% in black Africans, and 3.1% and 13% in the white population, respectively [30].

1.2.1. Metabolic risk factors for cardiovascular disease and type two diabetes

Established metabolic risk factors for CVD and T2DM include central obesity, hypertension, hyperglycaemia, IR, dyslipidaemia and a chronic low-grade inflammatory response [31]. The clustering of these factors, known as the metabolic syndrome, occurs more commonly in insulin resistant/hyperinsulinaemic individuals, and is associated with a further increased risk for CVD and T2DM [32]. As a result, diagnostic criteria for the metabolic syndrome have been developed in European populations to help identify individuals at increased risk for T2DM and CVD, and include thresholds for elevated blood pressure (BP), dyslipidaemia (characterised by high triglyceride concentrations (TG) and reduced high density lipoprotein cholesterol (HDL-C) concentrations), hyperglycaemia and obesity (assessed by waist circumference, WC) (Table 1.1) [31;33]. The International Diabetes Federation (IDF) estimates that individuals with the metabolic syndrome (classified as having greater than 3 of the above risk factors together with abdominal obesity) are 2-3 times more likely to
experience CVD events compared to people without the metabolic syndrome. In addition, individuals with the metabolic syndrome have a 5 fold greater risk of developing T2DM [33]. Insulin resistance, through direct effects of vessel walls and indirect effects on lipids and BP is regarded by some, to be the underlying factor linking the clustering of metabolic risk factors (metabolic syndrome) to CVD and T2DM [31;33;34]. Black American and South African women have been shown to be more insulin resistant than their white counterparts [12;35-38]. However, despite this, the prevalence of the metabolic syndrome is lower in black compared to white individuals. Data from the Profiles of Women with Insulin Resistance Syndrome (POWIRS) study [39], which compared age and body mass index (BMI)-matched black (n=102) and white (n=115) apparently healthy South African women, showed the prevalence of the metabolic syndrome (IDF criteria) to be higher in white (30.4%) compared to black (24.8%) women. Similarly, Kalk et al. found higher prevalence of the metabolic syndrome (IDF criteria) in white (74.1%) compared to black (46.5%) diabetic patients [2]. A higher prevalence of the metabolic syndrome has also been found when comparing apparently healthy white (23.5%) compared to black (8.2%) Americans [40].

### Table 1.1. Metabolic syndrome criteria for women

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Defining Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central obesity, given as a WC</td>
<td>≥80cm (IDF)</td>
</tr>
<tr>
<td></td>
<td>&gt;88cm (NCEP ATP III)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>≥1.7 mmol/L</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol</td>
<td>&lt;1.29 mmol/L</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>≥130/85 mmHg</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>≥5.6mmol/L</td>
</tr>
</tbody>
</table>

*IDF, International Diabetes Federation; NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III.*
Further investigation has shown that there is an atypical presentation of components of the metabolic syndrome in black individuals, with a very low percentage presenting with hypertriglyceridaemia compared to their white counterparts [2;41]. Furthermore, Goedecke et al. have shown that whilst increased TG and reduced HDL-C concentrations are associated with lower insulin sensitivity ($S_I$) in a cross-sectional sample of white South African women as has been previously shown in European populations, lipids were not related to $S_I$ in black women. The failure of association between lipids and $S_I$ has also been shown in black American women [42]. These findings suggest that the use of the current metabolic syndrome criteria may not be appropriate in black populations. However, due to the lack of prospective data identifying risk factors that predispose black populations to CVD and T2DM, the current cut-points for the individual risk factors established by the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III will be used to identify risk in my thesis [31].

1.2.2. Obesity and body fat distribution in relation to metabolic risk

Obesity is not only an independent risk factor for CVD [43], it also increases the incidence of other risk factors associated with CVD and T2DM, notably elevated BP, dyslipidaemia and IR [6;44]. Obesity is highly prevalent in South Africa, and results from the first South African Demographic and Health Survey (SADHS) conducted in 1998, showed that 56% of adult women and 26% of men were overweight or obese, with the highest prevalence shown in black women (58.5%), followed by women of mixed ancestry (52%) and then white women (49.2%) [1]. Cross-sectional studies in South Africa have shown that a high percentage of patients with T2DM and CVD are overweight or obese [2;45]. Whilst the prevalence of obesity is high in both black and white South African women, ethnic differences in the distribution of fat have been shown. Specifically, black South African and
American women have less central fat mass, specifically VAT, and more peripheral (femoral and gluteal) fat [11] compared to white women.

The distribution of body fat is an important determinant of the metabolic consequences of obesity. Abdominal accumulation of AT (central obesity), in particular VAT, is associated with a greater risk of cardiovascular complications [6] compared to accumulation of AT in the femoral-gluteal region (peripheral obesity), which is associated with a lower risk of metabolic complications [7]. Using imaging techniques (computer-tomography, CT and magnetic resonance imaging, MRI) to quantify abdominal VAT and subcutaneous AT (SAT), cross-sectional studies have demonstrated strong correlations between VAT and individual metabolic risk factors, including IR, dyslipidaemia and elevated BP, independent of total fat and abdominal SAT [46;47]. The added risk of VAT over SAT is thought to be due to the direct release of FFA and adipose-derived proteins, including pro-inflammatory cytokines, and decreased delivery of anti-inflammatory factors such as adiponectin from VAT into the hepatic portal system (known as the portal hypothesis) [48] resulting in reduced hepatic insulin clearance, increased gluconeogenesis and increased dyslipidaemia [49]. Indeed, visceral adipocytes have been shown to be more sensitive to lipolytic stimuli, and less sensitive to anti-lipolytic stimuli (such as insulin), compared with subcutaneous adipocytes [50-52].

Sumner et al. [53] compared the waist circumference (WC)-VAT relationship in black (n=186) and white (n=148) South African women and black women from America (n=99) and West Africa (n=23). White South African women (n=148) had a significantly higher increase in cross sectional VAT per unit increase in WC than all black populations. Further, no difference was found in the WC-VAT relationship between black South African, American and West African women (Figure 1.2). This is in agreement with earlier studies on
American and South African women. In small groups of obese black and white women matched for body mass index (BMI), van der Merwe and Puyandeera et al. have demonstrated that black women had significantly smaller VAT area (5 level slice) than white women (72mm vs. 140mm) [35;54]. Similar findings were shown by Conway et al. [55] when comparing obese black (n=9) and white (n=11) American women. Although, WC correlated significantly with VAT in both ethnic groups, black women had less VAT (cross sectional area at level of L2-L3) than white women, and similar levels of SAT. In this study, black American women had significantly lower fasting TG and glucose concentrations and lower diastolic BP than their white counterparts [55]. Conway et al. [55] concluded that obesity might not be associated with as severe health consequences in black compared to white women. Contrary to this, when examining ethnic differences in the relationship between VAT volume and blood lipids, BP, and IR (assessed by the homeostasis model of insulin resistance, HOMA-IR) in apparently healthy premenopausal (~ 37 years old) overweight black (n=30) and white (n=36) American women matched for SES, physical activity levels, age and BMI, Perry et al. [56] found no difference in insulin or glucose concentrations between ethnic groups, despite lower levels of VAT in the black women. However, black women had lower TG concentrations than white women, which were attributed to their lower VAT. The authors concluded that a critical level of VAT was not necessary to have a significant relationship with insulin or glucose in black populations.
Figure 1.2. Visceral adipose tissue vs. waist circumference: scatter plots and linear regression line for each group. Equations for white South Africans: $\text{VAT} = -185 + 3.18 \text{ WC}$; African Americans: $\text{VAT} = -111 + 1.65 \text{ WC}$; black South Africans: $\text{VAT} = -50 + 1.38 \text{ WC}$; black Africans in United States: $\text{VAT} = -115 + 1.88 \text{ WC}$. VAT, visceral adipose tissue; WC, waist circumference. Figure from Sumner et al. [53].

However, whilst there is consensus that VAT has a strong association with CVD risk factors, particularly dyslipidaemia and hyperinsulinaemia [47], abdominal SAT has also been shown to contribute to obesity-related co-morbidities, particularly IR. Specifically, both VAT and abdominal SAT volume were significantly associated with BP, fasting glucose, TG and HDL-C concentrations and increased odds of hypertension, impaired fasting glucose, T2DM and clustering of metabolic risk factors in older, apparently healthy women (n=1452) included in the Framingham Heart study [57]. Furthermore, when comparing the relationship of VAT and abdominal SAT volume with $S_1$ (frequently sampled intravenous glucose tolerance test, FSIGT), BP and lipid levels in a cross-sectional sample of obese non-diabetic black American women, Tulloch-Reid et al. [58] found that both VAT and abdominal SAT were negatively correlated with $S_1$, HDL-C, HDL particle size, and positively correlated with
TG and systolic BP. Statistical comparison of correlation coefficients between VAT and abdominal SAT revealed no differences in the strength of association with $S_I$ or lipids. In obese premenopausal black and white American women, Lovejoy et al. [59] reported lower VAT area (L4-L5) in black (n=23), but similar SAT, compared to white (n=15) women. Comparing the association of abdominal SAT and VAT with $S_I$ (FSIGT), blood lipids and BP, their results showed that for a lower level of VAT, black women had higher BP and fasting insulin concentrations and lower $S_I$ than white women. VAT correlated significantly with $S_I$, but not with BP or lipid profiles in both black and white women. Furthermore, in this study, abdominal SAT was significantly associated with $S_I$ in black but not white women. The authors concluded that ethnic differences may exist in the sensitivities of VAT and abdominal SAT to an insulin stimulus. In agreement with this, a recent study on normal-weight and obese black (n=30) and white (n=30) apparently healthy South African women showed that despite lower levels of VAT area (L4-L5), black South African women were more insulin resistant than their white counterparts [12]. While both VAT and abdominal SAT depots were similarly correlated to $S_I$ in white women, abdominal SAT was more closely related to $S_I$ in black women [12].

In addition to differences in VAT, some studies have shown greater amounts of abdominal SAT in black compared to white women. Notably, abdominal SAT is anatomically separated by a stromal fascia into superficial (SSAT) and deep subcutaneous adipose tissue (DSAT) [60], and ethnic differences in abdominal SAT are possibly due to a greater proportion of superficial SAT (SSAT) rather than deep SAT (DSAT).

Ethnic differences in body composition were investigated in a baseline analysis of overweight healthy middle aged black (n=55) and white (n=103) American women from the Healthy Transitions Study [61]. Adjusting for differences in body fatness (%), black women
had lower VAT (non-significant) and higher SSAT ($167cm^2$ vs. $142cm^2$) but not DSAT ($172cm^2$ vs. $162cm^2$) compared to white women. In agreement with these findings, Goedecke et al. found higher abdominal SAT in obese black compared to obese white women, with differences in SAT due to higher SSAT ($321cm^2$ vs. $244cm^2$) and similar DSAT ($269cm^2$ vs. $247cm^2$) [12]. Notably, in black women, the relationship between $S_I$ and abdominal DSAT was stronger than for SSAT (Figure 1.3). Abdominal DSAT has previously been shown to have as strong a positive relationship with IR as VAT, and therefore possibly metabolically distinct to superficial SSAT [61;62].

The relative size of VAT compared to abdominal SAT is very small, accounting for only 5-8% of total fat stores, whereas abdominal SAT accounts for up to 80% of total fat stores [47]. Further, Nielsen et al. showed that between 50-60% of hepatic FFA delivery is from the systemic circulation, with the contribution of VAT lipolysis to hepatic FFA delivery ranging from only 10% in lean (those with less VAT) to about 30% in obese (those with greater VAT) individuals. This suggests that abdominal SAT is probably the main source of increased circulating FFAs and hepatic FFA delivery [48].
Figure 1.3. Relationship in black and white South African women between the insulin sensitivity index ($S_I$) and a) body fat percentage, b) visceral adipose tissue area (VAT), c) deep subcutaneous adipose tissue area (SAT), d) superficial SAT. Solid regression lines are for black women and broken regression lines for white women. When the relationship is not significant, no regression line is shown. Figure from Goedecke et al. [12]

In contrast to abdominal obesity, the accumulation of gluteal SAT has been regarded as protective, with some [7;17;63;64], but not all [46;65;66], studies showing a positive association between increasing hip circumference and/or leg fat mass and favourable metabolic risk profiles. It has been suggested that the femoral-gluteal fat depot plays a protective role by acting as a ‘sink’ for circulating FFA [67]. In contrast to abdominal VAT and SAT, adipocytes in the femoral region are relatively insensitive to lipolytic stimuli and have a high sensitivity for anti-lipolytic stimuli [52;68]. Therefore, the femoral-gluteal SAT region is more likely to effectively take up FFA from the circulation and is less likely to
release them readily. As a result, ectopic fat storage in the liver, skeletal muscle and pancreas, as well as VAT accumulation may be prevented [69].

The added metabolic risk of VAT in comparison to abdominal and gluteal SAT, has in part, been attributed to their apparent higher expression and/or secretion of inflammatory mediators [70;71]. Numerous studies have shown that in comparison to abdominal SAT, abdominal VAT has greater expression and secretion of a number of key inflammatory mediators shown to be associated with IR, dyslipidaemia and elevated BP [71]. However the production of inflammatory proteins in SAT has also been linked to metabolic complications [72-74]. Although there is a paucity of data confirming this, DSAT has been shown to have an inflammatory gene expression greater than SSAT [75;76] and in the case of tumor necrosis factor (TNF)-α expression, similar to that of VAT [75]. It is further hypothesized that the protective effects of peripheral fat accumulation are mediated by a more favourable inflammatory profile [7], though there is a lack of evidence to support this. The inflammatory properties of different adipose tissue depots will be discussed in more detail in section 1.3.

In summary, there are ethnic differences in body fat distribution and the relationship between abdominal VAT and SAT with metabolic risk variables. Notably, advanced imaging techniques, such as CT, are not always available in a primary health care setting and exposure to radiation makes this a high-risk method of risk screening. On the other hand, anthropometric proxy measures of VAT, including WC, waist-height ratio (WHtR) and waist-hip ratio (WHR) are more cost-effective and less high risk, and are frequently used indicators of central obesity. However, as discussed previously, the same level of WC does not reflect the same amount of VAT or SAT between black and white women, and may have important implications in identification and prevention of risk factors for CVD and T2DM.
1.2.3. Thresholds of central obesity measures as determinants of metabolic risk variables

As discussed in section 1.2.1 and in accordance with the IDF [77] and the NCEP ATP III [31], central obesity is a major modifiable risk factor for CVD and T2DM, and should be a target for clinical interventions. Subsequently, thresholds of central obesity measures, including WC, WHR, WHtR and BMI, and less frequently VAT, are used for the early identification of individuals at increased risk for CVD and T2DM [78].

The most common cut-points used to identify increased risk are those of a WC of 80cm and 88cm, as recommended by the IDF and NCEP ATP III, respectively (Table 1.1). Furthermore, the IDF criteria specify that different WC thresholds should be used for different ethnic groups and/or populations, and have further defined ethnic specific WC cut-points for Europid, Chinese, South Asian and Japanese populations [77]. However, currently there is no central obesity threshold defined for Sub-Saharan populations, and it is recommended that a WC of 80 cm be used until more specific data is available. Considering the differences in the association between body composition and metabolic risk in different ethnic groups as discussed in section 1.2.2, using current thresholds derived from European populations for risk screening may lead to inadequate screening in high-risk ethnic groups.

Several cross-sectional studies have evaluated the use of IDF and NCEP ATPIII WC cut-points in black and white American populations. Okusan et al. [79] compared the ability of a WC >88cm for identifying the clustering of hypertension, T2DM and dyslipidaemia in black and white Americans from the National Health and Nutritional Survey (NHANES) III study. The odds ratio (OR) associated increased CVD risk due to a WC>88cm was higher in white (OR=6.21 for 2 or more risk factors and OR=14.2 for 4 or more risk factors) compared to black individuals (OR=5.2 for 2 or more risk factors, OR=5.8 for 4 or more risk factors) [79].
Although the effectiveness of these cut-points has not been explored in white South African women, Jennings et al. compared the sensitivity and specificity of the IDF and NCEP ATPIII WC thresholds for predicting IR (as estimated by HOMA-IR > 2.60) in a sample of young, apparently healthy black South African women [41]. There was no difference in the sensitivity or specificity between the WC cut-points. However, the IDF cut-point of 80cm had a positive predictive value of 86.1% compared to 78.4% for the NCEP ATPIII cut-point of 88cm. This suggests that lower obesity thresholds may be needed for early risk detection in black populations.

Approaches based on receiver operator characteristic (ROC) curve analyses are frequently used for defining obesity thresholds, though only a few studies in America and UK have investigated the ethnic-specific level of obesity that predicts incidence of T2DM (Table 1.2). Using data from NHANES 2003-2004 and HSE 2003-2004 [80], Diaz et al. explored thresholds of BMI, WC and WHR that best predicted the development of T2DM in black and white men and women. BMI thresholds did not differ across countries or between ethnicities, with BMI thresholds ranging between 26 and 28 kg/m². Conversely, WC thresholds were higher in American compared to British women, with a higher (~3cm) WC threshold in the black compared to white American women. In contrast, a lower WC (~3cm) threshold was found in black compared to white British women. The optimal WHtR thresholds were similar between black American and white and black British women, but were higher in white American women. WC thresholds for identifying the development of T2DM from the Atherosclerosis Risk in Communities (ARIC) cohort were lower than those identified in the NHANES cohort, but similarly, black women had higher WC thresholds compared to white women (101 vs. 95cm, respectively). BMI thresholds were also higher in black compared to white women, but no difference in WHR was found [81]. Sargeant et al. investigated the thresholds for BMI, WC, WHR and WHtR for predicting incident T2DM in Jamaican adults
of self-reported African ancestry [82]. Although BMI thresholds for black women in the different studies (ARIC, NHANES, HSE) were similar, those for WC and WHtR were much lower in the Jamaican cohort compared to those reported for black women from the ARIC, NHANES and HSE cohorts. These findings highlight the variability in obesity thresholds for identifying metabolic risk across ethnic groups and/or populations.

In addition to identifying the level of obesity that best predicts risk for CVD and T2DM, numerous investigations have sought to find the anthropometric measure (WC, WHR, WHtR or BMI) that can achieve the best discrimination of risk. Lee et al. conducted a meta analysis including more than 88 000 individuals from several countries (including Germany, Hong Kong, Taiwan, Jamaica, Thailand and Japan), to determine which of four indices of overweight and obesity (BMI, WC, WHtR or WHR) was the best discriminator of hypertension, T2DM and dyslipidaemia [83]. For hypertension, T2DM and dyslipidaemia, WHtR had the highest, and BMI the lowest pooled ROC area under the curve (AUC). However, only small differences were observed between WC, WHR and WHtR. Although they were unable to perform sub-group analysis of ethnic groups, results from NHANES, HSE and ARIC cohorts discussed previously suggest that WC and WHtR are the best measures for predicting T2DM in these populations, irrespective of ethnicity. T2DM and hypertension have been shown to be more prevalent in short-stature individuals compared with taller individuals [84] and when matched for WC, shorter individuals have been shown to have a higher risk for CVD [85]. It has therefore been suggested that WHtR may still be a better measure of metabolic risk than WC, especially in populations that vary in height [86;87]. This may be of particular relevance to the South African context, since black South African women are shorter than white South African women [11;12].
<table>
<thead>
<tr>
<th>Study Population</th>
<th>Outcome</th>
<th>Ethnicity</th>
<th>WC (cm)</th>
<th>WHR</th>
<th>WHtR</th>
<th>BMI (kg/m²)</th>
<th>Additional Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHANES and HSE [80]</td>
<td>Undiagnosed T2DM HbA1c &gt;6.1%</td>
<td>Black American (n=491)</td>
<td>108.9 (0.69)</td>
<td>0.59</td>
<td>27.7</td>
<td></td>
<td>ROC AUC for WC and WHtR significantly greater than ROC AUC for BMI in all groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White American (n=1486)</td>
<td>105.8 (0.71)</td>
<td>0.64</td>
<td>27.7</td>
<td>(0.65)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black British (n=279)</td>
<td>88.0 (0.68)</td>
<td>0.60</td>
<td>28.1</td>
<td>(0.59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>White British (n=4488)</td>
<td>91.4 (0.72)</td>
<td>0.60</td>
<td>26.7</td>
<td>(0.66)</td>
<td></td>
</tr>
<tr>
<td>ARIC [81]</td>
<td>T2DM (9 year follow up): Fasting Glucose ≥7mmol/L</td>
<td>Black (n=1817)</td>
<td>101 (0.69)</td>
<td>0.92</td>
<td>30.0</td>
<td>(0.66)</td>
<td>ROC AUC for WC significantly greater than ROC AUC for BMI in black women only</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White (n=5293)</td>
<td>95 (0.73)</td>
<td>0.91</td>
<td>27.2</td>
<td>(0.72)</td>
<td></td>
</tr>
<tr>
<td>Jamaica [82]</td>
<td>Incident T2DM (4 year follow up): Fasting glucose ≥7mM and/or 2hr glucose ≥11.1mM</td>
<td>Black (n=438)</td>
<td>84.5 (0.61)</td>
<td>0.80</td>
<td>0.54</td>
<td>29.3</td>
<td>(0.62)</td>
</tr>
</tbody>
</table>

NHANES, National Health and Nutritional Examination Survey; HSE, Health Survey for England; ARIC, Atherosclerosis Risk in Communities study; T2DM, type two diabetes mellitus; WC, waist circumference; WHR, waist-hip ratio; WHtR, waist-height ratio; BMI, body mass index; ROC AUC, receiver operator characteristic area under the curve
As discussed in section 1.2.2, for the same level of WC white women accumulate more VAT whereas black women accumulate more SAT [53]. Although VAT is considered to be a better marker of risk compared to SAT in some populations, both VAT and SAT are associated with risk in black populations [12]. However, it is not known what level of VAT, if any, best predicts increased risk for CVD and T2DM in black populations. Furthermore, as WC, is reflective of both VAT and SAT, WC may be superior to VAT for prediction of risk factors. However, only a few studies have examined the effectiveness of VAT compared to anthropometric measures for the identification of individuals at increased risk for CVD. In a cross-sectional sample of obese Korean women, WC and CT-derived VAT were equivalent in predicting a combination of at least two metabolic risk factors for CVD [78]. Further, in a subgroup of patients from the Diabetes Prevention Programme, Bray et al. compared the ability of VAT, SAT, BMI, WHR and WC for prediction of T2DM [88]. WC, WHR, BMI and VAT, but not SAT, were significant predictors of T2DM, with little difference between the discriminative ability of the other measures [78]. Although they adjusted for ethnicity, it is not known whether there were ethnic differences in the ability of CT-derived compared to anthropometric measures for identifying T2DM. Further investigation of the effectiveness of anthropometrics compared to more direct measures of VAT is therefore needed, particularly given the ethnic differences in the relationship between WC and VAT [53] and the differential relationship between AT distribution and metabolic risk in South African women [12].

In summary, the most optimal measure of central obesity, as well as the level of central obesity that best predicts metabolic risk factors for CVD and T2DM has not been explored in a South African population. These questions are explored in Chapter 2 of this thesis.
1.2.4. Impact of socioeconomic status on disease outcome and metabolic risk

A low SES has been shown to be associated with increased risk for non-communicable diseases, including CVD and T2DM [15;89;90]. Results from the Interheart study, a large case-control study designed to investigate risk factors associated with acute myocardial infarction across 52 countries, including Africa [91], showed that a low level of educational attainment (≤ 8yrs) was associated with higher odds of acute myocardial infarction compared to higher educational attainment (≥9yrs) [91]. Furthermore, the association between low SES and increased risk for myocardial infarction was present in both low/middle and high income countries. A lower educational attainment has also been shown to be associated with longer time to first diagnosis of T2DM [92].

Notably, studies in America have shown that ethnic minority groups (which include black populations) are more likely to be less well-educated than the majority white population [93;94]. As a result, it has been suggested that ethnic differences in disease outcome discussed previously may be due to differences in SES rather than ethnicity per se. This is highlighted by recent analysis of NHANES data [94]. Results of this study showed that SES, which was lower in black women, was inversely associated with estimated 10-year CVD and T2DM risk in both black and white American women. However, when comparing ethnic groups stratified for level of SES (low-high), only black women in the middle SES had higher odds (OR=2.4) of T2DM compared to white women. In support of these findings, matching black and white participants in the Boston Area Community Health survey according to SES status (assessed by education and income), T2DM prevalence no longer differed between ethnicities [93]. This study concluded that SES, which they classed as a potentially modifiable risk factor, was more important in identifying T2DM than the non-modifiable risk factor of ethnicity. This may be of particular importance in the South African
context, where the majority of blacks, although the majority population group, live in poorer areas [89]. As this is largely a result of the socio-political history of the country, data comparing health related variables between black and white South Africans matched for SES have yet to be shown.

Although the exact biological mechanisms are not fully understood, it is believed that negative behavioural/lifestyle factors (including smoking, alcohol consumption, contraceptive use, sedentary behaviour and poor dietary patterns) characteristic of low SES communities may, in part, mediate the relationship between low SES and increased CVD and T2DM risk [90]. Indeed, in the Interheart study [91], approximately half of the risk due to low SES was explained by modifiable behavioural/lifestyle variables, including smoking, higher WHR, lower consumption of fruit and vegetables and lower levels of exercise. In addition, results from NHANES data showed that irrespective of ethnicity, a large proportion of the association between SES and CVD risk was explained by health behaviours (physical activity and smoking) and central obesity characteristic of lower SES groups [94].

In summary, there is evidence to suggest that SES and behavioural/lifestyle factors impact on metabolic risk outcomes in both black and white populations. These findings highlight the importance of including SES and behavioural variables when investigating risk for CVD and T2DM, and will be explored in Chapter 3 of this thesis.

1.2.5. Summary

Differences in the prevalence of CVD and T2DM between ethnicities are possibly related to differences in risk factor burden, differential clustering of risk factors and SES and/or behavioural variables. Prospective studies are needed to identify which metabolic risk factors are important for the development of CVD and T2DM, especially in black populations.
Furthermore, it remains unclear whether traditional risk factor profiling adequately identifies all persons at high risk for CVD and T2DM, or whether additional novel risk markers, such as inflammatory proteins, are of added benefit for early risk identification.

1.3. INFLAMMATION IN OBESITY, CARDIOVASCULAR DISEASE AND TYPE TWO DIABETES

It is now well established that a state of chronic low-grade inflammation is present in obesity, T2DM and CVD [95-97]. Increased circulating concentrations of inflammatory proteins, such as proinflammatory cytokines, acute phase proteins, chemokines and adipocyte hormones, are found in obese individuals [98], and in patients with T2DM and CVD ([99]. In contrast, anti-inflammatory proteins, such as adiponectin, have been shown to be lower in obese individuals and in patients with CVD and T2DM [100;101]. As a result, it is hypothesised that this low grade inflammatory response may be central to the development of CVD and T2DM [96]. Indeed, some markers of inflammation have shown potential for improved disease risk prediction, and in some cases as potential therapeutic targets for clinical intervention [102;103].

Several factors, both environmental and genetic, have been shown to influence circulating concentrations of inflammatory proteins, and may impact on the relationship between inflammatory markers and metabolic risk factors for CVD and T2DM. Specifically, a low SES (assessed by lower income, asset ownership and education) and negative health behaviour/lifestyle variables (smoking, sedentary behaviour, poor dietary intake and contraceptive use) are associated with increased circulating inflammatory markers [104]. Genetic variants within inflammatory genes have been shown to influence circulating concentrations of inflammatory markers and in some instance are associated with risk factors.
for CVD and T2DM [105]. Furthermore, studies in America, the UK and South Africa have shown ethnic differences in inflammation at both a circulating and genetic level [13;106;107]. Studies exploring the relationship between circulating inflammatory markers and risk factor for CVD and T2DM in black and white populations are summarized in Table 1.3 and discussed in further detail below.

1.3.1. Circulating inflammatory proteins as markers of cardiovascular disease and type two diabetes risk

1.3.1.1. Acute Phase Reactant C-reactive protein (CRP)

Of the inflammatory proteins, the most extensive data have been shown with high sensitivity C-reactive protein (hsCRP). CRP is an acute phase protein secreted by the liver in response to inflammatory cytokines, mainly interleukin (IL)-6, IL-1 and TNF-α [97]. Cross-sectional studies have shown that hsCRP concentrations increase with increasing adiposity [108] and are associated with metabolic risk factors for CVD and T2DM, including IR, elevated BP and dyslipidaemia [97;108;109].

Studies in America have shown that a lower SES (assessed by education and income) is associated with increased circulating hsCRP concentrations [104;110]. This association has been shown to be largely due to behavioural/lifestyle variables which also influence hsCRP. Specifically, excessive alcohol consumption [111], smoking [112], sedentary behavior [113;114], poor dietary patterns [115] and use of oral contraceptives [116] have all been associated with increased circulating hsCRP concentrations. Furthermore, studies in America, UK and South Africa have shown that hsCRP concentrations are higher in black compared to white populations (Table 1.3). These differences have been shown to be largely due to differences in obesity and/or SES and behavioural/lifestyle factors. Data from the 1999-2000 NHANES study showed age-adjusted hsCRP concentrations were significantly higher in
black compared to white women, however these differences were not statistically significant after adjusting for WC, TG concentration and systolic BP [117]. Data from the Women’s Health Study [118] indicated that hsCRP concentrations were 44.6% higher in black compared to white women. However, adjusting for BMI reduced this difference to 10.2%. Despite differences in hsCRP concentrations between ethnicities, there is evidence to suggest that it remains a good overall marker of risk in both black and white populations, though associations with specific risk factors have been shown to differ between ethnicities. A comparison of the association between hsCRP and metabolic risk factors in women included in the POWIRS study [13] revealed that hsCRP levels correlated significantly with BP and cardiac output in both black and white women, yet only with TG in white women. Results from the Insulin Resistance Atherosclerosis Study (IRAS), demonstrated that the relationship between hsCRP and BMI, fasting insulin and SI were consistent across ethnic groups though the association was not as strong among black women [96].

Earlier prospective studies have shown that high concentrations of hsCRP (>3mg/L) were of added prognostic value for CVD risk in individuals with no known disease, the metabolic syndrome and diabetic patients [103;119;120]. As a result, it was suggested that hsCRP be added to the diagnostic criteria for metabolic syndrome [103]. However this has recently been challenged due to a number of studies showing that, after adjusting for traditional risk factors, circulating hsCRP concentrations do not offer a marked improvement in risk prediction for CVD [121]. Specifically, in a patient population scheduled for diagnostic coronary arteriography, traditional CVD risk factors (based on metabolic syndrome components) were associated with a higher degree of CVD irrespective of hsCRP concentration [121]. Similarly, after adjusting the NCEP ATP III criteria to include circulating hsCRP concentrations >3mmol/L, Hanley et al. found no improvement in the prediction of T2DM after 5 year follow up in participants from the IRAS study [122].
It must be noted that hsCRP is a non-specific indicator of inflammation [123], and it is therefore possible that higher hsCRP concentrations are caused by unknown inflammatory conditions. Therefore, other inflammatory markers involved in the initiation of inflammatory responses may be a more accurate reflection of pathways underlying CVD and T2DM.

1.3.1.2. Cytokines

1.3.1.2.1. Interleukin-18 (IL-18)

IL-18 is a pro-inflammatory and pro-atherogenic cytokine which amplifies the TH1 immune response by inducing the production of pro-inflammatory cytokines and chemokines [124]. In addition, IL-18 has been shown to induce the TH2 response, including IL-10 production [125]. IL-18 is produced by several cell types, including activated macrophages, epithelial cells, endothelial cells and adipocytes [126]. Circulating IL-18 levels have been shown to correlate with a number of metabolic risk factors for CVD, including WC, fasting TG, HDL-C, insulin and elevated BP and hyperglycaemia, in apparently healthy populations [127-129], and in patients with acute coronary syndromes [130]. Although the exact mechanisms linking IL-18 to disruption of metabolic pathways are not known, there is evidence to suggest IL-18 is involved in atherogenic pathways through activation of essential proteins involved in atherogenesis, e.g., vascular adhesion molecules (VCAM-1), chemokines (IL-8), cytokines (IL-6), and matrix metalloproteinases (MMP-1/-9/-13) [131]. Furthermore, upregulation of IL-18 gene expression has been shown to involve NFκB, which in turn has been implicated in disruption of insulin signalling pathways [132].

To my knowledge, the impact of SES on circulating IL-18 concentrations has not been explored. Furthermore, there is limited data on the impact of behavioural/lifestyle factors on circulating concentrations of IL-18, though both regular exercise [133] and a Mediterranean diet [134] have previously been shown to reduce circulating IL-18 concentrations. Two
studies have investigated ethnic differences in IL-18 concentrations [135;136]. Results from the Dallas Heart Study [136] revealed that subjects with higher concentrations of IL-18 (upper quartile for whole study group) were more likely to be of older age, male, white and obese. In agreement with this, circulating IL-18 concentrations were significantly higher in white compared to black hypertensive women participating in the Genetic Epidemiology Network in Arteriopathy (GENOA) study [135]. Black American ethnicity in this study was associated with lower IL-18 concentrations independent of age, sex, BMI, conventional risk factors and lifestyle factors (β=-0.17, P<0.001). However, the authors did caution that results of this study could not be extrapolated to younger, healthy populations. Ethnic differences in circulating IL-18 concentrations could potentially be explained by ethnic differences in the IL-18 gene. Ness et al. compared the distribution of functional single nucleotide polymorphisms (SNPs) in 9 cytokine genes, including IL-18, between black (n=179) and white (n=396) American women [106]. Their results showed that the G allele of the -137G/C polymorphism, which has previously been associated with higher IL-18 concentrations [137], had a higher frequency in black compared to white women. However it remains to be established if ethnic differences in the distribution of this polymorphism are associated with ethnic differences in circulating IL-18 concentrations.

Despite ethnic differences in IL-18 concentrations in the Dallas Heart study, no difference in the relationship between IL-18 and CVD risk was observed between ethnicities. Geometric mean concentrations of IL-18 rose progressively with increasing CVD risk (metabolic syndrome), with strong associations shown with HOMA-IR and glucose concentrations in both ethnic groups [136]. Furthermore, there is evidence to suggest that IL-18 is a good independent marker of CVD and T2DM risk. Data from the Women’s Health Study showed that baseline concentrations of IL-18 above a threshold of 442 pg/ml (90th percentile) were associated with future CVD events (6 year follow-up) in a population of apparently
healthy women [138]. This association remained significant after adjusting for age, obesity (BMI), smoking, T2DM, family history of CVD and hormone use. Hivert et al. found that high circulating IL-18 concentrations (>4.5pg/ml) were associated with future T2DM incidence in a healthy cohort of middle aged women from the Nurses’ Health Study [139], and this association was independent of traditional risk factors for T2DM, including obesity (BMI and WC), dietary intake, and other inflammatory proteins. These findings suggest that IL-18 is a promising independent marker of metabolic risk. However, currently there is limited data on the impact of SES on circulating IL-18 and its relationship with CVD and T2DM risk. Furthermore, these associations have not been explored in a South African population.

1.3.1.2.2. **Interleukin-6 (IL-6)**

The pro-inflammatory cytokine IL-6 is secreted by a number of cell types, and is an important regulator of the acute phase response, in particular CRP synthesis by the liver [140]. Circulating concentrations of IL-6 increase in proportion to fat mass [141], and are 2-3 times higher in obese T2DM patients compared to non-diabetic controls [142]. Peripheral administration of IL-6, that mimics the level in obesity, has been shown to induce hyperlipidaemia, hyperglycaemia and IR [143]. IL-6 is thought to promote IR through inhibition of insulin-stimulated glucose transport [144] and by increasing hepatic glucose production [145]. Notably, due to portal theory of metabolic risk, the metabolic impact of IL-6 release from VAT may be of particular importance since IL-6 has also been shown to increase hepatic triglyceride secretion and therefore contribute to hypertriglyceridaemia associated with VAT [146].

Circulating IL-6 concentrations are influenced by SES gradients [104], and similar to studies exploring CRP, this association has been shown to be largely due to behavioural/lifestyle
variables, including alcohol consumption [111;147], smoking [104;148] and sedentary behavior [104;113]. Circulating concentrations of IL-6 have been shown to be higher in black compared to white women [149;150], and these differences appear to persist after adjustment for differences in body fatness [135;151]. The distributions of IL-6 gene variants have also been shown to differ across ethnicities [106]. Results showed that the G allele of the -174G/C genotype, which has previously been associated with higher IL-6 concentrations [152], had a higher frequency in black compared to white women. Similar to IL-18, there is limited data on whether this is associated with ethnic differences in circulating IL-6 concentrations.

Few studies have investigated whether the association between IL-6 and metabolic risk is different between ethnic groups. Although Hyatt et al. [37] found no ethnic differences in IL-6 between overweight black and white American women, IL-6 concentrations correlated with S_1 in the white women only. However, the association with S_1 in the white women was not significant after adjusting for VAT, supporting VAT as being a significant contributor to IL-6 levels and that the association with risk is largely due to the effects of obesity. In agreement with this, inclusion of IL-6 into traditional risk factor models did not improve the prediction of T2DM development in a large prospective multi-ethnic cohort of post-menopausal women [153].

1.3.1.2.3. Tumor necrosis factor-α (TNF-α)

TNF-α, is a pleiotropic cytokine whose primary role is regulation of immune cells, which exerts its effects by binding to its receptors, tumor necrosis factor receptor (TNFR)1 and TNFR2, present in a large number of cell membranes [154]. Studies showing an association between circulating TNF-α concentrations and metabolic risk factors are conflicting. A few studies have found that circulating concentrations of TNF-α were increased in obese
compared to normal weight individuals [155;156], and associated with glucose uptake and HOMA-IR [157]. However, other studies have found no association between circulating TNF-α and increased CVD and T2DM risk [37]. Indeed, evidence for a mechanistic role of TNF-α in CVD and T2DM is shown mainly at the AT level, where it has been shown to inhibit glucose uptake in lean individuals [158], suggesting that most effects of TNF-α are exerted in a paracrine fashion.

Studies exploring the association between SES and behavioural factors with circulating concentrations of TNF-α are limited. In older men and women, low SES (education and income), smoking and alcohol consumption were associated with increased circulating TNF-α concentrations [110]. Evidence for ethnic differences in TNF-α are conflicting. A few studies [37;135;149] have found higher circulating concentrations of TNF-α in white compared to black American women, whereas Kalra et al. found that the concentration of TNF-α was higher in black Afro-Caribbean compared to whites [159]. Schutte et al. found that black South African women tended (p=0.06) to have lower circulating TNF-α concentrations compared to BMI and age-matched white South African women [160]. In the study by Hyatt et al. [37], adjusting for differences in VAT between black and white women reduced the ethnic difference in TNF-α concentrations (P=0.054). Despite ethnic differences, none of these studies showed an association between circulating TNF-α concentrations and metabolic risk factors in either ethnic group. These findings suggest that circulating TNF-α is not a reliable marker of risk in both black and white women. Instead it appears that local tissue effects of TNF-α may be more important and future research needs to establish whether ethnic differences in adipose tissue levels exist.
Table 1.3. Ethnic differences in circulating inflammatory markers.

<table>
<thead>
<tr>
<th>Study (Population/ intact and Ref)</th>
<th>Subject Characteristics</th>
<th>Inflammatory Marker</th>
<th>Ethnic Difference</th>
<th>Moderating factors</th>
<th>Association with risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women’s Health Study [118]</td>
<td>Black (n=475) and white (n=24 455) older (~55yrs) women</td>
<td>hsCRP</td>
<td>Black&gt;White</td>
<td>BMI decreased the difference from 44% to 10%</td>
<td>Not defined</td>
</tr>
<tr>
<td>SWAN [161]</td>
<td>Black (n=875) and White (n=1496) perimenopausal women</td>
<td>hsCRP</td>
<td>Black&gt;White</td>
<td>Decrease in difference from 82% in unadjusted model to 68% with SES and 18% with BMI. Fully adjusted resulted in 15% difference (P=0.01)</td>
<td>Significant associations with BMI, WHR, SBP, HDL-C and TG in both ethnicities. Association with TC and LDL-C only in white women</td>
</tr>
<tr>
<td>Texas City Stress and Health Study [150]</td>
<td>Black (n=150) and white (n=535); age 25-65yrs</td>
<td>hsCRP, IL-6, TNFR1, IL-10</td>
<td>Black&gt;White, No difference</td>
<td>No difference in CRP after adjustment for BMI</td>
<td>Not defined</td>
</tr>
<tr>
<td>POWIRS [13]</td>
<td>Black (n=102) and white (n=115) women; Matched for age (<del>31yrs) and BMI (</del> kg/m²)</td>
<td>hsCRP, Leptin, Adiponectin</td>
<td>Black&gt;White, Black&gt;White, No difference</td>
<td>BMI not ethnicity significant predictor of CRP, Leptin and fibrinogen significant predictors of CRP in separate models for black and white</td>
<td>Significant correlations with obesity and cardiovascular indices in both ethnic groups</td>
</tr>
<tr>
<td>Study (Population/cohort and Ref)</td>
<td>Subject Characteristics</td>
<td>Inflammatory Marker</td>
<td>Ethnic Difference</td>
<td>Moderating factors</td>
<td>Association with risk</td>
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<tr>
<td>CARDIA [104]</td>
<td>Black (n=857) and white (n=944) women</td>
<td>hsCRP</td>
<td>Black&gt;White</td>
<td>Not defined</td>
<td>Age-adjusted CRP and IL-6 increased with decreasing education and income in both ethnic groups. Relationship not significant after adjusting for health variables in white only</td>
</tr>
<tr>
<td>Dallas Heart Study [136]</td>
<td>2 231 black, white and Hispanic men and women</td>
<td>IL-18</td>
<td>Black&gt;White/Hispanic</td>
<td>Not defined</td>
<td>Significant associations with BMI, IR, glucose in both black and white</td>
</tr>
<tr>
<td>GENOA [135]</td>
<td>Black (n=936) and White (n=702) older (~64yrs) hypertensive women</td>
<td>hsCRP, IL-6, IL-18, TNFRI, TNFRII, Leptin, Adiponectin</td>
<td>Black&gt;White/Black</td>
<td>Differences remained significant after adjusting for age and BMI</td>
<td>Not defined</td>
</tr>
<tr>
<td>Health ABC [149]</td>
<td>Black (n=592) and White (n=757) older (~74yrs) women</td>
<td>hsCRP, IL-6, TNF-α</td>
<td>Black&gt;White</td>
<td>Not defined</td>
<td>CRP and IL-6 correlated with VAT and SAT in both ethnicities. TNF-α correlated with VAT and SAT in white only</td>
</tr>
<tr>
<td>Study</td>
<td>Subject Characteristics</td>
<td>Inflammatory Marker</td>
<td>Ethnic Difference</td>
<td>Moderating factors</td>
<td>Association with risk</td>
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<tr>
<td>Carroll et al. [151]</td>
<td>Black (n=54) and white (n=90) older (~56yrs) obese women</td>
<td>hsCRP</td>
<td>Black&gt;White</td>
<td>Difference remained significant after adjustment for age, VAT and SAT</td>
<td>SAT and VAT significantly correlated with CRP and IL-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-6</td>
<td>Black&gt;White</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalra et al. [159]</td>
<td>Black (n=78) and White (n=82) men and women</td>
<td>hsCRP</td>
<td>No difference</td>
<td>Not defined</td>
<td>Not defined</td>
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<td></td>
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<td>IL-6</td>
<td>Black&gt;White</td>
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<td></td>
<td></td>
<td>TNF-α</td>
<td>Black&gt;White</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferris et al. [162]</td>
<td>Black (n=19) and white (n=22) South African women; Similar BMI (~27kg/m²)</td>
<td>Adiponectin</td>
<td>White&gt;Black</td>
<td>Still significant after adjustment for age, BMI or WHR</td>
<td>Significant associations with BMI, IR and HDL-C in white only</td>
</tr>
<tr>
<td>Hulver et al. [163]</td>
<td>Non-obese and obese black (n=13, n=24) and white (n=12, n=36) women</td>
<td>Adiponectin</td>
<td>Non-obese White&gt;Non-obese Black</td>
<td>Not defined</td>
<td>BMI, insulin and HOMA-IR correlated with adiponectin in white women only</td>
</tr>
</tbody>
</table>

**Table 1.3. Continued**

*SWAN, Study of Women’s Health Across the Nation; POWIRS, Profile of Women with Insulin Resistance Syndrome; CARDIA, Coronary Artery Development in Young Adults; GENOA, Genetic Epidemiology Network of Arteriopathy; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IL-18, interleukin-18; IL-10, interleukin-10; TNFRI, tumor necrosis factor receptor 1; TNFR2, tumor necrosis factor receptor 2; TNF-α, tumor necrosis factor alpha; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; WHR, waist-hip ratio; SES, socio-economic status; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HOMA-IR, homeostasis model of insulin resistance.*
1.3.1.3. **Adipocyte Hormones**

1.3.1.3.1. **Adiponectin**

Adiponectin is produced exclusively by adipocytes [164] and has anti-inflammatory, anti-atherogenic and insulin-sensitising properties [165;166]. Circulating concentrations of adiponectin are positively correlated with $S_I$ [167] and are decreased in obese individuals and in patients with T2DM and CVD [100;101;168]. Adiponectin seems to regulate the expression of several anti- and pro-inflammatory cytokines, such as TNF-α, and the inhibitory controls of pro-inflammatory cytokine production are speculated to be disturbed with decreased synthesis of adiponectin [169].

In a cross-sectional investigation on the impact of SES and behavioural factors on circulating adiponectin concentrations in black American adults, Schootman et al. found higher adiponectin concentrations in individuals with better housing conditions, but no impact of income level, smoking status or alcohol consumption [170]. The majority of studies have shown higher adiponectin in white compared to black individuals [37;162;163;171]. There is evidence to suggest that ethnic differences in adiponectin are largely dependent of the level of obesity of the population studied. For example, in South African women in the POWIRS study [171], ethnic differences in adiponectin were only observed when comparing normal-weight white and black women, but not overweight or obese women. In agreement with these findings, higher circulating adiponectin concentrations were found in normal-weight but not obese white compared to black American women [163]. Results from this study also showed that the association between adiponectin levels and metabolic risk factors for CVD and T2DM was not consistent across ethnicities. Specifically, adiponectin concentrations were associated with a more favourable metabolic profile in the white women only, correlating with BMI, fasting insulin and HOMA-IR [163]. Similarly, in a sample of black and white
South African women, adiponectin concentrations correlated with HOMA-IR in the white women only [162].

In contrast to these findings, results from prospective studies suggest that circulating adiponectin concentrations are good predictors of T2DM irrespective of ethnicity and independent of other confounding/mediating factors. Specifically, results from the ARIC study showed higher adiponectin concentrations were associated with a lower incidence of T2DM, with a similar magnitude of association in black and white individuals [100]. In a prospective European cohort, increasing quintiles of adiponectin were significantly associated with the development of diabetes independent of age, level of education, smoking, physical activity level, alcohol consumption, diet and obesity (assessed by BMI and WC) [172].

1.3.1.3.2. Leptin

Leptin is a true marker of AT mass and is involved mainly in energy homeostasis, acting at the level of the central nervous system to regulate food intake and energy expenditure [173]. Leptin is also involved in the propagation of the inflammatory response by regulating the secretion of chemokines and cytokines from monocytes [174-176]. Leptin is secreted in response to fat cell hypertrophy [177], and circulating levels increase with obesity and are associated with individual risk factors for CVD [101;178].

Earlier studies in South African women showed no difference in circulating leptin concentrations between small samples of black and white women [54]. However more recently, leptin concentrations in normal-weight (BMI <25 kg/m²) and overweight (BMI: 25-30 kg/m²) women in the POWIRS study were significantly higher in black compared to white women [171]. In agreement, results from the GENOA study showed higher leptin in black compared to white women [135]. Despite these ethnic differences, circulating leptin concentrations have been shown to correlate significantly with metabolic risk factors in both
black and white women [171]. However, although leptin has been shown to be involved in blood pressure regulation [179], it is not known higher leptin concentrations found in black populations relates to a higher prevalence of hypertension. In addition, although leptin appears to be a good independent marker of CVD and T2DM risk in cross-sectional studies, analysis of prospective data from the British Women’s Heart and Health study [180] showed no association between circulating leptin concentrations and incident CVD.

1.3.1.4. Other inflammatory markers shown to differ by ethnicity

In addition to the inflammatory markers discussed above, circulating concentrations of several coagulation factors and adhesion molecules have been shown to differ between ethnicities. Black individuals have been shown to have higher concentrations of fibrinogen, factors VII and VIII and plasminogen-activator inhibitor-1, and lower concentrations of VCAM-1, endothelial leukocyte adhesion molecule-1, and intracellular adhesion molecule-1 (as reviewed in [181;182]). However the details of this are beyond the scope of this review.

In summary, circulating inflammatory markers have been shown to be associated with individual metabolic risk factors for CVD and T2DM. However, it still remains unclear whether inflammatory markers are of added diagnostic value beyond traditional risk criteria for the early identification of susceptible individuals at high risk for CVD and T2DM, especially when considering the potential confounding and/or mediating factors, such as ethnicity, SES and lifestyle variables that influence circulating concentrations of these proteins. Notably, ethnic differences in some circulating inflammatory proteins (such as IL-18 and CRP) have been shown to remain even after adjustment for these potential confounding/mediating variables. Whether genetic differences in inflammatory gene polymorphisms and/or tissue-specific production of inflammation can explain these differences is unknown. Indeed, disruption of normal metabolic processes within the AT is
considered to be one of the main sources of chronic low-grade inflammation present in obesity, CVD and T2DM.

1.3.2. Adipose tissue as an inflammatory organ

AT comprises several different cell types including adipocytes and immune cells, whose products, through autocrine, paracrine and endocrine mechanisms, are actively involved in metabolism (including energy homeostasis, adipocyte differentiation, and insulin sensitivity) and inflammation (including innate and adaptive inflammatory control, cardiovascular protection and vascular inflammation) [5;183]. As discussed previously, obesity is an independent risk factor for CVD and T2DM. There is increasing evidence showing that the pathogenic potential of excess AT is exerted partly via the increased release of FFA and the altered production of adipose-derived proteins, which include inflammatory cytokines, acute phase proteins and chemokines [5]. As a result, the chronic low-grade inflammation characteristic of obesity, T2DM and CVD is thought to have a putative origin in AT.

1.3.2.1. Adipose tissue inflammation in obesity, cardiovascular disease and type two diabetes

AT of obese individuals and patients with T2DM or CVD is characterized by increased expression and/or secretion of several inflammatory proteins [158]. Compared to lean, healthy controls, obese individuals have been shown to have greater expression of pro-inflammatory cytokines including TNF-α [156], IL-18 [184], IL-6 [72], chemokines such as C-C motif ligand (CCL) 2 and macrophage markers CD68 and CD14 [184;185]. Whilst the exact mechanisms still need to be elucidated, it is postulated that a disruption of normal adipogenic pathways, either leading to/or resulting from excessive increases in fat cell size (adipocyte hypertrophy), due to increase TG storage in the adipocyte, leads to an infiltration of immune cells into the AT (Figure 1.4). Skurk et al. [186] measured the mRNA expression
and protein secretion of several inflammatory markers from cultured human subcutaneous adipocytes. Their results showed that secretion of pro-inflammatory proteins, including leptin, IL-6, IL-8, TNF-α, CCL2 and colony stimulating factor (CSF)-1 were positively correlated with adipocyte size, whilst secretion of the anti-inflammatory protein IL-10 was decreased.

Increased inflammation in AT has been shown to be associated with increased risk for CVD and T2DM. Apovian et al. showed that individuals with inflamed SAT (characterised by increased gene expression of inflammatory proteins and presence of crown like structures) are more hyperinsulinaemic and insulin resistant compared to BMI matched individuals without inflamed SAT [187]. In addition, in a small cross-section sample of obese women, those who were insulin resistant (n=14) had greater SAT expression of macrophage marker CD68, cytokines IL-8, IL-6 and the chemokine CCL2 compared to those who were insulin sensitive [72]. Disruption of insulin signalling pathways by inflammatory proteins was first shown by Hotamisiligil et al. [188] who demonstrated that TNF-α inhibits the insulin receptor tyrosine kinase in AT of obese insulin resistant rats (Zucker model). Subsequently, activation of several other inflammatory kinases, including IKKβ, ERK, mTOR, and S6K have been linked to IR, as reviewed in [189].

Although the adipocyte fraction of AT has been shown to be directly involved in inflammatory protein release, the increased inflammation in obesity (with the exception of adipocyte derived hormones like leptin and adiponectin) mainly originates from the non-adipose fraction (stromal vascular fraction, SVF), which contains mainly immune cells [184;190]. Evidence for immune cell infiltration into AT was first shown in studies on obese mice, showing the percentage of macrophages in a given AT depot correlated positively with adiposity and adipocyte size, and microarray profiling of AT showed 30% of proteins present
within the AT encoded for macrophagic proteins [191;192]. Similar relationships have subsequently been shown in human SAT [193], with weight loss resulting in a regression in adipocyte hypertrophy and macrophage infiltration, accompanied with an improvement in inflammatory profile [194].

It is hypothesised that macrophage recruitment and infiltration into AT during the obese state is mainly a result of chemotactic signals originating from enlarged adipocytes. Chemotactic cytokines, such as CCL2 (also known as monocyte chemoattractant protein 1) are secreted from adipocytes in both mice [195;196] and human subjects [197], with a greater release from larger adipocytes [186]. The expression of chemokines in AT along with chemokine receptors in newly-recruited macrophages are increased in AT of obese individuals [185;198].

Furthermore, results from *in vitro* experiments on mouse and human macrophages suggest that AT macrophages are present in several different activation states in the tissue. Among them are the classic M1/proinflammatory polarisation state that initiates the inflammatory reaction and the M2/anti-inflammatory state that counteracts or terminates the inflammatory process [199]. From isolated mature adipocytes and SVF cells from SAT of healthy women undergoing elective fat removal surgery, Bourlier et al. [200] showed that AT expresses both M1 and M2 macrophages, evidenced by the expression of both pro-inflammatory (eg. TNF-α, IL-6, CCL2) and anti-inflammatory (eg. IL-10) factors. However, it appears that with increasing fat mass there is a phenotypic switch from M1 to M2 state, shown by a decrease in M1 markers. Bourlier et al. also found that treatment of AT macrophages with leptin led to decreased expression of M1 markers suggesting that leptin may be involved in the modulation of the AT macrophage phenotype with increasing adiposity. These findings suggest that the function and properties of AT macrophages are shaped by the local
environment in the tissue, and the relative presence of these states characterise the inflammatory phenotype.

Figure 1.4. Inflammation in obesity and potential link to insulin resistance in adipose tissue, muscle and the liver.

With increasing weight gain there is an expansion of adipose tissue mass and adipocyte size within the adipose tissue and ectopic tissues such as muscle. With this expansion, increased free fatty acids are released into the circulation and oxygen delivery to the adipocyte is decreased. The combination of nutrient excess and hypoxia results in an inflammatory response within the adipocyte. Through increased secretion of chemokines and cytokines, and down regulation of anti-inflammatory proteins, macrophages are recruited into the adipose tissue and resident immune cells are activated. The ensuing inflammatory response activates intracellular inflammatory pathways (JNK and IKK) in neighbouring cells and distal tissues through paracrine and endocrine mechanisms. This figure is adapted from Schenk et al. [201].
1.3.2.1.1. **Depot-specific adipose tissue inflammation**

As discussed in section 1.2.2, the distribution of AT influences its metabolism and thereby disease risk, independently of the adipose tissue stores. Subsequently, it is hypothesised that the differential effects of body fat distribution, in particular that of VAT and SAT, on metabolic outcome are, in part, mediated via a differential inflammatory profile. The added risk of VAT may be due to higher expression and/or secretion of key inflammatory proteins. Alvehus et al. [76] compared the gene expression of macrophage migratory inhibitory factor (MIF), CCR2, CCL2 in whole tissue samples of VAT and SAT from a small sample (n=17) of apparently healthy women. VAT appeared as a distinct depot, with significantly greater expression of MIF and CCR2 compared to SAT. In agreement with this, Liu et al. [202] compared paired samples of abdominal VAT and SAT from obese non-diabetic women (n=11). VAT had higher expression of CCL2, IL-6 and IL-8, whilst SAT had higher expression of leptin. Similarly, Samaras et al. compared inflammatory gene expression from paired samples of abdominal VAT and SAT in diabetic patients (n=6) and healthy age- and gender-matched controls (n=10). SAT had higher expression of leptin, adiponectin and NFkB [203].

Several studies have shown an increased AT macrophage infiltration in VAT compared to SAT, possibly explaining the higher inflammatory gene expression [194;204]. Comparing gene expression in the SVF from human VAT and SAT of morbidly obese patients undergoing bariatric surgery, O’Rourke et al. [204] showed that the number of CD14 positive AT macrophages and CD3 and CD56 positive T cells were higher in VAT compared to SAT, and VAT had higher expression of inflammatory genes characteristic of M1 macrophages. Furthermore, they showed that CD14 positive AT macrophages were the dominant source of
inflammatory cytokines compared to other immune cells, having a greater relative expression of IL-6, IL-8, CCL2 and IL-10.

In contrast, lower body obesity (fat stored in the femoral-gluteal region), which is associated with a lower risk of metabolic complications [64] has been suggested to be protective due to a more favourable metabolic profile [7]. However, this has not been comprehensively studied. Only two studies have explored differences in adiponectin gene expression between abdominal SAT and gluteal (n=5) [205] or thigh (n=3) [206] SAT. Both studies showed no depot-specific differences in adiponectin mRNA levels. Importantly, to our knowledge, it is not known if gluteal and abdominal SAT differ with regards to the expression of other inflammatory genes, and this will be explored in Chapter 5 of this thesis.

### 1.3.2.1.2. Ethnic differences in adipose tissue-derived inflammation

Given the depot-specific expression inflammatory proteins, ethnic differences in circulating inflammatory markers and the relationship between abdominal AT tissue depots and metabolic risk factors might be explained, in part, by differences in depot-specific inflammation. However, only a few studies have examined ethnic differences in the inflammatory profile of AT. In a sample of morbidly obese black (n=26) and white (n=46) women undergoing bariatric surgery, Madan et al. [207] reported no ethnic differences in mRNA levels or *in vitro* release of IL-6, IL-8 and prostaglandin E₂ from VAT. In line with this, Smith et al. [208] found that the mRNA levels of abdominal SAT CD68, leptin and retinol binding protein-4 were similar between obese black (n=29) and white (n=29) American women, matched for BMI and S₁.

In summary, the relationship between inflammation within AT and CVD and T2DM risk is, in part, mediated by the distribution of the AT stores. However, whether depot-specific differences in AT inflammation can explain ethnic differences in the relationship between AT
depots and metabolic risk factors for CVD and T2DM, especially in a South African population, is unknown, and will be explored in Chapter 5 of this thesis.

1.3.3. Summary

Chronic low-grade inflammation at a circulating, genetic and AT level is associated with increased risk for CVD and T2DM. These associations are partly mediated by differences in underlying genetic variation, body fat and its distribution, SES and lifestyle factors, as well as their interactions. Notably, the impact of these factors on the relationship between inflammation at a circulating, genetic and AT level, with risk for CVD and T2DM in South African women is not known.

1.4. LITERATURE CONCLUSIONS

Obesity is highly prevalent in South African women and is associated with increased risk for CVD and T2DM. Determining what level of obesity is associated with risk is important for early identification of individuals at increased risk for CVD and T2DM. Importantly, these associations need to be ethnic and population specific, as both body composition and metabolic risk factors for CVD, differ between ethnic groups. Currently, there are no recommendations for the level of obesity that best predicts women at increased risk for CVD and T2DM in South Africa. Furthermore, results from America and the UK suggest that basic anthropometric measures are able to predict an equivalent amount of risk as more precise measures of fat distribution such as CT or MRI. This may have important implications in the South African context, where resources are often limited.

It remains unclear whether all persons at high risk for CVD and T2DM are adequately identified solely by traditional risk factor profiling, and investigation into novel risk markers,
such as the inflammatory proteins, is therefore required. Importantly, it appears that not all circulating inflammatory proteins, such as TNF-α and IL-6, are of added benefit to risk prediction in both black and white populations. In contrast, the pro-inflammatory and pro-atherogenic cytokine IL-18 has been shown to provide added risk prediction of CVD beyond traditional risk facts, and this has been shown in both black and white American women. However, it is not known if SES and behavioural/lifestyle factors known to alter inflammation, impact on the relationship between circulating IL-18 and CRP concentrations and metabolic risk factors for CVD, particularly in South African women. A better understanding of the factors affecting the relationship between inflammatory markers and metabolic risk in different populations may be important for future disease detection and prevention.

Ethnic differences shown in the distribution of cytokine gene polymorphisms suggests that black populations have an up-regulation of inflammatory risk alleles. It is however, not known if differences in the distribution of these polymorphisms impact on circulating concentrations of inflammatory markers and metabolic outcomes. In particular, the IL-18 -137G/C polymorphism has previously been shown to be associated with CVD outcome, and the distribution of the risk allele of this polymorphism has been shown to be more frequent in black compared to white American women. Whether ethnic differences in this gene polymorphism are present in South African women, and whether this polymorphism is associated with ethnic differences in circulating IL-18 concentrations and metabolic risk factors for CVD is not known.

It has been suggested that depot-specific differences in the metabolic risk profile of abdominal AT-depots are possibly related to depot-specific differences in AT inflammatory gene expression. However, it is less clear if the protective effects of peripheral AT are due to
a lower inflammatory gene expression than that of abdominal depots. A better understanding of the inflammatory profiles of these depots may provide further insight into AT distribution in relation to disease risk. Furthermore, ethnic differences in inflammation and metabolic risk factors may be explained by ethnic and depot-specific differences in AT inflammation. However, to my knowledge it is not known if there are ethnic differences in the depot-specific AT inflammation or how this is related to metabolic risk in different populations.

1.5. AIMS AND OBJECTIVES

Accordingly, the primary aim of this thesis was to investigate the ethnic-specific association between inflammation at a circulating, genetic and AT level, and obesity and its related metabolic risk factors for T2DM and CVD in apparently healthy black and white premenopausal South African women. In order to achieve these aims, I completed four studies, which are presented as separate chapters as described below:

1. The first study (Chapter 2) tested the hypothesis that central obesity thresholds relating to metabolic risk factors for CVD and T2DM are different in black and white South African women. Therefore the aim of this study was to compare the discriminative ability of central obesity measures to identify black and white South African women with or without known metabolic risk factors for CVD and T2DM, including elevated BP, elevated TG and reduced HDL-C levels, and IR.

2. The second study (Chapter 3) tested the hypothesis that circulating inflammatory markers hsCRP and IL-18 would be of added benefit than central obesity (measured by waist circumference) for identifying black and white women at increased risk for CVD and T2DM. Therefore the aim of the second study was to investigate the association between circulating inflammatory markers, namely IL-18 and hsCRP with metabolic risk factors for CVD and T2DM in black and white South African women.
taking into account socioeconomic and behavioural/lifestyle factors known to influence both inflammation and CVD risk.

3. The third study (Chapter 4) tested the hypothesis that ethnic difference in circulating IL-18 levels and metabolic risk factors could be explained, in part, by ethnic differences in the distribution of the IL-18 -137G/C polymorphism. Therefore, the aim of the third study was to investigate whether ethnic variation in the distribution of the -137 G/C polymorphism within the IL-18 gene is associated with differences in serum IL-18 levels and metabolic risk factors for CVD in black and white South African women.

4. In the final chapter of this thesis (Chapter 5), three hypotheses were tested: a) SAT inflammatory gene expression is greater in black compared to white South African women; b) SAT inflammatory gene expression is greater in the abdominal compared to the gluteal depot; and c) a depot-specific inflammatory profile is associated with differential $S_I$ in black and white South African women. Therefore, the aim of the fourth study was to determine the ethnic- and depot-specific associations between AT inflammatory gene and protein expression with insulin sensitivity ($S_I$), a major risk factor for T2DM, in black and white South African women.
1.6. **THESIS OUTLINE**

An outline of the subject recruitment and sample sizes for each study are summarised in Figure 1.5. In total, the main cohort consisted of 241 black and 188 white South African women. However, not all measurements (such as circulating inflammatory markers, IL-18 genotyping, and fat biopsies) were performed on the whole cohort. As a result, sample sizes varied according to the different studies. The methods and statistical analysis are explained individually for each study in the relevant chapters. Importantly, the age and waist circumference (as a measure of abdominal obesity) of those subjects included in the individual studies did not differ significantly to the whole cohort.

![Figure 1.5. Thesis outline. Schematic showing the sample size of the whole thesis cohort and the sample sizes of the individual studies. CT, computer-tomography; DXA, dual x-ray absorptiometry; SES, socioeconomic status; hscRP, high sensitivity C-reactive protein; IL-18, interleukin-18; FSIGT, frequently sampled intravenous glucose tolerance test; DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue.](image-url)
CHAPTER TWO

ETHNIC-SPECIFIC OBESITY THRESHOLDS ASSOCIATED WITH METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASE AND TYPE TWO DIABETES IN SOUTH AFRICAN WOMEN

Data from this chapter have been published in part in: Evans J, Micklesfield LK, Jennings C, Levitt NS, Lamber EV, Olsson T, Goedecke JH. Diagnostic ability of obesity measures to identify metabolic risk in South African women. 2011 April Metabolic Syndrome and Related Disorders (In press).

2.1. ABSTRACT

Thresholds of VAT, and its anthropometric proxies including WC, WHR and WHtR, aid in the early identification of individuals at increased risk for CVD and T2DM. Importantly, ethnicity alters the relationships between body fat distribution and metabolic risk factors for T2DM and CVD. However, the effectiveness of central obesity measures for identifying women with or without metabolic risk factors for CVD and T2DM has not been explored in a South African population. The aim of the first study of this thesis was to compare the extent to which measures of central obesity can optimally differentiate black and white South African women with or without metabolic risk variables for CVD and T2DM, including elevated BP, dyslipidaemia and IR.

Body composition, including weight, height, WC, hip circumference, and computer tomography (CT)-derived VAT, BP, fasting insulin, glucose and lipids were measured in 241 black and 188 white South African women, with no known disease and not taking medications known to alter metabolism or inflammation. Receiver operator characteristic
(ROC) curve analyses were performed to determine the discriminative ability of WC, WHtR and VAT to identify subjects above metabolic risk thresholds. WC, WHtR and VAT were significant discriminators of all metabolic risk variables in both ethnic groups (P<0.05), however, for all metabolic risk variables the ROC area under the curve (AUC) was greater in white compared to black women (P<0.01). Compared to WC, the ROC AUC for VAT was greater for identifying elevated TG concentrations in white women (P<0.05), and elevated BP in black women (P<0.05). Conversely, the ROC AUC for WC was greater than VAT for identifying IR in both ethnicities (P<0.05). WC and VAT thresholds were lower in black compared to white women, whereas WHtR thresholds varied the least between ethnicities.

In conclusion, all central obesity measures were better at identifying elevated BP, dyslipidaemia and IR in white compared to black women. These findings highlight the need to explore other factors contributing to increased metabolic risk in black South African women. WHtR thresholds varied less between ethnicities than WC and VAT, and may therefore be more useful for ethnic comparisons of risk. Long-term prospective studies are required to examine whether these relationships are similar in groups with known disease or pathophysiology.

2.2. INTRODUCTION

Central obesity, particularly the accumulation of VAT, is linked to the development of several metabolic diseases, including hypertension, dyslipidaemia and IR [6;209]. Thresholds of WC, as a proxy measure for VAT, aid in the early identification of individuals who develop these diseases [81]. However, ethnic differences exist in the relationship between WC and VAT and in the relationship between VAT and metabolic risk variables. With increasing WC black women accumulate less VAT than white women [53] and present with
lower TG and TC concentrations than white women, yet paradoxically the former are more insulin resistant [10;12;210].

Although many studies have examined the validity of anthropometric measures as proxies for central obesity, few have taken it one step further and examined the ability of central obesity measures, both CT-derived VAT and anthropometric measures, to identify metabolic risk factors, particularly in Southern African populations [211;212]. In addition, recent studies in Europeans and Asians have shown that WHtR and WHR have a greater ability than WC alone to identify patients with hypertension, dyslipidaemia and T2DM [83;213]. This may be of particular relevance to the South African context, since black South African women are shorter and have greater peripheral fat than white South African women [11]. Similar to WC and VAT, the ethnic-specific associations between WHtR and WHR with known metabolic risk factors remains to be explored in a South African population.

Therefore, this first study tested the hypothesis that central obesity thresholds relating to metabolic risk factors for CVD and T2DM are different in black and white South African women. Specifically, the aims of this study were to compare the level of obesity (WC, WHR, WHtR and VAT) that identifies black and white South African women with or without elevated BP, dyslipidaemia and IR, and to determine which measure of obesity has the strongest association with metabolic risk thresholds.

2.3. METHODS

2.3.1. Subjects

The study population consisted of a convenience sample of 241 black and 188 white apparently healthy premenopausal South African women. Ethnicity was self-reported. Subjects were recruited from church groups, community centres and universities, and
included in the study if they were 18 to 45 years old, with no known disease, and not taking any chronic medications for the management of obesity, T2DM, dyslipidaemia, hypertension or inflammatory disorders, were not currently pregnant, lactating or post-menopausal. The Health Sciences Research Ethics Committee of the University of Cape Town gave the study ethics approval. Written informed consent was obtained from all subjects.

2.3.2. Body composition assessment

Basic anthropometric measurements including weight (in light clothing without shoes), height, waist (at level of the umbilicus; WC) and hip (largest gluteal area) circumference were taken. WC was divided by hip circumference to calculate WHR, and by height (cm) to calculate WHtR. CT-derived abdominal VAT area (Toshiba X-press Helical Scanner, Japan) was measured by a single slice scan at the level of L4-L5 lumbar vertebrae in 183 black and 143 white women.

2.3.3. Blood Pressure

After at least 5 minutes of seated rest, BP was measured 3 times at 1-minute intervals using an appropriate sized cuff and an automated BP monitor (Omron 711, Omron Health Care, Hamburg, Germany). An average of the last 2 readings was used in the analyses.

2.3.4. Blood sampling and analysis

Blood samples were drawn after an overnight fast (10-12 hours) for the determination of plasma glucose and serum insulin, TC, HDL-C and TG concentrations. Fasting plasma glucose concentrations were determined using the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA). Serum insulin concentrations were determined by a Microparticle Enzyme Immunoassay (MEIA) (AxSym Insulin Kit, Abbot, IL, USA). The intra- and interassay coefficients of variation for plasma glucose and serum
insulin concentrations were 0.6 and 2.5%, and 4.5 and 12.2%, respectively. Serum lipids were analyzed using the Roche Modular auto analyzer and enzymatic colorimetric assays were used to analyze TC, TG, and HDL-C concentrations.

2.3.5. Insulin Resistance (IR)

The homeostasis model of insulin resistance (HOMA-IR), an estimation of basal insulin sensitivity first described by Matthews et al. [214], was used to estimate insulin resistance by applying the following formula: HOMA-IR = fasting glucose (mmol/L) \times fasting insulin (mU/L) / 22.5.

2.3.6. Metabolic Risk factors

Thresholds for elevated BP (systolic BP≥130mmHg and/or diastolic BP≥85 mmHg), elevated TG concentrations (TG≥1.7mmol/L) and HDL-C concentrations (HDL-C≤1.29mmol/L) were based on the definition by NCEP ATPIII criteria [31]. Thresholds for TC/HDL-C ratio and HOMA-IR were based on the upper tertile for the whole cohort (TC/HDL-C≥3.27; HOMA-IR≥2.09) and comparable with those reported previously [215-217].

2.3.7. Statistical Analysis

Normally distributed data are presented as means ± SD with ranges in parentheses. Non-normally distributed parameters were logarithmically transformed for parametric analysis and are presented as geometric means with 95% confidence intervals. Analysis of covariance (ANCOVA), adjusting for age, was used to compare means for black and white women. Pearson correlations were used to explore associations between central obesity measures with metabolic risk factors. Chi-squared tests were used to compare the percentage of black and white women above the metabolic risk factor thresholds. ROC curve analyses were performed to determine the discriminative ability of WC, WHtR, WHR and VAT for subjects.
with IR, elevated BP, elevated TG, elevated TC/HDL-C ratio and reduced HDL-C concentrations. A ROC AUC <0.60 was considered to have poor discriminative ability [218]. In separate analyses for black and white women, a non-parametric test was used to compare the ROC AUC for VAT, WHtR and WHR of each metabolic risk variable with that obtained using WC [219]. The Youden index (J) [maximum (specificity-sensitivity)-1] [220] was calculated to explore thresholds for central obesity measures as determinants of metabolic risk variables. Data were analyzed with STATISTICA version 9 (StatSoft Inc., Tulsa, OK, USA).

2.4. RESULTS

2.4.1. Subject characteristics

White women were significantly older than black women, and as a result all subsequent analyses included age as a covariate (Table 2.1). There were no ethnic differences in body weight; however the white women were significantly taller, and had a lower BMI and WHtR than the black women. There were no ethnic differences in WC or WHR; however, black women had less VAT than white women.

2.4.2. Metabolic outcomes

Adjusting for age, there were no differences in systolic and diastolic BP between black and white women (Table 2.2). Fasting glucose concentrations were within normal ranges for both black and white women. However white women had higher mean fasting glucose and lower fasting insulin concentrations than black women, but there was no ethnic difference in HOMA-IR. White women had significantly higher TG, TC and HDL-C concentrations, but a similar TC/HDL-C ratio than black women.
Table 2.1. Subject characteristics and body composition of black and white South African women

<table>
<thead>
<tr>
<th></th>
<th>Black Women (n=241)</th>
<th>White Women (n=188)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27 ± 7 (18-45)</td>
<td>31 ± 8 (18-45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.6 ± 0.1 (1.5-1.8)</td>
<td>1.7 ± 0.1 (1.5-1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.2 ± 20.5 (43.0-140.1)</td>
<td>77.4 ± 20.1 (49.6-140.1)</td>
<td>0.366</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>29.7 ± 7.8 (17.7-53.5)</td>
<td>27.8 ± 7.0 (19.4-48.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>89.7 ± 18.2 (59.5-138.5)</td>
<td>90.2 ± 15.9 (65.0-138.2)</td>
<td>0.150</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>112.2 ± 15.8 (60.5-166.0)</td>
<td>110.8 ± 16.6 (88.0-165.0)</td>
<td>0.273</td>
</tr>
<tr>
<td>WHR</td>
<td>0.80 ± 0.08 (0.57-1.03)</td>
<td>0.81 ± 0.06 (0.54-0.99)</td>
<td>0.321</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.56 ± 0.12 (0.37-0.84)</td>
<td>0.54 ± 0.10 (0.38-0.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VAT (cm$^2$)</td>
<td>65.0 (44.8-94.3)</td>
<td>81.6 (59.8-126.4)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Values are unadjusted means ± standard deviation, with ranges in parentheses. VAT had a skewed distribution, therefore the median and interquartile range is displayed in parenthesis. All P-values adjusted for age. BMI, body mass index; WC, waist circumference; WHR, waist-to-hip-ratio; WHtR, waist-to-weight ratio; VAT, visceral adipose tissue.
Table 2.2. Metabolic outcomes of black and white South African women

<table>
<thead>
<tr>
<th></th>
<th>Black Women (n=241)</th>
<th>White women (n=188)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (range)</td>
<td>Mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110.5 ±14.3 (80.5-160.0)</td>
<td>110.8 ± 11.5 (80.5-156.0)</td>
<td>0.090</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74.9 ± 10.0 (47.5-103.5)</td>
<td>76.2 ± 10.5 (51.5-115.5)</td>
<td>0.439</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.4 ± 0.6 (3.0-5.5)</td>
<td>4.7 ± 0.4 (3.7-5.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>11.2 ± 7.7 (1.7-45.6)</td>
<td>8.6 ± 5.4 (1.5-29.8)</td>
<td>0.021</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.3 ± 1.7 (0.2-10.9)</td>
<td>1.4 (0.9-2.1)</td>
<td>0.145</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.7 ± 0.4 (0.3-2.3)</td>
<td>0.8 (0.6-1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>3.9 ± 0.9 (2.2-7.7)</td>
<td>4.7 ± 0.9 (3.2-8.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.3 ± 0.4 (0.6-2.5)</td>
<td>1.6 ± 0.4 (0.7-2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>2.9 (2.4-3.6)</td>
<td>2.7 (2.3-3.4)</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Values are unadjusted means ± standard deviation, with ranges in parentheses. HOMA-IR, TG and TC/HDL-C ratio had a skewed distribution, therefore the median and interquartile ranges are displayed in parenthesis. All P-values adjusted for age. BP, blood pressure; HOMA-IR, homeostasis model for insulin resistance; TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TC/HDL-C, total cholesterol-high density lipoprotein cholesterol ratio.

The percentage of subjects above each metabolic risk threshold is shown in Figure 2.1. There were no differences between ethnic groups in the proportion of women above the risk threshold for elevated systolic and diastolic BP (p=0.14 and p=0.21, respectively) and HOMA-IR (P=0.07). A significantly higher proportion of white women had elevated TG concentrations compared to the black women (P<0.01). Conversely, a higher proportion of
black women had reduced HDL-C concentrations compared to white women (P<0.001). As the very high proportion (over 45%) of black women with low HDL-C concentrations is most likely related to low TC concentrations in this population, we analysed TC/HDL-C ratio > 3.27 (upper tertile for whole cohort), which has been used previously as a diagnostic criteria for the metabolic syndrome [221]. There was no significant ethnic difference in the proportion of women with a TC/HDL-C ratio > 3.27 (P=0.294).

Figure 2.1. Percentage of black and white South African women presenting with metabolic risk factors. ■ Black women; ○ White women. SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; TC/HDL-C, total cholesterol-high density lipoprotein cholesterol ratio; HOMA-IR, homeostasis model of insulin resistance. * P<0.01.

2.4.3. Relationship between central obesity with metabolic risk factors

Correlations between obesity measures and metabolic risk factors for black and white women are presented in Table 2.3. In white women, WC, WHtR and VAT correlated significantly with all metabolic variables (P<0.001). Similarly, in black women, WC, WHtR and VAT
correlated significantly with all metabolic variables except TC concentrations. WHR correlated with all metabolic variables except for systolic and diastolic BP in white women and systolic BP and TC in black women.

2.4.4. Central obesity thresholds for metabolic risk factors

ROC AUC, sensitivities and specificities of optimal thresholds (based on the Youden index) of WC, WHtR and VAT for metabolic risk variables are presented in Table 2.4. WHR was a poor measure for discriminating between black and white women with or without metabolic risk (ROC AUC’s<0.60) and hence the data are not shown. Similarly, none of the central obesity measures were significant discriminators for elevated TG in black woman (ROC AUC<0.60), as a result no thresholds were established in black women.

For elevated blood pressure, dyslipidaemia and insulin resistance, the ROC AUC of WC, WHtR and VAT were significantly greater in white compared to black women (P<0.05). There were no differences in ROC AUC between WC and WHtR for all metabolic risk variables in both black and white women (P>0.05). In white women, compared to the AUC for WC, there was no difference in the VAT AUC for elevated BP and reduced HDL-C concentrations; however the VAT AUC was significantly greater for elevated TG and TC/HDL-C ratio and significantly smaller for HOMA-IR. In black women, the AUC for VAT was significantly greater than WC for elevated BP, but significantly lower for HOMA-IR, HDL-C concentrations and TC/HDL-C ratio.

The Youden index (J), a measure of the maximum sensitivity (the fraction of women below the risk variables threshold correctly identified by the test) and specificity (the fraction of women above the risk variable threshold correctly identified by the test) of a variable, was used to explore thresholds for WC, WHtR, WHR and VAT in relation to the distribution of
IR, elevated BP, TG concentrations, TC/HDL-C ratio and reduced HDL-C concentrations in black and white women (Table 2.4). When comparing ethnicities, WC thresholds were similar for HOMA-IR and TC/HDL-C ratio, but lower for BP and HDL-C concentrations in black compared to white women. VAT thresholds were also lower for HOMA-IR, HDL-C concentrations and TC/HDL-C ratio in black compared to white women, but were similar for BP. Conversely, WHtR thresholds were similar for all metabolic risk variables between black and white women. The sensitivities and specificities of these thresholds also differed according to ethnicity. Compared to black women, white women had higher sensitivities for optimal thresholds for elevated BP, HOMA-IR and TC/HDL-C ratio, and lower sensitivities for thresholds for reduced HDL-C. The specificities for all thresholds were lower in black compared to white women for all metabolic risk factors.

Thresholds and their sensitivities and specificities also varied across metabolic risk variables. In black women, the lowest WC, WHtR and VAT thresholds were for identifying reduced HDL-C concentrations. Conversely, the highest WC and WHtR thresholds in black women were for HOMA-IR and TC/HDL-C ratio, and highest VAT threshold was for elevated BP. In white women, lower WC thresholds were observed for elevated BP, elevated TG and TC/HDL-C ratio, whereas the highest thresholds of all central obesity measures were observed for reduced HDL-C.
Table 2.3. Pearson correlation coefficients (r) between central obesity measures and metabolic outcomes in black and white South African women

<table>
<thead>
<tr>
<th>WC (cm)</th>
<th>SBP</th>
<th>DBP</th>
<th>Glucose</th>
<th>Insulin</th>
<th>HOMA-IR</th>
<th>TG</th>
<th>TC</th>
<th>HDL-C</th>
<th>TC/HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>0.22*</td>
<td>0.42*</td>
<td>0.27*</td>
<td>0.47*</td>
<td>0.47*</td>
<td>0.41*</td>
<td>0.04</td>
<td>-0.37*</td>
<td>0.34*</td>
</tr>
<tr>
<td>White</td>
<td>0.24*</td>
<td>0.43*</td>
<td>0.27*</td>
<td>0.63*</td>
<td>0.63*</td>
<td>0.34*</td>
<td>0.29*</td>
<td>-0.40*</td>
<td>0.51*</td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>0.29*</td>
<td>0.23*</td>
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<td>0.38*</td>
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<td>0.25*</td>
<td>0.35*</td>
<td>0.39*</td>
<td>0.21*</td>
<td>0.21*</td>
<td>-0.32*</td>
<td>0.38*</td>
</tr>
<tr>
<td>WHtR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Black</td>
<td>0.23*</td>
<td>0.43*</td>
<td>0.28*</td>
<td>0.48*</td>
<td>0.48*</td>
<td>0.40*</td>
<td>0.05</td>
<td>-0.36*</td>
<td>0.35*</td>
</tr>
<tr>
<td>White</td>
<td>0.23*</td>
<td>0.44*</td>
<td>0.28*</td>
<td>0.63*</td>
<td>0.64*</td>
<td>0.36*</td>
<td>0.31*</td>
<td>-0.40*</td>
<td>0.51*</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>0.30*</td>
<td>0.42*</td>
<td>0.35*</td>
<td>0.40*</td>
<td>0.44*</td>
<td>0.28*</td>
<td>0.01</td>
<td>-0.29*</td>
<td>0.23*</td>
</tr>
<tr>
<td>White</td>
<td>0.29*</td>
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<td>0.32*</td>
<td>0.55*</td>
<td>0.56*</td>
<td>0.45*</td>
<td>0.39*</td>
<td>-0.46*</td>
<td>0.63*</td>
</tr>
</tbody>
</table>

WC, waist circumference; WHR, waist-hip ratio; WHtR, waist-height ratio; VAT, visceral adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model of insulin resistance; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol. TC/HDL-C, total cholesterol high density lipoprotein cholesterol ratio. *P<0.01
Table 2.4. WC, WHtR and VAT thresholds for metabolic risk variables in black and white South African women.

<table>
<thead>
<tr>
<th></th>
<th>Black women</th>
<th></th>
<th>White women</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ROC AUC ± SE</td>
<td>P value (vs. WC)</td>
<td>Optimal Threshold</td>
<td>Optimal Sensitivity (%)</td>
</tr>
<tr>
<td><strong>Systolic BP ≥ 130mmHg and/or Diastolic BP ≥ 85mmHg</strong></td>
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<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>0.70 ± 0.05</td>
<td>-</td>
<td>&gt; 80</td>
<td>88.6</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.71 ± 0.04</td>
<td>0.675</td>
<td>&gt; 0.57</td>
<td>73.1</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>0.76 ± 0.06</td>
<td>0.028</td>
<td>&gt; 107</td>
<td>83.3</td>
</tr>
<tr>
<td><strong>HOMA-IR ≥ 2.09 (upper tertile for whole group)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>0.76 ± 0.03</td>
<td>-</td>
<td>&gt; 94</td>
<td>66.7</td>
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<tr>
<td>WHtR</td>
<td>0.77 ± 0.03</td>
<td>0.720</td>
<td>&gt; 0.62</td>
<td>59.7</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>0.72 ± 0.04</td>
<td>0.033</td>
<td>&gt; 78</td>
<td>56.6</td>
</tr>
<tr>
<td><strong>HDL-C ≤ 1.29 mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>0.66 ± 0.03</td>
<td>-</td>
<td>&gt; 77</td>
<td>83.1</td>
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<tr>
<td>WHtR</td>
<td>0.67 ± 0.03</td>
<td>0.882</td>
<td>&gt; 0.47</td>
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<tr>
<td>VAT (cm²)</td>
<td>0.64 ± 0.04</td>
<td>0.039</td>
<td>&gt; 48</td>
<td>82.7</td>
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</table>
## Table 2.4. Continued.

<table>
<thead>
<tr>
<th></th>
<th><strong>Black women</strong></th>
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<th><strong>White women</strong></th>
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</thead>
<tbody>
<tr>
<td></td>
<td><strong>ROC AUC ± SE</strong></td>
<td><strong>P value (vs. WC)</strong></td>
<td><strong>Optimal</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Threshold* (%)</td>
</tr>
<tr>
<td>TC/HDL-C ≥ 3.27 (upper tertile for whole group)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>0.70 ± 0.04</td>
<td>-</td>
<td>&gt; 91</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.70 ± 0.04</td>
<td>0.771</td>
<td>&gt; 0.54</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>0.66 ± 0.04</td>
<td>0.048</td>
<td>&gt; 74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG ≥ 1.7mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>0.59 ± 0.06</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>WHtR</td>
<td>0.59 ± 0.06</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>0.59 ± 0.07</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

ROC, receiver operator characteristic; AUC, area under the curve; SE, standard error; WC, waist circumference; WHtR, waist-to-height ratio; VAT, visceral adipose tissue; BP, blood pressure; HOMA-IR, homeostasis model of insulin resistance; HDL-C, high density lipoprotein cholesterol; TC/HDL-C, total cholesterol-high density lipoprotein cholesterol ratio; TG, triglyceride. * J = (sensitivity + specificity)-1.
2.5. DISCUSSION

The main finding of this chapter was that all measures of central obesity (WC, WHtR and VAT) were better indicators of metabolic risk (elevated BP, dyslipidaemia and IR) in white compared to black South African women. This is in line with Stevens et al., who have previously shown that BMI, WC and WHR had a greater ability to predict diabetes in white compared to black American women [81]. Further, a recent study comparing French and Cameroonian nationals showed for the same increase in WC, French individuals had a greater increase in metabolic risk [212]. These findings may suggest that factors other than body composition, such as inflammation or environmental factors, contribute to an increased risk of metabolic disease in black women.

A second important finding is that there were significant differences in the discriminative ability between VAT and anthropometric measures for identifying black and white South African women above metabolic risk thresholds. Although several studies have compared the discriminative ability between anthropometric measures for identifying individuals above metabolic risk thresholds, few have compared anthropometrics to a direct measurement of VAT [78;88]. Notably, we found that WC was better than VAT for identifying black and white women with IR (upper tertile of HOMA-IR). Although WC is normally used as a proxy measure for VAT, it reflects both VAT and abdominal SAT. Further, we, and others, have shown a similar magnitude of association between IR and VAT and SAT in both black and white women [12;58;222]. These findings suggest that central accumulation of fat, whether it is visceral or subcutaneous, is important in the development of IR.

Conversely, VAT was significantly better than WC for identifying elevated TG concentrations and the upper tertile of TC/HDL-C ratio in white women, and for identifying elevated BP in black women. Our data support the findings of Vega et al. who have shown in a combined analysis of black and white American women that VAT had a predictive power
for TG/HDL-C ratio and systolic BP beyond that of total fat and abdominal SAT [223]. VAT is thought to have a greater contribution than SAT to increased metabolic risk through direct release of fatty acids into the hepatic portal system [49], thereby explaining the greater ability of VAT to identify white women with elevated TG concentrations and TC/HDL-C ratio. Furthermore, VAT is reported to be associated with increased sympathetic nervous system activity [224] and higher expression of angiotensinogen [225], both of which are associated with elevations in BP.

Despite WC and VAT differing significantly in their ability to discriminate between metabolic risk variables, the physiological relevance of this difference is unknown as the ROC AUC’s differed by a maximum of only eight percent. Given the high cost and risk associated with exposure to radiation with CT, and considering that WC and WHtR can be used to identify an equivalent amount of metabolic risk, we recommend using these simple anthropometric measures over VAT for identifying metabolic risk variables, particularly in developing countries with limited financial and human resources such as South Africa.

In agreement with my original hypothesis, the results also show that optimal central obesity thresholds were different across ethnicities. Recently, several reviews have highlighted the need for ethnic-specific thresholds of obesity measures to identify individuals at increased metabolic risk, and that there is currently insufficient evidence to do so in black sub-Saharan populations [211;226]. In general, WC and VAT thresholds were lower in black compared to white South African women. Notably, although adjusting WC for height did not affect the ability of the measure to identify risk, it did narrow the difference in WHtR thresholds between black and white women. In a cross-sectional study of central obesity thresholds across eight different ethnicities, Diaz et al. also showed that WHtR thresholds were narrower between ethnicities compared to those for WC and WHR [80]. Therefore, similar WHtR
thresholds, specific to the risk criterion being examined, could be used in studies where ethnic comparisons are made.

Cameron et al. have highlighted that defining obesity thresholds using the Youden index, even in longitudinal studies, is dependent on the prevalence of obesity in that population, at a given point in time [227]. As the prevalence of obesity increases in a population, so does the ‘optimal’ threshold derived from the Youden index. In line with this, Stevens et al. [81] have shown that the distribution of the central obesity measure impacts on the sensitivity and specificity of the measure, and hence the optimal threshold. Although the distribution of central obesity measures, in particular VAT, was different in the black and white women in this study, this did not seem to impact on the sensitivity and specificity of the measure as sensitivity was not consistently higher in the white compared to the black women, in whom the VAT distribution was skewed to the right. Long-term prospective studies are required to examine whether individuals from these ethnic groups who exceed the central obesity thresholds develop CVD and diabetes and/or whether these relationships are similar in groups with known disease or pathophysiology.

As the relationship between obesity and increased metabolic risk is continuous, creating specific thresholds may be arbitrary and only appropriate to the setting in which it is used [81]. Thresholds may vary according to the prevalence of the risk factor in each ethnic group, with a higher prevalence of a risk factor relating to a lower obesity threshold. For example, over 45% of black women had HDL-C concentrations below 1.29 mmol/L, and this corresponded to the lowest WC and VAT thresholds in this group. The low HDL-C concentrations in the black women may possibly reflect differences in lipid metabolism between ethnic groups [228]. Using a ratio of TC/HDL-C, the percentage of women above the threshold for risk (upper tertile) was reduced (compared to HDL-C) and the optimal thresholds were higher. Similarly in white women, compared to reduced HDL-C
concentrations, a higher percentage of women fell above the upper tertile of the TC/HDL-C ratio, and corresponding thresholds were reduced.

Notably, it is not known whether the risk factor thresholds used in the present study translate to equal risk in black and white women. Although this data is unavailable, the percentage of women above metabolic risk thresholds was comparable to other cross-sectional study cohorts from South Africa [3;13]. Due to the lower percentage of black women with elevated TG, we were unable to define meaningful central obesity thresholds for elevated TG in black women. It has previously been suggested that the thresholds for TG that relate to risk for CVD in black South Africans should be lowered [13], or that elevated TG is not associated with increased metabolic risk in black populations [42]. Supporting this, Kalk et al. have shown that the prevalence of elevated TG in diabetic black South Africans was also lower than that of white South Africans [2]. Importantly, prospective studies are required to determine the risk factors associated with CVD and T2DM in both ethnic groups.

In conclusion, in our sample of black and white South African women, all central obesity measures were better at identifying elevated BP, dyslipidaemia and IR in white compared to black women. These findings highlight the need to explore factors other than obesity that contribute to increased metabolic risk in black South African women. Overall, due to the cost, access and radiation exposure, CT is not recommended above the use of simple anthropometric measures for the determination of metabolic risk. As thresholds for WC varied more than WHtR between ethnicities, WHtR may be more useful for ethnic comparisons of thresholds for risk. Prospective studies including both black and white South African women are required to establish the level of obesity and other metabolic risk factors associated with the development of T2DM and CVD.
CHAPTER THREE

RELATIONSHIP BETWEEN CIRCULATING INFLAMMATORY MARKERS IL-18 AND hsCRP WITH METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASE AND TYPE TWO DIABETES IN SOUTH AFRICAN WOMEN

3.1. ABSTRACT

Chronic low-grade inflammation, characterized by altered circulating concentrations of inflammatory proteins including IL-18 and hsCRP, has been suggested as a key link between obesity, IR, T2DM and CVD. Several environmental factors including SES and behavioural/lifestyle factors such as smoking, alcohol, contraception and sedentary behaviours have previously been shown to influence circulating inflammatory marker concentrations. To my knowledge the impact of SES and behavioural/lifestyle factors on the relationship between circulating inflammatory markers and metabolic risk factors for CVD has not been explored in a South African population. Therefore the aims of the second study of this thesis were to investigate the association between SES, health behaviour/lifestyle factors and central obesity and circulating concentrations of IL-18 and hsCRP, and to determine the ethnic-specific associations between circulating concentrations of IL-18 and hsCRP and metabolic risk factors for CVD in black and white South African women, taking into account the impact of obesity, SES and behavioural/lifestyle factors known to influence both inflammation and CVD risk.

Central obesity (WC), BP, IR (as estimated from homeostasis model of insulin resistance, HOMA-IR), fasting lipids, level of SES (assessed by education, asset index and housing density), behavioural/lifestyle variables (smoking status, alcohol intake, contraceptive use and physical activity), and circulating concentrations of IL-18 and hsCRP were measured in 223 white and 177 black South African women. ANCOVA, adjusting for age and central
obesity (WC) was used to compare circulating concentrations of IL-18 and hsCRP between ethnic groups and across categories of SES and health behaviours. Multiple regression analysis with a forward selection procedure was performed to determine whether inflammatory markers are associated with metabolic risk factors independent of SES, behavioural/lifestyle variables and obesity (WC), in separate analysis for black and white women. Black women had significantly higher circulating concentrations of IL-18 compared to white women, independent of WC and age (P<0.01), however there was no ethnic difference in circulating hsCRP concentrations (P=0.32). Obesity, lower level of education, smoking and contraceptive use were associated with higher circulating concentrations of IL-18 and hsCRP (P<0.05), which in turn were associated with metabolic risk factors for CVD and T2DM in both black and white women. The associations between IL-18 (in black women only) and hsCRP (black and white women) and metabolic risk factors were independent of SES, contraception and smoking, but not WC.

In conclusion, SES, behavioural/lifestyle factors and central obesity were associated with increased circulating concentrations of IL-18 and hsCRP in both black and white South African women. The association between these inflammatory markers and risk was mainly mediated by the effects of adiposity, suggesting that the measurement of circulating inflammatory proteins provides no added benefit to WC for identifying women at increased risk for CVD and T2DM. Furthermore, these results support the hypothesis that AT may be involved in the early stages of the inflammatory response associated with CVD and T2DM.

3.2. INTRODUCTION

It is now well established that a state of chronic low-grade inflammation is present in obesity, T2DM and CVD [95]. Increased circulating concentrations of inflammatory proteins, such as proinflammatory cytokines, acute phase proteins, chemokines and adipocyte hormones, are
present in obese individuals [98], and in patients with T2DM and CVD [99]. Subsequently, markers of inflammation have shown potential for improved disease risk prediction, and in some cases, have been investigated as potential therapeutic targets for clinical intervention [103]. Circulating inflammatory proteins investigated as potential risk markers include the acute phase protein hsCRP and the pro-inflammatory cytokines IL-18, IL-6 and TNF-α. Increased circulating concentrations of these proteins have been shown to be associated with a number of metabolic risk factors for CVD and T2DM, including central obesity, dyslipidaemia, hypertension and IR [97;108;109;127;129;141;142;155].

Notably, circulating concentrations of hsCRP, IL-6 and TNF-α have been shown to be influenced by several environmental factors, including SES and associated negative health behaviours, such as smoking, alcohol abuse, poor dietary patterns, sedentary behaviour and contraceptive use [104;110;229], which in turn are known to influence CVD risk and outcome [90]. To my knowledge, IL-18 has not been explored in this context, nor has the impact of SES and behavioural/lifestyle factors on hsCRP been investigated in a South African population. Furthermore, in the case of hsCRP [122] and IL-6 [153], studies in America have shown that increased circulating concentrations of these proteins provided no improved prediction for T2DM and CVD beyond that of traditional risk factors, in particular SES and obesity. In contrast, data from the Women’s Health study [138] and Nurses’ Health study [139] have shown that high IL-18 concentrations were predictive of future CVD events and T2DM incidence, respectively, independent of possible mediating/confounding factors. However, neither of these studies investigated whether these findings were consistent across different ethnic groups. This may be of particular relevance as ethnic differences in disease risk, level of SES and circulating inflammatory markers have been shown to exist. Furthermore, based on the results from Chapter 2 of this thesis, central obesity measures explained a smaller proportion of risk in black compared to white women. Therefore, I
hypothesise that circulating inflammatory markers hsCRP and IL-18 would be of added benefit for identifying black and white women at increased risk for CVD and T2DM.

Accordingly, the aims of this chapter were to investigate the association between SES, health behaviour/lifestyle factors and obesity with circulating concentrations of IL-18 and hsCRP, and to determine the ethnic-specific associations between circulating concentrations of IL-18 and hsCRP with metabolic risk factors for CVD in black and white South Africans women, taking into account central obesity, socioeconomic and behavioural/lifestyle factors known to influence both inflammation and CVD risk.

3.3. METHODS

3.3.1. Subjects

This cross-sectional study consisted of a convenience sample of 223 black and 177 white apparently healthy premenopausal South African women. Further information on the inclusion and exclusion criteria is provided in the methods section of Chapter 2. The Faculty of Health Sciences Research Ethics Committee of the University of Cape Town gave the study ethics approval. Written informed consent was obtained from all subjects prior to participation.

3.3.2. Socioeconomic status and health behaviour/lifestyle measures

A questionnaire was administered that included measures of SES and behaviour/lifestyle factors. Three indicators of SES were assessed: education, asset index and housing density. Categories for education were none, grade 1-7, Grade 8-11, completed high school and post-high school education. An asset index score was based on 16 items reflecting individual and household wealth and resources. This included indoor access to running water and/or flushing toilet, electricity in the home, ownership of a TV, radio, motor vehicle, fridge, stove/oven,
washing machine, telephone, video machine, microwave, computer, cellular telephone, MNET or DSTV. Housing density was defined as the number of persons per room living in the household. For the purpose of this analysis, both asset index and housing density were categorized into quintiles.

Health behaviour variables included: Smoking status, categorized as smoker, ex-smoker or non-smoker; Alcohol consumption based on average weekly intake of alcohol and categorized as non drinker, ≤ 1 drink/day, or >1drink/day; physical activity (PA) levels (minutes/week of moderate intensity activity), were measured using the Global Physical Activity Questionnaire (GPAQ) [230], and based on a cut-point of 150min/week and categorized into Active Total PA (≥ 150 min/week), and Sedentary Total PA (< 150 min/week), as previously suggested [231]. Contraceptive use was defined as none, oral contraceptives or injectable contraceptives.

3.3.3. Body composition and metabolic outcomes

Central obesity (WC), BP, fasting plasma concentrations of glucose, and serum concentrations of insulin, TG, TC and HDL-C were measured. HOMA-IR was estimated from fasting insulin and glucose concentrations as previously described [214]. The measurements and biochemical analysis of these variables are described in detail in the methods section of Chapter 2.

3.3.4. Inflammatory markers

Serum concentrations of hsCRP (Immun Diagnostik AG, Bensheim, Germany) and IL-18 (Biosource, Nivelles, Belgium) were analyzed using commercially available ELISA kits according to the manufacturer’s protocols. Women with CRP >10 µg/ml (N=12) were excluded from the study.
3.3.5. **Statistical analysis**

Normally distributed data are presented as means ± SD with ranges in parentheses. Non-normally distributed parameters were logarithmically transformed for parametric analysis and are presented as geometric means with 95% confidence intervals. Differences in SES, health behaviour characteristics and circulating concentrations of hsCRP and IL-18 between black and white women were compared using chi-squared analysis for categorical variables and ANCOVA, adjusting for age, for continuous variables. In separate analyses for black and white women, general linear models were used to compare circulating concentrations of hsCRP and IL-18 across categories of SES and health behaviour variables, adjusting for age. P values for trend represent differences between lowest and highest SES categories. To determine whether inflammatory markers relate to metabolic risk factors independent of SES and behaviour/lifestyle variables, linear regression analyses were used. First, univariate analyses were performed to analyze the relationship between single determinants (hsCRP and IL-18) and metabolic risk factors (model 1). Second, a multiple regression analysis with a forward selection procedure was performed in which age, SES and behaviour/lifestyle variables were included as possible determinants (model 2). A third model (model 3) further included WC as a measure of central adiposity. Independent variables with P value .05 were left in the model. Data were analyzed using STATISTICA version 9 (Statsoft Inc., Tulsa, OK).

3.4. **RESULTS**

3.4.1. **Basic subject characteristics**

Socioeconomic, health behaviour and health status characteristics of black and white women are presented in Table 3.1. As described previously, black women were significantly younger than white women, and as a result all subsequent analysis included age as a covariate.
Marked differences in socioeconomic variables were observed between black and white women. Black women had lower levels of education, asset index and greater housing density than white women. A significantly smaller proportion of black women were current smokers, consumed alcohol or used oral contraceptives, whereas a larger proportion of black women used injectable contraceptives and were sedentary (PA < 150 min/week) compared to white women.

A higher proportion of black women had a family history of T2DM; however there was no ethnic difference in the proportion of women with family history of CVD (including stroke and heart attack). Similar to results reported in the previous chapter, there was no difference in WC between black and white women (89.9 ± 1.1 cm vs. 90.0 ± 1.2 cm, respectively, P=0.087). Further, there were no differences between ethnic groups in the proportion of women above the risk threshold for elevated systolic and diastolic BP (p=0.330 and p=0.357, respectively), HOMA-IR (P=0.570) and TC/HDL-C ratio (P=0.235). A significantly higher proportion of white women had elevated TG’s compared to black women (P<0.010). Conversely, a higher proportion of black women had reduced HDL-C concentrations compared to white women (P<0.001) (Figure 2.1).

For both ethnic groups, a higher percentage of smoking, sedentary behaviour, alcohol consumption, and metabolic risk factors were present in women with a low level of educational attainment and asset index (Appendix 1, Tables A1 and A2). No differences in health status or health behaviour variables were observed across quintiles of housing density in both black and white women.
Table 3.1. SES, health behaviour, health status and circulating inflammatory markers of black and white women

<table>
<thead>
<tr>
<th></th>
<th>Black Women (N=223)</th>
<th>White Women (N=177)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic and Socioeconomic variables</strong></td>
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<tr>
<td>Age (years)</td>
<td>27 ± 7</td>
<td>31 ± 8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Education</td>
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<td></td>
<td>&lt;0.01</td>
</tr>
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<td>None</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grade 1-7</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grade 8-11</td>
<td>35.2</td>
<td>5.0</td>
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</tr>
<tr>
<td>Completed High School</td>
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</tr>
<tr>
<td>Tertiary Education</td>
<td>17.6</td>
<td>72.2</td>
<td></td>
</tr>
<tr>
<td><strong>Housing Density</strong></td>
<td></td>
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<td>&lt;0.01</td>
</tr>
<tr>
<td>Q1 (&lt;0.59)</td>
<td>19.0</td>
<td>79.9</td>
<td></td>
</tr>
<tr>
<td>Q2 (0.60-0.83)</td>
<td>23.4</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>Q3 (0.84-1.18)</td>
<td>17.7</td>
<td>2.8</td>
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<tr>
<td>Q4 (1.19-1.59)</td>
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<tr>
<td>Q5 (&gt;1.60)</td>
<td>18.6</td>
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<td><strong>Asset Index</strong></td>
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</tr>
<tr>
<td>Q1 (&lt;5)</td>
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<tr>
<td>Q2 (5-7)</td>
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<tr>
<td>Q3 (7-10)</td>
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<td>Q4 (10-12)</td>
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<td>Current smoker</td>
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<tr>
<td>Ex-Smoker</td>
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<tr>
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<td>Non-drinker</td>
<td>66.8</td>
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<tr>
<td>≤1 drink/day</td>
<td>18.0</td>
<td>44.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&gt;1 drink/day</td>
<td>15.2</td>
<td>34.2</td>
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<tr>
<td><strong>PA</strong></td>
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<tr>
<td>Sedentary (&lt;150min/week)</td>
<td>84.9</td>
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<tr>
<td>Active (≥150min/week)</td>
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<td>Oral</td>
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<td>Family history of T2DM</td>
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<td>Median (Interquartile range)</td>
<td>Median (Interquartile range)</td>
<td></td>
</tr>
<tr>
<td>hsCRP (µg/ml)</td>
<td>2.38 (0.98-5.30)</td>
<td>2.52 (0.99-6.08)</td>
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<tr>
<td>IL-18 (pg/ml)</td>
<td>195.4 (107.3-315.9)</td>
<td>186.9 (111.2-267.5)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*P values adjusted for age. Q1-Q5, quintiles 1-5; PA, physical activity; T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease; IL-18, interleukin-18; hsCRP, high sensitivity C-reactive protein.*
3.4.2. **Ethnic differences in circulating inflammatory markers**

White women had significantly lower circulating IL-18 concentrations compared to black women (Table 3.1), however no difference in circulating hsCRP was observed between ethnic groups. Ethnic differences in IL-18 concentrations were independent of age and WC (P<0.01).

3.4.2.1. **Socioeconomic status (SES)**

In black women, lower education and asset index were associated with higher circulating concentrations of hsCRP and IL-18 (Figure 3.1). In white women, lower education and asset index were associated with higher hsCRP circulating concentrations, and lower education was associated with higher IL-18 concentrations.

3.4.2.2. **Behavioural/lifestyle variables**

In black women, the use of injectable contraceptives (29.3% of black women) was associated with significantly higher circulating concentrations of both hsCRP and IL-18 compared to women not on contraception (P<0.01 and P=0.048, respectively) and current smokers had significantly higher circulating hsCRP concentrations than non-smokers (P=0.038; Figure 3.2). In white women, the use of oral contraceptives (29.8% of white women) was associated with significantly higher hsCRP concentrations compared to women not on contraception (P=0.010) and current smokers had significantly higher circulating hsCRP and IL-18 concentrations than non-smokers (P<0.010 and P=0.032, respectively; Figure 3.2). Reported alcohol consumption and PA levels did not influence circulating hsCRP and IL-18 concentrations in either ethnic group.
Figure 3.1. Circulating hsCRP and IL-18 concentrations in black (a, b) and white (c, d) women across socioeconomic status categories of education, asset index and housing density. Values are age-adjusted means ± SE. CHS, completed high school; TE, tertiary education. * P<0.05 for trend.
Figure 3.2. Relationship between behaviour/lifestyle variables and circulating hsCRP and IL-18 concentrations in black women (a, b) and white women (c, d).  

Contraceptive Use*. Alcohol consumption.  Smoking status. Physical Activity status. * P<0.05 Injectable vs. no contraception; ** Oral vs. no contraception, ***Smoker vs. non smoker. Values are age-adjusted means ± SE.  

* Due to the small numbers in black and white women for oral contraceptive and injectable contraceptive use, respectively, only the relationship between inflammation and injectable contraceptives are reported for black women and inflammation and oral contraceptives are reported for white women.
3.4.2.3. **Central obesity**

Circulating concentrations of hsCRP and IL-18 correlated significantly with WC in both black and white women (Figure 3.3), but the strength of the association was greater for hsCRP than IL-18 in both groups.

![Figure 3.3. Correlations between WC and circulating hsCRP and IL-18 in black and white women.](image)

**3.4.2.4. Metabolic risk factors**

In black women, circulating concentrations of IL-18 and hsCRP were weakly, but significantly associated with all metabolic risk factors (Table 3.2, model 1). In white women, circulating concentrations of IL-18 were significantly associated with only diastolic BP, HOMA-IR and TC/HDL-C, while the associations between circulating hsCRP and all metabolic risk variables were significant (Table 3.3, model 1).

**3.4.3. Multivariate associations with metabolic risk factors for cardiovascular disease and type two diabetes**

**3.4.3.1. Black women**

In black women, the inflammatory markers alone accounted for less than 10% of the variance in metabolic outcomes. Addition of SES, smoking and contraceptive use to the model (Table 3.2, Model 2) explained an additional 5-22% of the variance in metabolic outcomes. The
associations between circulating IL-18 and hsCRP and all metabolic risk variables, except for systolic BP and TG in the case of CRP, were independent of age, SES, smoking and contraception (Table 3.2, Model 2). However, the association between circulating IL-18 and hsCRP and the majority of metabolic risk factors were not independent of WC (Table 3.2, model 3). In the model including lifestyle/behavioural variables and WC, IL-18 was only independently associated with diastolic BP, whereas hsCRP was only independently and negatively associated with HDL-C. WC was significantly and independently associated with all metabolic outcomes (except systolic BP), and explained the majority of the variance in metabolic outcomes, contributing an additional 6-25% to the total explained variance (Table 3.2, model 3). Independent of WC and inflammatory markers, low SES (education and/or housing density) was significantly associated with diastolic BP and serum lipid concentrations (Table 3.2, models 2 and 3), while the use of injectable contraceptives was significantly associated with all metabolic risk factors, and current smoking was significantly associated with diastolic BP.

3.4.3.2. White women

In white women, the inflammatory markers alone accounted for less than 18% of the variance in metabolic outcomes. Addition of SES, smoking and contraceptive use to the model (Table 3.3, Model 2) explained an additional 7-12% of the variance in metabolic outcomes. The associations between circulating hsCRP and all metabolic risk variables, except for TG, were independent of age, SES, smoking and contraception (Table 3.3, Model 2). However, the association between circulating hsCRP and the majority of metabolic risk factors were not independent of WC (Table 3.3, model 3). In contrast, the associations between IL-18 with diastolic BP, HOMA-IR and TC/HDL-C were not independent of age, SES and contraceptive use (Table 3.3, model 2). In the model including lifestyle/behavioural variables and WC, hsCRP was only independently and negatively associated with TG. WC was significantly and
independently associated with all metabolic outcomes (except systolic and diastolic BP), and explained the majority of the variance in metabolic outcomes, contributing an additional 7-28% to the total explained variance (Table 3.3, model 3). Independent of WC, inflammatory markers and low SES, age was significantly associated with BP, HOMA-IR and TC/HDL-C, and notably accounted for all the variance (13%) in systolic BP (Table 3.3, models 2 and 3). The use of oral contraceptives was significantly associated with TG and HDL-C, and SES (education) with HOMA-IR.
Table 3.2. Regression models for metabolic risk factors in black women, including serum IL-18, hsCRP, lifestyle/behavioural factors and body composition

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>IL-18</th>
<th>Model 2</th>
<th>IL-18</th>
<th>Model 3</th>
<th>IL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2=0.05$</td>
<td>$\beta \pm SE$</td>
<td>$R^2=0.23$</td>
<td>$\beta \pm SE$</td>
<td>$R^2=0.35$</td>
<td>$\beta \pm SE$</td>
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<tr>
<td>SBP</td>
<td>0.23 $\pm 0.07$</td>
<td>IL-18</td>
<td>0.14 $\pm 0.07$</td>
<td>Age</td>
<td>0.37 $\pm 0.07$</td>
<td>Age</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.39 $\pm 0.06$</td>
<td>0.17 $\pm 0.07$</td>
<td>0.16 $\pm 0.07$</td>
<td>Education</td>
<td>0.20 $\pm 0.07$</td>
<td>Contraception $^a$</td>
</tr>
<tr>
<td></td>
<td>0.17 $\pm 0.07$</td>
<td>0.16 $\pm 0.07$</td>
<td>0.15 $\pm 0.06$</td>
<td>Smoking</td>
<td>0.16 $\pm 0.06$</td>
<td>Contraception $^a$</td>
</tr>
<tr>
<td></td>
<td>WC</td>
<td>0.27 $\pm 0.06$</td>
<td>WC</td>
<td>0.27 $\pm 0.06$</td>
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<table>
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<th>hsCRP</th>
<th>Model 3</th>
<th>hsCRP</th>
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<td>$\beta \pm SE$</td>
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<td>$\beta \pm SE$</td>
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<tr>
<td>SBP</td>
<td>0.17 $\pm 0.07$</td>
<td>CRP</td>
<td>0.13 $\pm 0.06$</td>
<td>Age</td>
<td>0.28 $\pm 0.06$</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>0.37 $\pm 0.07$</td>
<td>Age</td>
<td>0.17 $\pm 0.07$</td>
<td>Contraception $^a$</td>
<td>0.17 $\pm 0.07$</td>
<td>Contraception $^a$</td>
</tr>
<tr>
<td>CRP</td>
<td>0.17 $\pm 0.07$</td>
<td>Age</td>
<td>0.17 $\pm 0.07$</td>
<td>Contraception $^a$</td>
<td>0.17 $\pm 0.07$</td>
<td>Contraception $^a$</td>
</tr>
<tr>
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<td>Age</td>
<td>0.17 $\pm 0.07$</td>
<td>Contraception $^a$</td>
<td>0.17 $\pm 0.07$</td>
<td>Contraception $^a$</td>
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<td>Contraception $^a$</td>
<td>0.17 $\pm 0.07$</td>
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<td>0.17 $\pm 0.07$</td>
<td>Contraception $^a$</td>
<td>0.17 $\pm 0.07$</td>
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<td>Model 3</td>
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<td><strong>HOMA-IR</strong></td>
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<td>R²=0.12</td>
<td>β±SE</td>
<td>R²=0.37</td>
<td>β±SE</td>
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<td>0.16 ± 0.07</td>
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<tr>
<td>CRP</td>
<td>± 0.07</td>
<td>0.16</td>
<td>± 0.07</td>
<td>0.16 ± 0.07</td>
<td>± 0.07</td>
<td>0.16 ± 0.07</td>
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<tr>
<td>WC</td>
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<td>± 0.07</td>
<td>0.57 ± 0.07</td>
<td>± 0.07</td>
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<tr>
<td>IL-18</td>
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<td>0.15 ± 0.07</td>
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<tr>
<td>CRP</td>
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<td>0.15</td>
<td>± 0.07</td>
<td>0.15 ± 0.07</td>
<td>± 0.07</td>
<td>0.15 ± 0.07</td>
</tr>
<tr>
<td>WC</td>
<td>± 0.07</td>
<td>0.17</td>
<td>± 0.07</td>
<td>0.17 ± 0.07</td>
<td>± 0.07</td>
<td>0.17 ± 0.07</td>
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<td>-0.18 ± 0.07</td>
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<td>-0.18 ± 0.07</td>
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<tr>
<td>CRP</td>
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<td>-0.21</td>
<td>± 0.07</td>
<td>-0.21 ± 0.07</td>
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<tr>
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<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
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<tbody>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>R²=0.06</td>
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<td>CRP</td>
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<td>0.22</td>
<td>± 0.07</td>
</tr>
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<td>WC</td>
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<td><strong>TG</strong></td>
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</tr>
<tr>
<td>CRP</td>
<td>± 0.07</td>
<td>0.27</td>
<td>± 0.07</td>
</tr>
<tr>
<td>Age</td>
<td>± 0.07</td>
<td>0.19</td>
<td>± 0.07</td>
</tr>
<tr>
<td>Education</td>
<td>± 0.07</td>
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<td>± 0.07</td>
</tr>
<tr>
<td>Contraception</td>
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<td>0.17</td>
<td>± 0.07</td>
</tr>
<tr>
<td>WC</td>
<td>± 0.07</td>
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<td>± 0.07</td>
</tr>
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<td><strong>HDL-C</strong></td>
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<td>CRP</td>
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<td>± 0.07</td>
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<td>HD</td>
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<td>-0.17</td>
<td>± 0.07</td>
</tr>
<tr>
<td>Contraception</td>
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<td>± 0.07</td>
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<tr>
<td>WC</td>
<td>± 0.07</td>
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Table 3.2. Continued

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<tr>
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</tr>
<tr>
<td>Age</td>
<td>0.16 ±</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>0.19 ±</td>
<td>0.07</td>
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</tr>
<tr>
<td>Contraception</td>
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</tr>
<tr>
<td>CRP</td>
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<td>0.07</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>0.23 ±</td>
<td>0.07</td>
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</table>

Only variables significant (P<0.05) to the model are shown. Model 1, unadjusted; Model 2, adjusted for age, education, asset index, housing density, smoking, contraceptive use (injectable contraceptives); Model 3; adjusted for age, education, asset index, housing density, smoking, contraceptive use (injectable contraceptives), waist circumference. IL-18, interleukin-18; hsCRP, high sensitivity C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model of insulin resistance; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; TC/HDL-C, total cholesterol-high density lipoprotein cholesterol ratio; HD, housing density.
Table 3.3. Regression models for metabolic risk factors in white women, including serum IL-18, CRP, lifestyle/behavioural factors and body composition

<table>
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<tr>
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<th>IL-18</th>
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<th>hsCRP</th>
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</thead>
<tbody>
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<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 3</td>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td><em>SBP</em></td>
<td>(R^2=0.02)</td>
<td>(\beta\pm SE)</td>
<td>(R^2=0.11)</td>
<td>(\beta\pm SE)</td>
<td>(R^2=0.13)</td>
<td>(\beta\pm SE)</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.14 ± 0.07(ns)</td>
<td>Age</td>
<td>0.34 ± 0.08</td>
<td>Age</td>
<td>0.33 ± 0.08</td>
<td>Age</td>
</tr>
<tr>
<td>DBP</td>
<td>(R^2=0.03)</td>
<td>(\beta\pm SE)</td>
<td>(R^2=0.15)</td>
<td>(\beta\pm SE)</td>
<td>(R^2=0.25)</td>
<td>(\beta\pm SE)</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.18 ± 0.07</td>
<td>Age</td>
<td>0.32 ± 0.08</td>
<td>Age</td>
<td>0.28 ± 0.08</td>
<td>Age</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>(R^2=0.06)</td>
<td>(\beta\pm SE)</td>
<td>(R^2=0.15)</td>
<td>(\beta\pm SE)</td>
<td>(R^2=0.43)</td>
<td>(\beta\pm SE)</td>
</tr>
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<td>IL-18</td>
<td>0.25± 0.07</td>
<td>Education</td>
<td>-0.32 ± 0.07</td>
<td>Education</td>
<td>-0.14 ± 0.06</td>
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<tr>
<td>HD</td>
<td>0.16 ± 0.07</td>
<td>WC</td>
<td>0.62 ± 0.07</td>
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<td>0.06</td>
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<tr>
<td>TG</td>
<td>(R^2=0.01)</td>
<td>(\beta\pm SE)</td>
<td>(R^2=0.11)</td>
<td>(\beta\pm SE)</td>
<td>(R^2=0.18)</td>
<td>(\beta\pm SE)</td>
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<tr>
<td>IL-18</td>
<td>0.06 ± 0.08(ns)</td>
<td>Education</td>
<td>-0.19 ± 0.07</td>
<td>Contraception</td>
<td>0.35 ± 0.07</td>
<td>Contraception</td>
</tr>
<tr>
<td>Contraception</td>
<td>0.32 ± 0.08</td>
<td>WC</td>
<td>0.30 ± 0.08</td>
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<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.3. Continued

<table>
<thead>
<tr>
<th></th>
<th>IL-18</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 3</td>
<td>Model 1</td>
</tr>
<tr>
<td><strong>HDL-C</strong></td>
<td>$R^2=0.02$</td>
<td>$\beta \pm \text{SE}$</td>
<td>$R^2=0.10$</td>
<td>$\beta \pm \text{SE}$</td>
</tr>
<tr>
<td>IL-18</td>
<td>$-0.12 \pm 0.07 (\text{ns})$</td>
<td>Education</td>
<td>$0.16 \pm 0.08$</td>
<td>Contraception&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WC</td>
<td>$0.07$</td>
<td></td>
</tr>
</tbody>
</table>

| **TC/HDL-C**        | $R^2=0.03$ | $\beta \pm \text{SE}$ | $R^2=0.13$ | $\beta \pm \text{SE}$ | $R^2=0.29$ | $\beta \pm \text{SE}$ | $R^2=0.09$ | $\beta \pm \text{SE}$ | $R^2=0.20$ | $\beta \pm \text{SE}$ | $R^2=0.29$ | $\beta \pm \text{SE}$ |
| IL-18               | $0.19 \pm 0.07$ | Age | $0.19 \pm 0.08$ | Age | $0.15 \pm 0.07$ | WC | $0.45 \pm 0.07$ | WC | $0.39 \pm 0.08$ | Age | $0.16 \pm 0.07$ |
|                     |       | Education | $-0.18 \pm 0.08$ | WC | $0.45 \pm 0.07$ | WC | $0.39 \pm 0.08$ | WC | $0.08$ |
|                     |       | HD       | $0.16 \pm 0.08$ |                  |                    | HD | $0.14 \pm 0.08$ |                  |                    |

Only variables significant ($P<0.05$) to the model are shown. Model 1, unadjusted; Model 2, adjusted for age, education, asset index, housing density, smoking, contraceptive use (*injectable contraceptives*); Model 3: adjusted for age, education, asset index, housing density, smoking, contraceptive use (*injectable contraceptives*), waist circumference. IL-18, interleukin-18; hsCRP, high sensitivity C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model of insulin resistance; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; TC/HDL-C, total cholesterol-high density lipoprotein cholesterol ratio; HD, housing density
3.5. DISCUSSION

In both black and white South African women, central obesity, low SES, smoking status and contraceptive use were associated with increased circulating concentrations of inflammatory markers hsCRP and IL-18, which in turn were associated with metabolic risk factors for T2DM and CVD. The association between IL-18 and CRP and metabolic risk factors was largely mediated by obesity and not SES or behavioural/lifestyle variables, supporting previous studies suggesting that AT is a putative source of inflammation characteristic of CVD and T2DM risk factors [201]. These findings suggest that compared to a simple WC measurement, there was no added benefit in measuring circulating inflammatory markers for added risk prediction in apparently healthy black and white South African women.

In the present study, circulating concentrations of IL-18 and hsCRP were significantly associated with metabolic risk factors in both black and white South African women. This is in agreement with several other cross-sectional studies in America showing an association between these inflammatory markers and metabolic risk factors for CVD [127-129]. IL-18 is involved in the induction of pro-inflammatory cytokines from mature TH1 cells and macrophages [232] and there is evidence to suggest IL-18 is involved in atherogenic pathways [131]. Upregulation of IL-18 gene expression involves NFκB, which in turn has been implicated in disruption of insulin signalling pathways [132]. CRP is secreted by the liver in response to inflammatory cytokines [97], and compared to IL-18 concentrations, which are likely representative of upstream events within the inflammatory cascade, increased hsCRP concentrations are more a reflection of overall inflammatory activity.
It has been suggested that circulating inflammatory markers, including IL-18 and hsCRP, may be of added use for early identification of individuals at increased risk for CVD and T2DM [103]. However, several environmental factors known to influence inflammation and CVD risk could impact on the use of inflammatory markers in a clinical setting. In both black and white women, low SES (assessed by education and asset index), smoking, contraceptive use (oral in white women and injectable in black women) and central obesity (WC) were associated with elevated circulating concentrations of IL-18 and hsCRP. Although the impact of SES and behavioural factors on hsCRP has been well documented in other populations [104;110;229;233], to my knowledge this is the first study investigating the impact of SES and behavioural/lifestyle factors on circulating IL-18 concentrations, and on hsCRP in South African women. Importantly, the association between circulating IL-18 and hsCRP concentrations and metabolic risk factors, although independent of SES and behavioural/lifestyle factors (with the exception of IL-18 in the white women), were not independent of central obesity. Furthermore, the amount of variance in metabolic risk factors explained by these inflammatory markers was relatively small (ranging between 2 and 9% in black and 1 and 18% in white women for all metabolic risk factors) compared to that of SES, smoking, contraceptive use and WC combined, which ranged between 12 and 26% in white women and 15 and 30% in black women. These results suggest that measuring circulating concentrations of IL-18 or hsCRP in this study provided no added benefit compared to a simple measurement of WC for identifying CVD and T2DM risk in these women. Furthermore, as the cost and methods for measuring inflammatory markers are high and invasive, they are not practical markers of risk, especially in a primary prevention setting.
Notably, in Chapter 2, I found that central obesity measures explained a smaller proportion of variance in metabolic risk factors in black compared to white women, and as a result suggested that other factors, including inflammatory markers and/or SES and behavioural/lifestyle variables, may help explain a greater proportion of risk. In contrast to this, the association between IL-18 and hsCRP and metabolic risk factors was attenuated by the inclusion of WC in the model. These results do support the hypothesis that AT may be a major source of increased circulating inflammatory markers associated with CVD and T2DM [201]. Indeed, although IL-18 is secreted by several cell types, adipose tissue has been shown to secrete IL-18 and the expression of IL-18 in adipose tissue and circulating concentrations are increased in obese individuals compared to lean controls [234]. Furthermore, WC was significantly and independently associated with the majority of metabolic risk factors in both black and white women, despite ethnic differences in abdominal VAT and SAT discussed in Chapter 2, supporting previous research, which has shown obesity to be an independent risk factor for CVD and T2DM [6;44]. Importantly, in addition to WC, age, SES and contraceptive use were independently associated with metabolic risk factors, and did add to the explained variance in risk in both black and white women. Specifically, age was a significant independent contributor to models for diastolic and systolic BP in both ethnicities; to TC/HDL-C and HOMA-IR models in white women and TG models in black women. The risk for CVD and T2DM increases with age [235] and although the women included in this study were relatively young, the white women were significantly older than the black women, which may explain why age contributed to more risk variables in these women. A low SES (education and/or housing density) was independently associated with diastolic BP and lipids in black women and with HOMA-IR in white women. These findings support previous studies linking low SES with increased risk for
CVD and T2DM [91]. White women were of higher SES compared to black women, and there was a smaller range in SES indices in the white women, which may explain the lack of association with other risk variables. However, the findings in this study are consistent with the current socio-political environment within South Africa, as the majority of blacks live in poorer communities compared to whites [89]. Furthermore, in black women, use of injectable contraceptives contributed significantly and independently to all metabolic risk models and in white women, use of oral contraceptives contributed significantly and independently to models for TG and HDL-C. Previous studies have shown that both oral contraceptive and injectable contraceptive are associated with increased risk for CVD and in particular have been shown to alter lipid metabolism by increasing triglyceride and decreased HDL-C-cholesterol concentrations [236]. These findings highlight the need for the prioritisation of the management of potentially modifiable risk factors, which include SES, contraceptive use and obesity, especially in primary prevention of CVD and T2DM in black and white women.

Despite being an established CVD risk factor [90], smoking only contributed significantly and independently to models for diastolic BP in black women. In addition, as physical activity levels and alcohol intake were not associated with IL-18 and hsCRP concentrations, they were not included in risk models, to which they may have contributed. Furthermore, behavioural/lifestyle factors used in this study were self-reported, and it is possible that more objective measurements would yield different results. Indeed, Hamer et al. have recently shown in a cross-sectional sample of black (n=192) and white (n=206) South African men and women, that the prevalence of self-reported smoking and alcohol consumption was lower when compared to objectively measured smoking (confirmed by serum cotinine) and alcohol (serum gamma glutamyl
transferase) consumption [237]. Accordingly, future studies should focus on more objective measures of SES and lifestyle.

In conclusion, central obesity, low SES, smoking status and contraceptive use were associated with increased circulating concentrations of inflammatory markers hsCRP and IL-18, which in turn were associated with metabolic risk factors for T2DM and CVD in apparently healthy black and white South African women. The association between IL-18 and hsCRP with metabolic risk factors was largely mediated by central obesity and not SES and behavioural/lifestyle factors. Based on these findings, measuring circulating hsCRP and IL-18 concentrations provides no added benefit to a simple WC measurement for identification of metabolic risk factors for CVD and T2DM in both black and white premenopausal women. Indeed, many of the effects of inflammatory proteins are mediated in a paracrine fashion, and therefore further research needs to explore the impact of variation in AT-specific inflammation on CVD risk factors in South African women.
CHAPTER FOUR

ASSOCIATION BETWEEN IL-18 -137G/C POLYMORPHISM WITH CIRCULATING IL-18 CONCENTRATIONS AND METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASE AND TYPE TWO DIABETES IN SOUTH AFRICAN WOMEN

Data from this chapter have been published in part in: Evans J, Collins M, Jennings C, van der Merwe L, Söderström I, Olsson T, Levitt NS, Lambert EV, Goedecke JH. The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease. 2007 Nov Eur J Endocrinol;157(5) 633-40.

4.1. ABSTRACT

Ethnic differences in circulating IL-18 concentrations may be explained, in part, by ethnic differences in the distribution of the G allele of the -137G/C polymorphism within the promoter region of the IL-18 gene. The G allele of this polymorphism has previously been shown to influence circulating IL-18 concentrations, and has a higher frequency in black compared to white American women. Therefore the aim of the third study of this thesis was to investigate the association between the -137G/C polymorphism within the IL-18 gene and circulating IL-18 concentrations and metabolic risk factors for cardiovascular disease in black and white South African women.

Body composition (kg of total body fat), fat distribution (VAT and subcutaneous adipose tissue, SAT), as well as BP, fasting glucose, insulin, lipid profile, IL-18 concentrations and IL-18 -137G/C genotype were measured in 206 black and 174 white South African women. The frequency of the G-allele was significantly higher in black compared to white women ($\chi^2=34.1$, ...
P<0.001). Circulating IL-18 concentrations remained higher in black compared to white women after adjusting for age, total body fatness, VAT and genotype. A significant independent association between the IL-18 -137 C-allele and BP was observed in black and white women. Black women with the C-allele had higher systolic (115.7 ± 1.2 vs. 110.2 ± 1.3 mmHg, P<0.001) and diastolic BP (78.7 ± 1.9 vs. 74.5 ± 1.1 mmHg, P<0.005) than women with the GG genotype. Conversely, white women with the C-allele had significantly lower systolic BP (108.9 ± 1.6 vs. 113.2 ± 1.6 mmHg, P<0.001) than women with the GG genotype.

In conclusion, ethnic differences in circulating IL-18 concentrations could not be explained by variation within the distribution of the IL-18 -137G/C polymorphism, suggesting the functional relevance of this polymorphism needs to be further explored in different populations. Although the -137 G/C polymorphism could not explain ethnic differences in circulating concentrations, the C-allele was independently associated with BP in both ethnic groups. However, the direction of this relationship was different in black and white women, with the C-allele associated with higher BP in black women and lower BP in white women. The mechanisms underlying this association are not known and require further investigation.

4.2. INTRODUCTION

As discussed in Chapter 3, increased circulating levels of the pro-inflammatory cytokine IL-18 were associated with metabolic risk factors for CVD, including central obesity, elevated BP, dyslipidaemia and insulin resistance (IR), in our sample of both black and white South African women, as well as other European cohorts [127;129]. Further, we showed that black South African women had higher circulating IL-18 levels compared to white women.
Ethnic differences in circulating IL-18 levels and CVD risk factors may be explained, in part, by genetic variation within the *IL-18* gene. A previous study in a European population showed that haplotype-based analysis of variants of the *IL-18* gene influenced circulating IL-18 concentrations and clinical outcomes in patients with cardiac disease, suggesting that IL-18 is involved in the development of atherosclerosis and its complications [238]. Furthermore, the G-allele of a potentially functional polymorphism at position -137 within the promoter region of the *IL-18* gene, previously associated with higher circulating IL-18 concentrations in monocytes [137;239], has a higher frequency in black compared to white American women [106]. However, it is not known if ethnic differences in genotype frequency are associated with ethnic differences in circulating IL-18 concentrations. Furthermore, a putative link between the *IL-18* -137 G/C polymorphism and variation in circulating IL-18 concentrations in association with metabolic risk factors for CVD in different ethnic groups has not been explored.

Therefore, I tested the hypothesis that ethnic difference in circulating IL-18 concentrations and metabolic risk factors could be explained, in part, by ethnic differences in the distribution of the IL-18 -137G/C polymorphism. Specifically, the aim of this study was to examine the association between the -137 G/C polymorphism within the *IL-18* gene with serum IL-18 concentrations and metabolic risk factors for CVD in black and white South African women.

**4.3. METHODS**

**4.3.1. Subjects**

The study population consisted of 206 black and 174 white premenopausal South African women. Only subjects successfully genotyped for the *IL-18* -137G/C polymorphism were included in this chapter. Subject recruitment and inclusion and exclusion criteria are described in
detail in the methods section of Chapter Two. The Faculty of Health Sciences Research Ethics Committee of the University of Cape Town gave the study ethics approval. Written informed consent was obtained from all subjects.

4.3.2. Testing Procedures

Weight, height, waist circumference, and hip circumference (at the largest part of hips) were measured as described in Chapter 2. Dual-energy X-ray absorptiometry (DXA) was used to measure whole body fat mass (Hologic QDR 4500 Discovery-W dual-energy X-ray absorptiometry, software version 4.40; Hologic, Bedford, MA) according to standard procedures. *In vivo* precision (% coefficient of variation) was determined for fat mass (1.7%) by measuring 30 individuals two times on the same day with repositioning. CT-derived VAT and SAT area were measured at the level of L4-L5 (as described in Chapter 2). BP, fasting plasma glucose, and serum insulin, IL-18, TC, TG and HDL-C, as well as IR, as estimated by HOMA-IR, were measured. The measurement of these variables is described in detail in the methods section of Chapters Two and Three.

4.3.3. DNA extraction and genotype analysis

After DNA was extracted from the venous blood samples (5 ml) using the method of Lahiri and Nurnberger [240], the -137 G/C polymorphism (rs187238) within the promoter of the *IL-18* gene was genotyped by using PCR-SSP. Two PCR reactions were performed. Each contained a common reverse primer 5′-AGG-AGGGCAAAATGCACTGG-3′, a control forward primer 5′-CCAATAGGACTGATTATTCCGCA-3′ and sequence specific forward primers: 5′-CCC-CAACTTTTACGGAAGAAAAG-3′ for reaction 1 (C-specific) and 5′-
CCCAACTTTTACGGAAG-AAAAC-3′ for reaction 2 (G-specific). The forward and reverse control primers were used to amplify a 446-bp fragment covering the polymorphic site to serve as an internal positive amplification control. PCR reactions were performed in a final volume of 50μl consisting of 50 mM KCl, 10 mM Tris–HCl pH 8.3, 1.5 mM MgCl₂, 0.25 mM dNTP, 20 ng genomic DNA and 0.5 U Taq polymerase. Final primer concentrations within the reaction mixtures were as follows: 0.3 μM of the control forward primer, 0.5 μM of the common reverse primer and 0.5μM of each sequence specific primer. PCR reactions were denatured for 2 minutes at 94°C. This was followed by five cycles of 94°C for 20 seconds and 68°C for 60 seconds and 25 cycles of 94°C for 20 seconds, 62°C for 20 seconds and 72°C for 40 seconds. The PCR fragments were resolved on 2% Agarose gels and stained with ethidium bromide for visualisation under ultra-violet light.

4.3.4. Statistical Analysis

Data is presented as means ± standard error. Chi-squared tests were used to compare genotype frequencies between black and white women. In separate analyses for black and white women, general linear models were used to compare medians of body composition variables and metabolic risk factors between individuals with the different genotypes, adjusting for age, body fatness (kg) and VAT. Multiple linear regression analysis were used to determined if IL-18 -137G/C genotype was associated with metabolic risk factors independently of age, circulating IL-18 concentrations, body fatness (kg) and VAT. Because of the rarity of the C-Allele, the GC and CC genotypes were combined in all genotype analysis and in relation to circulating IL-18 concentrations and metabolic outcomes. Data were analysed with the STATISTICA version 9
(StatSoft Inc., Tulsa, OK, USA) statistical programme. Hardy-Weinberg equilibrium was tested using the Genepop web version 3.4 program (http://wbiomed.curtin.edu.au/genepop/).

4.4. RESULTS

4.4.1. Subject characteristics

Black women (n=206) included in this analyses were significantly younger than the white women (n=174; 27 ± 1 vs. 31 ± 1 years, respectively. P<0.01), as a results all subsequent analysis included age as a covariate. The majority of the black subjects reported being of Xhosa ancestry (N=175, 85%), with the other 31% being of mixed (N=31) South African tribal ancestry. The white South African women were all of European ancestry.

4.4.2. Distribution of IL-18 genotype among ethnic groups

No black women were homozygous for the C-allele. The C-allele was less frequent and the G allele more frequent in both black and white women. IL-18 -137 genotype distributions (GG, GC and CC) were significantly different between black and white women (Figure 4.1, P<0.001). The frequency of the C-allele was significantly lower in black (n=50/412) compared to white (n=103/348) women (χ²=34.6, p<0.001). Similar results were obtained when genotype distributions of the black subjects of only Xhosa ancestry (n=170) were compared to the white women (P<0.001), indicating that there was no population stratification in the black women. IL-18 genotype distributions of the black and white women included in this study were in Hardy-Weinberg equilibrium (P=0.34 and P=0.57, respectively).
4.4.3. Association between IL-18 genotype with circulating IL-18 concentrations

There was no association between \textit{IL-18} -137G/C polymorphism with circulating IL-18 concentrations in black (\(P=0.99\)) and white women (\(P=0.67\)). Irrespective of genotype, circulating IL-18 concentrations were significantly higher in black compared to white women, even after adjusting for differences in age, total body fat (kg fat) and VAT (Figure 4.2, \(P=0.008\)).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure41.png}
\caption{Genotype frequency of the IL-18-137G/C polymorphism in black and white women. \(\text{\textbullet\hspace{1em}}\) Black women, \(\square\) White women * \(P<0.01\).}
\end{figure}
Figure 4.2. Ethnic differences in circulating IL-18 levels according to IL-18 -137G/C genotype. ■ Black women, □ White women. Black vs. White women * P<0.01. Data are presented as unadjusted means, with error bars indicating standard error.

4.4.4. Differences in IL-18 -137G/C genotypes, body composition and metabolic risk factors for cardiovascular disease and type two diabetes between black and white South African women.

Differences between IL-18 -137G/C genotype and body composition and metabolic risk factors for CVD, according to ethnicity, are shown in Tables 4.1 and 4.2. There were no significant differences in age, height, weight, body fatness or fat distribution, between genotype groups, in both black and white women (P>0.05).

Blood pressure differed significantly according to IL-18-137G/C genotype, in both black and white women. Black women with the C-allele had significantly higher systolic and diastolic BP compared to women with the GG genotype. Conversely, white women with the C-allele had significantly lower systolic BP than women with the GG genotype (Table 4.2). In black women,
*IL-18* genotype (GC/CC: $\beta=0.16$, $P=0.024$), age ($\beta=0.37$, $P<0.01$) and circulating IL-18 concentrations ($\beta=0.18$, $P=0.012$) were significant predictors of systolic BP (Adjusted $R^2=0.21$, $P<0.01$); and *IL-18* genotype (GC/CC: $\beta=0.15$, $P=0.023$), age ($\beta=0.27$, $P<0.01$), body fat (kg) ($\beta=0.25$, $P<0.01$) and circulating IL-18 concentrations ($\beta=0.22$, $P<0.01$) were significant predictors of diastolic BP (Adjusted $R^2=0.30$, $P<0.01$). In white women, *IL-18* genotype (GC/CC: $\beta=-0.18$, $P=0.023$), age ($\beta=0.29$, $P<0.01$) and body fat (kg) ($\beta=0.19$, $P=0.010$) were significant predictors of systolic BP (Adjusted $R^2=0.16$, $P<0.01$).

*IL-18* genotype was not associated with measures of insulin sensitivity or blood lipid parameters in either ethnic group.
Table 4.1. Subject characteristics for genotypes of the -137 G/C polymorphism within the *IL-18* gene in black and white South African women

<table>
<thead>
<tr>
<th></th>
<th>Black women</th>
<th>White women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG Genotype (N=156)</td>
<td>GC Genotype (N=50)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Means ± SE</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>156</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>156</td>
<td>1.60 ± 0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>156</td>
<td>74.6 ± 2.0</td>
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<tr>
<td>Body fat (%)</td>
<td>155</td>
<td>39.6 ± 1.3</td>
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<tr>
<td>WC (cm)</td>
<td>156</td>
<td>88.1 ± 0.1</td>
</tr>
<tr>
<td>WHR</td>
<td>156</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>120</td>
<td>70.7 ± 5.3</td>
</tr>
<tr>
<td>SAT (cm²)</td>
<td>120</td>
<td>371 ± 20</td>
</tr>
</tbody>
</table>

*WC*, waist circumference; *WHR*, waist-hip ratio; *VAT*, visceral adipose tissue area; *SAT*, subcutaneous adipose tissue area.
<table>
<thead>
<tr>
<th></th>
<th>Black Women</th>
<th>White Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG genotype (N=156)</td>
<td>GC or CC genotype (N=89)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Means ± SE</td>
</tr>
<tr>
<td>Blood Pressure</td>
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<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>156</td>
<td>110.2 ± 1.3</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
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<td>74.5 ± 1.1</td>
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<tr>
<td>Insulin (mU/L)</td>
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<tr>
<td>HOMA-IR</td>
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<td>2.32 ± 0.15</td>
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<td>Lipid Profile</td>
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<tr>
<td>Triglycerides (mmol/L)</td>
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<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>145</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>145</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>145</td>
<td>3.1 ± 0.1</td>
</tr>
</tbody>
</table>

BP, blood pressure; HOMA-IR, homeostasis model of insulin resistance; TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TC/HDL-C, total cholesterol-high density lipoprotein-cholesterol ratio.
4.5. DISCUSSION

The main and novel finding of this study was that the C-allele of the \(-137\ G/C\) polymorphism within the \(IL-18\) gene was associated with BP in apparently healthy black and white South African women. However, the direction of the relationship between genotype and BP was different between black and white women. In contrast to my original hypothesis, variation within the \(IL-18\)-137 G/C polymorphism could not explain ethnic differences in circulating IL-18 concentrations. This is despite black women having a higher frequency of the G allele, which has previously been associated with higher IL-18 concentrations.

\(IL-18\) genotype, circulating IL-18 concentrations, age and VAT were all significantly associated with BP, accounting for 22.6% of the variability in the black women and 16.8% in the white women; however the direction of association between genotype and BP was different between the two ethnicities. The C-allele was associated with higher BP in black women, whereas it was associated with lower BP in white women. The actual difference in BP between genotype groups was ~5mmHG. And though seemingly little, this is similar to results from weight-loss intervention studies, that have shown that 5kgs in weight loss corresponds to an average drop of 5.6mmHg in systolic BP [241]. The C-allele of this polymorphism has previously been associated with several inflammatory diseases including cancer [242] and type 1 diabetes [243-245]. However in a Finnish population, hypertension was a risk factor for sudden cardiac death and coronary atherosclerosis only in carriers of the -137 G allele, but not the C allele [246]. Although the exact reasons for the differential relationship between IL-18 genotype and BP between ethnic groups in the present study are unknown, the presence of strong linkage disequilibrium across the \(IL-18\) gene might suggest that the effects of other genetic markers
linked to the -137G/C genotype may underlie this association [238]. In support of this, Tiret et al. (2005) examined the genetic variability of four functionally important variants in the IL-18 system in relation to circulating IL-18 concentrations and cardiovascular mortality. Their analysis of other polymorphisms (G-887T, C-105T, S35S, A+183G and T+533C) within the *IL-18* gene did not show an association between any of the individual SNP’s and IL-18 concentrations or cardiovascular outcomes. However, a haplotype (GCAGT) of these polymorphisms was associated with circulating IL-18 concentrations and clinical outcome in patients with cardiac disease, but the association with cardiovascular risk was not independent of circulating IL-18 concentrations and explained less than 2% of the inter-individual variability in IL-18 concentrations [238].

Although the mechanisms are unclear, both endothelial cells and smooth muscle cells express the IL-18 receptor, which show enhanced expression in response to other inflammatory cytokines, namely, IL-1β, TNF-α, IL-6 and IFN-γ [131], and the potent vasoconstrictor, angiotensin II [247]. Stimulation of endothelial and smooth muscle by IL-18 produces biologically active IFN-γ, as well as IL-1β, IL-6 and TNF-α [124;248;249]. The reciprocal inducibility of these cytokines by IL-18 signalling suggests a positive feedback loop in the vasculature, which might contribute to dysregulation in the inflammatory response [131]. This is of particular relevance to BP regulation as high concentrations of TNF-α in the vascular lumen inhibit nitric oxide synthase activation and nitric oxide production [250], leaving unopposed vasoconstriction of endothelin-1 [251]. Alternatively, or in addition, IL-18 may exert its effects on BP via the RAS system, as IL-6, IL-1, TNF-α and IFN-γ have been shown to up regulate angiotensin gene expression [252].
Similar to findings by Ness et al., white women in the present study had a higher frequency of the C-allele compared to black women, however the genotype distribution was lower in our study cohort compared to theirs (White Americans: GG= 51.8%, GC=40.0%, CC=8.2% vs. white South Africans: GG=48.6%; GC=43.5%; CC=7.9%; and black Americans: GG=59.1%, GC=36.3%, CC=4.7% vs. black South Africans: GG=77%; GC=23%; CC=0%) [106], Notably, this discrepancy is similar to that observed between Zulus and African-Americans when comparing the distribution of the 592C/A polymorphism within the IL-10 gene [253]. However, despite the differences in distribution and even though both the -137 G/C polymorphism, and baseline IL-18 concentrations were associated with individual metabolic risk factors, there was no association between the -137 G/C polymorphism and circulating IL-18 concentrations in either ethnic group in this study. The IL-18 -137 G/C promoter polymorphism has been found to have clear promoter activity when blood monocytes were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomicin, yet non-significant differences in IL-18 expression was observed between alleles [137], suggesting that the functional relevance of this polymorphism warrants further investigation.

In conclusion, ethnic differences in circulating IL-18 concentrations could not be explained by differences in the distribution of the -137G/C polymorphism within the IL-18 gene in black and white South African women. However, differences in BP reported across IL-18 genotypes, together with the results from the previous chapter, strengthen the putative role of IL-18 as an inflammatory mediator of obesity-related CVD. Further research is required to verify the association between the -137 G/C polymorphism within the IL-18 gene and BP in a higher risk population, and identify other factors that may explain the higher IL-18 concentrations in black women.
CHAPTER FIVE

DEPOT- AND ETHNIC-SPECIFIC ASSOCIATIONS BETWEEN ADIPOSE TISSUE INFLAMMATION AND INSULIN SENSITIVITY

Data from this chapter have been published in part in: Evans J, Goedecke JH, Söderström I, Búren J, Alvehus M, Blomquist C, Jonsson F, Hayes PM, Adams K, Dave JA, Levitt NS, Lambert EV, Olsson T. Depot- And Ethnic-Specific Differences In The Relationship Between Adipose Tissue Inflammation And Insulin Sensitivity. 2011 Clin Endocrinol (Oxf);74:51-59.

5.1. ABSTRACT

Ethnic differences in circulating inflammatory markers may be mediated by ethnic differences in body fat distribution, as black women have less central fat, specifically VAT, and more peripheral fat than white women. Indeed the relationship between abdominal AT depots and $S_I$ has been shown to differ between black and white women, with abdominal SAT more closely related to $S_I$ in black women. It is not clear as to whether these differences are associated with differences in inflammatory gene expression between abdominal and gluteal SAT. We therefore tested the hypotheses that SAT inflammatory gene expression is greater in the abdominal compared to the gluteal depot and SAT inflammatory gene expression is associated with differential $S_I$ in black and white women.

$S_I$ (frequently sampled intravenous glucose tolerance test), and abdominal SAT, and gluteal SAT gene expression levels of 13 inflammatory genes were measured in a subgroup of normal-weight (body mass index (BMI): 18-25 kg/m$^2$) and obese (BMI >30 kg/m$^2$) black (n=30) and white (n=26) South African women. Black women had higher abdominal and gluteal SAT expression of macrophage markers, chemokines and cytokines compared to white women (P<0.01).
Multivariate analysis showed that inflammatory gene expression in the white women explained 56.8% of the variance in $S_i$ ($P<0.005$), compared to 20.9% in black women ($P=0.30$). Gluteal SAT had lower expression of adiponectin, but higher expression of inflammatory cytokines, macrophage markers and leptin than abdominal SAT depots ($P<0.05$).

In conclusion black South African women had higher SAT inflammatory gene expression levels than white women; however the relationship between SAT inflammation and $S_i$ was stronger in white compared to black women. Further research is required to explore the contributions of inflammation from other tissues, which may affect $S_i$ in black populations. Contrary to our original hypothesis, gluteal SAT had a greater inflammatory gene expression profile than abdominal SAT depots. The protective nature of gluteo-femoral fat therefore requires further investigation.

5.2. INTRODUCTION

As described in Chapter 3, chronic low-grade inflammation, with a putative origin in AT, has been suggested to be a key link between obesity and IR [97]. Expression of inflammatory genes within AT are increased in obese individuals [185] and AT mRNA levels of inflammatory cytokines, chemokines and adipokines have been shown to be associated with metabolic risk factors for CVD and T2DM [254]. As described in Chapter 2 and 3, abdominal obesity is associated with IR, and increased risk of T2DM and CVD [6], with the added increased metabolic risk of VAT versus abdominal SAT having been attributed to its apparent higher expression of inflammatory cytokines [70;71].

This may be of particular importance in explaining ethnic differences in circulating inflammatory markers described in Chapter 3 and the ethnic differences in the relationship between AT depots
and $S_I$ discussed in Chapter 2. Notably, previous studies have shown that while both VAT and abdominal SAT depots were similarly correlated to $S_I$ in white women, SAT was more closely related to $S_I$ in black women [12]. Further, in Chapter 2 I found that WC, which reflects both VAT and SAT, was better than VAT for identifying black and white women with IR (upper tertile of HOMA-IR), suggesting that certain properties of SAT contribute to IR, especially, in black women. Notably, DSAT, a sub-compartment within the abdominal SAT depot, has been reported to have an inflammatory gene expression greater than superficial SSAT [75] and in the case of TNF-$\alpha$ expression, similar to that of visceral AT [75]. Indeed, TNF-$\alpha$ expression correlated positively with IR in healthy normal-weight subjects [75]. Therefore, ethnic differences in the relationship between abdominal AT tissue depots and IR might be explained, in part, by differences in depot-specific inflammation.

In contrast, lower body obesity (fat stored in the femoral-gluteal region), which is associated with a lower risk of metabolic complications [64] has been suggested to be protective due to a more favorable metabolic profile [7]. Importantly, however, to my knowledge no studies have compared inflammatory gene expression between gluteal and abdominal SAT depots. Thus for this study I explored the process of AT inflammation from macrophage recruitment (macrophage marker and chemokine mRNA) to adipocytokines (cytokines and adipokines).

I therefore tested three hypotheses: 1) SAT inflammatory gene expression is greater in black compared to white South African women; 2) SAT inflammatory gene expression is greater in the abdominal compared to the gluteal depot; 3) a depot-specific inflammatory profile is associated with differential $S_I$ in black and white South African women.
5.3. METHODS

5.3.1. Subjects

The study consisted of a sub-sample of 15 normal-weight (BMI 18-25 kg/m$^2$) black, 13 normal-weight white, 15 obese (BMI >30 kg/m$^2$) black and 13 obese white South African women, who were recruited by advertisement as described previously in Chapter 2. The study was approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town and written informed consent to participate in the study was obtained from all subjects prior to participation in the study.

5.3.2. Testing procedures

5.3.2.1. Body composition assessment

As previously described in Chapter 2, 3 and 4, basic anthropometric measurements, including weight, height, WC and hip circumference were taken, and fat mass and gynoid region of interest [255] were measured using DXA (Discovery-W, Software version 4.40, Hologic Inc., Bedford, MA, USA). Abdominal VAT, DSAT and SSAT areas were measured using CT-scan (Toshiba X-press Helical Scanner, Japan) at the level of L4-L5 lumbar vertebrae, with the fascia superficialis used to discriminate DSAT vs. SSAT [222].

5.3.2.2. Fat biopsies

After a 4 hr fast, fat biopsies were obtained from the abdominal DSAT, SSAT and gluteal areas, using a mini-liposuction technique. After local anaesthesia with Lignocaine hydrochloride (2%, Intramed, Port Elizabeth, South Africa), a small incision was made directly above the umbilicus. 200-300 ml of normal saline with adrenaline (0.1%, Intramed, Port Elizabeth, South Africa) and
Lignocaine (0.75%, Intramed, Port Elizabeth, South Africa) was infused using an infiltration cannula (Lamis 14ga x 15cm, Byron Medical Inc., Tucson, AZ). An aspiration cannula (Coleman, 12ga x 15cm, Byron Medical Inc, Tucson, AZ) attached to a 10ml syringe was used to aspirate fat from above (SSAT) and below (DSAT) the fascia superficialis, under ultrasound guidance by a radiologist. Gluteal samples were obtained from the right upper quadrant using the same procedure. Approximately 2 ml of fat was extracted from each site and washed three times with normal saline or until no blood was visible. AT was then placed into vials and frozen immediately in liquid nitrogen and stored at $-80^\circ$ C for subsequent analyses.

### 5.3.2.3. Adipocyte area

Frozen SAT samples were cut into 25µm sections and stained with haematoxylin and eosin, as previously described [256]. Images of intact adipocytes were captured using an inverted fluorescent microscope (Zeiss Axiovert 200, Zeiss, Jena, Germany) connected to an Axiocam (black and white, Zeiss, Jena, Germany) with Axiovision software (Axiovision Rel 4.5, Zeiss, Jena, Germany). Computer image analysis (WCIF Image J software, Java, Maryland, USA) was used to measure adipocyte area as previously described [257]. Mean adipocyte area was calculated from the average area of 200 cells.

### 5.3.2.4. Insulin sensitivity ($S_I$)

After an overnight fast, subjects underwent an insulin-modified FSIGT to quantify insulin sensitivity. Baseline samples were drawn at -15, -5 and -1 min prior to the infusion of glucose (50% dextrose; 11.4 g/m2 body surface area) over 60 seconds commencing at time 0. At 20 min, human insulin (0.02 unit/kg; Actrapid, Novo Nordisk) was infused over 5 min at a constant rate. Plasma glucose and serum insulin concentrations were measured in the three baseline samples.
and the 32 samples drawn over 240 min following commencement of the glucose infusion.
Glucose and insulin data from the FSIGT were used to calculate the insulin sensitivity index (SI)
using Bergman’s minimal model of glucose kinetics [258].

5.3.2.5.  **Biochemical Analysis**

Plasma glucose concentrations were determined using the glucose oxidase method (YSI 2300
STAT PLUS; YSI Life Sciences, Yellow Springs, OH) and serum insulin by
immunochemiluminometric assays using the ADVIA Centaur (Bayer Diagnostics, Tarrytown,
NJ). Serum concentrations of leptin (Linco Research, St Charles, Missouri, USA), high
molecular weight (HMW) adiponectin (Linco Research, St Charles, Missouri, USA), hsCRP;
(Immun Diagnostik AG, Bensheim, Germany) and IL-18 (Biosource, Nivelles, Belgium) were
all analyzed using commercially available ELISA kits according to the manufacturer’s protocols.

5.3.2.6.  **RNA extraction, reverse transcription, and real-time PCR**

Total RNA was extracted from SAT biopsies using the RNeasy Lipid Tissue Mini and RNeasy
Lipid Tissue Midi Kit (QIAGEN, Hilden, Germany). The yield and purity were determined by
spectrophotometer (ND-1000 spectrophotometer; NanoDrop Technologies, Wilmington, DE)
and RNA integrity was analyzed by 1% agarose gel electrophoresis in the presence of ethidium
bromide.

Two micrograms of RNA were reverse transcribed, using TaqMan RT reagents (High Capacity
cDNA Reverse Transcription Kit, Applied Biosystems, Foster City, CA) and RNase inhibitor
(Applied Biosystems) at a final concentration of 1.0 U/ml. Subsequently, mRNA levels of
chemokines (Chemokine (C-C motif) ligand 2 (CCL2) and its receptor CCR2), macrophage
markers (CD14, CD68 and CD163), cytokines (colony stimulating factor 1 (CSF-1), macrophage
migration inhibitory factor (MIF), TNF-α, Interleukin (IL)-6, IL-10 and IL-18) and adipokines (leptin and adiponectin) were quantified (relative quantification standard curve method) using TaqMan Universal PCR Master Mix (Applied Biosystems) and the following TaqMan gene expression assays (Applied Biosystems): CD14 (Hs00169122_g1), CD68 (Hs00154355_m1), CD163 (Hs01016657_m1), CCL2 (Hs00234140_m1), CCR2 (Hs00356601_m1), CSF-1 (Hs00174164_m1), MIF (Hs00236988_g1), TNF-α (Hs00174128_m1), IL-6 (Hs00985639_m1), IL-10 (Hs00174086_m1), IL-18 (Hs99999040_m1), leptin (Hs00174877_m1), adiponectin (Hs00605917_m1), peptidylprolyl isomerase A (PPIA) (Hs99999904_m1), as well as ribosomal protein P0 (RPLP0) (Hs99999902_m1). Each sample was run in duplicate on an ABI Prism 7900HT sequence detection system (Applied Biosystems) and the relative expression level was calculated from the standard curve. Reference genes were evaluated by running PPIA and RPLP0 on the full study cohort. NormFinder algorithm identified PPIA as the best normalization gene, expression levels of which were not altered by obesity (p=0.476), ethnicity (p=0.075) or depot (p=0.143). Expression levels of the target genes were thus normalized to the PPIA endogenous control.

5.3.2.7. Measurement of protein expression in adipose tissue

Frozen SAT samples (250-350mg) were washed in phosphate buffer solution and homogenized in lysis buffer (20mM TrisHCL, 1mM EDTA, and 0.1mM phenylmethanesulfonyl fluoride pH 7.5). The homogenate was centrifuged for 30 minutes (+4°C, 14,000rpm), and the infranant used to determine, in duplicate, human leptin (Millipore Corporation, Billerica, MA, USA), IL-18 (BioSource Europe S.A. Nivelles, Belgium), CSF-1 and CCL2 (R&D Systems Europe, Ltd. Abingdon, United Kingdom), using commercial ELISA kits according to the manufacturer’s instructions. Final concentrations were calculated based on an internal standard curve.
5.3.3. Statistics

5.3.3.1. Univariate data analysis

As previously described [12], power calculation was based on data from Lovejoy et al. [59], which showed that we had 80% power to detect a difference of $2 \times 10^{-5}$ min/pmol/l in $S_I$ at $P < 0.05$ with 25 subjects per ethnic group. Normally distributed data are presented as means ± SD with ranges in parentheses. Non-normally distributed parameters were logarithmically transformed for parametric analysis and are presented as geometric means with 95% confidence intervals. Two-way ANCOVA, adjusting for age, was used to compare body composition, fat distribution, adipocyte area, $S_I$ and circulating inflammatory markers between normal-weight and obese, black and white women. To explore the differences in gene and protein expression between SAT depots, and how obesity and ethnicity alter this, two-way ANOVA, with repeated measures and Tukey’s post-hoc analysis was used. Ethnic comparisons were adjusted for age, total body fatness (fat kg) and VAT. Spearman’s correlation coefficients were used to explore the relationships between circulating inflammatory markers and inflammatory gene and protein expression levels. Data were analyzed using STATISTICA version 9 (Statsoft Inc., Tulsa, OK).

5.3.3.2. Multivariate data analysis

Partial least squares (PLS) regression, a multivariate linear projection method, was used to explore the association between SAT gene expression and $S_I$, in two separate models for black and white women. The predictor variables included in each model were total body fatness (fat kg) and gene expression levels of the 13 genes measured in the three different SAT depots (40 predictor variables in total). ANOVA was used to test the significance of each model. The VIP (Variables Important to the Projection) value reflects the importance of the variable in the model.
both with respect to the other predictor variables (gene expression levels) and the response variable, $S_I$. Confidence intervals were computed by jack knife from the cross validation using the standard formula [259]. A predictor variable was considered significant to the model if it had a combination of a VIP greater than 1, and the lower limit of the VIP confidence interval (CI) not below 0.5 [260]. Regression coefficients show the effect of the different genes on $S_I$. For the multivariate analysis commercial software was used (SIMCA 12.0 from Umetrics AB, Umeå Sweden).

5.4. RESULTS

5.4.1. Body composition, fat distribution, insulin sensitivity and circulating adipokines

Subject characteristics, as described previously [12], are shown in Table 5.1. In brief, black and white groups were well matched for BMI, total adiposity (DXA-derived fat mass), waist and hip circumferences, and DXA-derived measure of gluteal fat. However, obese black women had significantly less VAT, but more SSAT than obese white women, independent of total body fatness. There were no ethnic differences in adipocyte area, but mean gluteal adipocyte area was significantly greater than DSAT and SSAT adipocyte area in both normal-weight and obese women (P<0.01).

$S_I$ was lower in normal-weight and obese black compared to white women (P<0.001). Both obese black and white women had significantly lower $S_I$ than normal-weight women (P<0.05). In contrast to the findings in the larger cohort, reported in Chapter 3, no ethnic differences in circulating hsCRP, IL-18, leptin or adiponectin concentrations were observed. Circulating hsCRP and leptin concentrations were higher in obese versus normal-weight women, while
circulating adiponectin concentrations were lower (P<0.001). Plasma IL-18 concentrations did not differ between normal-weight and obese groups.

5.4.2. Subcutaneous adipose tissue gene expression

Gene expression levels of the chemokines, macrophage markers, cytokines and adipokines are shown in Figures 5.1-5.33. When examining the differential effects of ethnicity on SAT gene expression, we found that both normal-weight and obese black women had significantly higher expression of CCL2, CD68, CSF-1 and TNF-α in all three depots than white women. Additionally, black women had significantly higher abdominal DSAT and SSAT expression of leptin, and DSAT expression of CCR2, than white women. Further, MIF expression was significantly higher in obese black compared to obese white women in all three depots. Ethnic differences in gene expression were still significant after adjusting for age, total body fatness (kg fat) and VAT.

When exploring the effect of depot differences in SAT inflammatory gene expression, we found that gluteal SAT had significantly lower expression of adiponectin, but higher expression of CCR2, CD68, CD14, CD163, MIF, TNF-α, IL-18, IL-10 and leptin, than DSAT and SSAT (P<0.01). Further, DSAT had higher CSF-1 and IL-6 mRNA levels than both SSAT and gluteal SAT depot, as well as lower leptin and higher IL-10 mRNA levels than SSAT (P<0.01).

We then explored the effect of obesity on SAT gene expression. Adiponectin mRNA levels were lower in all depots in obese compared to normal-weight women. In contrast, the expression of leptin and all inflammatory markers, except IL-6 (unchanged), were higher in obese compared to normal-weight women, for all three AT depots (P<0.01).
Table 5.1. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Normal-weight</th>
<th></th>
<th>Obese</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black (n=15)</td>
<td>White (n=13)</td>
<td>Black (n=15)</td>
<td>White (n=13)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>24 ± 1\textsuperscript{a}</td>
<td>25 ± 1\textsuperscript{b}</td>
<td>28 ± 2\textsuperscript{a}</td>
<td>31.7 ± 2.1\textsuperscript{b}</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>23.0 ± 0.4\textsuperscript{a}</td>
<td>22.6 ± 0.4\textsuperscript{b}</td>
<td>38.1 ± 0.9\textsuperscript{a}</td>
<td>36.5 ± 1.6\textsuperscript{b}</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>76.3 ± 1.1\textsuperscript{a}</td>
<td>79.7 ± 1.6\textsuperscript{b}</td>
<td>113.4 ± 3.1\textsuperscript{a}</td>
<td>108.3 ± 4.3\textsuperscript{b}</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>98.7 ± 1.3\textsuperscript{a}</td>
<td>102.0 ± 1.1\textsuperscript{b}</td>
<td>125.9 ± 1.8\textsuperscript{a}</td>
<td>125.3 ± 4.1\textsuperscript{b}</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>17.4 ± 0.9\textsuperscript{a}</td>
<td>18.9 ± 1.4\textsuperscript{b}</td>
<td>43.8 ± 1.7\textsuperscript{a}</td>
<td>45.6 ± 14.9\textsuperscript{b}</td>
</tr>
<tr>
<td>Gynoid fat (kg)</td>
<td>1.74 ± 0.10\textsuperscript{a}</td>
<td>1.70 ± 0.15\textsuperscript{b}</td>
<td>4.07 ± 0.23\textsuperscript{a}</td>
<td>3.54 ± 0.20\textsuperscript{b}</td>
</tr>
<tr>
<td>DSAT (cm\textsuperscript{3})</td>
<td>70.1 ± 6.6\textsuperscript{a}</td>
<td>83.3 ± 11.4\textsuperscript{b}</td>
<td>269.9 ± 16.2\textsuperscript{a}</td>
<td>257.6 ± 23.1\textsuperscript{b}</td>
</tr>
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<td>SSAT (cm\textsuperscript{3})</td>
<td>99.4 ± 8.2\textsuperscript{a}</td>
<td>95.2 ± 7.3\textsuperscript{b}</td>
<td>321.4 ± 21.9\textsuperscript{a,d}</td>
<td>247.6 ± 14.9\textsuperscript{b,d}</td>
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<td>VAT (cm\textsuperscript{2})</td>
<td>56.9 \textsuperscript{a}</td>
<td>48.9 \textsuperscript{b}</td>
<td>107.6 \textsuperscript{a,d}</td>
<td>129.3 \textsuperscript{b,d}</td>
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<tr>
<td>Fat cell Size</td>
<td></td>
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<tr>
<td>DSAT (mm\textsuperscript{2})</td>
<td>4410 ± 409\textsuperscript{a}</td>
<td>3851 ± 145\textsuperscript{b}</td>
<td>5100 ± 320\textsuperscript{a}</td>
<td>5284 ± 277\textsuperscript{b}</td>
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<td>SSAT (mm\textsuperscript{2})</td>
<td>4218 ± 275\textsuperscript{a}</td>
<td>3710 ± 355\textsuperscript{b}</td>
<td>6174 ± 410\textsuperscript{a}</td>
<td>5752 ± 606\textsuperscript{b}</td>
</tr>
<tr>
<td>Gluteal (mm\textsuperscript{2})</td>
<td>5058 ± 552\textsuperscript{a}</td>
<td>4491 ± 185\textsuperscript{b}</td>
<td>6332 ± 206\textsuperscript{a}</td>
<td>5908 ± 336\textsuperscript{b}</td>
</tr>
<tr>
<td>Insulin Sensitivity (S\textsubscript{I})</td>
<td>1.60 \textsuperscript{a,c}</td>
<td>6.31 \textsuperscript{b,c}</td>
<td>1.11 \textsuperscript{a,d}</td>
<td>3.52 \textsuperscript{b,d}</td>
</tr>
<tr>
<td>Circulating inflammatory markers</td>
<td></td>
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<tr>
<td>hsCRP (mg/L)</td>
<td>2.8 ± 0.6\textsuperscript{a}</td>
<td>1.4 ± 0.3\textsuperscript{b}</td>
<td>6.4 ± 0.8\textsuperscript{a}</td>
<td>6.4 ± 1.0\textsuperscript{b}</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>6.5 ± 0.7\textsuperscript{a}</td>
<td>6.8 ± 0.8\textsuperscript{b}</td>
<td>3.7 ± 0.5\textsuperscript{a}</td>
<td>4.1 ± 0.6\textsuperscript{b}</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>16.4 ± 2.7\textsuperscript{a}</td>
<td>18.8 ± 3.3\textsuperscript{b}</td>
<td>57.9 ± 3.1\textsuperscript{a}</td>
<td>50.8 ± 2.9\textsuperscript{b}</td>
</tr>
<tr>
<td>IL-18 (pg/ml)</td>
<td>102.7</td>
<td>197.4</td>
<td>102.1</td>
<td>262.8</td>
</tr>
<tr>
<td></td>
<td>(39.0-279.18)</td>
<td>(105.7-209.6)</td>
<td>(70.7-249.7)</td>
<td>(200.1-336.1)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for normally distributed variables. VAT, S\textsubscript{I} and IL-18 had a skewed distribution, therefore the median and interquartile ranges are displayed in parenthesis. P-value adjusted for age. BMI, body mass index; DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; VAT, visceral adipose tissue area; hsCRP, high sensitivity C-reactive protein; IL-18, Interleukin 18. Matching superscripts represent differences between groups. \textsuperscript{a} Normal-weight black vs. obese black (P<0.001); \textsuperscript{b} Normal-weight white vs. obese white (P<0.001); \textsuperscript{c} Normal-weight black vs. normal-weight white (P<0.01); \textsuperscript{d} Obese black vs. obese white (P<0.01).
Figure 5.1. Adipose tissue chemokine gene expression. ■ Normal-weight black women; □ Normal-weight white women; △△ Obese black women; △△△ Obese white women. DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; GLUT, gluteal adipose tissue. mRNA levels are normalized to PPIA. Each bar represents mean ± SE. δ P<0.001, GLUT, vs. DSAT and SSAT. # P<0.001, Normal-weight vs. obese. * P<0.001, Black vs. white.
Figure 5.2. Adipose tissue macrophage marker gene expression. ■ Normal-weight black women; □ Normal-weight white women; ▢▪▪▪ Obese black women; □▪▪▪▪ Obese white women. DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; GLUT, gluteal adipose tissue. mRNA levels are normalized to PPIA. Each bar represents mean ± SE. δ P<0.001, GLUT, vs. DSAT and SSAT. # P<0.001, Normal-weight vs. obese. * P<0.001, Black vs. white.
Figure 5.3. Adipose tissue cytokine and adipokine gene expression. □ Normal-weight black women; □ Normal-weight white women; □□□ Obese black women; □□□□ Obese white women. DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; GLUT, gluteal adipose tissue. mRNA levels are normalized to PPIA. Each bar represents mean ± SE. δ P<0.001, GLUT vs. DSAT and SSAT. λ P<0.05, DSAT vs. SSAT and GLUT. # P<0.001, Normal-weight vs. obese. * P<0.001, Black vs. white.
5.4.3. Relationship between insulin sensitivity and subcutaneous adipose tissue gene-expression and circulating concentrations

Multivariate analysis was used to assess the relationship of SAT inflammatory gene expression and total body fatness (kg fat) to $S_I$, in 2 separate models for white and black women (Figure 5.4a and b). In both models, with the exception of adiponectin which showed a positive association with $S_I$, all other markers were negatively associated with $S_I$, irrespective of depot.

The model for white women was able to explain 56.8% of the variation in $S_I$ ($P<0.005$). Nineteen of the 40 predictor variables were considered significant to the model (VIP>1 and lower VIP-CI limit > 0.5). The majority of these were genes expressed in the abdominal depots, whereas only CCR2 and IL-18 expression in the gluteal depot added significantly to the model.

Conversely, the model for $S_I$ in black women was only able to explain 20.9% of the variation. Although the overall model was not able to account for a significant variation in $S_I$ in these women ($P=0.30$), 12 of the 40 predictor variables were considered significant (VIP>1 and lower VIP-CI limit > 0.5). Similar to the model for $S_I$ in white women, the majority of genes were those expressed in the abdominal depots.

Similar to the findings for gene expression, circulating hsCRP ($r=-0.59$, $P=0.002$), adiponectin ($r=0.49$, $P=0.045$) and leptin ($r=-0.62$, $p=0.007$) were significantly associated with $S_I$ in white, but not black women (hsCRP: $r=-0.17$, $p=0.35$; adiponectin: $r=0.03$, $p=0.86$; leptin: $r=-0.15$, $p=0.44$). Circulating IL-18 concentrations were not associated with $S_I$ in either ethnicity (Black women: $r=-0.11$, $p=0.57$; White women: $r=-0.31$, $p=0.08$).
Figure 5.4. Relationship between adipose tissue gene expression and insulin sensitivity ($S_I$) in white women (a) and black women (b). Deep subcutaneous adipose tissue, Superficial subcutaneous adipose tissue, Gluteal subcutaneous adipose tissue. Positive regression coefficients have a positive influence on $S_I$, whereas negative regression coefficients have a negative influence on $S_I$. * Variables significant to the model (combined VIP greater than 1 and lower VIP-CI limit greater than 0.5).
5.4.4. Subcutaneous adipose tissue protein expression

SAT protein levels are shown in Table 5.2. There were no differences between normal-weight and obese groups, or between ethnic groups in SAT leptin and CSF-1 protein levels in all 3 depots, nor for gluteal IL-18 protein levels. As with leptin mRNA levels, leptin protein levels in the gluteal depot were higher than in the abdominal DSAT and SSAT depots (p<0.001). Conversely, despite higher DSAT CSF-1 mRNA levels, DSAT CSF-1 protein levels were lower than in the SSAT and gluteal SAT depots (P<0.05).

SAT protein levels of CCL2 were below the detection level (<31.5pg/ml) in all depots. Similarly IL-18 protein levels were below the detection limit (<12.5pg/ml) for a majority of the abdominal DSAT (66%) and SSAT (64%) samples, and as a result, only data relating to the gluteal depot are presented.

5.4.5. Relationship between subcutaneous adipose tissue gene expression, protein levels and corresponding circulating adipokine concentrations

CSF-1 mRNA levels did not correlate with SAT CSF-1 protein levels (data not shown). Similarly, SAT leptin mRNA levels did not correlate with corresponding SAT protein levels, but did correlate with circulating leptin levels (DSAT: r=0.72, P<0.001; SSAT: r=0.73, P<0.001; Gluteal: r=0.56, P<0.001). Conversely, gluteal IL-18 mRNA levels correlated with gluteal IL-18 protein levels (r=0.35, p<0.05), but SAT IL-18 mRNA in all depots and gluteal IL-18 protein levels did not correlate with circulating levels (data not shown). In contrast, SAT adiponectin mRNA in all depots correlated significantly with circulating adiponectin levels (DSAT: r=0.56, P<0.001; SSAT: r=0.63, P<0.001; Gluteal: r=0.48, P<0.001).
Table 5.2. Adipose tissue protein expression levels in normal-weight and obese, black and white women

<table>
<thead>
<tr>
<th></th>
<th>Normal-weight</th>
<th>Obese</th>
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<tbody>
<tr>
<td></td>
<td>Black</td>
<td>White</td>
<td>Black</td>
<td>White</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=14</td>
<td>N=12</td>
<td>N=15</td>
<td>N=13</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.82 ± 0.49</td>
<td>1.36 ± 0.30</td>
<td>2.09 ± 0.44 (0.72-5.91)</td>
<td>1.20 ± 0.13 (0.61-2.02)</td>
<td></td>
</tr>
<tr>
<td>DSAT</td>
<td>(0.66-5.57)</td>
<td></td>
<td></td>
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<tr>
<td>SSAT</td>
<td>2.72 ± 0.70</td>
<td>1.92 ± 0.46</td>
<td>2.35 ± 0.38 (0.52-5.52)</td>
<td>2.42 ± 0.43 (0.78-5.67)</td>
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<tr>
<td>(0.52-6.89)</td>
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<tr>
<td>GLUT</td>
<td>4.63 ± 0.75(^a)</td>
<td>3.96 ± 0.49(^a)</td>
<td>4.01 ± 0.50(^a)</td>
<td>5.21 ± 0.67(^a)</td>
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<tr>
<td>(0.90-11.04)</td>
<td>(1.45-7.20)</td>
<td></td>
<td>(1.06-8.07)</td>
<td>(1.41-8.53)</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CSF-1 (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSAT</td>
<td>268.51 ± 45.48(^b)</td>
<td>302.92 ± 37.90(^b)</td>
<td>303.41 ± 35.98(^b)</td>
<td>285.53 ± 50.07(^b)</td>
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<tr>
<td>(76.15-671.45)</td>
<td>(140.15-572.71)</td>
<td></td>
<td>(91.53-564.80)</td>
<td>(119.87-631.57)</td>
<td></td>
</tr>
<tr>
<td>SSAT</td>
<td>474.03 ± 52.79</td>
<td>512.06 ± 42.70</td>
<td>394.10 ± 42.07</td>
<td>428.67 ± 45.35</td>
<td></td>
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<tr>
<td>(85.92-788.67)</td>
<td>(189.78-703.23)</td>
<td></td>
<td>(125.5-689.80)</td>
<td>(243.51-746.97)</td>
<td></td>
</tr>
<tr>
<td>GLUT</td>
<td>498.26 ± 79.03</td>
<td>461.26 ± 41.65</td>
<td>430.81 ± 61.11</td>
<td>490.27 ± 48.61</td>
<td></td>
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<tr>
<td>(129.64-1305.02)</td>
<td>(286.78-755.10)</td>
<td></td>
<td>(155.93-866.87)</td>
<td>(265.76-777.04)</td>
<td></td>
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<tr>
<td>IL-18 (ng/ml)</td>
<td></td>
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<tr>
<td>GLUT</td>
<td>45.86 ± 19.3</td>
<td>23.74 ± 6.88</td>
<td>52.40 ± 5.73</td>
<td>37.50 ± 9.56</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, with ranges in parentheses. DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; GLUT, gluteal subcutaneous adipose tissue; CSF-1, macrophage colony stimulating factor 1; IL-18, interleukin – 18. \(^a\)P<0.05, GLUT vs. DSAT and SSAT; \(^b\)P<0.05, DSAT vs. SSAT and GLUT.
5.4.6. Relationship between adipocyte size and circulating inflammatory markers and inflammatory gene expression

Adipocyte area in all three AT depots correlated positively with circulating hsCRP and leptin, and negatively with circulating adiponectin (P<0.005), however, the associations with the regional fat depots and adipocyte area were no longer significant after adjustment for total body fatness. Circulating IL-18 concentrations did not correlate with any measures of regional adiposity or with adipocyte area (P>0.05). Gluteal, deep SAT and superficial SAT gene expression correlated with adipocyte area in all three AT depots, showing a positive correlation for leptin, the macrophage markers, cytokines (excluding IL-6), and chemokines mRNA levels, and a negative association with adiponectin expression (p<0.001). However, after adjusting for age and total body fatness, the positive correlation between adipocyte area and gene expression for all genes measured was no longer significant (P>0.05).

5.5. DISCUSSION

This study reports two novel findings: Firstly, in support of the original hypothesis, black South African women had higher SAT inflammatory gene expression compared to white South African women. However this only explained a small non-significant proportion (less than 21%) of the variance in SI, whereas in white women, their SAT inflammatory gene expression profile explained 56% of the variance in SI. Secondly, inflammatory gene expression was higher in gluteal compared to abdominal SAT, independent of adiposity and ethnicity. This is in contrast to the hypothesis that gluteal AT is protective due to a reduced inflammatory profile [7].
5.5.1. Ethnic differences in gene expression

To my knowledge, this is the first study to show ethnic differences in SAT inflammatory gene expression. Black women had a higher expression of the chemokine CCL2 and its receptor CCR2, macrophage marker CD68 and cytokines TNF-α, CSF-1, MIF than white women; independent of age, total adiposity and VAT. The relevance of these findings are highlighted by studies showing that increased expression of CD68 is positively related to macrophage content within AT [71], and the recruitment of additional macrophages into AT is signalled by the release of chemo-attractants, such as CCL2 [261]. Activated macrophages secrete increasing amounts of cytokines such as TNF-α, MIF and CSF-1 in a feed-forward manner, further amplifying the inflammatory response. This is in line with reports from genetic studies showing that African-American and sub-Saharan populations are more likely to carry allelic variants associated with a higher inflammatory response [106].

Despite the higher inflammatory profile in black women, SAT inflammatory gene expression only accounted for 20% of the variance in $S_I$ in black women, compared to 56% of the variance in $S_I$ in white women. In white women, 10 of the 13 genes measured were significantly negatively associated with $S_I$. These included the chemokines CCL2 and CCR2, macrophage markers CD68, CD14 and CD163, and cytokines IL-18, CSF-1, TNF-α, IL-10 and leptin. Similarly, CCL2, CD68, CD14, IL-18, MIF and IL-10 were significantly negatively associated with $S_I$ in black women. Our findings support both murine [192;262] and human studies [72;73;75;263-265] that have examined the expression of these genes individually in relation to $S_I$, and support the presence of AT macrophage recruitment and proinflammatory activation during the development of IR.
Differences in the magnitude of association between SAT inflammatory gene expression and $S_I$ in black and white women were mirrored at a circulating level, with leptin, adiponectin and hsCRP concentrations correlating significantly with $S_I$ in white, but not black women. Although the results from Chapter 3 showed that hsCRP concentrations were significantly associated with HOMA-IR in both black and white women, the unadjusted $R^2$ was much higher in the white compared to the black women (0.19 vs. 0.06, respectively). In the present study, there were no differences in circulating concentrations of IL-18, as was found in the larger cohort in Chapter 3. Indeed, a possible explanation for these differences could be due to the smaller sample size in the present Chapter. Further, $S_I$ of the women in the current study was significantly different between ethnicities, whereas black and white women included in Chapter 3 had similar levels of IR, based on HOMA-IR. In support of this, a recent study that showed that African-American women were more insulin resistant than Caucasian women, independent of obesity, fat distribution and higher circulating inflammatory markers [37]. Notably, we have evaluated $S_I$ using FSIGT, which relates to peripheral skeletal muscle insulin sensitivity, while HOMA reflects the sensitivity of insulin-stimulated glucose uptake at basal levels. It therefore seems important to explore whether ethnic differences exist in insulin signalling in skeletal muscle. Of note, African-Americans have been shown to have greater intramuscular AT, which is associated with lower $S_I$ [266]. It is also possible that inflammation in VAT may be associated with $S_I$ in black women. Unfortunately, as we did not collect VAT we could not test this hypothesis. However, black women have less VAT than white women, and studies in America and South Africa have shown that in black women, SAT may be more closely associated with IR than VAT [12;58].
5.5.2. Depot differences in gene expression

The second novel and important finding was that gluteal SAT had higher mRNA levels of the chemokine CCL2, macrophage markers CD14, CD68, and CD163, inflammatory cytokines MIF, TNF-α, IL-18 and IL-10, and leptin, and lower expression of adiponectin, compared to the abdominal depots. This is in direct contrast to the hypothesis that gluteo-femoral SAT secretes less pro-inflammatory cytokines compared with abdominal SAT [7]. Accumulation of gluteal SAT has been regarded as protective, with some [7;64], but not all [65;66], studies showing a positive association between increasing hip circumference and/or leg fat mass and favourable metabolic risk profiles.

Despite lower expression levels compared to the gluteal depot, the majority of inflammatory genes contributing to the model for $S_1$ were from abdominal DSAT and SSAT, suggesting that inflammation in gluteal SAT does not contribute to IR as much as other sources. Inflammatory gene expression increases proportionately with adipocyte size [186] and the higher inflammatory profile in the gluteal depot may be an appropriate response due to the greater adipocyte size of the gluteal AT. This may also possibly explain the lower adiponectin and higher leptin expression in the gluteal compared to abdominal SAT. Indeed, Bruun et al. have demonstrated in humans in vitro that there is a cytokine-induced reduction in adiponectin mRNA [267]. This contrasts to two previous studies with limited sample size, which found no differences in adiponectin gene expression when comparing abdominal SAT and gluteal (n=5)[205] or thigh (n=3) [206] SAT.

Despite the consistent relationship among white women between mRNA and circulating concentrations of inflammatory markers with $S_1$, inflammatory mRNA levels (with the exception
of gluteal IL-18) did not correlate with protein levels. Previous studies have shown that marked disparity can exist between the relative gene expression levels and those of their corresponding proteins [268]. This could be due to methodological issues, including low sensitivity of detection and/or biological factors, such as post-transcriptional and translational regulation of the gene measured. Further research is therefore needed to identify which are the functionally relevant inflammatory mediators of $S_I$ in AT.

In conclusion, black South African women had higher inflammatory gene expression levels than white women but this only explained a small proportion of the variance in $S_I$. In contrast, in white women, the SAT inflammatory gene expression profile explained 56% of the variance in $S_I$. Further research is required to explore the contribution of inflammation in other tissues, which may affect insulin sensitivity in black populations, in particular the role of skeletal muscle, the major site for glucose disposal. Secondly, gluteal SAT had a greater inflammatory gene expression profile than abdominal SAT depots, which is in direct contrast to the hypothesis that gluteal SAT is ‘protective’ due to its lower inflammatory profile. The protective nature of gluteo-femoral fat therefore requires further investigation.
CHAPTER SIX

SUMMARY AND CONCLUSIONS

Inflammation is thought to be one of the main factors linking obesity to increased risk for CVD and T2DM. A better understanding of the role of inflammation in CVD and T2DM risk in different ethnic groups, who differ according to body fat distribution, SES and inflammation may help with improved disease prevention and future intervention strategies. Therefore the main objective of this thesis was to explore the ethnic-specific associations between obesity and inflammation, at a circulating, genetic and adipose tissue-specific level, with risk factors for CVD and T2DM in apparently healthy black and white South African women. The main findings of this thesis are summarized in Table 6.1 and further discussed below.

Obesity, in addition to being an independent risk factor for CVD and T2DM, is associated with other risk factors, namely elevated BP, dyslipidaemia and IR. As a result, several indices of obesity, including WC, WHtR and VAT are used for early detection of individuals at increased risk for CVD and T2DM. The level of obesity associated with risk differs according to ethnicity and population group, and currently there are no recommendations for a South African population. Further, it is important to establish which is the best measure of obesity that identifies individuals at increased risk for CVD and T2DM. Therefore, the first study of this thesis examined the discriminative ability (using ROC curves) of anthropometric measures (WC and WHtR) and CT-derived VAT to identify black and white women with elevated BP, dyslipidaemia and IR. The main finding was that central obesity measures were better at identifying risk in white compared to black women (ie. WC explained a greater proportion of
risk in the white compared to the black women). Furthermore, the level of obesity that predicted risk was generally lower in black compared to white women, however WHtR differed the least between ethnic groups and therefore may be useful for studies involving ethnic comparisons. In addition, there was little difference in the ability of anthropometric compared to a more high-cost CT-derived measure of VAT for identifying risk in both ethnicities and therefore recommend that anthropometric measures be used. To my knowledge, this is the first study to investigate obesity thresholds in black and white South African women and to compare the effectiveness of anthropometric indices to the gold standard CT measure.

As central obesity measures were less effective in identifying black compared to white women at increased risk for CVD and T2DM, novel risk markers, such as circulating inflammatory markers IL-18 and hsCRP, may provide improved prediction of CVD and T2DM risk, especially in black populations. However, several environmental factors, including SES and behavioural/lifestyle factors, which influence both inflammation and CVD and T2DM risk, need to be considered when investigating the independent association between inflammation and CVD risk. Therefore, the second study of this thesis investigated the ethnic-specific association between circulating concentrations of IL-18 and hsCRP and CVD and T2DM risk factors, taking into consideration SES, health behaviour/lifestyle factors and central obesity. Circulating concentrations of IL-18 and hsCRP were associated with CVD and T2DM risk factors in both black women, and these associations were largely independent of SES and behavioural/lifestyles factors, but were not independent of WC. These results suggest that the measurement of circulating inflammatory proteins is not of added benefit to a simple WC measure for identifying women at increased risk for CVD and T2DM. These results also support the hypothesis that AT may be a main source of chronic low-grade inflammation characteristic of CVD and T2DM. To
my knowledge, this is the first study to investigate the relationship between SES and behavioural/lifestyle factors and circulating IL-18 concentrations. In addition, this is the first study to investigate whether circulating IL-18 and hsCRP concentrations are associated with CVD and T2DM risk factors, independent of SES and behavioural/lifestyle factors in black and white South African women.

Ethnic differences in circulating IL-18 concentrations observed in Chapter 3 may be explained, in part, by ethnic differences in the distribution of the -137G/C polymorphism within the IL-18 gene. The G allele has previously been shown to be associated with variation in circulating concentrations and to be more frequent in black compared to white American women. Therefore, the third study aimed to investigate the association between the -137G/C polymorphism with circulating IL-18 concentrations and metabolic risk factors for CVD. Although the black women had a higher frequency of the G allele, this did not explain ethnic differences in IL-18 concentrations. Circulating IL-18 concentrations were higher in black compared to white women independent of age, genotype and body fatness (P<0.01). Further research examining the functional relevance of this polymorphism is therefore needed. In addition, the -137G/C genotype was associated with BP in both black and white women, with the G/C genotype associated with approximately 55mHg difference in BP compared to the GG genotype. However, the direction of this relationship was different between black and white women, as the C-allele was associated with higher BP in black women, whereas it was associated with lower BP in white women. This is the first study to investigate whether ethnic differences in the distribution of the IL-18 -137G/C genotype is associated with ethnic differences in circulating IL-18 concentrations, and to investigate the association between the -137 G/C polymorphism with CVD risk factors in apparently healthy South African women.
This thesis has identified that the association between circulating inflammatory markers with metabolic risk factors for CVD and T2DM in South African women was largely mediated by central obesity. Whether there are ethnic- and depot-specific differences in AT inflammatory gene and protein expression, and whether this is related to a differential $S_I$ in South African women is not known. Therefore in a subsample of black and white women, the final study investigated the ethnic and depot-specific association between inflammatory gene and protein expression of abdominal and gluteal AT with $S_I$. I report a higher inflammatory gene expression in black compared to white women. However, despite higher AT inflammatory gene expression in black women, this was not able to explain their lower $S_I$. Therefore, ethnic differences in $S_I$ might be due to metabolic effects of other insulin-sensitive tissues, such as the muscle, which have also been shown to have inflammatory properties. Furthermore, the gluteal depot had a higher inflammatory gene expression compared to the abdominal AT depots, suggesting that the protective properties of gluteal adipose tissue are not due to a more favourable inflammatory profile and therefore need to be investigated further. This study provides novel insight into the inflammatory profile of abdominal and gluteal SAT, and is the first to investigate the ethnic–specific differences in AT inflammatory gene expression.

The thesis has some limitations that should be noted. The subjects were not randomly sampled but recruited through advertisements in local newspapers and universities. Therefore, the data may not be representative of the black and white South African population. Notably, although the women included in this study reported having no known disease, it is possible that undiagnosed or undisclosed confounding factors such as HIV, TB, depression or other physiological states relating to stress, could have influenced the findings in this study. Type of contraceptive use differed between black and white women, with the majority of black women
being on injectable contraceptives, whereas the majority of white women were on oral contraceptives. Notably, both these forms of contraception have been linked to metabolic disturbances and alterations in fat distribution (as reviewed by Johan Verhaeghe [269]). The metabolic effects of contraceptives appear to be dose- and hormone specific. Therefore, ethnic differences in type of contraception used may have influenced results of this thesis, particularly in chapters 2, 4 and 5, where contraceptive use was not accounted for in the analyses. Importantly, these results need to be verified in other cohorts. Specifically, the women included in the study were relatively young (~27 years) and apparently healthy (no known disease), therefore the relationship between body composition, inflammation and metabolic risk needs to be explored in similar groups with known disease or pathophysiology. Furthermore, with the majority (over 70%) of black women being of Xhosa ancestry, it is also not known whether obesity and risk thresholds established in Chapter 2 are applicable to other Sub-Saharan populations. Due to the cross-sectional nature of the study, it is not known whether the risk factor thresholds used (NCEP ATPIII criteria derived from European cohorts [31]) translates to equal risk in black and white women. Ideally the determination of the WC cut-point of risk (Chapter 2), and establishing whether circulating inflammatory markers are of added benefit to risk prediction (Chapter 3), would be based on the results of prospective investigations that link WC and inflammation to the development of T2DM, CVD or death. However, evidence from large prospective studies in South Africa is currently not available. In Chapter 3, SES and behavioural/lifestyle factors were self reported by means of a questionnaire. Although this questionnaire has been validated in a South African population, there may be reporter bias and more objective measures of lifestyle need to be used in future studies. In Chapter 5 of this thesis, although the sub-sample of women included in the analysis was adequate to detect ethnic
differences in $S_t$, the findings of this study need to be confirmed in a larger sample. Furthermore, despite ethnic differences in gene expression levels, these findings were not mirrored at a protein level. Notably, inflammation acts via endocrine and paracrine effects, with the latter being difficult to quantify. Other sites of inflammation, particularly those associated with ectopic fat deposition (pancreas, skeletal muscle, liver, and heart), may also contribute to disease risk; to our knowledge, this has not been explored in multi-ethnic populations.

Despite these limitations, this thesis has important novel findings (Table 6.1) regarding the ethnic specific role of obesity and inflammation (at a circulating, genetic and AT-specific level) with metabolic risk factors for CVD and T2DM in apparently healthy black and white South African women. The practical implications of these findings are that basic anthropometric indices provided the most cost-effective marker of CVD and T2DM risk in both black and white South African women. Currently, due to the cost and methods for measuring inflammatory markers being high and invasive, they are not practical markers of risk, especially in a primary prevention setting. Notably, several modifiable risk factors, namely SES and contraceptive use, influenced both circulating inflammatory markers and CVD risk and should be targets for future disease prevention. Furthermore, although inflammation has previously been associated with increased risk for CVD and T2DM, ethnic differences in circulating inflammatory markers and expression of inflammatory genes in AT do not fully account for the disparity in disease risk between black and white women.

Based on the findings of this study, future research should investigate the contribution of inflammation from other tissues, which may affect metabolic risk factors, particularly in black populations. As a large proportion of risk remained unexplained, future studies should focus on other factors involved, in particular exploring more objective measures of lifestyle, such as
physical activity and dietary intake. Furthermore, prospective studies are also required to establish whether these relationships are similar in individuals who develop T2DM and CVD.
Table 6.1. Summary, implications and novel aspects by chapter

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Summary</th>
<th>Implications</th>
<th>Novel Aspects</th>
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<tr>
<td>Two</td>
<td>Measures of central obesity (WC, WHtR and VAT) were significantly associated with metabolic risk factors (elevated BP, dyslipidaemia and IR) for CVD and T2DM in both black and white South African women. The ability of central obesity measures to identify metabolic risk factors was stronger in white compared to black women (based on ROC AUC). Anthropometric indices of central obesity could identify an equivalent amount of metabolic risk as CT-derived VAT, in both black and white women. Thresholds for WHtR varied the least between ethnicities and may be more useful than WC or VAT for comparisons of risk between black and white women.</td>
<td>This finding supports the use of anthropometry as a measure of central obesity and for use as a diagnostic tool for identifying black and white South African women at increased risk for CVD and T2DM.</td>
<td>This was the first study to identify central obesity thresholds for identifying black and white South African women at increased risk for CVD and T2DM. This is also the first study to compare the ability of anthropometric obesity measures to CT-derived VAT for identifying black and white South African women at increased risk for CVD and T2DM.</td>
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Table 6.1 Continued

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<th>Chapter</th>
<th>Summary</th>
<th>Implications</th>
<th>Novel Aspects</th>
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<tr>
<td>Three</td>
<td>Low SES, smoking, contraceptive use and central obesity were associated with higher circulating concentrations of IL-18 and hsCRP. Circulating concentrations of the inflammatory markers IL-18 and hsCRP were associated with metabolic risk factors for CVD in both black and white women. WC, but not SES or behavioural/lifestyle factors attenuated the relationship between hsCRP and IL-18 and metabolic risk factors in black and white women. Although WC, SES and contraceptive use explained a significant proportion of risk, a large amount of variation remained unexplained by factors explored in this thesis.</td>
<td>These findings does not support the use of circulating inflammatory markers IL-18 and hsCRP for identification of CVD and T2DM risk in a primary prevention setting. However, they do support the hypothesis that chronic inflammation associated with metabolic risk has a putative origin in AT. Further attention needs to be given to managing potentially modifiable risk factors, including SES and contraceptive use, in primary prevention of CVD and T2DM in black and white women.</td>
<td>This was the first study to explore the impact of SES and behavioural/lifestyle factors on circulating concentrations of IL-18 and hsCRP and to explore the possible mediating effects of obesity, SES and behavioural/lifestyle factors on the relationship between IL-18 and hsCRP with metabolic risk factors for CVD and T2DM in black and white south African women.</td>
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Table 6.1 Continued.

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<th>Chapter</th>
<th>Summary</th>
<th>Implications</th>
<th>Novel Aspects</th>
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<tr>
<td>Four</td>
<td>The distribution of the C-allele (protective allele) of the -137G/C polymorphism within the IL-18 gene was lower in black compared to white South African women.</td>
<td>These findings suggest that further investigation into the functional relevance of the <strong>IL-18</strong> – 137 G/C polymorphism is required. The IL-18-137 C-allele was differentially associated with BP in black and white South African women.</td>
<td>This was the first study to investigate an association between the <strong>IL-18</strong> -137G/C polymorphism with metabolic risk factors for CVD and to investigate whether ethnic differences in circulating concentrations of IL-18 are influenced by ethnic differences in the distribution of this SNP.</td>
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Despite a higher frequency of the G-allele in black women, this could not explain their higher circulating IL-18 concentrations.

The IL-18-137 C-allele was differentially associated with BP in black and white South African women.
Table 6.1 Continued.

<table>
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<tr>
<th>Chapter</th>
<th>Summary</th>
<th>Implications</th>
<th>Novel Aspects</th>
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<tr>
<td>Five</td>
<td>Abdominal and gluteal SAT inflammatory gene expression was higher in black compared to white South African women. Abdominal and gluteal SAT inflammatory gene expression and circulating inflammatory markers were significantly associated with S_I in white (P&lt;0.01) but not black women (P=30). The gluteal SAT depot had a higher inflammatory gene expression compared to abdominal SAT depots, Abdominal SAT inflammatory gene expression was more closely related to S_I than gluteal SAT gene expression, supporting the higher risk attributed to central obesity.</td>
<td>These findings show that ethnic differences SAT inflammatory gene expression cannot explain ethnic differences in S_I. In addition they highlight the need for further research into the proposed protective nature of peripheral fat accumulation.</td>
<td>This was the first study to compared SAT inflammatory gene expression between black and white women and between abdominal and gluteal SAT depots.</td>
</tr>
</tbody>
</table>
REFERENCES


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APPENDIX 1

Table A1. Percentage of white women with negative health behaviour and health status characteristics across SES categories

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Alcohol Intake</th>
<th>Physical Activity</th>
<th>Contraception Use</th>
<th>Metabolic Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Current</td>
<td>&gt;1drink/day</td>
<td>Sedentary &lt;150min/week</td>
<td>Oral</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1-7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Grade 8-11</td>
<td>23.3</td>
<td>12.8</td>
<td>26.4</td>
<td>22.5</td>
</tr>
<tr>
<td>Completed High School</td>
<td>28.7</td>
<td>30.8</td>
<td>34.5</td>
<td>33.0</td>
</tr>
<tr>
<td>Tertiary</td>
<td>48.0</td>
<td>56.4</td>
<td>39.1</td>
<td>44.5</td>
</tr>
<tr>
<td><strong>Asset Index</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quintile (5-8)</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quintile (9-12)</td>
<td>3</td>
<td>8.9</td>
<td>14.2</td>
<td>26.4</td>
</tr>
<tr>
<td>Quartile (13-16)</td>
<td>4</td>
<td>37.4</td>
<td>33.0</td>
<td>33.4</td>
</tr>
<tr>
<td>Quintile (&gt;12)</td>
<td>5</td>
<td>53.7</td>
<td>52.8</td>
<td>39.9</td>
</tr>
</tbody>
</table>

Values are %. BP, blood pressure; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; TC/HDL-C, total-cholesterol/high density lipoprotein cholesterol ratio; HOMA-IR, homeostasis model of insulin resistance.
Table A2. Percentage of black women with negative health behaviour and health status characteristics across SES categories

<table>
<thead>
<tr>
<th></th>
<th>Smoking</th>
<th>Alcohol Intake</th>
<th>Physical Activity</th>
<th>Contraception Use</th>
<th>Metabolic Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Current</td>
<td>&gt;1drink/day</td>
<td>Sedentary (&lt;150min/week)</td>
<td>Oral</td>
<td>Injectable</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1-7</td>
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<td>0.0</td>
<td>22.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Grade 8-11</td>
<td>0.0</td>
<td>12.8</td>
<td>20.8</td>
<td>0.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Completed High School</td>
<td>48.6</td>
<td>32.8</td>
<td>28.6</td>
<td>39.0</td>
<td>46.0</td>
</tr>
<tr>
<td>Tertiary</td>
<td>51.4</td>
<td>54.4</td>
<td>28.6</td>
<td>61.0</td>
<td>48.0</td>
</tr>
<tr>
<td><strong>Asset Index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quintile 1</td>
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<td>7.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Quintile 2</td>
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<td>7.4</td>
<td>18.2</td>
<td>4.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Quintile 3</td>
<td>4.1</td>
<td>22.6</td>
<td>11.3</td>
<td>0</td>
<td>3.9</td>
</tr>
<tr>
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<td>35.0</td>
<td>38.2</td>
<td>62.2</td>
<td>46.9</td>
</tr>
<tr>
<td>Quintile 5</td>
<td>80.9</td>
<td>35.0</td>
<td>24.9</td>
<td>33.3</td>
<td>40.0</td>
</tr>
</tbody>
</table>

Values are %. BP, blood pressure; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; TC/HDL-C, total-cholesterol/high density lipoprotein cholesterol ratio; HOMA-IR, homeostasis model of insulin resistance.