THE IMPACT OF BODY FAT AND ITS DISTRIBUTION ON RISK FACTORS FOR CARDIOVASCULAR DISEASE IN BLACK SOUTH AFRICAN WOMEN

By

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This thesis is dedicated to my parents, Thomas and Sandra Jennings, my sister Ashley, my grandmother Leta Stormer and my husband Matthew for all of their love and support.

"The whole is greater than the sum of its parts"

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DECLARATION

I, **Courtney Lynn Jennings,** do herby 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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PUBLICATIONS

Peer reviewed publications related to this thesis

Rush EC, Goedecke JH, **Jennings CL,** Micklesfield L, Dugas L, Lambert EV and Plank L. BMI, fat and muscle difference in urban women of five ethnicities from two countries. International Journal of Obesity, 2007. Aug; 31(8):1232-9.

Evans J, Collins M**, Jennings CL**, Van der Merwe L, Soderstrom I, Olsson T, Lambert EV, Levitt NS and Goedecke JH. IL-18 genotype and serum levels are associated with metabolic risk factors for cardiovascular disease. European Journal of Endocrinology, 2007. Nov; 157(5):633-40.

Jennings CL, Lambert EV, Collins M, Joffe Y, Levitt NS and Goedecke JH. Determinants of a healthy metabolic phenotype in normal weight and obese black South African women. Obesity Research. *IN PRESS*.

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Abstracts related to this thesis

Central fat mass distinguishes between metabolically healthy and at risk black South African women. **Jennings CL**, Lambert EV, Collins M, Joffe Y, Levitt NS and Goedecke JH. International Association for the Study of Obesity, Obesity Reviews 7(Suppl. 2)(2006) pg. 348.

Central fat mass distinguishes between metabolically healthy and at risk black South African women. **Jennings CL**, Lambert EV, Collins M, Joffe Y, Levitt NS and Goedecke JH Diabetes UK 2006, Diabetic Medicine, 23(Suppl. 4) pg. 735.

Il-18 genotype and serum levels are associated with metabolic risk factors for cardiovascular disease. Evans J, Collins M**, Jennings CL**, Lambert EV, Levitt NS, Olsson T and Goedecke JH. Diabetes UK 2006, Diabetic Medicine, 23(Suppl. 4) pg. 732.

Other publications related to this thesis

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Goedecke JH, **Jennings CL** and Lambert EV. Technical review-A summary of research and policy on obesity in South Africa from 1994-2004. In: Fourie JM, Steyn K (eds.). Technical Report on Chronic Diseases of Lifestyle. Medical Research Council of South Africa, 2006.

International conference presentations related to this thesis

Central fat mass distinguishes between metabolically healthy and at risk black South African women. **Jennings CL**, Lambert EV, Collins M, Joffe Y, Levitt NS and Goedecke JH. International Association for the Study of Obesity, Sydney Australia, September 2006. Poster presentation.

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Local conference presentations related to this thesis

The atypical presentation of cardiovascular disease risk in black South African women; the influence of regional body fat distribution. **Jennings CL,** Lambert EV, Collins M, Levitt NS and Goedecke JH. Medical Research Council of South Africa, Capacity development Research Day, Cape Town, October 2007–Oral presentation.

Central fat mass distinguishes between metabolically healthy and at risk black South African women. **Jennings CL**, Lambert EV, Collins M, Joffe Y, Levitt NS and Goedecke JH. The 2nd Bi-annual Research Day, Faculty of Health Sciences, University of Cape Town, Cape Town. September 2006–Oral presentation. Recipient of University of Cape Town award for a highly recommended PhD oral presentation.

ABBREVIATIONS

AACE- Association of American Clinical Endocrinologists

AHA/NHLB1- American Heart Association/National Heart, Lung and Blood Institute

BIA- Bioelectrical impedance

BMI- Body mass index

CVD- Cardiovascular disease

cm- Centimeter

CT- Computer tomography

CHD- Coronary heart disease

CRD- Cortisone reductase deficiency

DSAT- Deep subcutaneous adipose tissue

DXA- Dual-energy x-ray absorptiometry

EGIR- European group for the study of insulin resistance

FFSTM- Fat-free soft tissue mass

FFA- Free fatty acid

FSIVGT- Frequently sampled intravenous glucose tolerance test

GPAQ- Global Physical Activity Questionnaire

GC- Glucocorticoid

GR- Glucocorticoid receptor

GR- Glucocorticoid receptor gene

GWAS- Genome wide association study

H6PD- Hexose-6-phosphate-dehydrogenase

H6PD- Hexose-6-phosphate-dehydrogenase gene

HDL-C- High density lipoprotein cholesterol

HOMA- Homeostasis assessment model

HPA-axis- Hypothalamic-pituitary-adrenal axis

IDF- International Diabetes Federation

ICC- Intra-class correlation coefficient

Kg- Kilogram

LOA- Limits of agreement

L- Liter

LDL-C- Low-density lipoprotein cholesterol

MRI- Magnetic resonance imaging

m- Meter

µg- Microgram

µl- Microliter

mg- Milligram

ml- Milliliter

min- Minute

ATP III- National Cholesterol Education Program Adult treatment panel III

NHANES- National Health and Nutrition Examination Survey

NIH- National Institute of Health

NIR- Near infrared interactance

OGTT- Oral glucose tolerance test

PAEE- Physical activity energy expenditure

PCOS- Polycystic ovary syndrome

QUICKI- Quantitative insulin sensitivity check index

SEE-Standard error of estimate

SD- Standard deviation

SAT- Subcutaneous adipose tissue

SSAT- Superficial subcutaneous adipose tissue

TC- Total cholesterol

tSSAT- Total body superficial subcutaneous adipose tissue mass

VAT- Visceral adipose tissue

WHO- World Health Organization

11-β-HSD-1- 11-β hydroxysteroid dehydrogenase type 1

HSD11B1- 11-β hydroxysteroid dehydrogenase type 1 gene

ABSTRACT

Obesity and obesity-related diseases are a large global problem in both developed and developing nations. In South Africa, a country currently undergoing epidemiological transition, the prevalence of obesity is high, particularly in urban black women. Early detection of overweight and obese individuals is essential for the management of obesity and its related co-morbidities; however, there is no ethnic-specific field measure of body fat percent validated for use in black South African women. Further, despite high levels of adiposity, these women have an atypical presentation of cardiovascular disease (CVD) risk factors, presenting with relatively low levels of visceral adipose tissue (VAT) and a favourable lipid profile compared to white women. As a result of this atypical presentation of CVD risk factors, a high prevalence of "healthy obesity" has been reported, although the determinants of this phenotype have not been systematically investigated. In addition, the applicability of commonly used diagnostic criteria for the determination of insulin resistance, which include enlarged waist circumference and dyslipidemia as components, has not been investigated in this population.

Therefore, the overall aim of this thesis was to investigate the impact of body fat and its distribution on the presentation and identification of CVD risk factors in relatively young black South African women, prior to the onset of CVD. More specifically, the objectives were; i) to determine if near infrared interactance (NIR) is a valid field measure of body fat percent in South African women; ii) to determine the agreement between International Diabetes Federation (IDF) and National Cholesterol Education Program (Adult treatment panel III) (ATP III) metabolic syndrome criteria and the degree to which these criteria can predict insulin resistance, and explore the extent to which these phenomena can be explained by body fat and its distribution; iii) to identify determinants of the "metabolically healthy obese" (MHO) and "metabolically obese normal weight" (MONW) phenotypes; and iv) to complete a preliminary investigation of the association between polymorphisms within genes that encode for proteins involved in tissue-specific glucocorticoid metabolism and obesity, body fat distribution and CVD risk factors in black South African women.

As obesity is associated with increased risk of cardiovascular disease, accurate quantification of body fatness is particularly important in health risk appraisal. However, in developing countries, "gold standard" measures of body fat percent such as underwater weighing and dual energy x-ray absorptiometry (DXA) are not always practical, as access to facilities and resources are limited. Therefore, a valid field measure of body fat percent is needed for the purpose of health risk appraisal. NIR is a potentially useful field measure of body fat percent that is currently used in South Africa for this purpose. However, NIR cannot be used with confidence in South Africa until it has been validated in different ethnic populations. Therefore, the first study in this thesis examined the validity of single-site NIR (Futrex-6100 A/ZL) as a measure of body fat percent compared to the criterion method of DXA in black and white South African women.

The subjects consisted of 172 normal weight (body mass index (BMI) ≤25 kg/m^2) and 157 obese (BMI ≥30 kg/m^2) urbanized black and white South African women (29±8 years) without known disease. Body fat percent measured by NIR correlated with that of DXA in both the normal weight black (r=0.55, P<0.001, N=102) and white (r=0.69, P<0.001, N=70) women and obese black (r=0.57, P<0.001, N=116) and white (r=0.57, P<0.001, N=41) women. In the obese black and white women, NIR body fat percent levelled off at 45-47%, while DXA measured up to 58% body fat. NIR generally under-predicted body fat percent compared to DXA in all of the women, but this difference was greatest in the black women (P<0.001). The mean differences between DXA and NIR were -4.46% (normal weight black), -0.29% (normal weight white), -3.62% (obese black) and -0.81% (obese white).

In conclusion, although NIR and DXA body fat percent were significantly correlated, NIR under-predicted body fat percent compared to DXA in the normal weight and obese women, but was more accurate in the white compared to the black women. Moreover, body fat percent measured using NIR appeared to level off at between 45-47%, resulting in bias in the limits of agreement between NIR and DXA with increasing levels of adiposity. Therefore, single-site NIR should be used with caution as a measure of body fat percent, particularly in black African women and women with very high levels of adiposity.

Previous research in African Americans and Afro-Caribbean populations has questioned the suitability of the metabolic syndrome diagnostic criteria as an indicator of CVD risk due to the relatively low levels of VAT and favourable lipid profiles found in these populations compared to white populations. However, this has not been investigated in a black South African population. Therefore, the second study in this thesis investigated the level of agreement between the IDF and ATP III metabolic syndrome criteria, which differ in their emphasis on central obesity, and the degree to which they predicted insulin resistance in black South African women. Further, the extent to which a diagnosis of the metabolic syndrome could be explained by body fat and its distribution was investigated.

Body composition (DXA scan), body fat distribution (waist circumference and computer tomography (CT) scan), blood pressure (BP), fasting glucose, insulin resistance (HOMA-IR), and lipid profiles were measured in 103 normal weight (BMI ≤25 kg/m^2) and 122 obese (BMI ≥30 kg/m^2) urbanized black South African women (27±7 years) without known disease. Insulin resistance was used as an indicator of CVD risk based on previous research and because the relationship between lipid levels and CVD risk has not been fully elucidated in individuals of black African origin. Insulin resistance was defined as the upper tertile of HOMA-IR. Agreement was high between the IDF and ATP III metabolic syndrome criteria (kappa=0.88); however, neither metabolic syndrome criteria accurately identified insulin resistance, as estimated by HOMA-IR (kappa=0.15 and 0.14 respectively). Waist circumference (>80 cm) had a 20% higher specificity and 66% higher positive predictive value for identifying insulin resistance compared to the metabolic syndrome criteria. Further, both waist circumference (>80 cm and >88 cm) and VAT (>100 cm^2) cut-points were accurate identifiers of insulin resistance (Kappa=0.31, 0.41 and 0.45, respectively). VAT was the largest contributor to a diagnosis of the metabolic syndrome, while deep SAT (DSAT) and superficial SAT (SSAT) were not significant contributors. Additionally, even the most insulin resistant subjects presented with lipid levels below the suggested IDF and ATP III metabolic syndrome cut-points. In conclusion, this thesis found that waist circumference was a better indicator of insulin resistance than the IDF or ATP III metabolic syndrome criteria in young black women. This has major implications for public health, since waist circumference is a non-blood

based indicator of insulin resistance in young black African women without known disease.

Subsets of metabolically "healthy" and "at-risk" normal weight and obese individuals have been previously identified, both in South Africa and in other countries. There are various criteria that are used to identify these phenotypes. In this thesis, a cut-point for insulin resistance based on previous studies was used to identify "healthy" and "at-risk" normal weight and obese phenotypes. The determinants of these metabolic phenotypes have not been investigated in a black South African population. Therefore, the third study in this thesis investigated the determinants of insulin resistant phenotypes in normal weight and obese black South African women.

In 103 normal weight (BMI ≤25 kg/m^2) and 122 obese (BMI ≥30 kg/m^2) premenopausal urban black South African women, body composition (DXA), body fat distribution (CT), BP, fasting glucose level, insulin resistance (HOMA-IR), and lipid profiles were measured. Questionnaires relating to family history, physical activity energy expenditure (PAEE) and socio-demographic variables were administered. The subjects were classified as "insulin sensitive" or "insulin resistant" according to HOMA-IR (≥1.95=insulin resistant).

We found that 22% of the normal weight women were insulin resistant and 38% of the obese women were insulin sensitive. Increased VAT (P=0.001) and decreased VAT/leg fat mass (cm^2/kg) (P<0.001), independent of total body fatness, distinguished between the phenotypes. Moreover, the insulin sensitive women were of higher socio-economic status, did more leisure and vigorous PAEE, and were less likely to use injectable contraceptives compared to their insulin resistant counterparts. Using a regression model, body fat distribution, body fat percent, age, leisure PAEE and use of injected contraception accounted for 35% of the variance in HOMA-IR in the normal weight women. When normal weight women who completed ≥ 150 minutes for PAEE were excluded from the analysis, the same variables explained 50% of the variance in HOMA-IR. In the obese women, 34% of the variance in HOMA-IR was explained by the same variables, excluding PAEE. No differences in smoking status, parity or family history of metabolic disease

were found between phenotypes. In conclusion, central fat distribution, total adiposity, socio-economic status, leisure PAEE and injectable contraceptive use distinguished between insulin sensitive and insulin resistant black South African women.

The results of the previous studies in this thesis highlighted the impact of central obesity on risk factors for CVD in black South African women. Additionally, it is interesting that despite high levels of adiposity, black South African women have relatively low levels of VAT. There are many factors that contribute to the centralisation of body fat, including altered glucocorticoid (GC) metabolism. Alterations in tissue-specific GC action influence the local regeneration of cortisol in adipose tissue and impact body fat distribution and CVD risk. Further, polymorphisms within the *GR*, *HSD11B1* and *H6PD* genes, which encode for glucocorticoid receptor (GR), hexose-6-phosphate dehydrogenase (H6PDH) and 11β hydroxysteroid dehydrogenase type 1 (11β-HSD-1) are all involved in tissue-specific GC metabolism and have been associated with obesity, body fat distribution and CVD. In the *HSD11B1* gene, an adenine insertion at nucleotide 4436 within intron 3 has been associated with increased BMI, waist/hip ratio and insulin resistance in a small study comprising African Americans. Further, a higher frequency of the A allele of the R>Q substitution at nucleotide 453 within exon 5 of the *H6PD* gene has been reported in African Americans compared to white Americans, and may impact H6PDH enzymatic activity, altering 11β-HSD-1 activity. The G allele of the G>C substitution at nucleotide 646 within intron 2 (*Bcl*I polymorphism) of the *GR* gene has been associated with differences in GC sensitivity, waist circumference and VAT, as well as with increased fasting insulin in white individuals. Despite these findings, none of these polymorphisms have been investigated in a black South African population. Therefore, the fourth study in this thesis was a preliminary investigation of the influence of polymorphisms in the *HSD11B1*, *H6PD* and *GR* genes on body composition, body fat distribution and risk factors for CVD in black South African women.

A sample of 103 normal weight (BMI ≤25 kg/m^2) and 121 obese (BMI ≥30 kg/m^2) premenopausal urban black South African women were genotyped for the *Bcl*I polymorphism within the *GR* gene, the Ins4436A polymorphism within the *HSD11B1* gene and the R453Q polymorphism within the *H6PD* gene. There were no significant

differences in genotype or allele frequencies between the normal weight and obese subjects. Further, there were no genotype effects on body composition, body fat distribution, insulin sensitivity, lipid profile or blood pressure. In conclusion, in this preliminary investigation, the *Bcl*I, Ins4436A and R453Q polymorphisms within the *GR*, *HSD11B1* and *H6PD* genes were not associated with obesity, fat distribution or risk fators for CVD in this cohort of normal weight and obese black South African women.

In summary, this thesis has important and novel practical implications regarding the presentation and identification of risk factors for CVD in young black South African women. The data showed that single-site NIR (Futrex-6100 A/ZL) was not a valid measure of body fat percent in black South African women and should be used with caution in health risk appraisal. Further, the results of this thesis highlight the atypical presentation of CVD risk factors in black South African women, which limits the ability of the IDF and ATP III metabolic syndrome criteria to identify insulin resistance. In fact, waist circumference, an inexpensive and practical field method for quantifying central obesity, was a better indicator of insulin resistance in young black women without known disease than a positive diagnosis of the metabolic syndrome using the IDF or ATP III diagnostic criteria. This thesis also identified modifiable determinants of insulin resistance, such as PAEE and method of birth control, in normal weight and obese black South African women. In conclusion, this thesis has major implications for public health practices in South Africa in relation to the diagnosis of CVD risk at a population level. These findings may assist in cost-effective early identification of CVD risk and expedite early intervention in this high risk population.

CHAPTER ONE

LITERATURE REVIEW

Note: Some sections of this literature review have been previously published in a technical review of obesity on which CL Jennings was a co-author

Goedecke JH, Jennings CL and Lambert EV. Technical review-A summary of research and policy on obesity in South Africa from 1994-2004. In: Fourie JM, Steyn K (eds.). Technical Report on Chronic Diseases of Lifestyle. Medical Research Council of South Africa, 2006.

1.1 THE GLOBAL PROBLEM OF OBESITY

Obesity is a growing global epidemic with an estimated one billion people overweight and at least three-hundred million people obese world-wide (1). In developed countries obesity is a major concern. For example, more than a quarter of the population in the USA is obese (2). Further, the obesity prevalence is as high as 25% in some European countries such as Finland, Sweden and Switzerland (3). However, obesity is not only a problem of developed nations, but is becoming an increasing problem in countries undergoing epidemiological transition such as South Africa, Mexico and South American countries (4;5;6;). The global prevalence of obesity in women is shown in Figure 1.1.

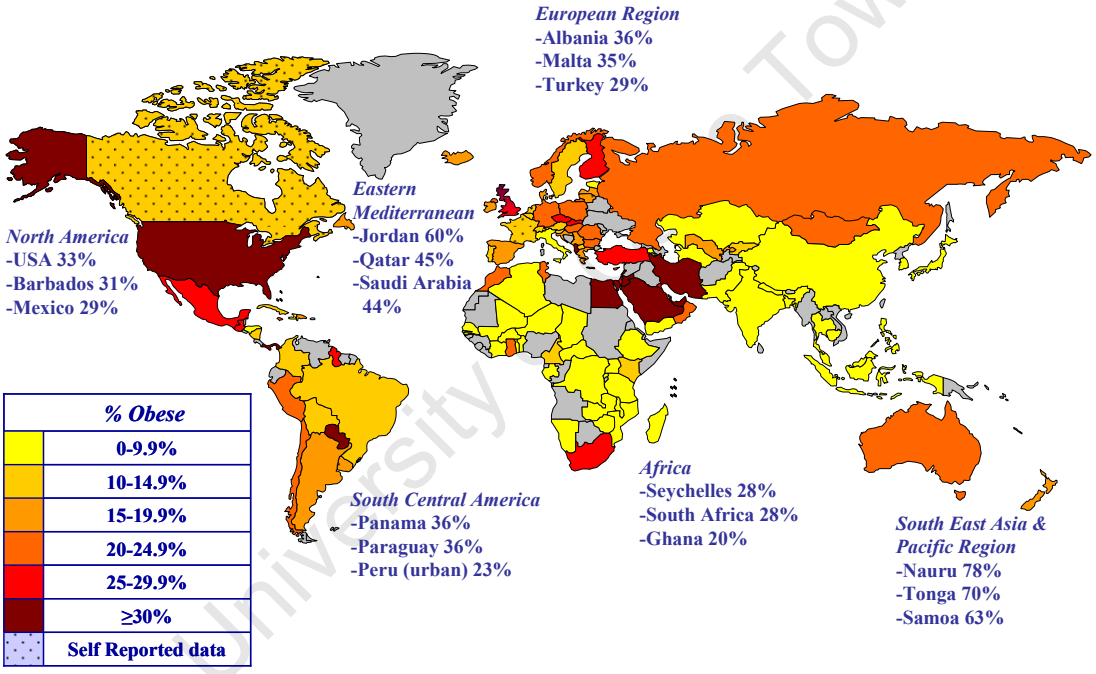

Figure 1.1. Map of the non-age standardized global prevalence of obesity (BMI ≥30 kg/m^2) in women. The countries with the highest prevalence of obesity are listed for each region. This figure is adapted from data on the International obesity Task Force website (www.IOTF.org, accessed September 7, 2007)

1.1.1 *The prevalence of obesity in South Africa*

The South African Demographic Health Survey, including 8,162 South African women and 5,665 South African men, aged 15 to 95 years old, demonstrated that the overall prevalence of overweight (BMI >25 kg/m^2) and obesity (BMI >30 kg/m^2) in South Africa was high, with more than 29% of men and 56% of women being classified as overweight or obese (4). Black women had the highest prevalence of overweight and obesity (58.5%), followed by white women (52.9%) and women of mixed ancestry (52.0%). Black urban women were found to have a significantly higher BMI than their rural counterparts (Figure 1.2), and BMI was found to increase with age in both rural and urban black women. Therefore, the primary focus of this review is black urban South African women.

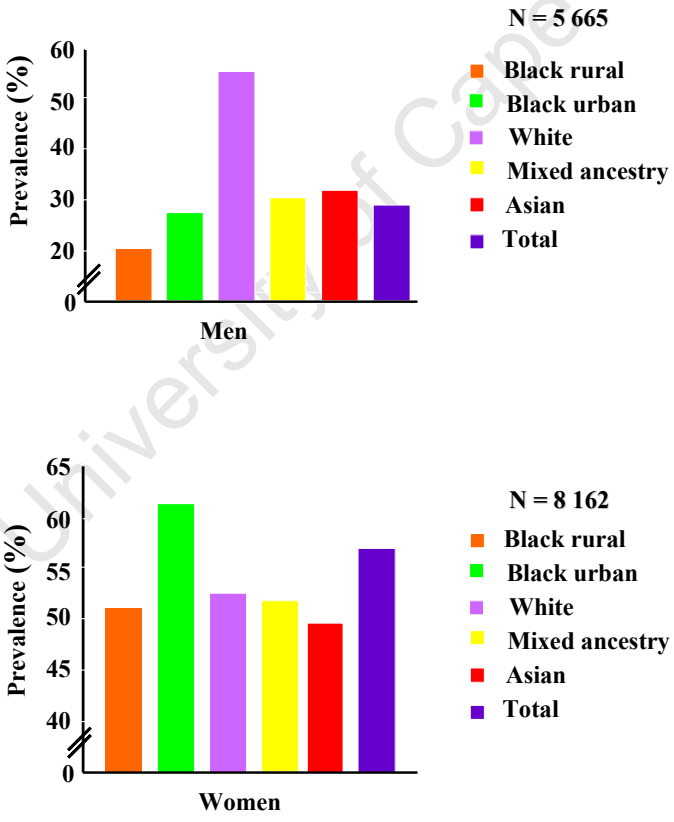

Figure 1.2. The anthropometric pattern of men and women (>15 years old) from the South African Demographic Health Survey, adapted from Puoane et al. (4)

In South Africa it is difficult to quantify the increase in prevalence of obesity prior to the South African Demographic Health Survey undertaken in 1998 (4). Prior to this survey, only regional, cross-sectional studies were conducted, from which a national prevalence cannot be extrapolated. Further research is needed to determine if the prevalence of obesity is increasing, as seen in other developing countries (6;7). For example, in Mexico, the prevalence of obesity in adult women increased from 9.4% in 1988 to 24.4% in 1999 (6). In Brazil, a tripling in the prevalence of overweight from 4.1% in 1974 to 13.9% in 1997 was reported in a sample including both genders and all ages. Similar findings have been reported in Mauritius where the prevalence of overweight or obesity (BMI >25 kg/m^2) increased from 26.1% to 35.7% in men and from 37.9% to 47.7% in women between 1987 and 1992 (8).

The increasing prevalence of obesity in developing countries results in an increasing prevalence of cardiovascular disease (CVD). CVDs are diseases related to the cardiovascular system. There are many conditions that fall into this category. For the purposes of this thesis, the definition of CVD was limited to those diseases that the metabolic syndrome was designed to identify, primarily heart disease and stroke. Additionally, the metabolic syndrome diagnostic criteria have been used to identify type 2 diabetes. Over 80% of hypertension and 60% of ischemic heart disease and stroke in South African women over the age of 30 years can be attributed to excess body weight (9). Further, in the year 2000, one in every ten female deaths in South Africa was related to excess body weight (9). Additionally, the Burden of Disease Study estimated that 36% of deaths in South Africa could be attributed to non-communicable disease such as stroke, hypertension, coronary heart disease (CHD) and type 2 diabetes (10).

1.1.2 *Cardiovascular disease (CVD) risk*

There are numerous genetic (11), socioeconomic (4) and lifestyle (12) factors that place individuals at increased risk of developing CVD. Some of these risk factors are discussed in this thesis (central body fat distribution, increased body fat percent, low physical

activity level, lifestyle factors, socioeconomic status, insulin resistance, ethnicity, genetics and family history of disease), while others fall outside the scope of my research (diet).

Due to the multi-factorial etiology of CVD, CVD risk can be defined in many ways. For the purposes of this thesis, insulin resistance (as measured by HOMA-IR) was the main outcome variable (used in Chapters Three and Four), which is a risk factor for type 2 diabetes and CVD. Insulin resistance was selected as the main outcome variable in this thesis as: a) it has been suggested to be the primary etiological factor for the metabolic syndrome components and consequently the underlying cause of CVD (13;14); b) it is an independent predictor of CVD and type 2 diabetes (15-18); c) has been used as a primary indicator of CVD risk in similar studies (19); and d) lipid levels may not be good indicators of CVD risk in black African women (19;20).

1.1.3 *Changes associated with urbanization that impact obesity and cardiovascular disease (CVD) risk*

South Africa is currently undergoing an epidemiological transition involving increased urbanization, associated with decreased physical activity energy expenditure (PAEE) and changes in dietary intake. These changes have contributed significantly to the progression of the obesity epidemic, particularly in the urban black population. In fact, South African studies report that black South Africans who have spent larger proportions of their lives in an urban setting tend to have less healthy lifestyles and a higher risk for chronic diseases of lifestyle when compared to those who are less urbanized (21;22). This could be a result of the changes in diet, PAEE and socio-economic variables associated with urbanization. These variables are discussed briefly.

1.1.3.1 *Dietary changes during urbanization*

Research has shown that although the diet of the black South African population meets prudent dietary guidelines, there has been a general trend toward an increase in fat intake and a decrease in carbohydrate intake in both rural and urban areas (23). In urban areas, carbohydrate intake decreased 10.9% and fat intake increased 59.7% between 1940 and 1990. These changes were more dramatic than those observed in Western countries undergoing rapid industrialization over longer periods of time (23). In rural areas, the change was less marked; with an 8% increase in fat intake and a 10% decrease in carbohydrate intake between 1970 and 1990. In a recent study, the high prevalence of obesity in a group of urban black community health workers was attributed, in part, to the intake of inexpensive fatty meats and large portion sizes (24). Moreover, a study undertaken in the North West Province of South Africa, including 1040 adult black women from 37 randomly selected sites in the region, reported a weak, but significant positive association between BMI, dietary energy and fat intake. This study also showed that the lowest fat intake was observed in the rural areas (46 g/day), whereas the highest fat intakes were reported in the urban areas (56 g/day) (25). This data therefore suggests that changes in dietary intake with urbanisation have contributed to the obesity epidemic in South Africa. More information on the dietary transition in South African is outside of the scope of this thesis but can be found in the work of Bourne (23) and Steyn et al. (26).

1.1.3.2 *Physical activity energy expenditure (PAEE) during urbanization*

Although there is a paucity of South African research in which PAEE has been studied in relation to obesity and CVD risk, the few published studies support the well-established observation that physical inactivity is an important determinant of obesity. For example, in the Transition and Health during the Urbanization of South Africans (THUSA) study, which included over 1,000 black women from the North West Province, physical inactivity showed the strongest association with measures of obesity when compared to other socio-demographic and dietary factors (25). Temple et al. (27) also reported an association between reduced PAEE and a rise in BMI in black South African women.

Physical inactivity has been shown to be a risk factor for CVD in black South African communities undergoing urbanization (28). The PAEE of black South African women living in urban areas of low socio-economic standing, where there is often a lack of access to resources and concerns over safety, is generally low (28). Similarly, a large American study found that low socio-economic status resulted in decreased access to exercise facilities and lower levels of PAEE (29). Kruger et al. (28) suggested that deterrents to physical activity in black South African women should be identified in order to promote PAEE and healthy lifestyles.

Further information on the impact of PAEE on health in communities undergoing epidemiological transition can be found in the work of E.V. Lambert (30).

1.1.3.3 *Socio-economic variables during urbanization*

Other variables associated with socio-economic status can also contribute to the development of obesity. Education, access to resources, body image and cultural differences, among other variables, have been identified as contributors to the obesity epidemic in South Africa. For example, a study in a disadvantaged community in the Western Cape attributed the rise in BMI to factors associated with rural-urban transition like increased access to electricity (27). In a sample of economically active South Africans, black ethnicity, low levels of education and a family history of obesity were implicated as risk factors for obesity (31). The South African Demographic Health Survey reported that low educational status was associated with a higher BMI in black South African women and that lower levels of education were related to incorrect perceptions of body weight (4).

In the South African context, body image may influence obesity status due to cultural values. Mvo et al. (32) found that an overweight body type had positive connotations within the black South African community, symbolizing happiness, beauty, affluence, health and a negative HIV/AIDS status (33). These positive connotations associated with a larger body size are already established in adolescence. For example, Caradas et al. (34)

found that the ideal body size desired by black South African girls was significantly greater than that of white South African girls. In the same study, dissatisfaction with present body size was significantly higher in white, compared to black girls. More recently, Mciza et al. (35) reported that white South African girls (9-12 years) had greater body size dissatisfaction compared to black South African girls.

Additionally, lifestyle factors, such as contraceptive use are influenced by socio-economic factors and impact CVD risk. Research on contraceptive use in Khayelitsha, a primarily black South African urban informal settlement, reported that 83% of women use progestin-based injected contraceptives (depot-medroxyprogesterone acetate and Norethindron enanthate). Of these women, 85% reported that their method of contraception was determined by the community healthcare provider (36), highlighting the impact of area of residence on the type of contraceptive administered. Moreover, injected progestin-based contraceptives have been shown to impact basal insulin levels and may compromise glucose tolerance (12). A longitudinal study in 174 premenopausal American women demonstrated that the administration of depot-medroxyprogesterone acetate for a period of 30 months increased adiposity, as well as the central to peripheral mass ratio compared to subjects not on birth control (37). Contraceptive administration and use in Khayelitsha illustrates the interaction between environment and CVD risk.

1.2 CO-MORBIDITIES ASSOCIATED WITH OBESITY

There are a large number of clinical problems associated with being obese, which can be categorized into those that are associated with excess adipose tissue and those that are associated with the metabolic effects of the increased adiposity (38). Those diseases associated with increased fat mass including osteoarthritis, sleep apnoea and psychological problems are beyond the scope of this thesis (38-40). Further information on these conditions and other fat mass related health problems can be found elsewhere (38;40).

There are numerous diseases associated with the metabolic effects of increased adiposity including CHD (41;42), hypertension (43), type 2 diabetes (44;45) and certain types of cancer (39;41;46). The metabolic effects of excess fat mass are described in brief below.

1.2.1 *The metabolic effects of excess fat mass*

Adipose tissue is not just a storage area for excess energy, but is also a dynamic endocrine organ (47). Adipose tissue exerts its metabolic effects through the secretion of free fatty acids (FFA), which in excess can interfere with metabolism and result in hypertriglyceridemia, hyperinsulinemia and glucose intolerance (48). In human and animal models, increased FFA levels have been implicated in the pathology of insulin resistance in muscle (49), liver (50), endothelial cells (51) and in the dysfunction of pancreatic β-cells (52).

Apart from FFA, adipose tissue also secretes numerous adipokines, which have specific autocrine/paracrine (local) and endocrine (systemic) functions. Some examples are; leptin, which regulates appetite and energy expenditure (53); adiponectin, which is associated with improved insulin sensitivity (53); and various cytokines such as tumour necrosis factor-α and interleukin-6, which are pro-inflammatory and involved in glucose and lipid metabolism (53). Additionally, adipose tissue can respond to afferent signals through nuclear receptors, such as peroxisome proliferator-activated receptor-γ, which is involved in the induction of adipocyte differentiation and insulin sensitivity; glucocorticoid

receptors (GR), which influence adipose tissue differentiation and metabolism; and progesterone and oestrogen, which regulate body fat distribution and metabolism (47;53). Through these mechanisms, adipose tissue is involved in energy metabolism, immune function and neuroendocrine function (53). Further information on adipose tissue as an endocrine organ can be found in the review by Kershaw (53).

The anatomic location of adipose tissue affects its metabolism and hence impacts the development of CVD. Visceral adipose tissue (VAT) is associated with increased risk of CVD compared to subcutaneous adipose tissue (SAT). VAT drains directly into the portal system, resulting in increased delivery of FFA and adipokines to the liver. In contrast, SAT secretes FFA and adipokines systemically, but is a larger depot and therefore produces the majority of circulating FFA and adipokines (54). Although visceral adipocytes are smaller than subcutaneous cells (55), they are more lipolytically active due to the increased density and sensitivity of β–adrenergic receptors (56). Further, VAT is less sensitive to the lipogenic effects of insulin and more sensitive to catecholamines compared to SAT, resulting in increased lipolysis in VAT (57). Moreover, the expression of leptin, adiponectin and lipoprotein lipase is higher in SAT, while VAT has increased expression of angiotensinogen, cytokines (TNF-alpha and IL-6) and GR, as well as higher 11β-hydroxysteroid dehydroganse-type 1 (11β-HSD-1) enzyme activity compared to SAT (58-62). Additionally, ectopic storage of fat in liver and muscle is associated with increased risk of CVD. For example, individuals with lipodystrophy, or inadequate adipose tissue mass, present with insulin resistance, perhaps due to ectopic fat in the liver, muscle and pancreatic β-cells (63). A similar scenario is seen in some obese individuals with type 2 diabetes (64). Further information on the metabolic impact of ectopic fat can be found in the review by Wajchenberg (65).

Additionally, recent research has reported that there may be ethnic differences in the metabolic effects of fat stored viscerally and in muscle. For example, Hanley et al. (66) found a stronger negative association between VAT and circulating adiponectin levels in African Americans compared to Hispanics. Further, Yim et al. (67) demonstrated that the association between intramuscular triglyceride levels and TC was stronger in white Americans compared to African Americans.

In summary, excess adipose tissue associated with obesity, exerts its metabolic effects via the release of FFA and through the action of adipokines and its receptors. The anatomic location of adipose tissue also influences CVD risk, with VAT being associated with greater risk than SAT. The metabolic affects and association with CVD of different fat depots may vary with ethnicity. The association of obesity with disease risk, including type 2 diabetes, hypertension and CHD is described below in brief.

1.2.2 Obesity and type 2 diabetes

Obesity and type 2 diabetes are closely associated in both men and women of all ethnic groups (44;68). The risk of type 2 diabetes increases with the extent and duration of overweight and the degree of central adiposity (38). In the Nurses Health Study, including 114,281 female nurses, the risk of type 2 diabetes increased 40-fold when BMI increased from ≤22 kg/m^2 to 35 kg/m^2 (44). A similar relationship was observed in men in the Health Professionals Follow-Up Study that included a cohort of 51,529 men. In this study, it was found that the relative risk of developing type 2 diabetes was 42.1 in men with a BMI of ≥35 kg/m^2 compared to those with a BMI of <23.0 kg/m^2 (68). According to government reviews in the U.K., obesity (BMI ≥30 kg/m^2) is associated with a relative risk of 5.2 and 12.7 for type 2 diabetes in men and women, respectively (69). In a recent reanalysis of the South African Demographic Health Survey data, it was reported that over 90% of type 2 diabetes in South African women over the age of 30 could be attributed to a BMI >21 kg/m^2 (9).

Based on age-adjusted type 2 diabetes prevalence estimates and population statistics from all countries of the world, King et al. (70) predicted there will be a 42% increase in the prevalence of type 2 diabetes in developed countries (from 51 to 72 million) compared to a 170% increase in developing countries (from 84 to 228 million) from 1995 to 2025. Therefore, in the year 2025, 75% of people with type 2 diabetes could reside in developing countries, as compared with 62% in 1995. Similar findings were reported by Wild et al. (71) who predicted that between the years 2000 and 2030, the prevalence of type 2 diabetes will increase by 97% in Sub-Saharan Africa, vs. 37% world wide.

With regard to type 2 diabetes in South Africa, no large-scale risk assessment study has been undertaken from which the prevalence of type 2 diabetes can be determined. Studies done in the early 1990's found that black South African women were twice as likely to present with type 2 diabetes compared to white women (7.0% vs. 3.6%) (72;73). Levitt et al. (22) found that the prevalence of type 2 diabetes in urban black people living in Cape Town was 7% when age-adjusted to world population figures.

1.2.3 Obesity and hypertension

Obesity is associated with a significant increased risk of hypertension. According to a population based survey including 195,005 randomly selected American adults, obesity was associated with a relative adjusted risk of 3.5 for hypertension (74). In the Nurses' Health Study (75), the relative risk for developing hypertension in women who gained 5.0-9.9 kg was 1.7, compared to 5.2 in women who gained over 25 kg. In the Framingham study it was estimated that being overweight may account for 28% of cases of hypertension in women (76).

The association between obesity and hypertension is also well established in South Africa (43;77;78). In the South African Demographic Health Survey, it was determined that the risk of hypertension was almost two times greater (odds ratio 1.97) in obese compared to normal weight individuals (43). In recent reanalysis of the same data, over 80% of hypertension cases in South African women between the ages of 30 and 59 years were attributable to excess body fat (9). According to the World Health Organization (WHO) guidelines for hypertension (140/90 mmHg), after adjusting for age, approximately 21% of the adult South African population were hypertensive (43).

Further, Steyn et al. (43) found that rural black women had a significantly decreased risk of hypertension when compared to white women. However, when socio-demographic variables were taken into account, there were no appreciable differences in the prevalence of hypertension among the South African population groups.

Similarly, a large variability in hypertension prevalence in both black African (14-44%) and white populations (27-55%) has been reported in a study including individuals of European and African descent from eleven different countries (N=85,000), highlighting the impact of environmental factors on hypertension (79). Interestingly, in the same study, the correlation between BMI and hypertension was stronger in African populations (r=0.60) than in European populations (r=0.30). Similarly, in a large study including African Americans, black ethnicity was an independent predictor of hypertension (80). However, these results should be interpreted with caution, as obesity is associated with lifestyle variables such as physical inactivity and socio-economic variables, which could affect CVD risk, possibly independently of obesity.

The prevalence of hypertension in black African women is expected to increase as the obesity epidemic progresses. For example, Kearney et al. (81) estimated that there were 41.6 million women with hypertension in Sub-Saharan Africa, and that this prevalence will increase to 77.1 million women by 2025.

1.2.4 Obesity and coronary heart disease (CHD)

Obesity is also associated with an increased risk of CHD with a relative risk of approximately 2.8 and 3.4 for men and women, respectively (75). Research in U.S. women found that the risk of developing CHD increased 3.3 fold when BMI was >29 kg/m^2 (41). In South African women between the ages of 30 and 59 years, over 60% of ischemic heart disease and stroke were attributable to excess body weight (9). However, data from the INTERHEART study (82) suggests that obesity is no longer an independent risk marker of CHD after adjustment for other known risk factors. In the same study, waist and waist/hip ratio were both independent risk markers for CHD. This supports the importance of central obesity vs. total obesity as investigated in this thesis.

Lipid profile abnormalities are associated with both CHD and obesity and include elevated total cholesterol (TC) levels, LDL-C and triglyceride levels, and decreased high density lipoprotein cholesterol (HDL-C) (38). In South Africa, 4.8 million people (11%) present

with hypercholesterolemia and 3.1 million people (7%) present with elevated LDL-C levels (83). Both of these conditions result in an increased risk of CHD.

Interestingly, although they have a higher prevalence of obesity, black South Africans appear to be less prone to hypercholesterolemia (78;84;85) and elevated LDL-C levels than individuals who are white or of mixed ancestry (83). Moreover, it has been reported that black South Africans have a higher proportion of protective HDL-C to TC compared to other ethnic groups (86). More favourable lipid profiles have also been reported in black compared to white individuals living in the United Kingdom (87); this also applies to black Africans compared to Europeans with type 2 diabetes (88).

This favourable lipid profile may protect black South Africans against CHD. For example, Seedat et al. (89) found that in an urban Zulu population, the prevalence of CHD was only 2.4%. In another study by the same author, the frequency of myocardial infarction was much lower in South African black compared to white individuals. In fact, in 5000 autopsies on black South Africans with non stroke-related deaths, only 2.7% of individuals died of myocardial infarction (90). Other studies have reported similar findings (91;92).

However, the majority of these studies were carried out over 20 years ago. More recently it was reported that although black South Africans have favourable lipid profiles compared to white South Africans, TC levels have increased with urbanization (93). Conversely, Joffe et al. (94) found no differences in lipid values in a cohort of black South African women tested in 1976 compared to a cohort of black South African women tested in the mid 1990's. However, in the INTERHEART Africa Study, data clearly showed that black Africans who are exposed to risk factors such as central obesity, smoking and hypertension or type 2 diabetes are at risk of developing acute myocardial infarction (82). Therefore, the risk of CHD in this population should not be under-estimated, particularly with increasing levels of urbanization.

1.2.5 Summary

South Africa is currently undergoing an epidemiological transition which has resulted in decreased PAEE, increased dietary fat intake and socio-demographic changes. Consequently, the prevalence of obesity and obesity-related disease is high, particularly in urban black South African women. Based on data from other developing countries, it is likely that the obesity epidemic will continue to grow as epidemiological change progresses. As excess fat has metabolic implications and impacts disease risk, the burden of obesity-related diseases such as type 2 diabetes, hypertension and CHD is also likely to increase. In this context it is important to identify individuals at risk of obesity in order to promote lifestyle change and provide support for the prevention and treatment of obesity and its associated morbidities.

1.3 THE MEASUREMENT OF OBESITY AND ADIPOSITY

As discussed in the previous section of this review, increased adiposity has been shown to increase the risk of developing metabolic disease (38), hence quantification of body fat percent is becoming an increasingly important aspect of health risk appraisal and research. Traditional methods used to measure body composition such as underwater weighing are based on the premise that the body is composed of two compartments; fat mass and fat free mass (95). The limitations of the two-compartment model are that it assumes fixed densities of fat mass and fat free mass, despite individual differences in fat free mass density and/or variation in the amount of bone minerals. DXA is a useful measure of body composition which is based on a three-compartment model, yielding estimates of fat mass, fat free soft tissue mass (FFSTM) and bone mass (96). However, DXA does not take into account differences in the composition of FFSTM. A four-compartment model of body composition has been developed which includes a measure of body water (isotope dilution), in addition to bone mineral content (DXA) and body density (underwater weighing) (97). Further, FFSTM hydration can be calculated from the four-compartment model (98). The four-compartment model is theoretically more valid than the three- and two-compartment models, since it accounts for the variability in bone mineral density and total body water (97).

However, use of expensive and complicated lab techniques for measuring body composition is not always practical in large research studies or in health risk assessment. Therefore, field measures of body composition such as bioelectrical impedance (BIA) (99), near infrared interactance (NIR) (96), skin fold thickness (100) and BMI (101) have been suggested as proxy measures of body fat percent. These field methods, as well as underwater weighing and DXA, which are often used in research and as criterion methods, are reviewed in more detail below.

1.3.1 Body mass index (BMI)

Obesity is now recognized by the WHO as a disease entity, for which BMI has been used as a measurement proxy (101). BMI is calculated as weight (kg) / height (m^2). According to the WHO, normal weight is defined as a BMI of <25 kg/m^2, overweight as a BMI of 25.0-29.9 kg/m^2, and obesity as a BMI of ≥30 kg/m^2 (102). BMI has been used as a measure of adiposity in many population-based studies due to its ease of measurement and practicality. Additionally, BMI is associated with body fat percent and increased risk of CVD in individuals of European and African origin (103). For these reasons, BMI is the most commonly used measure of adiposity.

There are several important limitations to the use of BMI that should be considered prior to its use. Universal BMI standards fail to consider the proportional composition of an individual (104) and assume that BMI is independent of factors such as gender, ethnicity, age and FFSTM. However, research has shown that this is not the case. The relationship between BMI and body fat percent is influenced by body build (105), age, gender (106) and ethnicity (107). In the Heritage Family cohort, Jackson et al. (106) reported that the relationship between BMI and body fat percent was not linear and was not independent of age and gender. In fact, body fat percent in women was 10.4% higher than that in males for the same BMI.

Recent studies have highlighted the influence of ethnicity on BMI (106;108). Jackson et al. (106) reported that when race was not accounted for, BMI under-estimated body fat percent in black women and over-estimated body fat percent in white women. Moreover, Deurenberg et al. (108) performed a meta-analysis investigating the relationship between body fat percent and BMI across ethnicities, including African American, European, Chinese, Ethiopian, Indonesian, Polynesian and Thai populations. They found that the relationship between body fat percent and BMI varied by ethnic group and that a universal cut-point for obesity was not appropriate for all population groups. For example, at the same level of body fat, age and gender, African Americans and Polynesians had BMIs 1.3 kg/m^2 and 4.5 kg/m^2 lower than white subjects, respectively. In individuals of African

origin residing in Nigeria, Jamaica and the United States, Luke et al. (109) found that BMI was a relatively good predictor of body fat percent. However, they also found that mean body fat percent varied substantially at a similar level of BMI between populations. Similarly, a study in urban women of five different ethnicities from South Africa and New Zealand reported that the relationship between BMI and body fat percent varied by ethnicity (110).

As the relationship between BMI and body fat percent is not independent of ethnicity, age, gender and body build, population-specific cut-points for BMI are required. Further, BMI should be used with caution as a proxy measure for body fat percent, especially in South Africa's multi-ethnic population.

1.3.2 Underwater weighing

Underwater weighing is regarded as one of the most reliable techniques for the quantification of body density (95) and has been the criterion measure for the validation of other techniques (99;111-113). It quantifies body volume by comparing the mass of an individual weighed in air to his or her mass underwater. Body density is determined from body volume, after adjusting for residual lung volume and the gas left in the gastrointestinal tract. Equations are then used to derive body fat percent from body density (114;115).

Although underwater weighing is a criterion method for the quantification of body composition, it is a complex and expensive technique that is impractical for use in the field. Moreover, there are multiple sources of technical and biological error inherent in the method that limit the use of underwater weighing in certain individuals, e.g. calculation of residual lung volume (116) and differences in bone density. Lohman et al. (117) reported that underwater weighing techniques could have a standard error of estimate as high as 2.7%, primarily due to population specific differences in FFSTM.

1.3.3 Dual energy x-ray absorptiometry (DXA)

DXA is a useful measure of body composition that yields estimates of three components of body composition; namely bone mass, FFSTM and adipose tissue mass (96), as shown in Figure 1.3. DXA is based on the principle that photon attenuation is a function of tissue composition, and it assumes that bone, adipose and FFSTM mass are distinguishable by their x-ray attenuation properties (118). Technical information regarding DXA is outside of the scope of this review but can be found in the review by Pietrobelli et al. (119).

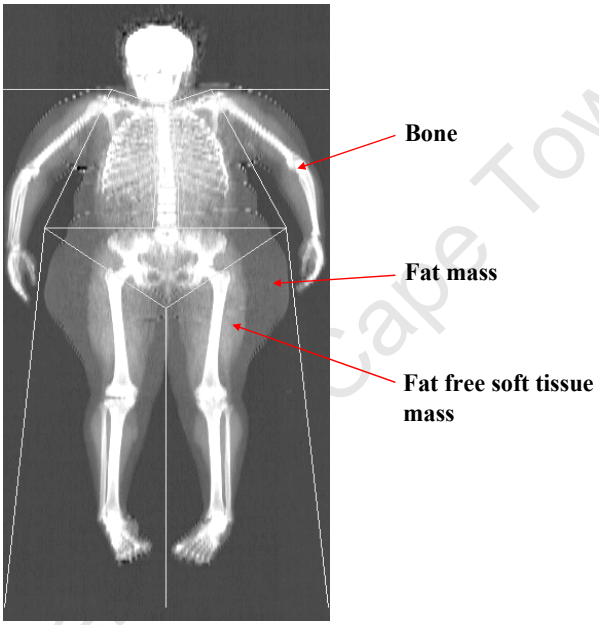

Figure 1.3. DXA scan of an obese woman with the bone, adipose tissue and FFSTM compartments indicated by the arrows

It is well documented that DXA is a precise and accurate tool for measuring body composition (120) and has been validated for this purpose in women of various ages (121-123). For example, in premenopausal white women, body fat percent measured with DXA was precise and highly correlated to that measured with underwater weighing, (r=0.91, SEE=2.4) (121).

However, there are concerns regarding inter-machine variability (124), software versions (125) and the measurement of obese individuals (126;127). Measurement of very obese

individuals with DXA is complicated by the limited size of the DXA platform, which may not be large enough to accommodate all obese subjects. As a solution, Tataranni et al. (126) found that a DXA scan of half the body of a very obese individual could be used to determine total body composition accurately. Alternatively, Micklesfield et al. (personal communication, manuscript in review) have shown that the arm-replacement scanning method, in which the data from one arm is used for both arms, is also a valid method for scanning obese individuals who do not fit on the scan platform. An additional limitation to DXA is that the technique assumes that body compartment layers are consistent as it cannot measure adipose tissue and FFSTM behind bone (118).

Nonetheless, DXA is a valid and useful tool for measuring body composition (121-123). As such, it is often used as a criterion measure for the validation of other body composition techniques (96). In addition to whole body composition, DXA is also used to predict regional body composition (123;128) and central adiposity (129;130). For example, Glickman et al. (131) found that total mass, fat mass and FFSTM in the L1-L4 DXA scan region were highly correlated to computer tomography (CT)-derived measures and that DXA showed high repeatability compared to CT (Intraclass correlation coefficient (ICC) =0.94, 0.97 and 0.89, respectively), but under-predicted central fat mass (limits of agreement (LOA)=0.40-1.94 kg). When comparing abdominal fat measured by DXA to that measured by magnetic resonance imaging (MRI), Park et al. (132) reported that DXA regions L2 to L4, and L2 to the upper iliac were highly correlated with MRI-derived total VAT area (r=0.85 and 0.84, respectively) and MRI- derived single-slice VAT area (L4-L5) (r=0.87 for both regions) in non-obese men. Similar results have been reported in overweight and obese individuals (129). Further, Houtkooper et al. (133) reported that DXA was as sensitive a method as underwater weighing for measuring small changes in body composition in postmenopausal women after an exercise intervention.

In summary, DXA is a valid and reliable tool for measuring total body fat percent and regional body fat distribution, as well as for tracking changes in body composition. To my knowledge, no ethnic differences in the measurement of body fat percent by DXA have been reported. DXA is a more practical tool for measuring body composition compared to underwater weighing, as subjects are not required to submerge in water, and must simply

relax on the scanning table in a hospital gown. As such, DXA is also used as a criterion measure of body composition. In terms of measuring regional adiposity, DXA uses a low level of radiation compared to a CT scan. For these reasons DXA is a very useful tool for research. However, DXA is relatively expensive, requires the skills of a trained technician, and is impractical for use in field work. Therefore, for field studies or for large-scale health risk appraisal, accurate proxy measures of body composition are needed, particularly in developing countries with limited resources.

1.3.4 Bioelectrical impedance (BIA)

BIA is a "doubly-indirect" proxy method of body composition that uses a low frequency electric current (800 μA) to estimate total body water by measuring the resistance (impedance) and reactance of the tissues in ohms (Ω). In normally hydrated individuals, total body water can be used as an indicator of fat and FFSTM (96). Generally, when measuring body fat percent using BIA, the subject relaxes in a recumbent position and electrodes are placed on the individual's hand and foot. However, Rush et al. (134) found that standing BIA using previously validated recumbent equations was reliable for use with appropriate adjustment in a multi-ethnic cohort.

Despite the practicality of the BIA, its validity and reliability varies dramatically by study. In female athletes, BIA was a repeatable (r=0.98) and valid measure of body composition compared to DXA. However, Brodie et al. (135) reported large differences in body fat percent measured by three BIA techniques vs. underwater weighing in normal weight women (8.1% difference) and moderately obese individuals (8.1% difference). In obese women, Heyward et al. (136) also reported a weak correlation (r=0.56) between BIA and underwater weighing. In a comparison of body fat percent measured by BIA and DXA in obese African American women, the majority of equations used to determine body fat percent from BIA under-estimated adiposity compared to DXA (137).

These somewhat conflicting results could be related to the BIA equations used or differences in pre-test conditions, since hydration status impacts the accuracy of BIA.

Therefore, pre-test guidelines for food and drink intake, exercise and diuretic medications which effect hydration status are generally required with use of BIA. Although BIA may be a useful tool in the field, its use is limited by these strict pre-test parameters. Another limitation to the use of BIA is that changes in skin temperature affect impedance (138), which has implications for testing in the field.

1.3.5 Skin fold thickness

Quantification of SAT by the measurement of skin fold thickness is often used in research as it involves minimal equipment, is inexpensive and is relatively quickly and easily administered. SAT is measured at different anthropometric sites using hand-held callipers. Total adiposity can be calculated from skin fold thickness values using validated equations (100;139). With proper technique, quantification of body fat percent using skin fold thickness has been shown to be reliable and valid in various populations (140;141). Body density, as calculated using the equations developed by Durnin and Womersley (100), was reported to have a standard error of estimate (SEE) of between 3.5-5.0% compared to underwater weighing (142).

However, there are many limitations to the use of skin fold thickness as an estimate of body fat percent. The skin fold thickness method operates on the assumption that SAT is representative of total body fat and that FFSTM is uniform in all populations. Therefore, differences in body fat distribution affect the validity of the measurement. This has resulted in the development of over 100 population-specific equations with validity coefficients for women ranging from r=0.72 to 0.84, compared to criterion methods (95). Limitations to the use of skin fold thickness are also related to inter-tester reliability (117), confusion surrounding appropriate equations for specific populations, difficulty in identifying the correct anthropometric sites for measurement (143), inability to measure obese individuals accurately (144) and requiring subjects to undress during measurement.

1.3.6 Near infrared interactance (NIR)

NIR is another "doubly-indirect" field method for the determination of body composition. In this technique, an infrared light wand is placed on the biceps while the subject is seated. The degree of infrared light absorbed and reflected is related to both the composition of the tissues through which the light is being passed and the specific wavelength being emitted by the light. The amount of light reflected by body tissue is measured as optical density from which body fat percent can be calculated (145).

NIR is relatively inexpensive, portable, and does not require the services of a technician for use and interpretation. Additionally it is non-invasive and does not require the subject to be mobile or to undress. NIR is not affected by skin temperature or hydration status like BIA. For these reasons NIR could be a very useful tool for measuring body composition in situations where methods like DXA, underwater weighing and BIA are not viable. In South Africa, where resources are limited and testing is often completed in the field, NIR could potentially function as an important research tool.

Research on the validity of NIR is somewhat conflicting. NIR has previously been shown to accurately estimate body fat percent in non-obese individuals (136;146), while a more recent study reported that NIR over-estimated body fat percent in normal weight individuals compared to underwater weighing (111). In contrast, in obese individuals, NIR has been shown to consistently under-predict body fat percent (147). The degree of underestimation increased with increasing levels of adiposity (148).

There are few studies that have explored the validity of NIR in different ethnic groups. Studies in American Indian women (112), as well as African American Division I college football players (113) found that NIR under-reported body fat percent compared to underwater weighing. In other small studies comprising African Americans, body fat percent measured by NIR was compared to that measured by DXA and underwater weighing, the prediction error of NIR was very high ranging from 3.9-4.6% (149-151). However, to my knowledge, no large scale studies on NIR in African populations have been undertaken. Concerns about the impact of skin tone and levels of SAT on the

relationship between optical density and body fat percent have been raised and suggested as probable causes of the NIR's inaccuracy in certain populations (112;152). Additionally, the recommended range for NIR use is between 5-45% body fat. This range encompasses the majority of individuals but may be a limitation in South Africa due to the high levels of obesity, particularly in urban black South African women. In addition, the suggested range for NIR use was not based on peer reviewed research and should therefore be viewed with caution.

In summary, NIR is a very practical and attractive technique for measuring body fat percent in the field in the South African context, but is somewhat under-researched, particularly in African individuals. Due to the limited data on the use of NIR as a measure of body composition in large ethnically diverse samples, further research is needed to investigate the validity and reliability of this potentially useful tool.

1.3.7 Summary

Since South Africa has a high prevalence of obesity, identification of at-risk individuals is important, but is complicated by limited resources. Although underwater weighing is considered the "gold standard" measure of body composition, it is not always practical or appropriate for use in research or in health risk appraisal. DXA is a valid and reliable method of measuring body fat, bone and FFSTM, although it is not always feasible for use in large studies or for field research. Therefore, field methods such as BMI, skin fold thickness, BIA and NIR are used to estimate level of adiposity, although research has highlighted serious limitations to the use of these techniques. NIR could potentially be a useful field measure of body composition as it does not require strict pre-test conditions and is easy and inexpensive to administer. However, it has not been validated in a black South African population with large ranges of body fatness. Therefore, the validity of NIR as a measure of body fat percent in black and white South African women is investigated in this thesis in Chapter Two.

1.4 OBESITY AND RISK FACTORS FOR CARDIOVASCULAR DISEASE (CVD)

The clustering of obesity-related risk factors for CVD, including dyslipidemia, impaired glucose tolerance, insulin resistance, hypertension and central obesity (38) are collectively referred to as the metabolic syndrome. A schematic diagram of the relationship between these features and CVD is shown in Figure 1.4.

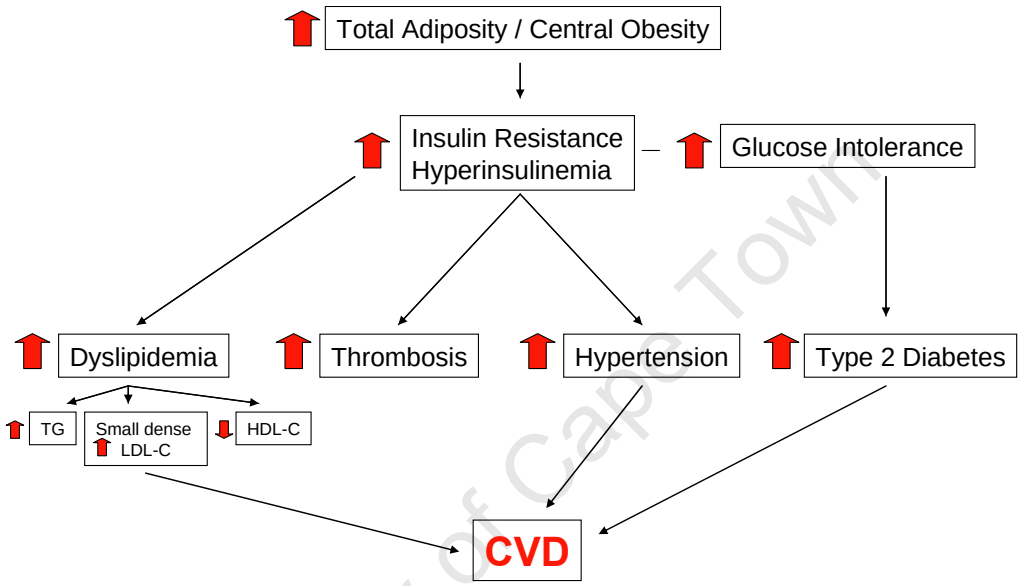

Figure 1.4. Schematic representation of the clustering of risk factors for CVD (153). CVD, cardiovascular disease; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol. This Figure was adapted to present CVD from Bakris' (153) original figure presenting macrovascular disease.

Diagnostic criteria for the metabolic syndrome have been developed to identify individuals at increased risk of CVD. However, consensus on a universal definition for the metabolic syndrome has not been reached and several different definitions are currently in use. These metabolic syndrome definitions contain similar components with varying cut-points and criteria for a positive diagnosis. The major metabolic syndrome criteria currently in use are: i) National Cholesterol Education Program Adult treatment panel III (ATP III); ii) International Diabetes Federation (IDF); iii) World Health Organization (WHO); iv) European group for the study of insulin resistance (EGIR); v) Association of American Clinical Endocrinologists (AACE); and vi) American Heart Association/National Heart,

Lung and Blood Institute (AHA/NHLBI). The components and criteria for a positive diagnosis of metabolic syndrome are presented below in Table 1.1.

Table 1.1. **The most commonly used diagnostic criteria for the metabolic syndrome in women**

	ATP III (154)	IDF (155)	WHO (modified) (156)	EGIR (157)	AACE (modified) (158)	AHA/ NHLBI (159)
IR			presence	presence		
IFG (mmol/L)	≥ 5.6	≥ 5.6	≥ 6.1	≥ 6.1	≥ 6.1	≥ 5.6
IGT (mmol/L)			≥ 7.8 or T2D		> 7.8 (no T2D)	
Waist (cm)	> 88	≥ 80		≥ 80		> 88
WHR			>0.85			
BMI (kg/m^2)			> 30		≥ 25	
BP (mmHg)	≥ 130/85	≥ 130/85	≥ 140/90	≥ 140/90	≥ 130/85	≥ 130/85
TG (mmol/L)	≥ 1.7	≥ 1.7	≥ 1.7	≥ 2.0	≥ 1.7	≥ 1.7
HDL-C (mmol/L)	< 1.29	< 1.29	< 1.0	< 1.0	< 1.29	< 1.1
Other			microalbuminuria			
Diagnosis criteria	≥ 3 components	waist, plus ≥ 2 other components	IR or IFG or IGT, plus ≥ 2 other components	IR, plus ≥ 2 other components	clinical judgment	≥ 3 components

ATP III, National Cholesterol Education Program (Adult treatment panel III); IDF, International Diabetes Federation; WHO, World Health Organization; EGIR, European group for the study of insulin resistance; AACE, Association of American Clinical Endocrinologists; AHA/NHLBI, American Heart Association/National Heart, Lung and Blood Institute; IR, insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; Waist, waist circumference; WHR, waist/hip ratio; BMI, body mass index; BP, blood pressure; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; T2D, type 2 diabetes.

For the purposes of this review, further discussion will focus primarily on the IDF and ATP III criteria and their components as they are most commonly used in research and clinical practice in South Africa (160-162). Moreover, the IDF and ATP III metabolic syndrome criteria are practical for large scale health appraisal and will therefore be investigated in Chapter Three of this thesis.

Dyslipidemia, impaired fasting glucose, hypertension and central obesity, as measured by waist circumference, are components of all of the different metabolic syndrome diagnostic criteria. These variables are well established risk factors for CVD in white populations.

However, there are fewer studies that have investigated the associations of some of these factors with CVD risk in black populations, and the applicability of current metabolic syndrome cut-points in this population may be limited. For example, African Americans have a higher prevalence of obesity, insulin resistance, type 2 diabetes and CVD than white Americans (163;164), despite a lower prevalence of the metabolic syndrome according to the ATP III definition (165-167). McNeil et al. (168) reported a similar prevalence of the metabolic syndrome in African American and white women. However, the African American women had a higher prevalence of elevated BP (30%), enlarged waist circumference (20%), and impaired fasting glucose (16%) compared to the white women. In contrast, lipid levels were more favourable in the African women compared to the white women.

Both African Americans and black South African women are notable for their favourable lipid profiles (19;166;169-172) and present with low triglyceride and TC levels, as well as low TC/HDL-C ratios. This favourable lipid profile could be due in part to the relatively low levels of VAT in black African women compared to white women (173-175). These ethnic differences may render current metabolic syndrome cut-points and the weighing of the components inappropriate for use in black African women.

Additionally, there are both direct and proxy methods for measuring the components of the metabolic syndrome. The strengths and weaknesses of these different measurement techniques must be kept in mind when designing a research study, interpreting data or diagnosing risk in a clinical setting. Therefore, measurement of the individual metabolic syndrome components, including central adiposity, insulin resistance, impaired fasting glucose, impaired glucose tolerance, dyslipidemia and hypertension, and their association with CVD are reviewed below. Where possible, this review has focused on research that has examined the ability of these variables to predict risk in black Africans.

1.4.1 Centralisation of body fat

In 1947, Vague (176) suggested that obesity is not a homogeneous condition and that body fat distribution also impacts metabolic risk. Subsequently, research has demonstrated that the distribution of adipose tissue influences its metabolism and thereby disease risk, more so than total adiposity (177). As discussed previously in section 1.21, increased central obesity in particular is a major determinant of CVD (178). Therefore, enlarged waist circumference is a component of the IDF and ATP III metabolic syndrome criteria (Table 1.1). Central obesity, as measured by waist circumference, may be particularly relevant in black African individuals as demonstrated by the INTERHEART Africa study, in which central obesity was a significantly stronger risk factor for acute myocardial infarction in the African sample compared to the global sample (82).

1.4.1.1 Central vs. peripheral body fat distribution and associations with cardiovascular disease (CVD)

Waist circumference is composed of both the VAT and SAT depots (179), which were discussed in section 1.21 of this review. In a recent study on the Framingham cohort (177), VAT and SAT were both shown to be individually associated with components of the metabolic syndrome including elevated BP, TC, fasting glucose and HDL-C levels, as well as with a positive diagnosis of the metabolic syndrome; however, VAT showed a stronger association. Other evidence also suggests that VAT plays an important role in the development of CVD. For example, Bonora et al. (180) and Solini et al. (181) observed that obese women with visceral obesity had lower rates of glucose disposal and glucose oxidation and higher rates of lipid oxidation compared to their peripherally obese counterparts, independent of total adiposity. Moreover, in a study comprised of African Americans with type 2 diabetes, Banerji et al. (182) reported that VAT explained 35% of the variance in insulin-mediated glucose disposal, while SAT was not significantly associated with insulin resistance. Similarly, in 718 African American and 844 white American women, VAT and not SAT was associated with a positive diagnosis of the

metabolic syndrome (183). Interestingly, in the same study increased VAT in normal weight women was more closely associated with a positive diagnosis of the metabolic syndrome compared to increased VAT in obese women (odds ratio 3.3, 95% CI: 2.4-4.6, vs. odds ratio 2.4, 95% CI: 2.0-3.0 respectively).

However, a role for SAT in the pathogenesis of the metabolic syndrome and CVD has also been suggested (184-187). For example, in a study on relatively young (± 33 years) non-diabetic, African American women and men, Tulloch-Reid et al. (188) found that in women, SAT was more closely correlated to insulin resistance (r^2=0.67) than VAT (r^2=0.50). The difference between Tulloch-Reid et al. and Banerji et al.'s (182) findings on the relative roles of VAT and SAT in insulin resistance in African Americans may be due to differences in their study populations (type 2 diabetics vs. non-diabetic subjects). Additionally, differences in the types of SAT depot (deep vs. superficial) may explain the different findings in these studies. The different SAT depots are described in detail below.

Smith et al. (189) described two separate SAT compartments which are separated by the fascia superficialis, termed superficial subcutaneous adipose tissue (SSAT) and deep superficial subcutaneous adipose tissue (DSAT) (Figure 1.5). SSAT is characterized by small tight packed fat lobules compared to DSAT which has larger fat lobules distributed irregularly. DSAT is more variable in size than SSAT, particularly in obese individuals (190).

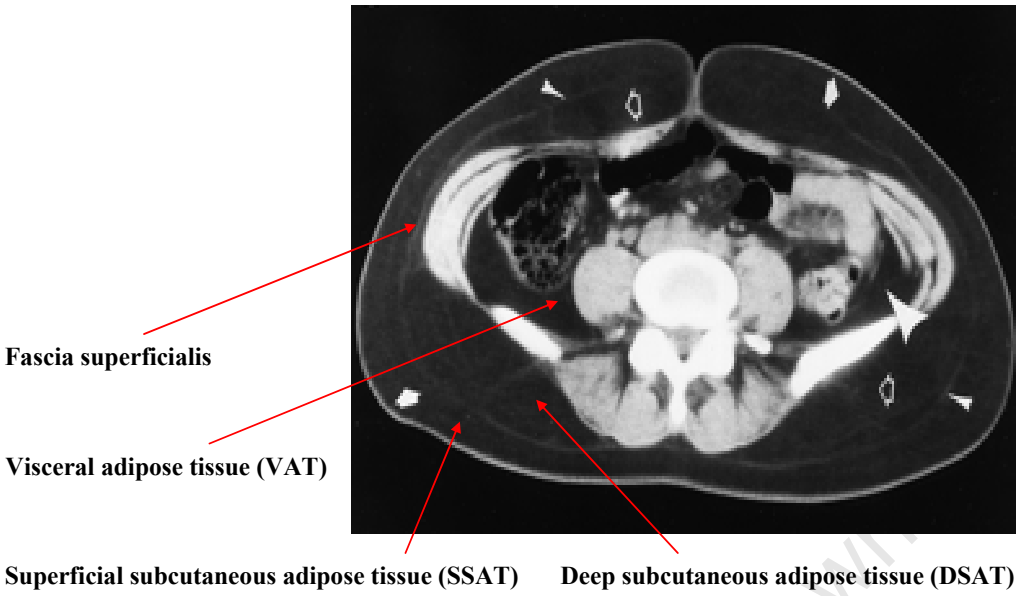

Figure 1.5. Computer tomography (CT) scan at the L4-L5 lumbar region highlighting VAT, SSAT and DSAT adipose tissue depots separated by the fascia superficialis (191)

DSAT and SSAT may influence disease risk differently. For example, research in white populations has shown that DSAT is as closely associated with insulin resistance as VAT (191), particularly in male subjects, while there was no association between SSAT and insulin resistance (189). This may be explained by greater rates of lipolysis in DSAT compared to SSAT (192) as well as by differences in adipokine expression (193). Therefore, determining the relative importance of the adipose tissue compartments in the development of CVD is essential for our understanding of the pathology of obesity-related co-morbidities in black populations.

In contrast to central obesity, adipose tissue deposition in the periphery may act as a 'reservoir' for circulating FFA, reducing ectopic fat deposition in muscle, liver and the visceral depot, thereby attenuating insulin resistance (194) and reducing hepatic LDL-C production (195), which improves metabolic health. Indeed, in African and white Americans from the Dallas Heart Study, central fat mass was associated with increased insulin resistance, lipid levels and C-reactive protein, while peripheral fat mass was metabolically protective (196). Further, Snijder et al. (197) demonstrated a protective association between increased hip circumference and decreased fasting glucose, and triglyceride levels and increased HDL-C levels in different populations from the Pacific

and Indian Ocean islands after adjusting for BMI, waist circumference and age. Similarly, a larger hip circumference was associated with lower levels of TC and LDL-C, triglycerides, fasting plasma glucose, two-hour plasma glucose, systolic and diastolic BP in Iranian women (N=5720) (198). Other studies have also demonstrated the protective effect of peripheral fat (64;199).

1.4.1.2 Body fat distribution in black African women

Studies undertaken in the United States of America have found that the relationship between body fat distribution and risk factors for disease are different between African Americans and white Americans (173;175;200;201). Ethnic differences in regional body fat distribution have previously been reported in American women, with African Americans presenting with less VAT compared to their white counterparts (173;175;201-203). Similarly, a series of studies completed at the University of the Witwatersrand in South Africa, comparing small groups of 8-15 obese black and white women, demonstrated that black women had significantly less VAT than white women (~72 vs. 140 cm^2) (204;205), assessed by CT scan, when matched for BMI (174).

Despite lower levels of VAT in black women, results from the South African Demographic Health Survey, including over 7,000 South African women, found that black women and women of mixed ancestry had a greater waist circumference and waist/hip ratio compared to white and Indian women. In fact, more than twice as many black than white women had a waist circumference greater than 85 cm (35.3% vs. 17.4%, respectively) (4). Recent studies on black Africans have also shown high levels of central obesity as assessed by waist circumference, as well as associations between waist circumference and CVD. For example, in a large study (N=1010) comprised of African and white Americans, waist circumference was a better indicator of CVD than body fat percent (206). In South Africa, Rheeder et al. (207) reported that an enlarged waist circumference, was associated with elevated fasting insulin and triglyceride levels as well as low HDL-C levels in urban black hypertensive women. There is also evidence to suggest that waist circumference is an important and commonly occurring component of

the metabolic syndrome in African individuals (160;161;166). In a longitudinal study of African and white American adolescent girls, central obesity as measured by waist circumference was strongly associated with development of the metabolic syndrome (208).

The combination of increased waist circumference and relatively low levels of VAT in black South African women may have implications for the diagnosis of CVD risk using the IDF and ATP III metabolic syndrome criteria. For example, increased waist circumference may not be reflective of increased VAT area, which could impact CVD risk, reducing the relevance of metabolic syndrome criteria waist circumference cut-points. Research has shown that despite relatively low levels of VAT, African Americans are more insulin resistant and have higher BP compared to white Americans, suggesting that the role of VAT in CVD may differ by ethnicity (201). The relationship between VAT and waist circumference and subsequent CVD risk requires further investigation in the black South African population and is explored in Chapter Three of this thesis.

1.4.1.3 Measurement of central obesity

Quantification of central obesity as well as VAT and SAT is important for both research and for health risk appraisal. Waist circumference is a field method often used in large-scale studies and risk screening to quantify central obesity, while CT scanning techniques and magnetic resonance imaging (MRI) are often used in research to directly measure VAT and SAT volumes or areas in relation to disease risk. These methods are discussed below.

1.4.1.3.1 Waist circumference

Waist circumference is often used as a proxy measure of central obesity since it is a very practical field measure, that is unrelated to height (209) and correlates closely with BMI and waist/hip ratio (210). Waist is also correlated with VAT (211) and is independently associated with the components of the metabolic syndrome (212) and risk for CVD (211). Changes in waist circumference have been shown to reflect changes in risk factors for chronic diseases, such as CVD (213). Additionally, a recent study by Janiszewski et al. (214) (N=5882) reported that waist circumference predicted type 2 diabetes beyond that explained by traditional risk factors for CVD such as lipid profile, BP and glucose level.

Cut-points for waist circumference for men and women are used to diagnose central obesity as part of the criteria for the metabolic syndrome. Commonly used cut-points for this purpose are the ATP III value of >88 cm (154) and the IDF value of >80 cm (155). Additionally, ethnic-specific cut-points for European, South Asian and Chinese (≥80 cm for all three groups) as well as Japanese women (≥90 cm) have been included in the IDF metabolic syndrome criteria (155). However, to my knowledge there are no cut-points for individuals of African origin, and cut-points for white populations are currently in use to identify central obesity in this population (215). This may be problematic due to the relatively low levels of VAT in black compared to white South African women (216). Therefore, the relationship between waist circumference and VAT area is investigated in Chapter Three of this thesis.

Although waist circumference appears to be a simple and practical tool for the assessment of risk, there are limitations to its use, such as large discrepancies in measurement technique. For example, studies examining the relationship between waist circumference and risk factors for CVDhave used at least fourteen different methods to quantify waist circumference. Moreover, even when the four most common approaches were compared, there were significant differences in the absolute values for waist circumference (217). Additionally, as waist circumference encompasses entire abdominal fat, it cannot distinguish between VAT and SAT compartments which have been shown to have different effects on disease risk.

1.4.1.3.2 Computer tomography (CT) scan

MRI and CT scans are "gold standard" techniques for measuring body fat distribution (218). As access to MRI for research is limited in developing countries like South Africa, CT scan techniques which are often used in South Africa research are the focus of this review and are described below.

Measurement of total abdominal adipose tissue, VAT and SAT volumes, using multiple-slice scan technique is very reproducible with low levels of error (0.4%, 1.2% and 0.5%, respectively) (219). Since multiple-slice CT scans expose subjects to high levels of radiation and are expensive, single-slice CT scans are often used for research purposes. Abdominal adipose tissue area, derived from a single scan at the L4-L5 lumbar region, is highly correlated to volume derived from multiple-slice scan techniques (189), and allows differentiation of SAT and VAT (220). Studies in white populations reported that the L4-L5 area is a highly reproducible measure of total abdominal fat (1.2% error), VAT (3.9% error) and SAT (1.9% error) in premenopausal normal weight and obese women (221). Although other researchers have found that scanning at other lumbar regions (5 cm above the L4/L5 region) may be a more accurate representation of VAT volume in women (222), the L4-L5 region is the most commonly used region, and it is generally recommended for a single-slice CT scan (189;219).

There is limited data available on the most appropriate site for single-slice CT scanning of African women. Sumner et al. (223) compared the L2-L3 and the L4-L5 single-slice VAT areas to VAT volume determined by multiple-slice technique in African American men and women. In women, they found that although the VAT area was not different between the two sites (P=0.42), the L2-L3 VAT area was more closely correlated to VAT volume, compared to the L4-L5 VAT area (r=0.97 and 0.90, respectively), although both correlations were highly significant (P<0.01 for both). Demerath et al. (224) suggested that the L3 site was most closely correlated to VAT volume in both African and white American men and women. Despite these findings, the majority of studies comprising African American women have used the L4-L5 lumbar region for single-slice CT scans to quantify VAT area, which also allows comparison with white populations (60;173). Since VAT is closely associated with CVD, VAT cut-points have been developed to identify individuals at increased of developing CVD. These cut-points range from 50-132 cm^2 and vary by gender, CT scan technique and ethnicity (Table 1.2). To my knowledge, no VAT cut-points have been determined for use in black African women.

Table 1.2 VAT area (cm^2) cut-points for the identification of women at risk of developing CVD

Reference	Population	CT imaging site	Cut-point (cm^2)
Despres and Lamarch et al. 1993 (225)	Normal weight and obese young French Canadians	L4-L5	≥130
Williams et al. 1996 (226)	Normal weight and obese white American	L4-L5	≥110
Anderson et al. 1997 (227)	Normal weight and obese Chinese with T2D	L4-L5	≥132
Saito et al. 1998 (228)	Normal weight and obese Japanese	Umbilicus	≥90
Tanaka et al. 2004 (229)	Overweight and obese Japanese	L4-L5	≥100 (cross-sectional data) ≥60 (longitudinal data)
Kim et al. 2006 (230)	Normal weight and obese Koreans	L4-L5	≥103.8
Hayashi et al. 2007 (231)	Normal weight Japanese Americans	Umbilicus	≥ 51.1 in women ≤56 years old ≥ 86.3 in women >56 years old

CT, computer tomography; L, lumbar; T2D, type 2 diabetes

Since VAT areas are not independent of age (231), gender (223) or ethnicity (173), VAT area cut-points to identify CVD are population specific. The relationship between VAT and CVD risk in black South African women is investigated in Chapters Three and Four of this thesis.

1.4.2 Insulin resistance, impaired fasting glucose and impaired glucose tolerance

Insulin sensitivity is defined as the ability of insulin to stimulate glucose uptake and inhibit hepatic glucose output. Insulin resistance is a CVD risk factor and central feature of the metabolic syndrome (232). In fact, the term "insulin resistance syndrome" has been used to refer to the concept that the combination of insulin resistance and associated hyperinsulinemia is associated with other CVD risk factors which cluster together. Through this association, insulin resistance and hyperinsulinemia increase the risk of developing CVD (233). Commonly used metabolic syndrome diagnostic criteria often include impaired glucose tolerance or impaired fasting glucose as components, as previously described in Table 1.1. According to the WHO, impaired glucose tolerance is a pre-diabetic condition that occurs when blood glucose levels are elevated to between 7.8-11.0 mmol/L two hours post challenge in an oral glucose tolerance test (OGTT) (156). Impaired fasting glucose is diagnosed when fasted blood glucose level is either 5.6-6.9 mmol/L or 6.1-6.9 mmol/L, depending on the diagnostic criteria being used (154-156;159).

Insulin resistance, impaired glucose tolerance and impaired fasting glucose are all well established risk factors for CVD and the development of type 2 diabetes (15;38;234-236). In fact, there is longitudinal evidence to suggest that insulin resistance is a risk factor for CVD, independent of obesity (237). With regard to impaired glucose tolerance and impaired fasting glucose, Barr et al. (235) reported all cause mortality hazard ratios of 1.5 (95% CI: 1.1-2.0) for impaired glucose tolerance and 1.6 (95% CI: 1.0-2.4) for impaired fasting glucose in a large longitudinal study (N=10,428). However, in another longitudinal study including middle-aged Italians, up to 59% of individuals with impaired glucose tolerance had normal fasting glucose levels (238). Further, Cheng et al. (239) found that

fasting plasma glucose level was inadequate at identifying insulin resistance in young African American women. Despite these limitations to the use of impaired fasting glucose as an indicator of risk, the IDF and ATP III metabolic syndrome criteria include impaired fasting glucose as a diagnostic component since it is practical and cost-effective to measure.

Insulin resistance has been associated with the development of type 2 diabetes, dyslipidemia and hypertension (15;38;234). Therefore, insulin resistance may be the underlying biological link between the CVD risk factors associated with the metabolic syndrome (13). Insulin resistance alters blood lipid profile by causing an increase in circulating triglyceride levels and a decrease in HDL-C levels. Briefly, insulin resistance results in a decrease in inhibition of lipolysis in adipocytes, resulting in increased release of FFA, which are transported to the liver and stabilize ApoB, the major apolipoprotein of very low density lipoprotein. Moreover, insulin resistance reduces the production and function of lipoprotein lipase, resulting in a lower clearance rate and increased circulating triglyceride levels (240). Very low density lipoprotein remnant proteins can form atheromas as well as affect HDL-C through the transferral of triglycerides via cholesteryl ester transfer protein, resulting in increased clearance of HDL-C and less HDL-C available to receive cholesterol from the vasculature (241).

With regard to the association between hypertension and insulin resistance, studies have shown that first degree relatives of hypertensive individuals are generally more insulin resistant compared to individuals without a family history of hypertension (242). Further, the hyperinsulinemia associated with insulin resistance predicts the eventual onset of hypertension in population-based studies (243). Therefore, it is likely that insulin resistance plays a role in the pathogenesis of hypertension.

1.4.2.1 Insulin resistance in black African women

Despite having less VAT, obese black South African women have been reported to be more insulin resistant and have been described as having relative insulinopenia compared

to their white counterparts (174;244). In addition, *in vitro* and *in vivo* studies found greater adipose tissue lipolysis and a greater degree of adipose tissue insulin resistance in obese black compared to obese white South African women (244;245). These findings are similar to those in African American women. Lovejoy et al. (173) found that African American women were more insulin resistant than white women, despite having less VAT. However, the insulin resistance in these women was associated with hyperinsulinemia (200). A more recent South African study did not find any ethnic differences in insulin or glucose levels in obese white and black women during a standard oral glucose tolerance test, despite ethnic differences in VAT (216). However, another paper by the same author reported that insulin levels were higher in obese black women compared to obese white women 30-minutes post administration of a mixed meal (246). The authors suggested that this higher 30-minute insulin response in obese black women might reflect a higher insulinotropic effect of FFAs or glucose. It is also possible that the obese black women had a relatively higher 30-minute insulin response as they were more insulin resistant compared to the obese white women.

The high prevalence of type 2 diabetes and insulin resistance in black women, despite low levels of VAT is unexpected, since VAT and insulin resistance are closely associated (247). However, it is possible that SAT may play a metabolic role linking obesity and insulin resistance in black African women. Indeed, a recent study found that SAT was more closely associated with insulin resistance than VAT in African American women (188). Therefore, investigation and quantification of SAT, specifically DSAT, which may have a greater metabolic impact than SSAT (189;191), could explain this apparent paradox. The influence of VAT, SSAT and DSAT on insulin resistance is investigated in Chapter Three of this thesis.

1.4.2.2 Measuring insulin sensitivity

Many tests have been developed to quantify β-cell function and insulin sensitivity (248-250). However, the methods used to measure insulin sensitivity vary considerably by study and are filled with inconsistencies and errors (251;252). A further complication is

individual variation in insulin sensitivity in healthy and at-risk individuals. For example, Yeni-Komshian et al. (253) found that in healthy non-diabetic men (N=490), insulin-mediated glucose disposal varied by 6 to 8-fold. Finally, there are no universal criteria defining insulin sensitivity and resistance, complicating the comparison of studies. Despite these challenges, quantification of insulin sensitivity is important clinically and in research, and several methods are currently in use.

Clinical tests such as the euglycaemic hyperinsulinemic clamp (254) which is considered the "gold standard" and the frequently sampled intravenous glucose tolerance test (FSIVGT) (255;256) are ideally used to quantify insulin sensitivity and β-cell function. They are particularly useful as they are dynamic measures in response to a glucose load. In the euglycaemic hyperinsulinemic clamp, glucose uptake is stimulated by infused insulin, while glucose is kept steady at a euglycemic level (5 mmol/L) with variable glucose infusion. This test measures mean glucose infusion rate at steady state, which is adjusted for lean body mass, resulting in an insulin sensitivity index termed *M* (mg/min/kg of lean body mass). Despite the proven efficacy of clamp methods and their status as "gold standards" (257), the technical expertise required, cost and lengthy, invasive protocol limit their use in large population-based studies.

The FSIVGT is an accurate method for the quantification of both insulin sensitivity and β-cell function (255;256). Insulin sensitivity is determined using individual dynamic data and curve-fitting equations (minimal model analysis). The minimal model calculates the insulin sensitivity index (Si), a variable that has been validated against clamp techniques in several studies, including those of Bergman et al. (258) ($r=0.89$, $P<0.001$) and Saad et al. (259) ($r=0.62$, $P<0.001$). The FSIVGT is less complex than clamp techniques, but still requires technical expertise, multiple blood samples, complicated calculations with the minimal model analysis, and it is time consuming and invasive. Therefore, as with the clamp, use of FSIVGT may not be practical for large studies.

Therefore, the OGTT (260) which is a dynamic, but less time consuming measure of insulin sensitivity, is often used in research and as a diagnostic tool to identify impaired fasting glucose and type 2 diabetes. The OGTT measures insulin sensitivity and β-cell

function after consumption of a glucose drink. This test is less invasive compared to the clamp and FSIVGT methodologies and is used to diagnose both impaired glucose tolerance and type 2 diabetes. However, use of the OGTT as a measure of insulin sensitivity may be limited, in that the rate of glucose appearance is unknown, and the glucose response in type 2 diabetes might not be strong enough to allow estimation of insulin sensitivity. Despite these factors, insulin sensitivity measured by OGTT has been shown to correlate closely with that measured by clamp methodologies (261;262).

Further information on dynamic measures of insulin sensitivity, including clamp, FSIVGT and OGTT methodologies can be found in the research of Ferrannini et al. (263), Pacini et al. (256), Stumvoll et al. (262) and Mari et al. (261). The homeostasis assessment model (HOMA), the modified HOMA2 model (249), quantitative insulin sensitivity check index (QUICKI) and modified QUICKI (250) are often used to quantify insulin sensitivity and β-cell function in larger studies where more complex methods are impractical. These methods are not dynamic, but are very practical as they only require fasting glucose, FFA and insulin samples. HOMA and QUICKI are briefly described below.

1.4.2.2.1 Homeostasis model assessment (HOMA)

The HOMA model of insulin resistance (HOMA-IR) and β-cell function (HOMA-B) are derived from a mathematical model of the interaction between fasting plasma glucose and insulin levels (248;249) and reflect the sensitivity of glucose production and uptake by insulin at basal levels. HOMA-IR is often used to measure insulin resistance in population-based studies and has been reported in over 500 publications (251). HOMA is a “paradigm” model as opposed to a “minimal model”, in that rather than using equations to describe dynamic data, HOMA has a theoretical base that is then adjusted to population norms (251). The physiological basis to the model is that basal glucose level is regulated by insulin-dependent hepatic glucose output. Insulin is dependent on the response of β-cells to glucose, and also signals glucose uptake. The feedback loop between the β-cell and liver is central to the HOMA model.

The original HOMA model (248) consists of a number of equations that simulate the functions of organs and tissues involved in glucose homeostasis. These equations result in estimates of C-peptide, glucose and insulin levels at differing levels of insulin sensitivity and β-cell function. HOMA-IR and HOMA-B are calculated as shown below. K is a constant used to scale HOMA so that it has the value of 1 (or 100%) with mean normal basal glucose and insulin (264):

$$\text{HOMA-IR} = \text{Glucose (mmol/L)}_{\text{basal}} \times \text{Insulin (uU/mL)}_{\text{basal}} / K \ (K=22.5)$$

$$\text{HOMA-B} = K \times \text{Insulin (uU/mL)}_{\text{basal}} / \text{Glucose (mmol/L)}_{\text{basal}} - 3.5 \ (K=20)$$

Computer models for HOMA (249) have been created and incorporate variations in hepatic and peripheral glucose resistance, proinsulin secretion, renal glucose loss and modifications to the insulin secretion curve. This newer model is referred to as HOMA2 and may be more accurate for the assessment of insulin resistance and β-cell function compared to HOMA-IR, since it is based on more recent assays specific for measuring fasting insulin (251). However, historically HOMA-IR is more often used in the literature than HOMA2.

Validation studies of HOMA-IR against clamp methodologies have yielded conflicting results, ranging from a strong to an insignificant association between the measures (261;265;266). It has been suggested that the lack of agreement between HOMA-IR and clamp studies could be related to differences in the methods (basal levels vs. a dynamic measure in response to a glucose load), and not a failure of HOMA-IR (266). It is also likely that the lack of agreement between HOMA-IR and clamp methodologies could be due to high variability in fasting insulin levels, and the lack of standardization of insulin assays, as described earlier (253).

Interpretation of HOMA-IR is somewhat ambiguous. A HOMA-IR value of 1 is considered “normal insulin sensitivity” (248), but there is no consensus on a definition of insulin resistance using HOMA-IR. Some studies (267;268) have used a cut-point of 1.95 for HOMA-IR to define insulin resistance ,which is based on a glucose disposal cut-point of 8.0 mg/min/kg fat-free mass, derived from euglycaemic hyperinsulinemic clamp data generated by a large multi-centre study (269). Insulin resistance has also been quantified

as a HOMA-IR cut-point of 1.69 (270) and the upper tertile or quartile of HOMA-IR of a particular study cohort (19;237;271).

Despite the lack of consensus on a definition of insulin resistance, HOMA-IR has been shown to be an independent predictor of CVD. For example, Jeppesen et al. (272) found that HOMA-IR was an independent predictor of CVD in a Swedish population. Similarly, in Italian type 2 diabetes patients, a one unit increase in HOMA-IR was associated with an odds ratio of 1.31 for CVD at the studies baseline (P=0.002) and odds ratio of 1.56 for incident CVD at follow-up 4.5 years later (P<0.001) (273). In a more recent longitudinal study, Bonora et al. (15) reported that HOMA-IR was associated with BMI and was an independent predictor of CVD in the general population. HOMA-IR has also been associated with vascular endothelial dysfunction in middle-aged men and women (274). In a multi-ethnic cohort from the Women's Health Initiative and Observational Study including black African, white, Hispanic and Pacific Islanders, Song et al. (275) reported that HOMA-IR was significantly associated with risk of type 2 diabetes in all ethnic groups. The relative risk per standard deviation increment in HOMA-IR was 3.40 (95% CI: 2.95-3.92). Finally, the HOMA-IR and HOMA2 protocol is very simple, only requiring a fasted glucose and insulin sample making it practical for large population-based studies.

1.4.2.2.2 Quantitative insulin sensitivity check index (QUICKI)

Katz et al. (276) reported that taking both the logarithm and reciprocal of the insulin-glucose product from HOMA resulted in good prediction of insulin sensitivity when compared to clamp studies. The index derived is referred to as QUICKI, and is therefore a non-linear transformation of HOMA.

$$QUICKI = 1/ \log Glucose\ (mmol/L)_{basal} + \log Insulin\ (uU/mL)_{basal}$$

Both Katz et al. (276) and Perseghin et al. (250) found that incorporation of fasting FFA level into the QUICKI equation resulted in improved estimation of insulin sensitivity, with

greater discriminatory power, in non-obese individuals when compared to the clamp-based indices as a criterion (250).

$$\text{Revised QUICKI} = 1/\left[\log \text{Glucose (mmol/L)}_{basal} + \log \text{Insulin (uU/mL)}_{basal} + \log \text{FFA (mmol/L)}_{basal}\right]$$

1.4.3 Dyslipidemia

Increased serum TC, LDL-C and triglyceride levels, as well as decreased HDL-C levels have been associated with increased risk of CHD (277;278). Due to their association with CVD risk, TC, triglyceride and HDL-C cut-points are included in the IDF and ATP III metabolic syndrome diagnostic criteria (Table 1.1). For example, in a longitudinal study including a multi-ethnic sample of men (N=12,467), TC level was linearly related to CHD mortality, and it was reported that a 0.50 mmol/L increase in TC resulted in a 17% increase in risk of mortality. Similarly, after a sixteen to twenty-five year follow up of 1,617 men who were apparently healthy at baseline, Stamler et al. (279) reported that increased TC level substantially increased absolute risk of CVD death. Other longitudinal studies have reported comparable findings in women and men of different ethnicities (277;280). For example, Lowe et al. (280) found that increased lipid levels were an independent predictor of mortality in 8,686 women from the Chicago Heart Association Detection Project in Industry. Further, in American Indians with type 2 diabetes, decreased HDL-C and increased LDL-C were important independent predictors for incident CHD. In the same study, HDL-C/TC and HDL-C/LDL-C ratios accurately predicted CHD four years after the baseline of the study (278).

Although elevated TC and LDL-C levels have been shown to impact CVD risk, numerous large studies such as the Framingham heart study, have highlighted the role of increased triglyceride and decreased HDL-C levels, in particular, in the development of CVD (281;282). Further, a meta-analysis of population-based prospective studies demonstrated that triglyceride levels were an independent risk factor for CVD (283). Moreover, in a prospective study on women (N=1405), Bass et al. (284), reported that triglyceride and HDL-C levels were better predictors of CVD risk compared to TC and LDL-C levels.

1.4.3.1 Lipid profile in black African women

As described above, the relationship between dyslipidemia and CVD is well characterized. However, African American, Afro-Caribbean and black South African women are notable for their favourable lipid profiles (19;20;86;166;169-172;285-287). For example, the INTERHEART Africa study showed that black Africans may have a less atherogenic lipid profile compared to white individuals and individuals of mixed ancestry (82). Similarly, Seftel et al. (86) reported that black South African males presented with fewer risk factors for CHD, including low HDL-C levels, compared to white men, Indian men and men of mixed ancestry. This has important clinical consequences as current lipid cut-points for CVD risk may be inappropriate for use in black African individuals, and could therefore mask risk. For example, in 85 black South African patients with significant CHD as determined by coronary angiography, lipid levels were within the ATP III recommended ranges (288).

HDL-C and triglyceride levels are components of every major criterion for the metabolic syndrome, which may be problematic in African individuals. For example, Jones et al. (289) found that HDL-C was more protective against CHD in white Americans compared to African Americans. Further, in a study of 254 Nigerian men and women with type 2 diabetes, there was no association between low HDL-C level and a positive diagnosis of the metabolic syndrome, waist circumference, BMI or microalbuminuria. From this data, Isezuo et al. (290) concluded that HDL-C level may not be a reliable diagnostic tool of metabolic syndrome for Africans with type 2 diabetes. This is investigated in black South African women in Chapter Three of this thesis.

Moreover, international studies have reported that the association between triglyceride levels and obesity is weaker in African compared to white Americans (291). In South Africa, triglyceride levels are lower in the black compared to the white population (85), which may complicate the use of universal triglyceride level cut-points across ethnic groups. Additionally, triglyceride levels are often used as an indicator of insulin resistance (292) as described previously. However, Sumner et al. (19;169;170) have consistently shown that triglyceride levels are not indicative of insulin resistance in African Americans.

Other researchers have has also shown that black Africans are more insulin resistant, but have lower triglyceride levels compared to white individuals (164). In South Africa, similar findings were reported in small studies by Van der Merwe et al. (174;205;245).

The disparate relationship between insulin resistance and triglyceride levels in white individuals compared to black African individuals may be due to differences in lipoprotein lipase activity (169) or genetic factors (166). Alternatively, differences in body fat distribution may explain the differing relationship. Black African women have a more protective pattern of body fat distribution, with relatively low levels of central fat and increased peripheral fat deposition compared to white women (130). Indeed, increased peripheral fat deposition, independent of BMI, age and waist circumference, is protective against insulin resistance, type 2 diabetes and hypertriglyceridemia (194;197;293).

Research has yet to determine if black African individuals are at increased risk of CVD disease at lower triglyceride levels compared to white individuals, and what role insulin resistance plays in this relationship. Some studies showed a significant association between insulin resistance and lipids, despite favourable lipid levels in African Americans (294). Alternatively, it is also possible that triglyceride levels are not indicative of CVD risk in this population (170). However, in the POWIRS cohort, black South African women had lower triglyceride, LDL-C and TC levels compared to white women, but presented with a less favourable cardiovascular profile as measured by BP, total peripheral resistance and Windkessel compliance (20). Moreover, Vezi et al (287) reported that although 90% of black South African type 2 diabetes patients displayed some form of dyslipidemia according to the ATP III metabolic syndrome criteria, mean triglyceride levels were only 1.8 mmol/L in the women, despite advanced type 2 diabetes. Further, in the only prospective study on lipid levels in black South African individuals (N=89 CHD patients and 356 controls), Loock et al. (295) reported that a low HDL-C/LDL-C ratio and elevated TC level were associated with increased risk of CHD over a follow-up period of ten years (odds ratio 2.82 and 2.53 respectively). Together, these data suggest that black South African women may be at increased risk of developing CVD at lower triglyceride levels compared to white women.

Furthermore, lipid levels in black South African women appear to be affected by urbanization, although this has not been confirmed by all studies (94). Oosthuizen et al. (93) demonstrated in 1,854 black South Africans from the THUSA study, that the more urbanized subjects had increased lipid values compared to the rural subjects, although lipid values were within levels recommended for other ethnic populations. As the South African black population is becoming increasingly urbanized, clarification of the relationship between triglyceride and HDL-C levels and CVD is needed.

1.4.4 Elevated blood pressure (BP)

Elevated BP is a risk factor for CVD and has been associated with an increase risk of CVD in longitudinal studies (280). In fact, risk of CVD doubles for every 20/10 mmHg increase in BP, starting from 115/75 mmHg (296). Therefore, elevated BP is a component of the IDF and ATP III metabolic syndrome diagnostic criteria (Table 1.1). It has been suggested that systolic BP may be a better indicator of CVD than diastolic BP (297). Elevated BP may be a particularly important CVD risk factor in black Africans. For example, a family history of hypertension was one of the CVD risk factors most strongly associated with acute myocardial infarction in the INTERHEART Africa study. Further, this relationship was stronger in the African sample of this study compared to the global sample (odds ratio 3.44 vs. 2.49, P=0.002) (82). Norman et al. (298) recently reported that 9% of all deaths in SA in 2000 were attributable to hypertension. In the same study it was reported that 27,620 South African women died from ischemic heart disease, stroke, hypertension and other CVDs in 2000 and that these conditions resulted in a total of 210,271 disability adjusted life years.

1.4.5 Prevalence of the metabolic syndrome

As described in the previous sections of this review, total adiposity, central obesity, insulin resistance, glucose intolerance, impaired fasting glucose, dyslipidemia and elevated BP, are established risk factors for CVD. Of these factors, central adiposity, dyslipidemia,

elevated BP, and impaired fasting glucose are included in the IDF and ATP III metabolic syndrome criteria (Table 1.1). These metabolic syndrome criteria differ in their components and criteria for a diagnosis. As a result, variability in their agreement has been reported. For example, Lorenzo et al. (299) reported close agreement between the IDF and ATP III metabolic syndrome criteria in women from Mexico, Peru, Spain and the USA (k-values of 0.86–0.93). However, in men from the same study, agreement between the IDF and ATP III metabolic syndrome was more variable (k-values of 0.43-0.69). In both men and women, the IDF criteria reported a higher prevalence of the metabolic syndrome compared to the ATP III criteria.

Further, in light of the ethnic differences in lipid profile and body fat distribution, as well as differences in metabolic syndrome criteria, ethnic variation in the prevalence of the metabolic syndrome is not unexpected. For example, Meigs et al. (300) reported a variation of up to 24% between the prevalence of the metabolic syndrome using the WHO and ATP III criteria in the San Antonio and Framingham offspring cohorts, due to differences in gender and ethnicity. Many other studies have also demonstrated differences in the prevalence of the metabolic syndrome due to ethnic and inter-criteria variation. In fact, there are over 4000 studies investigating the prevalence of the metabolic syndrome in different populations. Some of these more recent studies that are comprised of women of African origin, that utilized the ATP III or IDF metabolic syndrome criteria and therefore considered pertinent to this review, have been summarized in Table 1.3.

Table 1.3. Prevalence of the metabolic syndrome by ATP III and IDF criteria in women of different ethnic origin

	Study Population	N	Subjects	ATP III (%)	IDF (%)
Hong et al. 2007 (301)	ARIC	6149	Middle-aged white American	29	
	ARIC	2161	Middle-aged African American	40	
Ford et al. 2005 (302)	NHANES 1999 -2002	122	African American 20-39 years old	22.0	23.7
		112	African American 40-59 years old	37.4	39.3
		94	African American ≥60 years old	57.4	60.6
		241	White American 20-39 years old	16.5	17.6
		281	White American 40-59 years old	33.1	37.1
		370	White American ≥60 years old	56.4	60.9
Tillin et al. 2005 (286)	London residents	563	White 40-69 years old	14.4	
	London residents	291	South Asian 40-69 years old	31.8	
	London residents	345	African-Caribbean 40-69 years old	23.4	
McNeill et al. 2005 (168)	ARIC	1764	African American	27.5	
		5132	White American	22.5	
Park et al. 2003 (167)	NHANES 1988-1994	1811	African American 40-43 years old	20.9	
		2955	White American 46-49 years old	22.9	
		1666	Mexican American 38-40 years old	27.2	

Table 1.3 continued.

	Study Population	N	Subjects	ATP III (%)	IDF (%)
Patt et al. 2003 (303)	Project Joy cohort	62	African American BMI $\leq$24.9 kg/m^2	2	
		113	African American BMI 25-29.9 kg/m^2	9	
		128	African American BMI 30-34.9 kg/m^2	24	
		93	African American BMI $\geq$35 kg/m^2	29	

ATP III, National Cholesterol Education Program (Adult treatment panel III); IDF, International Diabetes Federation; ARIC, Atherosclerosis risk in communities; NHANES, National Health and Nutrition Examination Survey; BMI, body mass index.

Despite the large body of literature, there are significantly less data on the prevalence of the metabolic syndrome in Sub-Saharan Africans. These studies are summarized below in Table 1.4. In the available studies, the reported occurrence of the metabolic syndrome ranges widely from 0% in rural women from Cameroon to 60% in middle-aged black South African CHD patients. In addition to the use of different definitions of the metabolic syndrome, the subject populations of these studies are very diverse with regard to age, health status and degree of urbanization which complicates comparison. In black South Africans with CHD, Ntyintyane et al. (160;161) reported a similar prevalence of the metabolic syndrome by ATP III (60%) and IDF (58%) criteria. However, this study was limited by its sample size (N=40) and included few female subjects (N=7). Conversely, Fezeu et al. (304) reported that the prevalence of the metabolic syndrome varied by definition in Africans from Cameroon (N=1353), which they attributed in part to the differing cut-points for waist circumference and emphasis placed on central obesity.

Interestingly, Schutte et al. (305) reported a higher proportion of white compared to black South African women with the metabolic syndrome. This is unexpected as black South African women have a higher prevalence of obesity and central obesity than white South African women (4). This could be due to the relatively small sample size in Schutte et al.'s study (N=102 and 115 black women and white women respectively) which was not

randomly sampled and may therefore not be representative of the South African population. Alternatively, this ethnic difference in the prevalence of the metabolic syndrome may be because the metabolic syndrome under-predicts CVD risk in black South African women in a similar manner to that described in African Americans (306). As discussed previously in section 1.4.3.1, the inclusion of the current lipid cut-points in the metabolic syndrome criteria may be inappropriate in black African women and result in under-estimated of CVD risk. Further research is needed in black South African women to determine the influence of central obesity on the metabolic syndrome and CVD risk. Additionally, to my knowledge, no studies have investigated the agreement between the IDF and the ATP III metabolic syndrome criteria, which place difference emphasis on central obesity or the impact of body fat distribution in black South Africans. Further, the agreement between the IDF and ATP III metabolic syndrome criteria and insulin resistance has not been previously investigated in a black South African population without known disease. These questions are explored in Chapters Three and Four of this thesis.

Finally, no longitudinal data on the metabolic syndrome criteria in black Sub-Saharan Africans is available. Further research in this area is needed to determine if insulin resistance is central to the development of the metabolic syndrome in individuals of African origin.

Table 1.4. Prevalence of the metabolic syndrome by ATP III and IDF criteria in Sub-Saharan African populations

Study	N	Population	Metabolic Syndrome Definition	Metabolic syndrome (%)
Ker et al. 2007 (307)	93	Black South African executive employees	ATP III	30.0
Fezeu et al. 2007 (304)	374	Middle-aged rural women from Cameroon	WHO	1.8
	513	Middle-aged urban women from Cameroon	WHO	5.9
	374	Middle-aged rural women from Cameroon	ATP III	0.0
	513	Middle-aged urban women from Cameroon	ATP III	0.2
	374	Middle-aged rural women from Cameroon	IDF	0.3
	513	Middle-aged urban women from Cameroon	IDF	1.5
Schutte et al. 2007 (305)	102	Black South African women	IDF	24.8
	115	White South African women	IDF	30.4
Naran et al. 2007 (162)	426	Urban black South Africans without T2D	ATP III	14.8
Ntintyane et al. 2007 (161)	40	Middle-aged black South Africans with CHD, without T2D	IDF	57.5
Ntyintyane et al. 2006 (160)	40	Middle-aged black South Africans with CHD, without T2D	ATP III	60.0
Isezuo et al. 2005 (308)	254	Middle-aged Nigerian men and women with T2D	WHO	59.1

ATP III, National Cholesterol Education Program (Adult treatment panel III); IDF, International Diabetes Federation; WHO, World Health Organization; T2D, type 2 diabetes; CHD, coronary heart disease.

1.4.6 Metabolic syndrome criteria as an indicator of cardiovascular disease (CVD) risk, insulin resistance and type 2 diabetes.

Despite their differences, both ATP III and IDF metabolic syndrome criteria have been shown to predict future risk of CVD in European populations (165;309). In other ethnic groups the metabolic syndrome criteria was also associated with increased risk of developing CVD. For example, in Chinese men and women the IDF and ATP III metabolic syndrome criteria were both associated with CVD (IDF odds ratio: 1.6, 95% CI 1.3 to 2.1 vs. ATP III criteria odds ratio: 1.1, 95% CI 0.7 to 1.8) (310). Further, in African Americans (N=244) and white Americans (N=304) the prevelence of coronary artery disease was higher in subjects with the metabolic syndrome than in those without the metabolic syndrome. In the same study, the association between coronary artery disease and the metabolic syndrome appeared to be more significant in the white (P=0.017) compared to the black subjects (P=0.046). Unfortunately correlation coefficients were not provided in this study and it is therefore not possible to determine the strength of this possible ethnic difference. Despite the association between the metabolic syndrome and CVD in African women, research is still needed to determine if the current ATP III and IDF metabolic syndrome criteria under-estimate CVD risk in this population.

Research on the ability of the ATP III and IDF metabolic syndrome criteria to identify insulin resistance and type 2 diabetes is less clear. Longitudinal studies in multi-ethnic populations report that metabolic syndrome criteria are good predictors of the onset of type 2 diabetes (311;312), while cross-sectional studies in healthy white populations show that metabolic syndrome criteria have a low sensitivity for identifying insulin resistance (313;314). For example, Cheal et al. (313) reported that the sensitivity of the ATP III metabolic syndrome criteria to identify insulin resistance, as measured by steady state plasma glucose during an insulin suppression test, was poor in apparently healthy white subjects (46%). Liao et al. (314) reported similar findings in white, non-diabetic individuals, where the sensitivity of ATP III criteria to identify insulin resistance, as measured by euglycemic-hyperinsulinemic clamp, was as low as 20%.

However, in a longitudinal study in Mexican and white Americans, the IDF metabolic syndrome criteria were as good a predictor of type 2 diabetes as the OGTT-derived two-hour glucose value (sensitivity 70.3% vs. 70.8%, respectively) (312). Similar results were reported in another longitudinal study comprising African, Hispanic and white Americans, where the ATP III and IDF metabolic syndrome criteria significantly predicted the five-year incidence of type 2 diabetes in individuals who were non-diabetic at the study baseline (311). The difference in findings could relate to the outcome measure (insulin resistance vs. type 2 diabetes), the method used to quantify insulin resistance (HOMA-IR vs. clamp studies), the study design (cross-sectional vs. longitudinal) or ethnicity (primarily white vs. multi-ethnic populations).

1.4.7 The clinical significance of the metabolic syndrome

The contrasting findings described in section 1.4.6 highlight the current debate regarding the clinical significance of the metabolic syndrome. On the one hand, studies have shown that the metabolic syndrome is a good predictor of CVD and type 2 diabetes, and that regarding related metabolic features as a syndrome, rather than separately, results in better assessment of patient risk (155;315). However, other research suggests that the metabolic syndrome criteria are no better than other tools (such as the Framingham 10 year risk index) used to predict CVD (316), and that the association between CVD and the metabolic syndrome is explained entirely by the presence of its individual components (317;318). Additionally, all of the current metabolic syndrome criteria assume that their individual components are equally reflective of CVD and not additive. It has also been suggested that the current cut-points are more arbitrary than scientifically based (319).

The clustering of the individual components of metabolic syndrome has also been questioned since different ethnic groups present with different clustering of metabolic risk factors. For example, in a large ethnically diverse cohort, Kraja et al. (320) found that African Americans were 50% more likely to present with central obesity, as defined by the ATP III metabolic syndrome criteria compared to Japanese individuals. However, African Americans were half as likely to present with elevated triglyceride levels compared to

white Americans. In African Americans, the primary features contributing to a diagnosis of metabolic syndrome using the ATP III criteria, were central obesity, low HDL-C and elevated BP, while triglyceride levels were less important (320). Lin et al. (321) also reported racial differences in the prevalence of components of the metabolic syndrome among middle-aged and elderly diabetics and non-diabetics in white, African American and Hispanic populations. They suggested that equal weighting of each component of the metabolic syndrome is problematic, and highlighted the need for further research exploring the presentation of the features of metabolic syndrome in different ethnic groups.

1.4.8 Summary

The diagnostic components that make up the IDF and ATP III metabolic syndrome criteria are established risk factors for CVD, based on prospective studies. The prevalence of the metabolic syndrome and the agreement between the IDF and ATP III criteria varies by population. The ability of the metabolic syndrome to predict CVD in multi-ethnic populations is fairly clear; however, there is controversy in the literature surrounding its ability to predict type 2 diabetes and insulin resistance. Additionally, the diagnostic value and clustering of CVD risk factors may not be the same in all populations. This is particularly true in black African individuals who present with increased CVD, but have a lower prevalence of the metabolic syndrome, compared to their white counterparts. Differences in blood lipid profile and body fat distribution may explain this finding. Further research is needed to explore the relevance of the metabolic syndrome and its diagnostic criteria in the black South African population. Further, the agreement between the IDF and ATP III criteria in black South African women has not been determined. These questions are investigated in Chapter Three of this thesis.

1.5 ALTERNATIVE METABOLIC PHENOTYPES

As obesity is a complex disorder resulting from both environmental (225) and genetic factors (322), obese individuals with varying metabolic phenotypes have been described. Obese individuals that are resistant to the negative health consequences associated with excess adiposity have been termed "metabolically healthy obese" (MHO) (271;323). Conversely, obesity is not a pre-requisite for CVD, and normal weight individuals prone to CVD have also been described and termed "metabolically obese normal weight" (MONW) (324). The characterization, prevalence and known determinants of the MHO and MONW phenotypes are described in brief below.

1.5.1 "Metabolically healthy obese" (MHO)

The criteria used to define this obese sub-group are not universally consistent (171;172;267;323;325), but MHO individuals are typically characterized according to cut-points for insulin sensitivity (<75th percentile of HOMA-IR and <1.95 HOMA-IR) and lipid levels (triglycerides ≤1.7 mmol/L, TC ≤5.2 mmol/L, HDL-C l ≥1.3 mmol/L and LDL-C ≤2.6 mmol/L) (326). More recently, the criteria have included cut-points for inflammatory markers (fibrinogen <4.00 g/L and white blood cell count <10,000 cells /dL) (325). Using a combination of these criteria, North American and European studies suggest that up to 37% of obese individuals can be classified as MHO (323;325;327).

MHO individuals appear to be resistant to the normal complications associated with obesity (237;271;323). Moreover, Meigs et al. (237) recently reported that in the absence of metabolic abnormalities, obesity was a relatively weak risk factor for incident type 2 diabetes in a longitudinal, population based study. However, despite clinical recognition of MHO individuals, the determinants of their protective metabolic phenotype are not well understood. In a study by Brochu et al. (327) including 43 sedentary obese postmenopausal white women, MHO women had 49% less VAT compared to their at-risk counterparts, a finding later corroborated by others (271). An earlier onset of obesity (327), and more favourable inflammation profile (271) have also been suggested as

determinants of the MHO phenotype. Yet, in a large study (N=681) of obese Italian men and women, onset of obesity and inflammation profile, as well as family history of obesity were not different between MHO and at-risk obese individuals (325). Interestingly, weight loss (3% of initial body weight) improved the lipid and inflammatory profiles of Japanese at risk obese women, but not their MHO counterparts (328).

In South Africa, in a sample of 50 urban and 40 rural obese black women, 78% of the former and 87% of the latter were considered **"**healthy**"** based on the prevalence of less than two adverse metabolic sequelae (BP ≥160/95 mmHg, TC ≥5.2 mmol/L, triglyceride level ≥1.8 mmol/L, glucose level ≥7.8 mmol/L) (171;172). These obese women's apparent **"**health**"** was attributed to their low socio-economic status, low fat intake (171) and in rural women, high levels of physical activity associated with manual labour (172). In retrospect, this high level of "health" was likely related to the criteria used to characterize MHO, which were more indicative of actual disease than disease risk.

High levels of PAEE may also be a determinant of the MHO phenotype. Studies on Japanese Sumo wrestlers reported that while maintaining high levels of physical activity, obese Sumo wrestlers had normal levels of VAT, despite consuming 5000-7000 kcal per day. Upon retirement and a subsequent decrease in PAEE, these same wrestlers developed CVD risk factors, including insulin resistance. Unfortunately, VAT was not measured post retirement (329). In contrast, Brochu et al. (327) reported no differences in PAEE between MHO and at-risk obese women measured by doubly-labelled water and indirect calorimetry.

In South Africa, low VAT and a preferential distribution of fat in the periphery in black compared to white women (130;205) might be an additional explanation for the 'healthy obese' phenotype in black women described previously (171;172). Indeed, centralisation of body fat is an integral component of the metabolic syndrome (38) and is a major determinant of the obese 'at-risk' metabolic phenotype (271;323;327). Conversely, peripheral adiposity, with larger hip and thigh circumferences, is associated with improved glucose tolerance and reduced incidence of type 2 diabetes (197). Further, Goodpaster et

al. (183) reported that increased peripheral fat mass in black and white obese individuals was associated with a lower prevalence of the metabolic syndrome. As black South African women have lower levels of VAT, but higher levels of insulin resistance compared to white South African women, the factors that contribute to the MHO phenotype may be different between ethnic groups. Since South Africa is currently undergoing an epidemiological transition, it is also possible that socio-economic factors could be a determinant of the MHO phenotype. However, the determinants of the MHO phenotype have not been previously investigated in South Africa and further research is needed for this purpose. Determinants of the MHO phenotype in black South African are explored in Chapter Four of this thesis.

1.5.2 "Metabolically obese normal weight" (MONW)

The MONW phenotype, which was first described by Ruderman in the 1980's (324), falls outside of traditional metabolic syndrome criteria. For example, MONW are often young and are a normal weight (based on BMI), yet display risk factors for CVD such as insulin resistance and dyslipidemia. In a recent longitudinal population-based study, 8% of subjects were MONW, and the phenotype was associated with a three to four-fold adjusted relative risk for type 2 diabetes and CVD (237). Further, in Spanish (N=1,311) and Mexican (N=1,918) subjects, the metabolic syndrome was present in normal weight as well as obese subjects (330). However, due to their normal BMI, MONW individuals are often undetected by traditional health screening techniques.

MONW are generally characterized by their level of insulin resistance (268;270) or level of VAT (331), although cut-points for identification vary, making comparison between studies difficult. In small studies comprising white populations, MONW accounted for 13%-18% of the normal weight population (268;326). When metabolic syndrome criteria (ATP III) were applied to a large cross-sectional study (N=3747), 11% of normal weight African American and Hispanic American women and 6% of white American women were classified as MONW (332).

In South Africa in the 1980's, 18% of 50 urbanized normal weight black women were characterized as "at-risk" as they presented with two or more adverse metabolic sequelae (BP ≥160/95 mmHg, TC ≥5.2 mmol/L, triglyceride level ≥1.8 mmol/L, glucose level ≥7.8 mmol/L) (171). However, use of these criteria, which are more indicative of disease, rather than disease risk, could result in under-estimation of the number of black South African MONW.

High levels of adiposity and VAT, despite a low BMI, have consistently been shown to be determinants of the MONW phenotype (268;270;331;333-335). In fact, increased VAT is more associated with increased CVD risk in normal weight compared to obese subjects (183). High triglyceride levels, perhaps due to increased VAT, have also been reported in MONW (331;336), although not confirmed in all studies (270). In Japanese MONW men, oxidative stress was higher ($P<0.01$) and adiponectin levels were lower ($P<0.01$) compared to the levels found in healthy normal weight Japanese men (337). Although not consistent, it has been suggested that PAEE is lower in MONW compared to their healthy counterparts (268;326). For example, a study on Venezuelan adolescents (N=167) reported that low levels of PAEE and an energy rich diet, high in saturated fat, resulted in dyslipidemia and increased insulin levels in young adults, even when BMI was less than 21 kg/m^2 (338). Dvorak et al. (268) found no differences in cardiorespiratory fitness, total daily energy expenditure or resting metabolic rate, but found that MONW women had lower levels of PAEE (-412 kcal), measured by doubly labeled water, compared to their healthy counterparts. Further research is needed to explore the impact of PAEE on the MONW phenotype.

The determinants of the MONW phenotype have not been previously investigated in the black South African population. However, as with the MHO phenotype, ethnic differences in body fat distribution and insulin resistance, as well as socio-economic and socio-cultural differences, may influence the determinants of the MONW phenotype. Further research is needed to identify determinants of this phenotype in black South Africans, particularly as research suggests that several of these determinants may be modifiable. This is explored in Chapter Four of this thesis.

1.5.3 Summary

MHO and MONW individuals have previously been described in the black South African population. In a longitudinal study, both the MHO and MONW phenotypes impacted risk (in opposite directions) of developing CVD and type 2 diabetes over time (237). It is currently not clear what the determinants of these alternative phenotypes are in black African women. It is therefore important to characterize MONW and MHO individuals in South Africa, particularly as black South African women have relatively low levels of VAT compared to white South Africans, which has been shown to be the most consistent determinant of both the MONW and MHO phenotypes. Further research is needed to identify the determinants of these phenotypes, particularly the modifiable factors that can be targeted in future interventions. These questions are explored in Chapter Four of this thesis.

1.6 BODY FAT DISTRIBUTION AND GLUCOCORTICOID (GC) METABOLISM

Altered glucocorticoid (GC) metabolism may play a key role in the pathogenesis of central obesity, insulin resistance and the metabolic syndrome (339). This may be of particular relevance in black South African women, who have a higher prevalence of obesity and central adiposity (based on a waist circumference of >88cm) (4), and a higher incidence of insulin resistance than white South African women (174). A schematic diagram of systemic and tissue-specific GC metabolism is shown in Figure 1.6 and is explained in brief below.

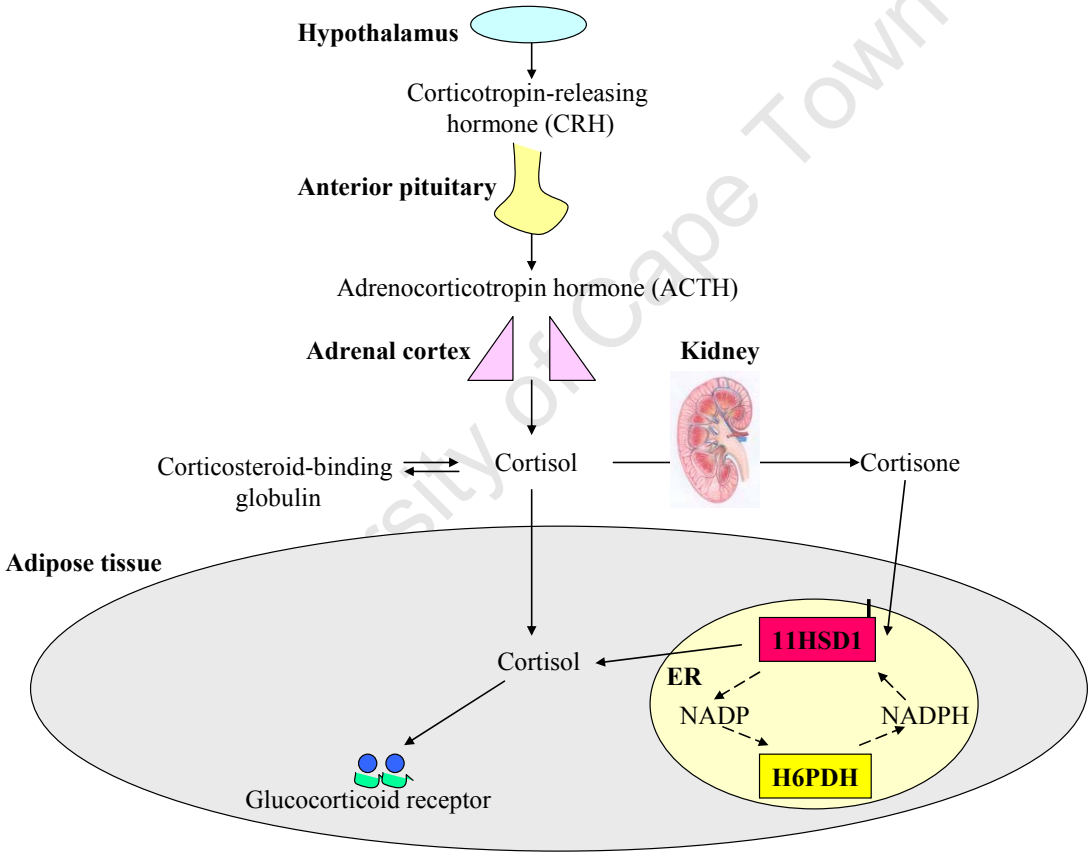

Figure 1.6. Schematic diagram of systemic and tissue-specific GC metabolism (340;341). The facilitation of 11β-hydroxysteroid dehydrogenase's conversion of cortisone to cortisol by NADPH, produced by H6PDH, is shown by dashed arrows. ER, endoplasmic reticulum; 11HSD1, 11β-hydroxysteroid dehydrogenase type 1; H6PDH, hexose-6-phosphate dehydrogenase.

GCs are steroid hormones that influence a wide variety of physiological functions including metabolism, stress and immune responses (342). Cortisol binds to nuclear GC receptors (GR) in adipocytes and other cell types and affects the transcription of target genes (343). Cortisol is only active when it is not bound to corticosteroid binding globulin, a protein that may mediate the access of cortisol to target tissues (344). Circulating cortisol levels are regulated by the hypothalamic-pituitary-adrenal axis, a major part of the neuroendocrine system. In response to stress, corticotropin-releasing hormone (CRH) is secreted by the hypothalamus, which stimulates the secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland. ACTH stimulates the synthesis of cortisol from cholesterol in the adrenal cortex, and cortisol inhibits CRH and ACTH production. This negative feedback loop allows for homeostatic adjustment of circulating cortisol levels (342).

Systemic cortisol levels influence a multitude of metabolic processes and play a major role in glucose homeostasis. In the liver, cortisol enhances the expression of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase and fructose-2,6-bis-phosphatase, resulting in increased hepatic glucose output (344). In adipose tissue, cortisol inhibits the uptake of blood glucose and has a permissive effect on catecholamines, resulting in increased lipolysis and circulating FFA levels. Cortisol also promotes adipocyte differentiation, resulting in increased fat mass (345). In muscle, cortisol decreases the uptake of blood glucose (342) and increases peripheral insulin resistance.

Sustained cortisol excess is associated with features of the metabolic syndrome. For example, clinical administration of GCs to treat inflammatory disease has been associated with obesity, insulin resistance and dyslipidemia (346). Further, the presence of the metabolic syndrome in patients with Cushing's syndrome, a rare condition characterized by excess circulating cortisol (347), suggests that GC metabolism may play a key role in the pathogenesis of central obesity, insulin resistance and the metabolic syndrome. The metabolic effects of cortisol on adipose tissue, the liver, muscle, the pancreas and the endothelium are described in more detail in Figure 1.7. Additional information on the

metabolic effects of GCs including impact on renal disease and inflammation can be found elsewhere in the literature (342;344).

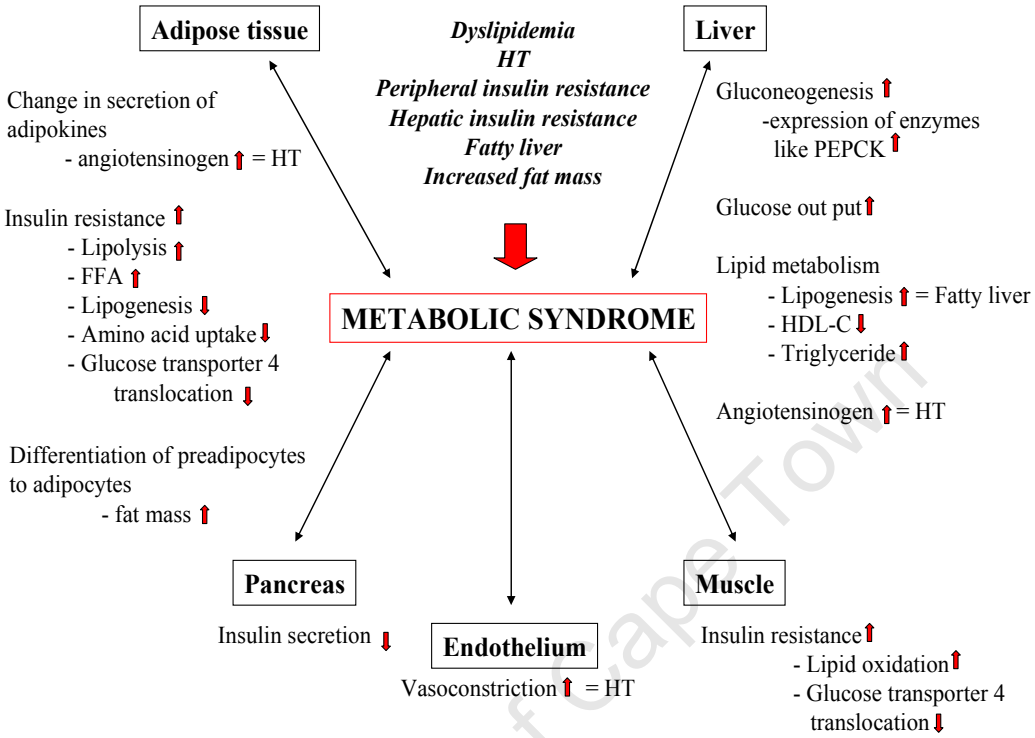

Figure 1.7. A schematic representation of the metabolic effects of GCs on adipose tissue, the pancreas, the liver, muscle and the endothelium as they relate to the components of the metabolic syndrome, as defined by IDF and ATP III criteria, and hence increased CVD, shown in italics. HT, hypertension; PEPCK, phosphoenolpyruvate carboxykinase; HDL-C, high density lipoprotein cholesterol. This figure was adapted from the work of Aron et al. (342) and Wang et al. (344).

Although, circulating cortisol concentrations in obese individuals are normal or even reduced (348), GC action is not only determined by systemic levels of GC. Tissue-specific pre-receptor regulatory mechanisms play a crucial role in determining access of cortisol to GR (349). The pre-receptor regulator of GC metabolism, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1), is an important enzyme in adipose tissue, the liver, and skeletal muscle and it catalyses the inter-conversion of inert 11-keto derivatives (cortisone) to active 11-hydroxycorticosteroids (cortisol) (350). Conversely, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2), which is predominantly expressed in the kidney, works in the opposite direction converting cortisol to cortisone in order to

prevent renal GC excess and activation of mineralocorticoid receptors by GCs. Since the focus of this thesis is on GC action in adipose tissue, further discussion of 11β-HSD-2 is outside of the scope of this review.

In vivo, 11β-HSD-1 converts cortisone to cortisol in the presence of the co-enzyme NADPH, which is produced in the adipocyte by hexose-6-phosphate dehydrogenase (H6PDH) (340). GR activation is amplified by 11β-HSD-1 when the enzyme works in the reductase direction (cortisone to cortisol). When cortisol binds to GR, GR regulates the transcription of GC responsive genes such as leptin, lipoprotein lipase, tumour necrosis factor-α and PEPCK. A summary of the regulation of GC-responsive genes is reviewed by Wang (344). Increased 11β-HSD-1, H6PDH and GR within adipose tissue may expose the tissue to increased concentrations of cortisol without any increase in circulating cortisol concentrations or cortisol secretion (351), thus creating a tissue-specific "Cushing's syndrome" (339) (Figure 1.8).

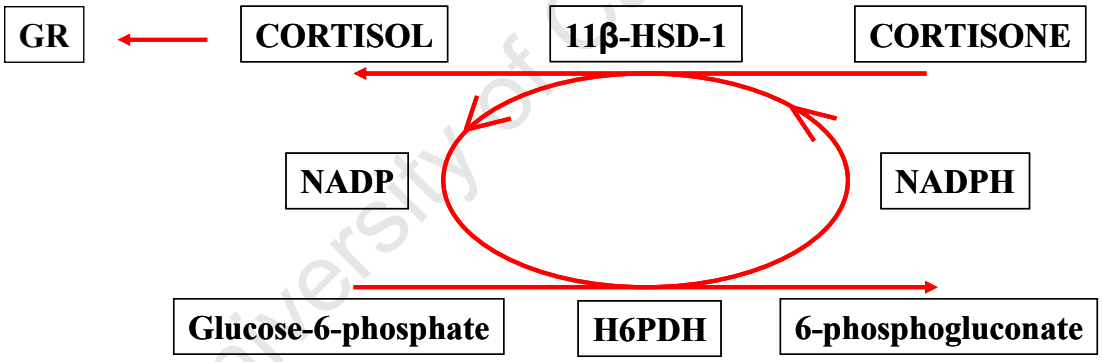

Figure 1.8. A schematic diagram of the relationship between 11β-HSD-1 and H6PDH in the lumen of endoplasmic reticulum in an adipocyte. Glucose-6-phosphate enters the lumen via the glucose-6-phosphate transporter and is either metabolized by glucose-6-phosphatase to glucose and inorganic phosphate, or used by H6PDH to produce glucose-6-phosphogluconate. This reaction converts NADP to NADPH, which is used by 11β-HSD-1 to convert cortisone into cortisol. Increased concentrations of cortisol in adipose tissue results in increased fat mass and central obesity (352;353). 11β-HSD-1, 11β hydroxysteroid dehydrogenase type 1; H6PDH, hexose-6-phosphate dehydrogenase; GR, glucocorticoid receptor.

1.6.1 The influence of tissue-specific glucocorticoid action on obesity, body fat distribution and cardiovascular disease risk

Compelling evidence for the importance of tissue-specific GC action in the development of obesity and features of the metabolic syndrome has been provided by murine models. Masuzaki et al. (354;355) found that transgenic mice over-expressing 11β-HSD-1 in fat tissue were 16% heavier and more viscerally obese, insulin resistant and glucose intolerant than wild type mice. Conversely, 11β-HSD-1 knockout mice had decreased intracellular GC concentrations and were protected from obesity, type 2 diabetes and dyslipidemia, even when fed a high fat diet (356;357). Moreover, whole body selective 11β-HSD-1 inhibitors have been shown to decrease obesity and triglyceride levels, as well as improve insulin sensitivity and glucose tolerance in mice (358;359).

The studies in humans have reported less consistent results, but have shown that 11β-HSD-1 mRNA and activity in VAT and SAT were associated with obesity (61;349;360-362), enlarged waist circumference, VAT accumulation (60;362;363) and features of the metabolic syndrome including increased BP, triglyceride and fasting blood glucose levels (60;360-362;364). Since VAT has increased 11β-HSD-1 mRNA (193;363) and activity (60;363) compared to SAT, it has been suggested that intracellular regeneration of cortisol in VAT may result in "Cushing's disease of the omentum" (363). In support of this hypothesis, the correlation between omental adipocyte cell size and 11β-HSD-1 expression in VAT suggests that local regeneration of cortisol might play a role in hypertrophy of the omentum (362). Commensurate with the findings in 11β-HSD-1, GR expression is higher in VAT than in SAT (365). In contrast to 11β-HSD-1, GR mRNA levels in SAT and VAT have been shown to be negatively associated with body fatness and VAT (60), suggesting a down-regulation of GR with obesity. However, Boullu-Cioca et al. (365) found that GR-alpha, the active isoform of the receptor, was not down-regulated in VAT with obesity, enabling VAT to retain full capacity to respond to increased local concentrations of cortisol. In a recent study, there was a trend for higher H6PDH expression in VAT compared to SAT, but no associations with obesity or the metabolic syndrome were reported (60). Preliminary results from our laboratory have demonstrated ethnic differences in GC metabolism in SAT. Although there were no ethnic

differences in 11β-HSD-1 mRNA levels or activity, black women had lower GR mRNA, but higher H6PDH mRNA levels than white women (Goedecke et al., personal communication). Since tissue-specific GC action in VAT and SAT has been linked to obesity, VAT accumulation and CVD risk factors, eighteen pharmaceutical companies to date have filed patents for 11β-HSD-1 inhibitors. Further, extensive research investigating tissue-specific inhibitors is underway (341).

Studies have also begun to investigate the association of polymorphisms within genes involved in local GC action with obesity, body fat distribution and risk factors for CVD. Although no studies have been undertaken in South Africa, polymorphisms within these genes may alter tissue-specific GC action. This concept is described below.

1.6.2 Genetic polymorphisms associated with glucocorticoid metabolism

Polymorphisms within genes that encode for proteins involved in systemic and tissue-specific GC metabolism have been associated with altered GC action, obesity, body fat distribution and CVD. For the purposes of this thesis, only polymorphisms within genes involved in tissue-specific GC metabolism are described. In particular, polymorphisms within the *HSD11B1, H6PD* and *GR* (also known as *NR3C1*) genes which encode for 11β-HSD-1, H6PDH and GR respectively, are reviewed. The *HSD11B1*, *H6PD* and *GR* genes have been mapped to human chromosomes, 1q32-q41, 1p36 and 5q31.1, respectively (www.ncbi.nlm.nih.gov).

1.6.2.1 Polymorphisms within the HSD11B1 gene

Polymorphisms within the *HSD11B1* gene have been associated with differences in BMI, body fat distribution, BP and insulin resistance (11;366-368). Studies have focused primarily on Pima Indians (11;368) and white populations (366), in which two microsatellites and/or three single nucleotide polymorphisms within the *HSD11B1* gene have been explored. These studies are summarized below.

In a sample of Scottish men and women from the MONICA study, and Danish men with juvenile onset obesity from the ADIGEN study, Draper et al. (366) found no association between two polymorphic dinucleotide $(CA)_n$ repeats within intron 4 (CA_{19} repeat 2.7 kb 3' of exon 4 and CA_{15} repeat 3 kb 5' of exon 5) and BMI or waist/hip ratio. To my knowledge, there are no other studies investing these microsatellites with regard to obesity or body fat distribution. Due to the lack of evidence and for technical reasons, these microsatellite polymorphisms were not investigated in this thesis.

Most research has focused on single nucleotide polymorphisms within the *HSD11B1* gene. For example, the A allele of the G>A substitution at nucleotide position 3334051 within intron 3 (rs846910) was associated with hypertension (11), type 2 diabetes and fasting insulin level, but not obesity, in Pima Indians (368). The T allele of the G4478T polymorphism (rs12086634) within intron 3 of the *HSD11B1* was associated with enhanced cortisol clearance in a study comprising normal weight and obese white polycystic ovary syndrome (PCOS) patients (N=102) and controls (N=98) (369). In Pima Indians (N=800), the G4478T polymorphism was associated with an increased risk of developing type 2 diabetes (odds ratio 1.79, 95% CI: 1.14-2.8) (368). Conversely, Robitaille et al. (370) did not find any association between the G4478T polymorphism and obesity, waist circumference or CVD risk factors in either middle-aged French Canadian men with the metabolic syndrome or control subjects. To my knowledge, these polymorphisms have not been investigated in a black African population with regard to obesity, body fat distribution or CVD. These polymorphisms were not investigated in this thesis for technical reasons and because the G4478T polymorphism is in 100% linkage disequilibrium with the Ins4436A polymorphism within the *HSD11B1* gene (368), which is investigated in Chapter Five of this thesis.

The insertion of adenine at nucleotide position 4436 within intron 3 (Ins4436A polymorphism, rs17850941) of the *HSD11B1* gene has been investigated in populations of several different ethnic origins. The results of these studies are summarized in Table 1.5. To my knowledge, the Ins4436A is the only *HSD11B1* polymorphism that has been investigated in relation to obesity, centralisation of body fat and CVD in individuals of African origin (367). In apparently healthy overweight and normal weight African

American and white American children (approximately 10 years old), Gelernter-Yaniv et al. (367) found that the 11 individuals homozygous for the A allele of the Ins4436A polymorphism (A/A) within the *HSD11B1* gene had increased BMI, waist/hip ratio and insulin resistance, compared to subjects with the wild type (W) allele. However, this result should be interpreted with caution due to the small sample size and since the black and white subjects were not analyzed separately. Further, in Pima Indians (N=800) the Ins4436A polymorphism was in 100% linkage disequilibrium with the G4478T polymorphism within the *HSD11B1* gene, which is associated with type 2 diabetes as described above (368).

In contrast, a study of middle-aged French Canadian men (370) found no association between the A allele of the Ins4436A polymorphism within the *HSD11B1* gene and BMI, body fat distribution, BP, lipid profile or insulin sensitivity. Similarly, in a mutational screening of the whole coding sequence and exon-flanking regions of the *HSD11B1* gene in apparently healthy normal weight (N=4, aged 27-31 years old) and obese (N=8, aged 33-62 years old) Italian women, the A allele of the Ins4436A polymorphism was not associated with central obesity (371). Notably, in both of these studies the frequency of the A/A genotype was too low to be analyzed separately from the W/A subjects. This could explain the conflicting findings in the literature.

The results of the studies investigating the Ins4436A polymorphism within the *HSD11B1* gene are therefore not consistent and the data is limited, particularly in black African populations. However, due to the associations between the A allele of this polymorphism and obesity, body fat distribution and CVD risk factors reported in white and African American children and Pima Indians, this polymorphism is investigated further in Chapter Five of this thesis. Based on previous studies, it is not clear if the Ins4436A polymorphism is functional since its influence on expression and activity has not been investigated. Further, the frequency of this polymorphism ranges widely from 18% in white and African American children to 54% in Pima Indians. The frequency has not been reported in Sub-Saharan Africans and is also investigated in Chapter Five of this thesis.

Table 1.5. **Summary of studies investigating the Ins4436A polymorphism (rs17850941) within the *HSD11B1* gene in relation to obesity, central obesity and CVD.**

Study	N	Population	Ins4436A allele frequency (%)	Finding
Caramelli et al. 2001 (371)	12	Normal weight and obese Italian women	A=21 W=79	No associations between the Ins4436A polymorphism and BMI in the normal weight or obese women. There were no individuals homozygous for the A allele (A/A).
Gelernter-Yaniv et al. 2003 (367)	263	Healthy normal weight and overweight white and African American children	A=18 W=82	Subjects with the A/A genotype had a greater body mass (P<0.005), waist/hip ratio (P<0.05) and were more insulin resistant (HOMA-IR) (P<0.05) than subjects with the W allele.
Nair et al. 2004 (368)	800	Pima Indians	A=54 W=46	The Ins4436A polymorphism was in 100% linkage disequilibrium with the G/T 4478 polymorphism in *HSD11B1* (rs12086634). The G allele of the G4478T polymorphism was associated with an increased odds of developingT2D (odds ratio: 1.79, 95% CI: 1.14-2.8, P=0.01) and a lower glucose disposal rate (euglycemic-hyperinsulinemic clamp) (P=0.04) but not with BMI.
Robitaille et al. 2004 (370)	217 36	French Canadian male control subjects French Canadian men with metabolic syndrome	A=36 W=64	No genotype associations with BMI, waist circumference, VAT, HDL-C, TG, BP, fasting glucose, fasting insulin or HOMA-IR.

A, adenine insertion; W, wild type; T2D, type 2 diabetes; BMI, body mass index; VAT, visceral adipose tissue; HDL-C, high density lipoprotein lipase cholesterol; TG, triglycerides; BP, blood pressure; HOMA-IR, homeostasis model of insulin resistance.

1.6.2.2 Polymorphisms within the H6PD gene

Genetic studies investigating the association between GC metabolism within adipose tissue and the *H6PD* gene have focused primarily on R453Q (rs6688832; G>A), a nonsynonymous single nucleotide polymorphism, within exon 5 of the gene. This polymorphism is usually investigated in combination with polymorphisms within the *HSD11B1* gene, and studies have focused on its association with cortisone reductase deficiency (CRD) and PCOS (372-376). CRD is a condition characterized by the inability of 11β-HSD-1 to convert cortisone to cortisol (372). As PCOS and CRD patients present with a similar phenotype, it has been hypothesized that 11β-HSD-1 activity may be reduced in PCOS patients (373). Since H6PDH drives 11β-HSD-1's conversion of cortisone to cortisol via the production of coenzyme NADPH, polymorphisms in the *H6PD* gene might affect 11β-HSD-1's oxo-reductase activity. The impact of impaired H6PDH enzyme activity on 11β-HSD-1's oxo-reductase activity is shown below in figure 1.9. Studies investigating the R453Q polymorphism within the *H6PD* gene are summarized in Table 1.6 and are described in brief below.

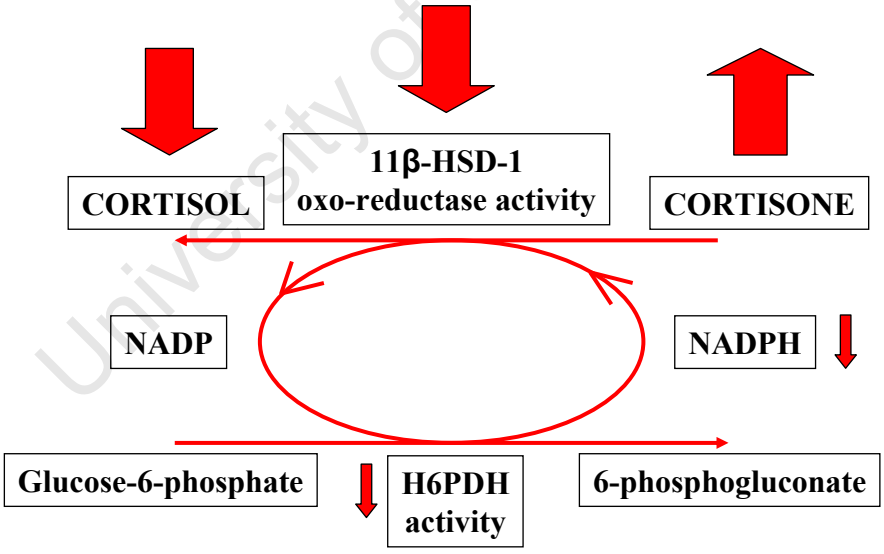

Figure 1.9. Schematic diagram of impact of impaired H6PDH enzyme activity on the production of NADPH. Decreased H6PDH enzyme activity would decrease the NADPH available to 11β-HSD-1 for the conversion of cortisone to cortisol in adipose tissue. Cortisol levels would then be decrease. 11β-HSD-1, 11β hydroxysteroid dehydrogenase type 1; H6PDH, hexose-6-phosphate dehydrogenase.

The R453Q polymorphism within the *H6PD* gene has been associated with changes in H6PDH enzyme activity (372) and differences in circulating cortisol concentrations (373). A study comprised of Indo-Asian and European CRD patients and control subjects demonstrated a 50% reduction in H6PDH enzymatic activity when the A allele of the R453Q polymorphism, which encodes for glutamine (Q) at amino acid position 453, was expressed in cultured cells (372). In another study comprised of Spanish PCOS patients and control subjects, the G allele of this polymorphism was associated with PCOS (373). Since PCOS is associated with decreased 11β-HSD-1 oxo-reducatase activity, the results of this study indirectly suggest that the G allele of the R453Q polymorphism decreases H6PDH enzyme activity. This runs counter to expectation since Draper et al. (372) associated the A allele of this polymorphism with decreased H6PDH enzyme activity. However, to my knowledge, the influence of the R453Q polymorphism on H6PDH enzyme activity has not been investigated in a healthy population.

Only one study has investigated the R453Q polymorphism within the *H6PD* gene in individuals of African origin. In African, Hispanic and white Americans from the Dallas Heart Study (N=1776, 533 and 1241 respectively), White et al. (374) reported that the frequency of the A allele was significantly higher in African Americans (47%) than in white Americans (26%) or Hispanic Americans (34%). This is particularly interesting since Draper et al. (372) associated the A allele of R453Q polymorphism within the *H6PD* gene with decreased H6PDH enzyme activity. Impaired H6PDH enzyme activity would decrease 11β-HSD1's conversion of cortisone to cortisol, lowering the concentration of cortisol in adipose tissue, which could potentially decrease VAT. Based on the findings of White et al. (374) and Draper et al. (372), this thesis hypothesized that the relatively low levels of VAT seen in black compared to white South African women, could be associated with a higher frequency of the A allele of the R453Q polymorphism within the *H6PD* gene in black women.

Despite the association between the R453Q polymorphism within the *H6PD* gene and changes in H6PDH enzyme activity and cortisol levels previously reported (372;373), there is no evidence that this polymorphism affects obesity, body fat distribution or CVD risk factors (374). For example, in both the Indo-Asian and European CRD patients and controls, as well as in the Spanish PCOS patients and controls described above, there were

no associations between obesity, body fat distribution or CVD risk factors and R453Q genotype (372;373). This was confirmed in two large studies including elderly white men from the Rotterdam and Frail old men studies (N=6152) (376) as well as in PCOS patients and control subjects from the United Kingdom (N=1018) (375). However, the data available on African individuals is limited (374) and to my knowledge, no studies have been undertaken in a Sub-Saharan African population.

Further, Draper et al. (372) reported that polymorphisms in the *HSD11B1* and *H6PD* genes interacted to cause CRD. However, the influence of the interaction between the Ins4436A polymorphism within the *HSD11B1* gene (described in section 1.5.5.1) and the *R453Q* polymorphism within the *H6PD* gene on tissue-specific GC action has not been previously investigated. Consequently; i) the influence of the R453Q polymorphism within the *H6PD* gene on obesity, centralisation of body fat and CVD risk factors, and ii) its interaction with the Ins4436A polymorphism within the *HSD11B1* gene is investigated in black South African women in Chapter Five of this thesis.

Table 1.6. Summary of studies that have investigated the R453Q polymorphism (rs6688832) within the *H6PD* gene

Study	N	Population	R453Q allele frequency (%)	Finding
Draper et al. 2003 (372)	157	Indo-Asian and Scottish CRD patients and controls.	A=21 G=79	The A allele decreased H6PDH enzyme activity A gene-gene interaction between *HSD11B1* & *H6PD* resulted in decreased 11β-HSD-1 mRNA levels and H6PDH enzyme activity *in vitro*.
White et al. 2005 (374)	1241	American white	A=26 G=74	No genotype influence on BMI, waist/hip ratio, VAT, GC sensitivity or insulin sensitivity was found.
	533	Hispanic American	A=34 G=66	African Americans had a higher frequency of the A allele compared to white and Hispanic Americans.
	1776	African Americans	A=47 G=53	
San Millan et al. 2005 (373)	116	Spanish PCOS patients	A=22 G=78	PCOS patients with the G/G genotype had increased cortisol levels compared to patients with the A allele. No differences were found in the control subjects. The G allele was associated with PCOS. No gene-gene affect on CRD
	76	Healthy Spanish controls	A=32 G=68	
Draper et al. 2006 (375)	1018	British white individuals	A=21 G=79	No genotype influence on BMI, waist/hip ratio or testosterone was reported. No gene-gene affect on CRD
Smit et al. 2007 (376)	6152	White elderly men	A=23 G=77	No genotype influence on BMI, waist/hip ratio, BP, fasting glucose, glucose tolerance or adrenal androgen production was found. No gene-gene affect on CRD

H6PD, hexose-6-phosphate dehydrogenase; 11β-HSD-1, 11β hydroxysteroid dehydrogenase type 1; BMI, body mass index; VAT, visceral adipose tissue; GC, glucocorticoid; BP, blood pressure; CRD, cortisone reductase deficiency; PCOS, polycystic ovary syndrome.

1.6.2.3 Glucocorticoid receptor (GR) polymorphisms

A significant volume of research has investigated polymorphisms within the *GR* gene in relation to obesity, body fat distribution and CVD risk factors. These studies have focused primarily on the *Bcl*I, N363S and ER22/23EK polymorphisms, which have all been associated with features of the metabolic syndrome (377-379). The mutant allele of the *GR* ER22/23EK polymorphism has been associated with down-regulation of GC action (377;380), while the mutant alleles of the N363S and *Bcl*I polymorphisms have been associated with up-regulation of GC action (42;381;382). This suggests that the ER22/23EK mutant allele may be "protective", while the N363S and *Bcl*I mutant alleles might be associated with increased CVD. A schematic diagram of the N-terminal part of the *GR* gene and the location of these polymorphisms is shown in Figure 1.10.

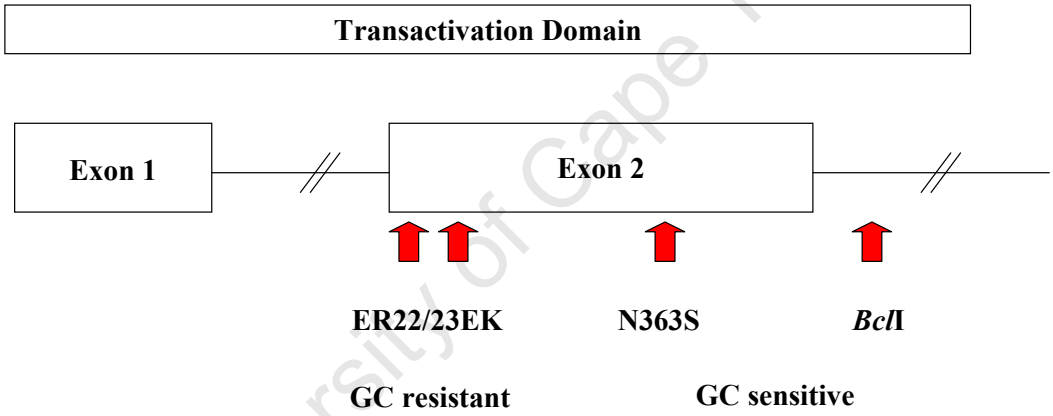

Figure 1.10. A schematic diagram of the locations of functional polymorphisms within the transactivational domain of the *GR* gene (378). GC, glucocorticoid.

The mutant allele of the functional ER22/23EK polymorphism, which consists of two linked point mutations in codons 22 and 23 of the *GR* gene, decreases GC action (383). The mutation in codon 23 from AGG to AAG results in an amino acid change from arginine (R) to lysine (K). The mutation in codon 22 is silent (384). The majority of research has shown an association between the mutant allele of this polymorphism and an improved metabolic profile (377;383;385), although not all studies reported positive findings (386). Since the focus of this review is on factors that increase the risk of developing CVD and up-regulate GC action, this polymorphism will not be discussed further, but readers are referred to the review by Van Rossum for more information (378).

The AAT to AGT codon change at position 363 in exon 2 of the *GR* gene results in an asparagine (N) to serine (S) amino acid change referred to as the N363S (rs6195) polymorphism. The G (S amino acid) allele of the N363S polymorphism within the *GR* gene has been associated with changes in transactivating capacity and sensitivity to GC (378;387) as well as increased BMI (388), waist/hip ratio (389) and CVD risk factors in some studies (390), but not others (389;391). The allele frequency of the G allele (S amino acid) ranges from 0.7% in South Asians (392) to 27% in Australians (393). However, the G allele (S amino acid) is not present in black Africans, and other ethnic groups, such as Chinese and Japanese individuals (www.ncbi.nlm.nih.gov) (394;395). This polymorphism will therefore not be investigated further in this thesis. Additional information on the N363S polymorphism can be found in the review by Marti et al. (396) and the work of Rosmond et al. (397;398).

The G allele of the G>C substitution at -646 within intron 2 of the *GR* gene, referred to as the *Bcl*I polymorphism (IVS2-646 G>C), has been associated with increased GC sensitivity (399;400), total adiposity (401;402), central obesity (391;401;403) and CVD risk factors (381;401;404), although the molecular mechanism of this intronic polymorphism is not known (378). Studies investigating this polymorphism with regard to obesity, body fat distribution and CVD are summarized in Table 1.7 and described below.

Evidence is conflicting regarding the association between the *Bcl*I polymorphism within the *GR* gene and obesity. For example, in middle-aged Swedish men (N=262) Rosmond et al. (401) found that the G allele was associated with increased BMI. This finding was not supported by studies in healthy Scottish men (N=64) (399), non-diabetic normal weight and obese British women (N=99) (381) or obese Italians (N=279). In the same study comprising obese Italians, there was also no association between the G allele of this polymorphism and CVD risk factors. The G allele of the *Bcl*I polymorphism, however, has been associated with increased fasting insulin levels and HOMA-IR in non-diabetic normal weight and obese British women (N=99) (381), as well as with increased intima and media thickness of the left common carotid artery in Greek patients with CHD (N=105) (404).

The association of the G allele of the *Bcl*I polymorphism in the *GR* gene with central obesity measured by anthropometry and CT scan is questionable. For example, Rosmond et al. (401) reported that the G allele of this polymorphism was associated with increased waist/hip ratio in middle-aged Swedish men. This was not corroborated by other studies in obese Italians (373) or elderly white men and women (379). The influence of the G allele of the *Bcl*I polymorphism within the *GR* gene on VAT (measured by CT scan) is somewhat clearer (403), although some inconsistent findings have been reported (405). In a large sample of men and women from the Quebec family study (N=742), Ukkola et al. (403) found that the G allele of the *Bcl*I polymorphism was associated with increased VAT ($P<0.001$) independently of total adiposity. In middle-aged Canadian men and women (N=152), Buemann et al. (42) reported similar findings in men and also found that in the lowest tertile of body fat percent, the G allele explained 41% and 35% of the variance in VAT in men and women respectively. Conversely, in a study comprising 12 sets of male identical twins, overfeeding was associated with increased VAT in subjects with the C/C genotype of the *Bcl*I polymorphism (405). Further, Di Blasio showed no associations between the *Bcl*I polymorphism and differences in body fat distribution in obese Italians with obesity-related disease (406). To my knowledge, the *Bcl*I polymorphism in the *GR* gene has not been investigated with regard to obesity, body fat distribution or CVD risk factors in a black African population.

As shown in Table 1.7, the frequency of the G allele of the *Bcl*I polymorphism within the *GR* gene varies greatly in white populations (25-60%) (402;405). In a methodological paper comprising a small multi-ethnic sample, the frequency of the G allele of the *Bcl*I polymorphism within the *GR* gene was 80% for Africans (N=42) and 71% for Europeans (N=38) (407).

In summary, despite inconsistent findings, a review of the literature suggests that a relationship may exist between the G allele of the *Bcl*I polymorphism within the *GR* gene and VAT accumulation and CVD in men and women. The *Bcl*I polymorphism's relationship with obesity and anthropometric measures of central obesity is less established. It should also be noted that the majority of associations between the *Bcl*I polymorphism and obesity have been reported in individuals homozygous for the G allele (381;399;403;404). Review of the literature indicates that this polymorphism has not been

previously investigated in a black African population in relation to obesity, central obesity and CVD risk factors. Therefore, since black South African women present with high levels of central obesity and insulin resistance, Chapter Five of this thesis investigates the *Bcl*I polymorphism within the *GR* gene in this population.

Table 1.7. Summary of studies investigating the *BclI* polymorphism in *GR* gene in relation to GC sensitivity, body composition and CVD

Study	N	Population	*BclI* allele frequency (%)	Finding
Weaver et al. 1992 (381)	99	Non-diabetic, obese and normal weight British women	G=57 C=43	In the obese women, the G/G genotype was associated with increased fasting insulin level (P=0.012) and HOMA-IR (P=0.012). No association was found between the G allele and obesity.
Buemann et al. 1997 (42)	152	Middle-aged Canadian men and women	G=33 C=67	The G allele was associated with increased VAT independently of total adiposity in men. In the lowest tertile of body fat percent the G allele explained 41% and 35% of the variance in VAT in men and women, respectively.
Panarelli et al. 1998 (399)	64	Healthy Scottish men	G=49 C=51	No association was found between the G allele and BMI. Increased sensitivity to the GC Budesonide (used to treat asthma and inflammatory bowel disease) was reported in G/G individuals.
Rosmond et al. 2000 (401)	262	Middle-aged Swedish men	G=47 C=64	Salivary cortisol levels, BMI (P<0.001), waist/hip ratio (P=0.015) and central obesity (P=0.002) were elevated in subjects with the G allele.
Ukkola et al. 2001 (403)	742	Quebec Family Study	G=37 C=63	Subjects with the G/G genotype had increased VAT (P<0.001) compared to subjects with the C allele, independently of total body fat. Subjects with the G/C genotype had a higher BMI (P=0.012) and more total SAT (P<0.001) than subjects with a G/G or G/C genotype.
Ukkola et al. 2001 (405)	24	12 pairs of male identical twins	G=25 C=75	Subjects with the C/C genotype experienced a greater increase in body weight (P=0.002), VAT (P=0.04), TC (P=0.007), LDL-C (P=0.003) and systolic BP (P=0.036) compared to subjects with the G/C genotype with overfeeding

Table 1.7 continued.

Study	N	Population	*BclI* allele frequency (%)	Finding
Tremblay et al. 2003 (402)	173	Men and women from the Quebec Family Study	G=60 C=40	Women that were heterozygous for the B*cl*I polymorphism (G/C) had a larger increase in SAT over the 12 years of the study, compared to women with the G/G or C/C genotype.
Van Rossum et al. 2003 (379)	2154	Elderly men and women (Rotterdam study)	G=36 C=64	Individuals with the C allele had a larger waist/hip ratio (P=0.02) than individuals with a G/G genotype.
	370	Elderly Dutch men	G=33 C=67	
Stevens et al. 2004 (400)	216	British white	G=38 C=62	The G allele of B*cl*I in conjunction with the variant alleles of two polymorphisms within intron B of the *GR* gene was associated with increased sensitivity to GCs.
Alevizaki et al. 2007 (404)	105	Greeks with CHD	G=58 C=42	Individuals with the G/G genotype had a greater intima and media thickness of the left common carotid artery compared to individuals with the G/C or C/C genotype.

HOMA-IR, Homeostasis model of insulin resistance; BMI, body mass index; VAT, visceral adipose tissue; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; BP, blood pressure; SAT, subcutaneous adipose tissue; CHD, coronary heart disease; GC, glucocorticoid.

1.6.3 Summary

Excess circulating cortisol has many negative metabolic effects, as demonstrated by the pathological condition of Cushing's disease and the adverse side effects associated with the clinical administration of GCs to treat inflammatory diseases. However, in obese individuals, cortisol levels are normal or even reduced. Therefore, research has investigated the influence of alterations in tissue-specific GC metabolism on obesity, centralisation of body fat and CVD. Research in humans has found that 11β-HSD-1 mRNA levels and activity were associated with obesity and VAT accumulation, as well as with features of the metabolic syndrome, including increased triglyceride levels, fasting insulin, BP and central obesity. Through the provision of NADPH to 11β-HSD-1, H6PDH may drive the conversion of cortisone to cortisol within adipose tissue. GR is the vehicle through which cortisol exerts its influence and GR expression is increased in VAT compared to SAT, possibly resulting in increased local cortisol concentrations and "Cushing's disease of the omentum". Further, ethnic differences in GR expression in the SAT of black and white women have been reported in our laboratory. Polymorphisms within the *HSD11B1*, *H6PD* and *GR* genes have been associated with altered GC action, centralisation of body fat, obesity and increased risk of CVD. Investigation of polymorphisms within these genes may aid in explaining why black South African women have a higher prevalence of obesity, central obesity and insulin resistance, despite decreased levels of VAT compared to white South African women.

Although there are various polymorphisms within the *HSD11B1*, *H6PD* and *GR* genes that alter tissue-specific GC action, mutant alleles of these polymorphisms that up-regulate GC action have been associated with increased obesity, central obesity and CVD. Based on previous research, three polymorphisms in particular merit further investigation in black South African women. These are i) the Ins4436A polymorphism within the *HSD11B1* gene, which was associated with obesity, centralisation of fat and insulin resistance in an African American and white American population (A allele; at-risk allele); ii) the R453Q polymorphism within the *H6PD* gene, which was associated with dramatic changes in H6PDH activity *in vitro* (both alleles have been associated with changes in enzyme activity, Figure 1.10); and iii) the *Bcl*I polymorphism within the *GR* gene, which was

associated with altered GC sensitivity, increased obesity, central obesity and CVD (G-allele; at-risk allele). The association of these polymorphisms with obesity, body fat distribution and CVD risk factors is explored in a preliminary study in Chapter Five of this thesis.

1.7 LITERATURE CONCLUSIONS

The prevalence of obesity in South African women is disproportionately high in black women. This is possibly a result of epidemiological and nutritional transition which has resulted in a decrease in PAEE, as well as socio-demographic and dietary changes. Consequently, as urbanization progresses, the obesity epidemic is expected to continue and the burden of obesity-related diseases such as type 2 diabetes, hypertension and CHD are likely to increase. In this context it is important to identify individuals at risk of obesity and CVD in order to promote lifestyle change and to prevent and manage obesity and its co-morbidities. Common risk factors for obesity-related CVD include increased adiposity, central fat distribution, elevated BP, insulin resistance, glucose intolerance and dyslipidemia. However, both the methods used to measure body fatness and the criteria used to identify individuals at-risk of CVD may not be applicable in all populations.

Quantifying body fat percent is important for research and for the prevention and management of obesity. However, criterion methods for the measurement of body fat percent such as underwater weighing and DXA are not always practical in large field studies or for health risk appraisal. Therefore, field techniques such as NIR have been used to measure body fat percent for research purposes and in clinical assessment in South Africa. However, the validity of NIR has been shown to vary with ethnicity and with the level of adiposity. Although a very practical tool, NIR has not been validated in the ethnically diverse South African population. Until NIR has been validated, it cannot be used with confidence for health risk appraisal or for research purposes.

In order to quantify CVD risk at the population level, the ATP III and IDF metabolic syndrome diagnostic criteria have been developed. These criteria vary slightly in their requirements for a positive diagnosis of metabolic syndrome, but have both been shown to predict CVD in white populations. Although the applicability of these criteria across ethnicities has been challenged, no studies have investigated the appropriateness of the metabolic syndrome criteria in black South African women prior to the onset of disease. In African American and Afro-Caribbean populations, the ATP III and IDF metabolic syndrome criteria have been shown to under-predict CVD risk. This has been attributed to

the favourable lipid profiles of black African women, which may influence the clustering of risk factors for the metabolic syndrome. These ethnic differences in lipid profile could be related to lower levels of VAT in black African women compared to white women, despite high levels of central obesity. Decreased levels of VAT may render waist circumference cut-points inappropriate for black South African women. Also, the influence of SAT, particularly DSAT, may be important in determining CVD risk in black Africans. The influence of the various abdominal fat depots on CVD risk factors and insulin resistance in particular, has not been investigated in a black South African population.

"Healthy obese" individuals have previously been reported in black South African women and internationally, and termed "metabolically healthy obese" (MHO). Conversely, normal weight individuals have been identified who present with CVD despite maintaining a "healthy weight" and have been termed "metabolically obese normal weight" (MONW). Research on white and Japanese individuals has highlighted body fat distribution and PAEE among other factors, as determinants of these phenotypes. Low levels of VAT have consistently been linked to the MHO phenotype and high levels of VAT have been consistently linked to the MONW phenotype. Significant epidemiological change (urbanization and increased dietary fat intake) has occurred since the first documentation of "healthy obese" individuals in South Africa. However, the occurrence and determinants of the MONW and MHO phenotypes have not been systematically investigated in the black South African population.

Central obesity has been associated with CVD and a positive diagnosis of the metabolic syndrome. Studies have demonstrated an association between tissue-specific GC action and obesity, centralisation of fat and CVD. Therefore, variability of the *HSD11B1*, *GR* and *H6PD* genes that encode for 11β-HSD-1, GR and H6PDH respectively merit investigation. Research has highlighted the influence of the mutant alleles of the Ins4436A, *Bcl*I and R453Q polymorphisms within the *HSD11B1*, *GR* and *H6PD* genes respectively, on sensitivity to GCs, obesity, central obesity and CVD. The A/A genotype of the Ins4436A polymorphism within the *HSD11B1* gene has previously been associated with increased BMI and level of insulin resistance in a small sample of African

Americans. Further, the frequency of the A allele of the R453Q polymorphism within the *H6PD* gene is higher in African compared to white Americans, and is associated with decreased H6PDH enzyme activity. This could explain the low levels of VAT in black compared to white South African women. The G allele of the *Bcl*I polymorphism within the *GR* gene is strongly associated with increased sensitivity to GCs, obesity, centralisation of fat and CVD. However, none of these polymorphisms have been previously investigated in a black South African population. Investigation of the Ins4436A, R453Q and *Bcl*I polymorphisms within the *HSD11BI*, *H6PD* and *GR* genes respectively, may help explain why black South African women have a higher prevalence of obesity, central obesity and insulin resistance, despite lower levels of VAT compared to white South African women.

1.8 AIMS AND OBJECTIVES

Accordingly, the primary aim of this thesis was to investigate the impact of body fat and its distribution on the presentation and identification of CVD risk factors in black South African women. The first objective of this thesis was to investigate the validity of NIR as a measure of body fat percent in white and black South African women. Further, this thesis aimed to investigate the applicability of the ATP III and IDF metabolic syndrome criteria in black South African women and identify determinants of alternative metabolic phenotypes. The final aim was to investigate the association between the mutant alleles of polymorphisms within genes involved in GC metabolism, body fat distribution and CVD risk factors. As a result, the following specific objectives were formulated for this thesis:

1 To determine if NIR is a valid field measure of body fat percent in South African women (Chapter Two).

2 To determine the agreement between the IDF and ATP III metabolic syndrome criteria and the degree to which these criteria can predict insulin resistance, a risk factor for CVD and type 2 diabetes, in black South African women. To explore the extent to which these phenomena can be explained by body fat and its distribution (Chapter Three).

3 To identify the determinants of the MHO and MONW phenotypes in black South African women (Chapter Four).

4 To complete a preliminary investigation of the association between the A allele of the Ins4436A polymorphism within the *HSD11B1* gene, the G allele of the *Bcl*I polymorphism within the *GR* gene, and the A allele of the R453Q polymorphism within the *H6PD* gene with obesity, body fat distribution and CVD in black South African women (Chapter Five).

In order to achieve the aims of this thesis, the following women were recruited for each of the Chapters as specified below in Figure 1.11:

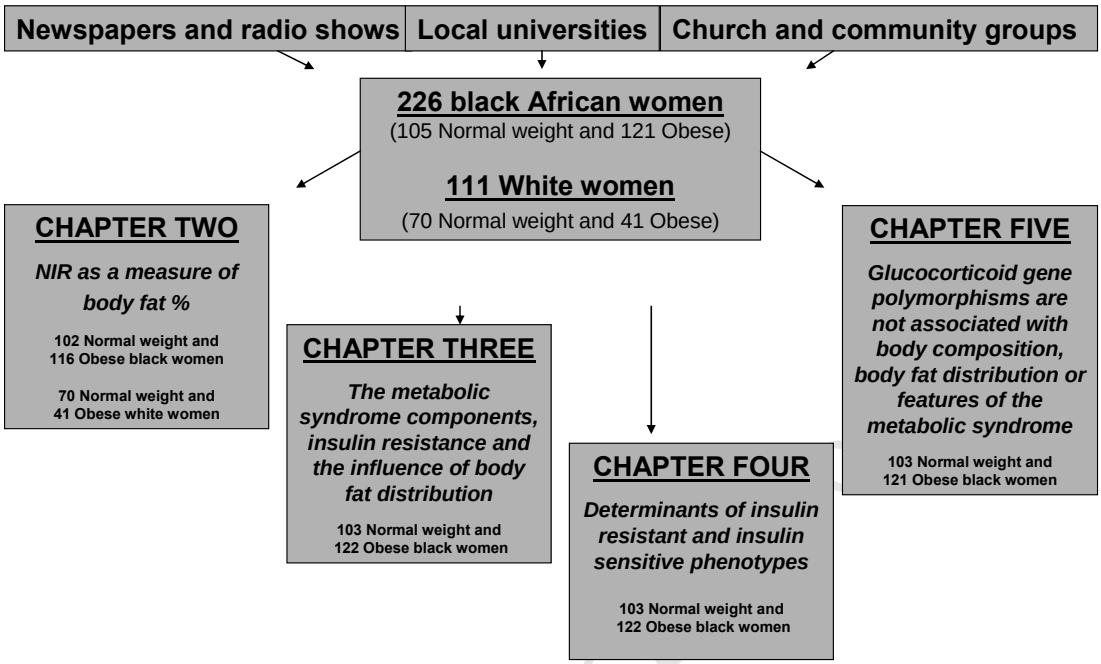

Figure 1.11 Illustration of the number of subjects in each chapter of this thesis. The number of subjects in each chapter differs slightly based on the variables being studied. In Chapter Two, only subjects who underwent both NIR and DXA testing were included in the analysis. In Chapters Three and Four, only subjects with HOMA-IR were included in the study. In Chapter Five, only subjects with DNA samples were included in the study.

CHAPTER TWO

THE VALIDITY OF NEAR INFRARED INTERACTANCE AS A MEASURE OF BODY FAT PERCENT IN WOMEN

2.1 INTRODUCTION

Obesity has become a global epidemic in both developed and developing countries (102). In South Africa, urbanized women in particular, are at an increased risk of developing obesity, with more than 58% of black and 49% of white women classified as overweight or obese, based on BMI (4). Since increased adiposity is associated with increased CVD risk (38), quantification of body fat percent is becoming an increasingly important aspect of health risk appraisal and research.

Traditionally, BMI (kg/m^2) has been used as a proxy measure of adiposity in health screening and in large epidemiological studies. However, the relationship between BMI and body fat percent is influenced by factors such as muscularity and ethnicity and therefore varies by population (130;408-410). There are more precise methods of quantifying body fat percent, such as DXA (120) and underwater weighing (411). However, these methods are not always viable for large scale health screening and research, due to the high cost and limited access to facilities, particularly in developing countries. Therefore, field methods of quantifying body composition, such as BIA (412) and NIR have been developed (145).

BIA is relatively inexpensive, portable and does not require the services of a radiographer for use and interpretation, making it an attractive field measure of body fat percent. However, although BIA has been validated for use in different populations (99;134;413), strict pre-test conditions must be observed since hydration status and changes in skin temperature have been shown to affect the validity of the measurement, which may be problematic when testing in the field (138). NIR is another practical field measure of body fat percent which does not require the same strict pre-test conditions as BIA. For these reasons NIR may be a very useful tool to measure body composition in situations where methods such as DXA and underwater weighing and BIA are not practical.

The validity and limits of agreement of NIR as a measure of body fat percent have been shown to be population specific. For example, a recent study reported that NIR tended to

over-estimate body fat percent in lean individuals and under-estimate body fat percent in individuals with higher levels of adiposity, compared to underwater weighing (111). Ethnicity may also affect the validity of NIR. Few studies have explored the validity of NIR in different ethnic groups, although concerns have been raised regarding the impact of skin tone and levels of SAT on the relationship between optical density and body fat percent, making these factors possible confounders for use of NIR in certain populations (112). For example, studies in American Indian women (112) as well as African American Division I college football players (113) found that NIR under-reported body fat percent compared to underwater weighing. Other small studies comprised of African Americans reported that NIR under-predicted body fat percent compared to criterion methods with a total error of between 3.9%-4.6%.

The data investigating the use of NIR as a measure of body fat percent in black Africans is limited and suggests that NIR may under-predict body fat percent more in black compared to white subjects (149;150). Moreover, black South African women have decreased VAT (205) and increased peripheral fat distribution compared to white South African women (130), which could affect the validity of the measurement. Therefore, the aim of this study was to determine the validity of NIR as a measure of body fat percent, compared to body fat percent measured by DXA in a cohort of normal weight and obese, black and white South African women.

2.2 METHODS

2.2.1 Subjects

The study population consisted of black and white normal weight (BMI ≤25 kg/m^2) (N=102 and 70, respectively) and black and white obese (BMI ≥30 kg/m^2) (N=116 and 41, respectively) urban South African women aged 18 to 45 years old. Racial identity was self reported. Subjects who identified both of their parents as black, or both of their parents as white, were included in the study. White and black subjects were recruited from church groups, community centres and universities, and were included in the study if they met the following criteria: i) aged 18 to 45 years; ii) normal weight (BMI ≤25 kg/m^2) or obese (BMI ≥30 kg/m^2); iii) free from known disease and not taking medication for diabetes, hypertension, HIV/AIDS or any other metabolic disorder, iv) not pregnant or lactating.

Permission to undertake the study was given by the Health Sciences Research Ethics Committee of the University of Cape Town. Written informed consent was obtained from all subjects prior to participation.

2.2.2 Body composition assessment

Weight (in light weight clothing without shoes) and height were measured. Body fat percent was measured by single-site NIR (Futrex-6100 A/ZL, Futrex inc. Gaithersburg, MD, USA) as described previously (414). Detailed information on the method of measurement and site of measurement is provided in the Appendix of this thesis. Briefly, the subject's gender, weight, height and age were entered into the NIR computer for the calculation of body fat percent. The subjects were asked to sit with their dominant arm relaxed on an examination table. After calibration, the light wand of the NIR was placed on the surface of the subject's biceps at the mid point between the elbow and the shoulder. The NIR device reading is determined via an infrared light which penetrates approximately 1 cm into the tissue of the measurement site. Lean tissue reflects the light and adipose tissue absorbs it. Six optical density readings are recorded and averaged by the NIR device. Scans are made over a range of wavelengths from 700-1100 nm. The final

averaged optical density is used to derive total body fat percent based on optical density, gender, height, weight and age (www. Futrex.com).

In the present study, a NIR light shield was used to block out any surrounding light which could affect the measurement. The shield was positioned so that the bottom of the light wand protruded approximately 3 mm. The NIR light shield was removed from the light wand, the machine was zero adjusted and the calibration checked prior to testing each subject.

In addition, whole body composition was assessed using DXA (Hologic QDR 4500 Discovery-W dual-energy x-ray absorptiometer, software version 4.40, Hologic Inc., Bedford, MA, USA) according to standard procedures. *In vivo* precision (%CV) was determined for FFSTM (0.7%) and fat mass (1.7%) by measuring thirty individuals twice on the same day with re-positioning. When a subject did not fit onto the DXA scan platform (N=16), the arm-replacement method was used (Micklesfield et al., personal communication) to measure total body composition. This method requires the radiographer to use the data from one arm for both arms. This method has been shown to be more accurate for the measurement of fat mass and FFSTM than the half-body method proposed by Tataranni et al. (126) (Fat mass: arm-replacement method r=1.0, limits of agreement (LOA)=-0.24-0.33 kg vs. half-body replacement method r=0.99, LOA=-0.06-2.09 kg and FFSTM: arm-replacement method r=1.0, LOA=-0.62-2.0 vs. half-body method r=0.99, LOA=0.39-4.13 kg).

Basic anthropometry was always completed before measurement of body fat percent by DXA and NIR. The order of testing for DXA and NIR was randomized by subject.

2.2.3 Statistical analysis

The data were analyzed using the STATISTICA Version 7 statistical program (StatsSoft Inc., Tulsa, OK, USA). All data are expressed as means ± standard deviations (SD). Pearson product moment correlations were used to describe the relationships between the two techniques. The 95% confidence intervals around the correlation coefficients were

calculated using a spreadsheet for this purpose downloaded from www.newstats.org (accessed 1st July, 2007). Limits of agreement were determined using the technique of Bland and Altman (415) to compare the level of agreement between body fat percent measured by DXA and NIR. One-way ANOVA was used to determine if the amount of bias in NIR compared to DXA was different by group. Significance was accepted when $P<0.05$. Estimated sample size was calculated from the data of normal weight white women assuming a minimum difference of 3% body fat between techniques and a standard deviation of 4% within a group. This calculation predicted a minimum sample size of 28 subjects per group (probability of a type I and II error =5% and 20% respectively). However, as the variation within the black obese and normal weight subgroups was not known, this sample size was increased to guard against a potential lack of statistical power.

2.3 RESULTS

2.3.1 Basic subject characteristics

The basic subject characteristics of the normal weight and obese, black and white subjects are described in Table 2.1. The obese subjects were older and had a greater body weight, higher BMI and increased adiposity compared to the normal weight subjects. There were no differences in height between the normal weight and obese women of the same ethnicity.

Table 2.1. Basic characteristics of the black and white, normal weight and obese South African women.

	Normal weight (BMI ≤25 kg/m^2)		Obese (BMI ≥30 kg/m^2)		Total model ANOVA
	Black (N=102)	White (N=70)	Black (N=116)	White (N=41)	P-value
Age (yrs)	$24 \pm 6^{*\ddagger+}$	$30 \pm 7^{*\#}$	$30 \pm 8^{\dagger+}$	$35 \pm 8^{\dagger\ddagger\#}$	*<0.001*
Height (cm)	$161.0 \pm 5.0^{*\ddagger}$	$167 \pm 6.0^{*\S}$	$160.0 \pm 6.0^{\dagger\S}$	$167 \pm 6.0^{\dagger\ddagger}$	*<0.001*
Weight (kg)	$57.6 \pm 6.2^{\ddagger+}$	$61.2 \pm 6.9^{\S\#}$	$92.0 \pm 14.6^{\S+}$	$95.1 \pm 14.1^{\ddagger\#}$	*<0.001*
BMI (kg/m^2)	$22.3 \pm 1.8^{\ddagger+}$	$22.0 \pm 1.9^{\S\#}$	$36.1 \pm 5.1^{\dagger+\S}$	$34.3 \pm 4.0^{\dagger\ddagger\#}$	*<0.001*
Body fat % DXA	$30.1 \pm 4.7^{*\ddagger+}$	$27.9 \pm 5.6^{*\#\S}$	$45.2 \pm 4.4^{\S+}$	$44.2 \pm 2.5^{\ddagger\#}$	*<0.001*
Body fat % NIR	$25.7 \pm 4.2^{*\ddagger+}$	$27.6 \pm 4.7^{*\#\S}$	$41.6 \pm 4.4^{\dagger\S+}$	$45.1 \pm 3.7^{\dagger\ddagger\#}$	*<0.001*

BMI, body mass index; ANOVA, analysis of variance; DXA, dual-energy x-ray absorptiometry; NIR, near infrared interactance. Values are expressed as unadjusted means ± standard deviation (SD). P-values are adjusted for age. *P<0.05 normal weight black vs. normal weight white, †P<0.05 obese black vs. obese white, ‡P<0.05 normal weight black vs. obese white, §P<0.05 normal weight white vs. obese black, $^\#$P<0.05 normal weight white vs. obese white, $^+$P<0.05 normal weight black vs. obese black.

The white subjects were older, taller and heavier than the black subjects (P<0.03). There were no ethnic differences in BMI in the normal weight women (P=0.20); however, BMI was higher in the black obese women compared to the white obese women (P=0.04). The normal weight white women had a lower body fat percent compared to the normal weight black women (P<0.01). Body fat percent measured by DXA was greater in the black

compared to the white women (P<0.01), while body fat percent measured by NIR was greater in the white compared to the black women (P=0.03).

2.3.2 Bias table for the limits of agreement between body fat percent measured by NIR and DXA

The bias in the limits of agreement between body fat percent measured by DXA and NIR are presented in Table 2.2. NIR under-predicted body fat percent compared to DXA in all groups. The degree of under-prediction was greater in the black compared to the white women (P<0.001) but was not different between the normal weight and obese black women (P=0.54) or the normal weight and obese white women (P=0.99).

Table 2.2 Bias table for the limits of agreement between body fat percent measured by NIR and DXA

	Mean difference (NIR-DXA)	95% confidence intervals	SEE
Normal weight black (BMI ≤25 kg/m^2)	-4.46 ± 0.81	-5.17 to -3.55	3.84
Obese black (BMI ≥30 kg/m^2)	-3.62 ± 0.75	-4.37 to -2.87	3.56
Normal weight white (BMI ≤25 kg/m^2)	-0.29 ± 1.00	-1.29 to 0.70	4.19
Obese white (BMI ≥30 kg/m^2)	-0.81 ± 0.98	-1.79 to 0.16	3.09

DXA, dual-energy x-ray absorptiometry; NIR, near infrared interactance; SEE, Standard error of the estimate; BMI, body mass index.

2.3.3 Bland Altman limits of agreement

The Bland-Altman limits of agreement between body fat percent by NIR and DXA are shown in Figures 2.1A and 2.1B for the black and white subjects respectively. In both the white and black obese subjects, the graphs show heteroscedasticity, in that NIR under-predicted body fat percent in obese subjects with very high levels of adiposity (>45% body fat). At lower levels of body fat percent there was no observable pattern in the variation between body fat percent measured by NIR and DXA. NIR was more accurate in normal weight and moderately obese white subjects (BMI >29.9 kg/m^2, <45% body fat), but under-predicted body fat percent in normal weight black women.

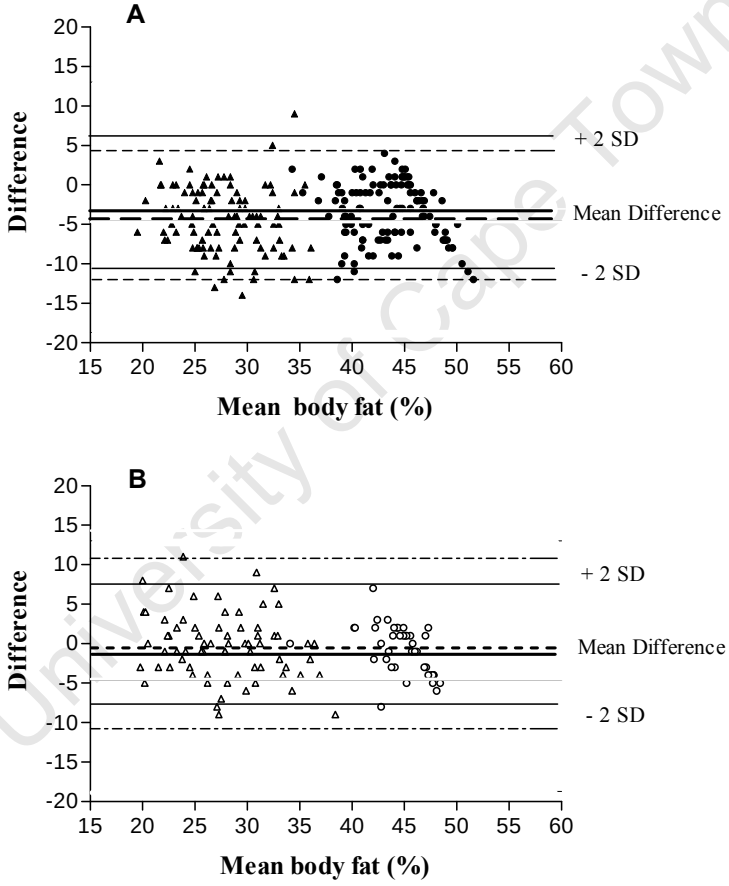

Figure 2.1. The Bland Altman LOA between body fat percent measured by NIR and DXA in the A) normal weight and obese black and B) normal weight and obese white South African women. Obese subjects are indicated by circles and normal weight subjects are indicated by triangles. The LOA are represented by the dashed lines for the normal weight women and solid lines for the obese women. Normal weight black women LOA=-13.60 to 4.74, obese black women LOA=-12.37 to 5.23, normal weight white women LOA=-11.49 to 10.91 and obese white women LOA=-8.21 to 6.59.

2.3.4 Bivariate relationship between NIR and DXA as measures of body fat percent

The bivariate relationship between NIR and DXA as measures of body fat percent is shown in Figure 2.2. Body fat percent from NIR correlated significantly to that of DXA in both the normal weight and obese black women (r=0.55, 95% CI: 0.40-0.67, P<0.001 and r=0.58, 95% CI: 0.44-0.68, P<0.001, respectively) and the normal weight and obese white women (r=0.69, 95% CI: 0.53-0.79, P<0.001 and r=0.57, 95% CI: 0.31-0.74, P<0.001, respectively). In the obese subjects, NIR body fat percent did not increase further than 45-46% while DXA continued to measure up to 58%. Eighteen percent of white women and 26% of black women had >45 body fat percent. This resulted in a large under-prediction of body fat percent by NIR in both black and white individuals with very high levels of adiposity (>45% body fat).

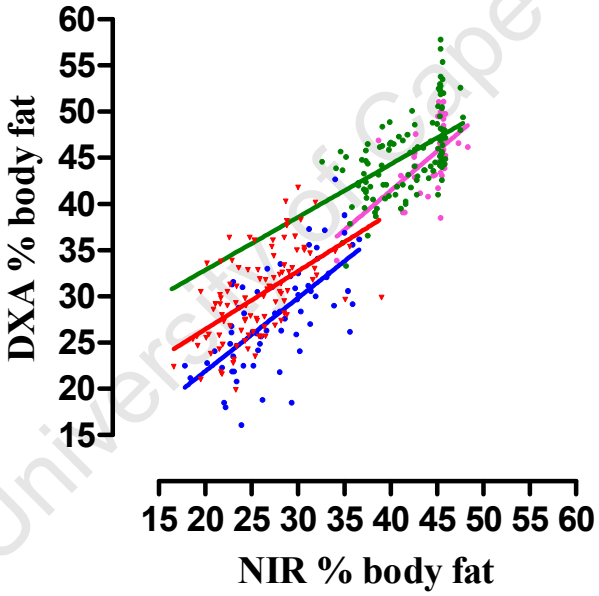

Figure 2.2. The correlation and individual regression lines for body fat percent measured by DXA vs. NIR for the normal weight and obese, black and white South African women. The normal weight black women are shown in red, the obese black women are shown in green, the normal weight white women are shown in blue and the obese white women are shown in pink.

Normal weight black r=0.55, 95% CI=0.40-0.67, P<0.001, DXA body fat %=13.864+0.629^NIR body fat %
Obese black r=0.57, 95% CI=0.44-0.68, P<0.001, DXA body fat %=21.369+0.573^NIR body fat %
Normal weight white r=0.69, 95% CI=0.53-0.79, P<0.001, DXA body fat %=5.744+0.803^ NIR body fat %
Obese white r=0.57, 95% CI=0.31-0.74, P<0.001, DXA body fat %=7.323+0.859^ NIR body fat %.

2.4 DISCUSSION

Although single-site NIR is a practical field method for the measurement of body composition, it under-predicted body fat percent in normal weight and obese, black and white South African women compared to DXA. The degree of under-predication was much greater in the black compared to the white subjects, and in subjects with very high levels of adiposity (>45%). Body fat percent measured by NIR appeared to level off at approximately 45%, while DXA measured up to almost 60% body fat. The degree of under-prediction of body percent by single-site NIR could be related to several methodological limitations. These limitations are related to impact of skin colour (152), body fat distribution patterns (112) and measurement site (416) on NIR.

There is a paucity of data available on the use of NIR in black Africans and the available literature on the validity of the instrument is somewhat conflicting. This is particularly true of the data in normal weight populations. For example, two studies have reported that NIR accurately estimated body fat percent in non obese and lean individuals compared to underwater weighing (136;146), while a more recent study reported that NIR over-estimated body fat percent in lean individuals compared to underwater weighing (111). In contrast, NIR under-predicted body fat percent in the normal weight subjects in the present study compared to DXA. However, the degree of under-prediction was notably greater in the normal weight black compared to white women (-4.46% vs. -0.29% mean difference between NIR and DXA, respectively).

The research on obese individuals is more clear in that NIR has been consistently shown to under-predict body fat percent (147). Moreover, the degree of underestimation of body fat percent in very obese individuals increases with increasing levels of adiposity (148). In the present study, in agreement with previous research, NIR under-predicted body fat percent in the very obese black and white South African women. In individuals with high adiposity, the difference between NIR and DXA body fat percent was as great as 12%, highlighting that NIR may be inappropriate for use in a very obese population.

NIR most closely approximated DXA in the normal weight white South African women, possibly due to their lower levels of adiposity compared to the other groups. Since obesity was defined by BMI, this difference could be due to the relatively high level of adiposity in the normal weight black compared to white subjects ($P<0.01$). For example, McLean et al. (147) reported that in individuals with >30% body fat, NIR under-estimated adiposity by more than 4%, which is in agreement with the findings of this study.

The correlation between body fat percent measured by NIR and DXA was very poor for all four of the groups ($r=0.55$ to 0.69) compared to recommended validity coefficients ($r=0.80$) (417). Although the correlation coefficients were similar, NIR had a smaller mean bias in the normal weight and obese white women compared to the normal weight and obese black women. This could be due to the relatively small sample of obese white women in this study, but could also be related to previously reported ethnic differences in the validity of NIR. Indeed, Hicks et al. (112) found that single-site NIR (Futrex-5000 NMR) was not an accurate measure of body fat percent ($r^2=0.36$, SEE=5.50) in 146 American Indian women (age=34.3±10 years, BMI=25.5±4.3 kg/m^2). In agreement with the findings of the present study, Hicks et al. (112) reported that NIR under-estimated adiposity by an average of 4.4% in American Indian women. However, both the normal weight black women in this study and the American Indian population studied by Hicks et al. (112) had high levels of adiposity for a normal BMI (black South African women, BMI 22.3 kg/m^2, body fat 30.1% and American Indian women, BMI 25.5 kg/m^2, body fat 35.4%). Therefore, the under-estimation of body fat percent by NIR could be due to increased adiposity rather than ethnic differences. However, differences in adiposity do not explain why NIR was more accurate in the white compared to black obese South African women who had similar ranges (31-51% vs. 33-57%, respectively) and levels of adiposity (~44% vs. ~45%, respectively).

This inconsistency could be related to ethnic differences in body fat distribution between the black and white women. Hicks et al. (112) reported that NIR under-predicted body fat percent in American Indian women due to differences in body fat distribution compared to white populations, on which NIR (Futrex-5000) equations were determined. Moreover, by incorporating hip circumference, biceps and chest optical density, physical fitness, age and

height into a regression equation, 78.9% of the variance in body fat percent in the American Indian women could be accounted for compared to only 36% using single-site NIR (biceps optical density) and 62% using multi-site NIR (abdominal, sub-scapular, thigh, chest and biceps optical density) (112). Since black women have decreased centralisation of body fat and increased peripheral body fat deposition compared to their white counterparts (130), perhaps use of the biceps as the NIR measurement site, which was shown to be the best predictor of total body fat percent (r=0.85, SEE=0.01) in middle-aged white men (416), is not appropriate in black women.

This finding is not unexpected when one considers the lessons learned from other body composition methods that extrapolate total adiposity from a measurement of one body segment. For example, the skin fold thickness method operates on the assumption that SAT is representative of total body fat and that FFSTM is uniform in all populations. Therefore, differences in body fat distribution affect the validity of the measurement. This has resulted in the development of over 100 population-specific equations with validity coefficients for women ranging from r=0.72 to 0.84, compared to criterion methods (95). Like skin fold thickness, it is likely that population-specific equations for single site NIR should be derived and validated prior to its use due to ethnic variations in body fat distribution patterns.

Additionally, research has shown that skin colour may affect the relationship between optical density and body fat percent which could also explain the ethnic differences in the validity of NIR in black and white South African women. For example, in a cohort of white, black, Hispanic and American Indian men (N=150), skin tone explained a significant amount of the variability in optical density at the biceps site (15.8%), beyond that explained by skin fold thickness alone (40.8%). Moreover, individuals with darker skin colours tended to have a smaller change in optical density, indicative of decreased subcutaneous adiposity compared to those with lighter skin colours (152). Therefore, darker skin colour could impact the relationship between body fat percent and optical density and confound the use of NIR in this population.

To my knowledge, this is the first study investigating the use of NIR in black and white South African women, and it is the largest study to date investigating NIR as a measure of body composition in black African individuals. The results of this study suggest that single-site NIR should not be used in black African women and individuals with high levels of adiposity. Further, investigation of ethnic-specific field techniques for quantifying body composition in individuals with varying levels of adiposity is required for the purposes of both health risk assessment and research.

CHAPTER THREE

THE ATYPICAL PRESENTATION OF THE METABOLIC SYNDROME COMPONENTS IN BLACK SOUTH AFRICAN WOMEN; THE RELATIONSHIP WITH INSULIN RESISTANCE AND THE INFLUENCE OF REGIONAL ADIPOSE TISSUE DISTRIBUTION

3.1 INTRODUCTION

In 1988, Gerald Reaven noted that insulin resistant individuals presented with a clustering of risk factors for CVD, including glucose intolerance, high TG levels, low HDL-C levels and elevated BP, compared to insulin sensitive individuals (13). Consequently the term 'syndrome X' was proposed as a conceptual framework to illustrate the relationship between insulin resistance and CVD. More recently, the components of syndrome X, including central obesity, have been used as criteria for the diagnosis of the 'metabolic syndrome' (154;155), a clustering of risk factors associated with increased risk of type 2 diabetes and CVD (14;418).

The IDF (155) and the ATP III (154) have developed independent diagnostic criteria to identify individuals with the metabolic syndrome. Both organizations include fasting glucose, triglycerides, HDL-C and BP as diagnostic components, with identical cut-points. However, waist circumference cut-points differ between the two diagnostic criteria (IDF >80cm and ATP III >88 cm). Further, the IDF criteria require the presence of central obesity, in addition to two or more components, while ATP III criteria require any three components for a positive diagnosis of the metabolic syndrome. Despite this difference in diagnostic criteria, both IDF and ATP III criteria have been shown to predict future risk of CVD, though largely in white populations (309). In contrast, the ATP III criteria have been shown to have low sensitivity for identifying insulin resistance in Caucasian populations (313;314), despite the presumption that insulin resistance is the underlying factor in the metabolic syndrome (13) and is associated with increased risk for CVD and type 2 diabetes (419).

There has been considerable debate regarding the clinical significance of the metabolic syndrome diagnostic criteria as well as the applicability of these clinical criteria across different ethnic groups (306). For example, African Americans have a higher prevalence of obesity, insulin resistance, type 2 diabetes and CVD than white Americans (163) despite a lower prevalence of metabolic syndrome according to the ATP III definition

(166). Although there are no national prevalence data for type 2 diabetes, insulin resistance or the metabolic syndrome in South Africa, epidemiological studies conducted during the 1990's suggest a relatively high occurrence of type 2 diabetes in black South African women (22). On the other hand, both African Americans and black South African women are notable for their favourable lipid profiles (19;86), perhaps due in part to their relatively low levels of VAT compared to white women (173;174). These ethnic differences may render current metabolic syndrome cut-points and the weighting of the components inappropriate for use in some population groups.

The high prevalence of type 2 diabetes and insulin resistance in black women, despite low levels of VAT is unexpected, since VAT and insulin resistance are closely associated (247). However, central obesity, as measured by waist circumference, has been associated with elevated fasting insulin and triglyceride levels, as well as low HDL-C levels in urban black hypertensive South African women (207). Therefore, abdominal SAT may play a metabolic role linking obesity and insulin resistance in black African women. Indeed, a recent study in African American women found that SAT was more closely associated with insulin resistance than VAT (188). Quantification of SAT, specifically DSAT (189), may assist with the interpretation of anthropometric measures of risk in black African women.

The applicability of the current IDF and ATP III metabolic syndrome criteria and the association of the metabolic syndrome components with insulin resistance have not been explored in a black South African population. Thus, the aims of this chapter were: i) to examine the level of agreement between the IDF and ATP III metabolic syndrome criteria in a cohort of relatively young black South African women; ii) to investigate the degree to which the IDF and ATP III metabolic syndrome criteria predict insulin resistance, as estimated by HOMA-IR; and iii) to investigate the extent to which insulin resistance and a diagnosis of the metabolic syndrome may be explained by body fat and its distribution. Insulin resistance (HOMA-IR) was used as the main outcome variable as: a) it has been suggested to be the primary etiological factor for the metabolic syndrome components and consequently the underlying cause of CVD (13;14); b) it is an independent predictor of CVD and type 2 diabetes (15-18); c) has been used as a primary indicator of CVD risk in

similar studies (19); and d) lipid levels may not be good indicators of CVD risk in black African women (19;20).

3.2 METHODS

3.2.1 Subjects

The study population consisted of 103 normal weight (BMI ≤25 kg/m^2) and 122 obese (BMI ≥30 kg/m^2) premenopausal urban black South African women. Subjects were recruited from church groups, community centres, universities and through the local press, and were included in the study if they were: i) 18-45 years old; ii) had no known diseases and were not taking medications for type 2 diabetes, hypertension, HIV/AIDS or any other metabolic diseases; iii) not pregnant or lactating; and iv) of black South African ancestry. Three of the obese subjects were classified as having type 2 diabetes mellitus based on a fasting glucose level of ≥7.0 mmol/L. As exclusion of these subjects did not change the findings of the study, they were included in the analysis. Permission to undertake the study was given by the Research Ethics Committee of the Faculty of Health Science of the University of Cape Town. Written informed consent was obtained from all subjects prior to participation.

3.2.2 Body composition assessment

Weight, height, waist circumference (at the level of umbilicus) and hip circumference (at the largest part of hips) were measured. Waist and hip circumferences were measured to the nearest 0.5 cm. DXA was used to measure regional and whole body composition as described in Chapter Two. Regional body fat distribution, including central fat mass, appendicular fat mass and appendicular skeletal muscle mass, was determined as described previously (130).

In addition, a single slice computerised tomography (CT, Toshiba X-press Helical Scanner, Japan) scan was taken at the level of the L4-L5 lumbar vertebrae to determine VAT, SSAT and DSAT. DSAT and SSAT were differentiated by the fascia superficialis as described previously (189). VAT and DSAT cross-sectional areas were converted to mass (kg) using regression equations derived by Smith et al. (189). Total body superficial

subcutaneous adipose tissue (tSSAT) kg was determined by subtracting VAT (kg) and DSAT (kg) from total body fat kg in order to obtain independent measures of single adipose tissue depots, rather than collinear multiple compartments for statistical analysis (189). A cut-point of VAT>100 cm^2 was used to describe VAT as an indicator of CVD risk (229).

3.2.3 Blood pressure

After at least five minutes of seated rest, BP was measured three times at one-minute intervals using an appropriate sized cuff and an automated BP monitor (Omron 711, Omron Health Care, Hamburg, Germany). The last two readings were averaged for the analyses.

3.2.4 Blood sampling and analysis

Blood samples were drawn after an overnight fast (10-12 hours) for the determination of plasma glucose, serum insulin, FFA, triglycerides, TC, HDL-C and LDL-C levels. Fasting plasma glucose levels were measured using the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA) and the intra-assay coefficient of variation was 0.67%. Serum insulin levels were measured by a Micro-particle Enzyme Immunoassay (MEIA) (AxSym Insulin Kit, Abbot, IL, USA) and the intra- and inter-assay coefficients of variation were 3.2% and 2.3%, respectively. Serum FFA levels were measured using a commercial kit (FFA Half-micro test; Roche, Mannheim, Germany) and the intra-assay coefficient of variation was 2.08%. Serum TC, triglyceride and HDL-C levels were measured on the Roche Modular Auto Analyzer using enzymatic colorimetric assays. LDL-C was calculated using the Friedewald equation (420).

3.2.5 Measuring insulin resistance

Insulin resistance was estimated using the homeostasis assessment model (HOMA-IR) (248) and HOMA2 (249). As the results obtained using the two methods did not differ, HOMA-IR was used to facilitate comparison with previous similar studies (19). Subjects were divided into tertiles by HOMA-IR values (Tertile 1 ≤1.07, Tertile 2 =1.08 to 2.59 and Tertile 3 ≥2.60). Insulin resistance was defined as being in the upper tertile as commonly practiced (19;292;313). The upper tertile cut-point in this study was very similar to that reported by Sumner et al. (HOMA-IR of 2.73) in an African American population (19). HOMA-IR ranged from 0.25-10.90.

3.2.6 Metabolic syndrome criteria

The ATP III (154) and IDF (155) metabolic syndrome criteria for women were employed to describe the sample. Metabolic syndrome as defined by the IDF criteria is diagnosed when central obesity (waist circumference) and an additional two or more of the following components are present: impaired fasting glucose (≥5.6 mmol/L), elevated blood pressure (systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg), raised triglycerides (≥1.7 mmol/L) and decreased HDL-C (≤1.29 mmol/L). The ATP III criteria include the same components, but do not weight the components of the metabolic syndrome. Metabolic syndrome is diagnosed when any three of the components are present. The cut-points for waist circumference (80 cm vs. 88 cm) are also different between the IDF and ATP III criteria.

3.2.7 Statistical analysis

In order to explore the metabolic effects of extreme ranges in BMI while maintaining a unimodal distribution for all other parameters (body fat percent, waist circumference, VAT, SAT, BP, HOMA-IR and lipid profile), women with a BMI of either ≤25 or ≥30 kg/m^2 were sampled. The data were analyzed using the STATISTICA Version 7 statistical program (StatsSoft Inc., Tulsa, OK, USA) and expressed as unadjusted means ± SD. Data

were normalized by log transformation if required. Analysis of variance (ANOVA) and the Bonferroni post-hoc test were used to determine differences in basic subject characteristics and metabolic outcomes between the HOMA-IR tertiles. Analysis of covariance (ANCOVA) was performed to investigate body fat distribution patterns between the HOMA-IR Tertiles. The ANCOVA analyses were adjusted for fat mass (kg) (421). Linear trends were determined to describe the patterns found in the prevalence of the metabolic syndrome components by HOMA-IR tertiles. In addition, kappa statistics (k) (422) were used to determine the specificity and sensitivity of the ATP III and IDF metabolic syndrome criteria, using the highest tertile for HOMA-IR as the insulin resistant group and the remainder as insulin sensitive. κ -values of <0.20, 0.21-0.40, 0.41-0.60 and >0.60 were considered as poor, fair, moderate, and good agreement, respectively. VAT (kg), DSAT (kg) and tSSAT (kg) were used in logistic regression analysis to determine their contribution to a positive diagnosis of the metabolic syndrome as defined by the IDF and ATP III. Statistical significance was accepted as $P<0.05$.

3.3 RESULTS

3.3.1 Basic subject characteristics and metabolic outcomes

The normal weight subjects were younger than the obese subjects, with mean ages of 24±6 years (range 18-43 years) and 30±8 years (range 19-45 years), respectively (P<0.01). By design, BMI (22±2 kg/m^2, range 18-25 kg/m^2 vs. 34±6 kg/m^2, range 30-54 kg/m^2, P<0.01), weight (57±6 kg, range 43-73 kg, vs. 93±14.6 kg, range 67-140 kg, P<0.01), waist circumference (73±6 cm, range 59-90 cm vs. 103±12 cm, range 74-139 cm, P<0.05**)** and total adiposity (30±5%, range 21-42% vs. 45±4%, range 33-58%, P<0.01) were lower in the normal weight women than in the obese women. There were no differences in height between the normal weight and obese women. Despite the subjects being either normal weight or obese, measures of body fatness and HOMA-IR were unimodally distributed. For subsequent analyses, subjects were subdivided into tertiles of HOMA-IR in order to explore factors associated with HOMA-IR between these groups.

The basic characteristics and metabolic outcomes of the normal weight and obese subjects by HOMA-IR tertile are presented in Table 3.1. Age and height did not differ between tertiles (P=0.40 and 0.65, respectively), but all measures of body fatness (weight, BMI, body fat percent and waist circumference) increased with increasing tertile (P≤0.001). By design, HOMA-IR was highest in Tertile 3 (P≤0.001). The increase in HOMA-IR across tertiles could not be attributed to changes in fasting glucose levels, but was rather due to increases in fasting insulin levels (2-4 fold increase from Tertile 1 to Tertiles 2 and 3). Triglyceride levels were relatively low in all tertiles (<0.9 mmol/L), but were significantly higher in Tertile 3 compared to Tertiles 1 and 2 (P≤0.001). Conversely, HDL-C was higher in Tertiles 1 and 2 compared to Tertile 3 (P=0.03). HDL-C in Tertile 3 was the only metabolic outcome that met ATP III or IDF metabolic syndrome cut-points. Notably, TC, LDL-C, TC/HDL-C and TG/HDL-C were not different between the tertiles, while systolic and diastolic BP rose with increasing tertile.

Table 3.1. Basic characteristics and metabolic outcomes of subjects according to tertiles of insulin resistance (HOMA-IR).

	Tertile 1 HOMA-IR ≤1.07 (N=75)	Tertile 2 HOMA-IR=1.08-2.59 (N=74)	Tertile 3 HOMA-IR ≥2.60 (N=76)	Total model ANOVA P-value
Age (years)	28 ± 8	27 ± 8	26 ± 7	0.403
Height (cm)	161 ± 0	160 ± 0	161 ± 0	0.646
Weight (kg)	67.3 ± 16.7‡	73.3 ± 19.9†	89.4 ± 19.7†‡	*<0.001*
BMI (kg/m^2)	25.1 ± 5.6‡	26.8 ± 6.7†	32.7 ± 8.2†‡	*<0.001*
Body fat (%)	34.3 ± 7.3‡	36.3 ± 9.1†	42.2 ± 7.2†‡	*<0.001*
Waist circumference (cm)	80 ± 14*‡	88 ± 17*†	101 ± 17†‡	*<0.001*
Fasting glucose (mmol/L)	4.1 ± 0.5‡	4.4 ± 0.5†	4.9 ± 0.5†‡	*<0.001*
Fasting Insulin (mU/L)	4.5 ± 1.0*‡	8.7 ± 2.4*†	20.3 ± 6.5†‡	*<0.001*
HOMA-IR	0.79 ± 1.87*‡	1.68 ± 0.43*†	4.50 ± 1.55†‡	*<0.001*
HOMA2 % B	95.6 ± 51.4	125.8 ± 42.6	213 ± 89.5	*<0.001*
HOMA2 % S	226.3 ± 77.8	113.9 ± 29.1	47.2 ± 15.48	*<0.001*
TG (mmol/L)	0.65 ± 0.30‡	0.69 ± 0.31†	0.87 ± 0.44†‡	*<0.001*
TC (mmol/L)	3.9 ± 1.0	3.9 ± 0.7	3.8 ± 1.0	0.499
HDL-C (mmol/L)	1.4 ± 0.5‡	1.4 ± 0.5†	1.2 ± 0.3†‡	*0.030*
LDL-C (mmol/L)	2.4 ± 0.9	2.2 ± 0.7	2.3 ± 0.8	0.359
TC/HDL-C	3.6 ± 4.2	3.6 ± 4.9	3.5 ± 1.1	0.989
TG/HDL-C	0.66 ± 1.17	0.62 ± 0.57	0.82 ± 0.49	0.276
Systolic BP (mmHg)	105 ± 13*‡	111 ± 15*	112 ± 11‡	*<0.001*
Diastolic BP (mmHg)	69 ± 10*‡	75 ± 10*	76 ± 9‡	*<0.001*

BMI, body mass index; HOMA-IR, homeostasis model insulin resistance (248);HOMA 2 % B, homeostasis model 2 beta cell function (249); HOMA 2 % S, homeostasis model 2 sensitivity (249); TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; BP, blood pressure. Values are expressed as means ± standard deviation. Data and p-values are unadjusted. *P<0.05 between Tertiles 1 and 2, †P<0.05 between Tertiles 2 and 3 and ‡P<0.05 between Tertiles 1 and 3.

3.3.2 Frequency of the ATP III and IDF metabolic syndrome components

In the total subject population, the frequency of the metabolic syndrome was 13.9% and 11.0% for the IDF and ATP III criteria, respectively. In the normal weight women the frequency of the metabolic syndrome by either criteria (IDF=3.8%, ATP III=0%) was lower compared to the obese women (IDF=20.8%, ATP III=19.2%). When examining the occurrence of the metabolic syndrome risk components in subjects by HOMA-IR tertiles, two important patterns emerged (Table 3.2). Firstly, there was a gradient of increasing waist circumference and BP across the tertiles (P<0.001 and P=0.024, respectively). Secondly, the occurrence of impaired fasting glucose and low HDL-C was similar in Tertiles 1 and 2, and increased dramatically in Tertile 3 (P=0.017 and P=0.006, respectively). This pattern was also seen in triglyceride levels although it was not significant (P=0.181), due to the rarity of hypertriglyceridemia. The overall prevalence of impaired fasting glucose (3.9%) and elevated triglyceride level (3.9%) was remarkably low, even in Tertile 3 (9.1% and 6.7%, respectively). The overall occurrence of the metabolic syndrome followed a similar pattern and increased three-fold in Tertile 3 (>20%) compared to Tertiles 1 and 2 (<10%) (P=0.003 and P=0.006, for ATP III and IDF respectively).

Table 3.2. Percentage of individuals presenting with components of the metabolic syndrome according to the ATP III and IDF criteria

	All subjects (N=225)	Tertile 1 HOMA-IR ≤1.07 (N=75)	Tertile 2 HOMA-IR =1.08-2.59 (N=74)	Tertile 3 HOMA-IR ≥2.60 (N=76)	Linear Trend P-value	X^2 P-value
Waist circumference (cm)						
ATP III: ≥88	49.4	25.3	43.4	77.6	*0.001*	*<0.001*
IDF: ≥80	61.6	37.3	61.8	85.5	*<0.001*	*<0.001*
Fasting plasma glucose (mmol/L)						
IDF and ATP III: ≥5.6	3.9	1.3	1.3	9.1	*0.013*	*0.017*
Lipids (mmol/L)						
IDF and ATP III, TG: ≥1.7	3.9	2.7	1.3	6.7	0.193	0.181
IDF and ATP III, HDL-C: ≤1.29	46.0	38.9	37.8	64.9	*<0.001*	*0.006*
Blood pressure (mmHg)						
IDF and ATP III: ≥130/85	20.7	12.0	23.7	25.0	*0.024*	0.139
Metabolic syndrome (%)						
ATP III (≥3 components)	11.0	5.3	6.7	21.1	*<0.001*	*0.003*
IDF (central obesity ≥2 components)	13.9	6.8	10.7	23.7	*0.002*	*0.006*

ATP III, National Cholesterol Education Program (Adult Treatment Panel III); IDF, International Diabetes Federation; TG, triglycerides; HDL-C, high density lipoprotein cholesterol.

3.3.3 Sensitivity and specificity of the ATP III and IDF metabolic syndrome criteria

We subsequently investigated the sensitivity and specificity of the ATP III and IDF metabolic syndrome criteria for predicting the highest level of HOMA-IR (Tertile 3) and the agreement between IDF and ATP III criteria (Table 3.3). The sensitivity for predicting the upper tertile of HOMA-IR was higher for the ATP III than the IDF criteria (62% vs. 56% respectively) although the specificity did not differ. The positive predictive value of the IDF criteria was slightly higher than that of the ATP III criteria (23% vs. 20%). Despite these differences, the ability of both the ATP III and IDF metabolic syndrome to predict insulin resistance (HOMA-IR Tertile 3) was very poor (κ=0.15, 0.14 respectively). As the low HDL-C levels may be an artifact of low TC levels in this population, when the ATP III and IDF HDL-C cut-points were replaced with the TC/HDL-C cut-point (>4.4 mmol/L) (423). Using this cut-point, the sensitivity of both metabolic syndrome criteria to predict HOMA-IR improved by almost 30%, but remained low. The agreement between a TG/HDL-C ratio of >3.0 mmol/L, which has been associated with insulin resistance (292), and the upper tertile of HOMA-IR was also very poor (κ=0.03). However, the ability of VAT and waist circumference cut-points to predict HOMA-IR was much greater (κ=0.45, 0.40 and 0.31 for VAT, ATP III waist circumference and IDF waist circumference respectively). Further, both VAT and waist circumference cut-points had a higher positive predictive value (40.9%, 78.4% and 86.1% for VAT, ATP III waist circumference and IDF waist circumference respectively). Nonetheless, agreement between the IDF and ATP III metabolic syndrome criteria was very high (κ=0.88).

Table 3.3. The specificity (%) and sensitivity (%) of measures of central obesity and the ATP III and IDF metabolic syndrome criteria for predicting the upper Tertile of HOMA-IR and each other.

Criteria	Sensitivity (%)	Specificity (%)	Positive predictive (%)	Negative predictive (%)	κ
IDF and ATP III metabolic syndrome criteria as predictors of HOMA-IR Tertile 3					
ATP III	61.5	68.7	20.3	93.2	0.15
IDF	56.3	68.7	22.8	90.5	0.14
Waist circumference and VAT cut-points as predictors of HOMA-IR Tertile 3					
ATP III waist (>88 cm)	55.4	85.2	78.4	66.2	0.40
IDF waist (>80 cm)	48.6	87.4	86.1	51.6	0.31
VAT (>100 cm^2)	64.3	69.8	40.9	85.7	0.45
IDF and ATP III metabolic syndrome criteria adjusted to include TC/HDL-C ratio (>4.4 mmol/L) rather than HDL-C					
ATP III	85.7	68.5	15.2	98.7	0.16
IDF	85.7	68.5	15.2	98.7	0.16
IDF compared to ATP III metabolic syndrome criteria					
IDF vs. ATP III	100.0	97.0	81.3	100.0	0.88

K, kappa; ATP III, National Cholesterol Education Program (Adult treatment panel III); IDF, International Diabetes Federation; HOMA-IR, homeostasis model of insulin resistance (248); VAT, visceral adipose tissue; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol.

3.3.4 Body fat distribution and the metabolic syndrome

Since body fat and its distribution may impact the risk factors for the metabolic syndrome as well as HOMA-IR, the body fat distribution of the subjects was investigated by HOMA-IR tertiles (Table 3.4.). After adjustment for total body fat percent, VAT, SSAT and DSAT areas increased with increasing tertile, whereas DXA derived peripheral fat mass (kg) was not different between the tertiles.

Table 3.4. Body fat distribution of the subjects according to tertiles of insulin resistance (HOMA-IR).

	Tertile 1 HOMA-IR ≤1.07 (N=75)	Tertile 2 HOMA-IR=1.08-2.59 (N=74)	Tertile 3 HOMA-IR ≥2.60 (N=76)	Total model ANOVA P-value
VAT (cm^2)	63.7 ± 5.1‡	69.6 ± 4.7†	81.1 ± 4.6†‡	*0.031*
SSAT (cm^2)	207.3 ± 8.5*‡	211.4 ± 8.5*†	226.2 ± 8.3†‡	0.048
DSAT (cm^2)	129.9 ± 9.3*‡	150.1 ± 8.5*†	170.8 ± 8.3†‡	*0.002*
Peripheral fat mass (kg)	16.6 ± 0.4	16.2 ± 0.4	15.3 ± 0.4	0.695

VAT, visceral adipose tissue; SSAT, superficial subcutaneous adipose tissue area; DSAT, deep subcutaneous adipose tissue area. Data and P-values are adjusted for body fat (kg). Corresponding superscripts indicate a significant difference between the HOMA-IR tertiles (P<0.05). *P<0.05 between Tertiles 1 and 2, †P<0.05 between Tertiles 2 and 3 and ‡P<0.05 between Tertiles 1 and 3.

In order to explore the contributions of the different adipose tissue depots to a positive diagnosis of the metabolic syndrome, the abdominal CT-derived cross-sectional areas were converted into masses and tSSAT (kg) was calculated, independently of VAT and SAT mass to avoid collinearity. In logistic regression analysis, VAT (kg), and tSSAT (kg) were significant independent contributors to a positive diagnosis of the metabolic syndrome by IDF (P<0.001) and ATP III (P<0.001) criteria. Within the model, VAT (kg) was the largest contributor for both ATP III and IDF criteria (odds ratio: 1.9, 95% CI: 1.3-2.7, P<0.001 and odds ratio: 2.1, 95% CI: 1.4-3.2, P<0.001, respectively), followed by tSSAT (kg) (odds ratio: 1.1, 95% CI: 1.0-1.2, P=0.006 and odds ratio: 1.1, 95% CI: 1.0-

1.2, P=0.002, respectively). In contrast, DSAT (kg) did not contribute significantly to the model (P=0.13 and 0.10, for ATP III and IDF respectively).

3.3.5 The association between visceral adipose tissue (VAT) and waist circumference

As VAT was the most significant determinant of the metabolic syndrome, VAT area was regressed against waist circumference as a means of illustrating the relationship between the physiological marker of risk (VAT) and its clinical proxy (waist circumference) (Figure 3.1). Although waist circumference and VAT area were significantly correlated, the association was extremely variable. For example, VAT ranged from 30-90 cm^2 for individuals with a waist circumference of 80 cm. Similarly, for individuals with a VAT of 100 cm^2, waist circumference ranged from 92-119 cm. Agreement between waist circumference and VAT cut-points was stronger using the ATP III compared to the IDF waist cut-point (k=0.52 and k=0.38 respectively). Approximately 24% of the subjects met both waist circumference (IDF and ATP III) and VAT cut-points. Of the subjects that met both waist circumference and VAT cut-points, 65% were in HOMA-IR Tertile 3. Despite this, waist circumference by either IDF or ATP III criteria, was a good predictor of HOMA-IR (IDF sensitivity=48.6%, specificity=87.4%, positive predictive value=86.1%, and ATP III sensitivity=55.4%, specificity=85.2%, positive predictive value=78.4%). A VAT cut-point of >100 cm^2 was a more sensitive predictor of HOMA-IR, although it had a low positive predictive value (sensitivity=64.3%, specificity=69.8%, positive predictive value=40.9%).

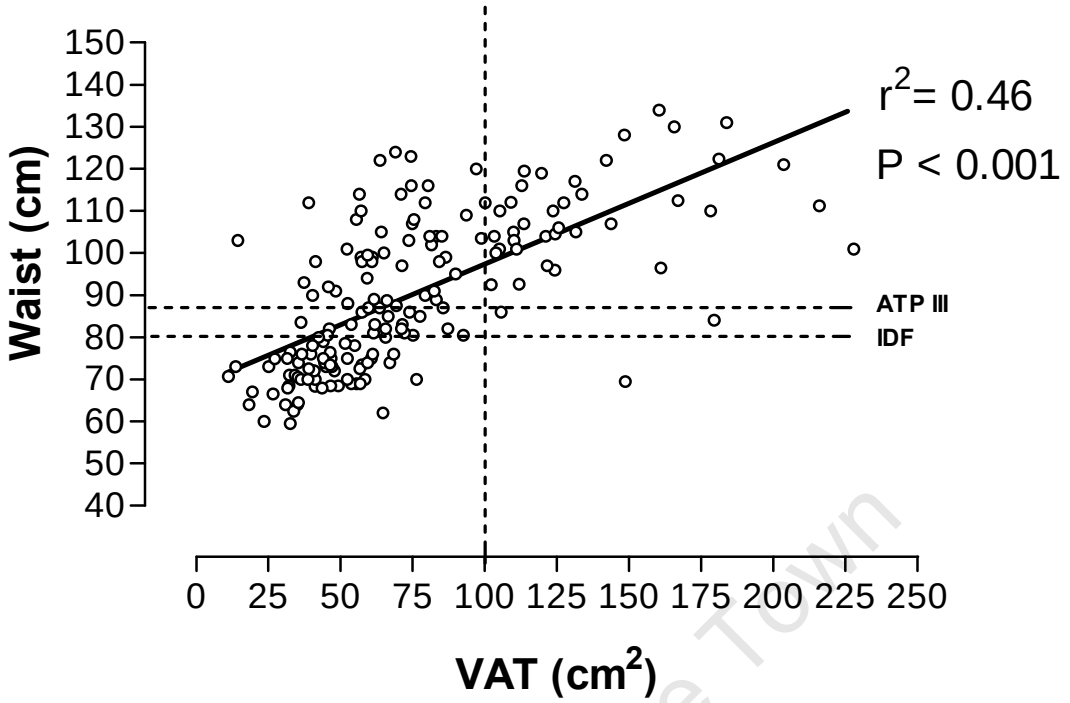

Figure 3.1. Correlation of waist circumference (cm) and VAT area (cm^2). Suggested ATP III and IDF metabolic syndrome waist circumference cut-points for central obesity are indicated by the dotted lines (88 cm and 80 cm respectively). A VAT cut-point of 100 cm^2 (229) is also indicated by a dotted line.

3.4 DISCUSSION

The main findings of this chapter were that although there was a high level of agreement between the IDF and ATP III criteria (κ=0.88), neither set of criteria were able to adequately detect insulin resistance, as estimated by HOMA-IR (κ=0.14) in this cohort of relatively young black women. This may be explained by the atypical clustering of risk factors in the black African women compared to that suggested by the ATP III and the IDF expert panels (154;155). In fact, cut-points for waist circumference (ATP III, κ=0.40 and IDF, κ=0.31) and VAT (κ=0.45) were better indicators of HOMA-IR than the metabolic syndrome criteria.

The metabolic syndrome stems from the premise that a cluster of related risk factors (glucose intolerance, high TG levels, low HDL-C levels and elevated BP) occur more commonly in insulin resistant/hyperinsulinemic individuals, that increase CVD risk (13). Although insulin resistance (HOMA-IR Tertile 3) was associated with increased waist circumference, high fasting glucose and TG levels, low HDL-C levels and elevated blood pressure in this sample of young black SA women , the ATP III and IDF metabolic syndrome diagnostic criteria had a low sensitivity (62%) and specificity (69%) for identifying insulin resistance. Only 21.1 and 23.7% of insulin resistant women in this study were diagnosed with the metabolic syndrome using the ATP III and IDF criteria, respectively. Although the sensitivity and specificity for measuring insulin resistance was low in our study population, it was higher than that reported in apparently healthy Caucasians. For example, Cheal et al. (313) reported a 46% sensitivity of the ATP III metabolic syndrome criteria to identify insulin resistance (as measured by steady state plasma glucose from an insulin suppression test); while Liao et al. (314) found that the sensitivity of ATP III metabolic syndrome criteria to identify insulin resistance (as measured by euglycemic-hyperinsulinemic clamp) was 20%.

Similarly two separate studies in African Americans reported lower sensitivity (30-36%), but much higher specificity (90-96%) for diagnosing insulin resistance using the ATP III

criteria (170;424). Furthermore, the positive predictive value for insulin resistance using ATP III criteria was dramatically lower (20%) in our study compared to the African American studies (85%). These differences may relate to the relative youth of our subjects, the method of quantifying insulin resistance (Si vs. HOMA-IR) or the inclusion of both genders in the African American studies. Conversely, in this study, waist circumference cut-points had a 20% higher specificity and a 66% greater positive predictive value for determining HOMA-IR compared to the metabolic syndrome. The majority (77.6% and 85.5% for ATP II and IDF, respectively) of insulin resistant subjects were identified using the metabolic syndrome waist cut-point alone, suggesting that waist circumference may be a better indicator of insulin resistance in young black South African women than the metabolic syndrome criteria. Therefore, in keeping with reports in African Americans (170;424), we demonstrated that the clinical criteria used to diagnose the metabolic syndrome may not be a dependable measure of insulin resistance, and hence CVD risk in black Africans. Moreover, these findings suggest that insulin resistance is not the primary pathogenic feature of the syndrome in this population.

The low sensitivity and specificity of the metabolic syndrome in predicting insulin resistance in this study may relate to ethnic differences in the prevalence of the different components of the metabolic syndrome. Indeed, it has been consistently shown that black South African women have low lipid levels compared to their Caucasian counterparts (86). In the present study, more than 45% of the subjects (65% insulin resistant subjects in Tertile 3 and 38% in Tertiles 1 and 2) had HDL-C levels below the ATP III and IDF cut-point, but, HDL-C levels were low, possibly as an artifact of low total cholesterol (86), rather than being indicative of increased disease risk**.** In the 1970's, a similar phenomenon was reported in Tarahumara Indians, who had very low HDL-C and low TC levels, but presented with no CVD (425). Consequently we used the TG/HDL and TC/HDL-C ratios as measures of risk and found that only 0% and 20% of the insulin resistant women exceeded the suggested cut-points of >3 and <4.4, respectively (292;423). Further, when TC/HDL-C ratio was substituted for HDL-C in the metabolic syndrome criteria, the sensitivity of both the ATP III and IDF definitions improved by almost 30% (86%), although agreement with HOMA-IR remained poor (κ=0.16).

Furthermore, Sumner et al. (19) have consistently shown that TG levels in African Americans are not reliable markers of insulin resistance. Although we demonstrated a marked difference in TG levels in the insulin resistant subjects (Tertile 3) compared to the subjects in Tertiles 1 and 2, the relationship between HOMA-IR and TG levels was weak (r=0.22, P=0.001). Only 7% of the insulin resistant subjects met the IDF and ATP III metabolic syndrome cut-point for TG levels, which could result in under-diagnosis of insulin resistance using the metabolic syndrome criteria in black South African women. Sumner and Cowie (19) also found a positive association between TG levels and HOMA-IR in African Americans, Caucasian Americans and Hispanics, but higher HOMA-IR values were observed for lower TG levels in the African Americans compared to other ethnicities. They further found that although elevated TG levels were indicative of insulin resistance, the converse was not true in African Americans, resulting in an under-diagnosis of the metabolic syndrome (19). Genetic studies in Caucasian and black African men suggest that racial differences in lipid levels, such as TG and HDL-C, are at least partially related to genetic differences. For example, in a small study by Vega et al. (426), the protective allele of the -514T/C polymorphism in the hepatic lipase gene (*LIPC*) which was associated with lower levels of hepatic lipase activity and increased HDL-C levels, was three times more common in black African (N=43) compared to Caucasian men (N=45) between the ages of 20 and 40 years.

The atypical presentation of risk in black South African women could be related to the relatively low levels of VAT reported in African compared to white women (173). In agreement with previous research, the black South African women in this study had lower levels of VAT relative to values reported in studies consisting of white women. For example, the insulin resistant black South African women had a VAT of 86 cm^2 at a BMI of 32.7 kg/m^2, whereas Lovejoy et al. (173) reported a VAT of 117 cm^2 at a BMI of 29.6 kg/m^2 in premenopausal white women. Lower VAT levels at similar levels of adiposity in black African women (173) may limit the usefulness of metabolic syndrome criteria in this population in two ways: i) altering the lipid profile, complicating the use of triglyceride and HDL-C cut-points as described above; and ii) confounding the association between VAT and waist circumference, reducing the applicability of waist circumference cut-points.

Despite lower levels of VAT, elevated waist circumference was the most prevalent and important component of the metabolic syndrome in the black South African women. In the regression analysis, VAT was a much larger contributor to a positive diagnosis of the metabolic syndrome than DSAT or tSSAT. However, the relationship between VAT and waist circumference was highly variable. In fact, for a particular waist circumference, VAT ranged almost 200 cm^2, and for a particular VAT area, waist circumference ranged up to 65 cm. This variability could make determination of ethnic-specific waist circumference cut-points problematic in this population.

When the subjects who were centrally obese according to waist circumference (>80 cm) and VAT (>100 cm^2) (229) cut-points were compared to those who had large waist circumferences (>80 cm) but low levels of VAT (<100 cm^2), subjects with both a large VAT area and waist circumference were older and had higher triglyceride levels, BP and HOMA-IR. After adjustment for age, these differences in metabolic outcomes remained, highlighting the additional negative impact of increased VAT level on health in centrally obese black South African women. Although centrally obese women with lower levels of VAT were at reduced risk compared to those with high VAT, centralisation of fat still conferred risk, in that serum triglyceride levels, BP and HOMA-IR were elevated compared to the women who were not centrally obese. Therefore, in terms of health risk appraisal, waist circumference is a good indicator of disease risk in this population, regardless of the variability in VAT level. In fact, when waist circumference (>80 cm) and VAT (>100 cm^2) cut-points were compared as indicators of insulin resistance (>75th percentile of HOMA-IR), waist circumference had a 40% greater positive predictive value-- although the sensitivity and specificity were higher for the VAT cut-point (15 and 7% respectively).

In summary, despite the high level of agreement between the IDF and ATP III metabolic syndrome criteria, both were poor predictors of HOMA-IR in young black South African women. This is likely because the metabolic syndrome criteria were primarily developed for white populations and do not take into account ethnic differences in lipids and the relatively low levels of VAT found in black African women. Although VAT was a stronger predictor of a positive diagnosis of the metabolic syndrome than DSAT or

tSSAT, there was large variability in the relationship between VAT and waist circumference. However, further analysis revealed that although increased levels of VAT influenced the degree of disease risk in centrally obese women, waist circumference was still a good indicator of risk in young black South African women. In fact, due to the atypical presentation of the metabolic syndrome criteria in this population, central obesity (waist circumference >80 cm and >88 cm), was a better indicator of insulin resistance compared to IDF or ATP III metabolic syndrome criteria.

This raises questions regarding the clinical significance of the metabolic syndrome criteria, and suggests that in black South African women, the current metabolic syndrome diagnostic criteria may not be greater than the sum of its parts. Further, waist circumference was a sensitive marker of insulin resistance in young black South African women without known disease. Although the data in this chapter are cross-sectional, increased waist circumference has previously been reported to be a predictor of CVD risk factors in longitudinal studies in diverse ethnic groups (427). This finding has important implications for public health in black African women, particularly in countries with limited resources, as waist circumference is an easily measurable risk factor for disease that does not involve blood sampling. In order to better identify individuals at risk of CVD, further research using prospective studies are required to determine ethnic-specific cut-points for waist and lipid levels indicative of risk in black African women.

CHAPTER FOUR

DETERMINANTS OF INSULIN RESISTANT AND INSULIN SENSITIVE PHENOTYPES IN NORMAL WEIGHT AND OBESE BLACK SOUTH AFRICAN WOMEN

Note: This chapter was accepted for publication in Obesity Research

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4.1 INTRODUCTION

In Chapter Three of this thesis, only 13% of the subjects were classified as having the metabolic syndrome using the ATP III and IDF criteria, despite high levels of adiposity and insulin resistance, as estimated by HOMA-IR. As a result of these and other similar criteria being used to identify CVD risk, a high prevalence of "healthy obesity" has been described in black South African women (171;172). Although the association between obesity and increased risk of metabolic and cardiovascular disease is well characterised (38), not all obese individuals present with cardiovascular risk factors. As such, a subset of apparently healthy obese individuals (BMI ≥30 m/kg^2) described as "metabolically healthy obese" (MHO) has been identified. The criteria used to define this obese sub-group are not universally consistent (171;172;237;267;323;325) but they are typically characterized according to cut-points for insulin sensitivity (HOMA-IR <1.95, equivalent to a glucose infusion rate of 8.0 mg/min/kg fat-free mass from euglycaemic hyperinsulinemic clamp studies) (269;327) and lipid levels (triglyceride level ≤1.7 mmol/L, TC ≤5.2 mmol/L, HDL-C ≥1.3 mmol/L and LDL-C ≤2.6 mmol/L) (326). More recently, MHO have been identified using the highest 25% of HOMA-IR (237) and criteria have expanded to include cut-points for inflammatory markers (fibrinogen <4.00 g/L and white blood cell count <10 000 cells/dL) (325). Using a combination of these criteria, North American and European studies suggest that up to 37% of obese individuals can be classified as MHO (237;323;325;327).

The concept of "healthy obesity" is not new to South Africa. In a sample of 50 urban and 40 rural obese black women, Walker et al. (171;172) reported that 78% of the former and 87% of the latter were considered "healthy" based on the prevalence of less than two adverse metabolic sequelae (BP ≥160/95 mmHg, TC ≥5.2 mmol/L, triglyceride level ≥1.8 mmol/L, glucose level ≥7.8 mmol/L). These obese women's apparent "health" was attributed to their low socio-economic status, low fat intake (171) and in rural women, high levels of physical activity associated with manual labour (172).

Relatively low levels of VAT and a preferential distribution of fat in the periphery in black compared to white women (130;205) might be an additional explanation for the 'healthy obese' phenotype in black South African women. Indeed, centralisation of body fat is an integral component of the metabolic syndrome (38;130) and is a major determinant of the obese 'at-risk' metabolic phenotype (268;270;271;323;327). Conversely, peripheral adiposity, with larger hip and thigh circumferences, is associated with improved glucose tolerance and reduced incidence of type 2 diabetes (197).

Obesity however is not a prerequisite for metabolic disease risk. An 'at-risk' normal weight phenotype has also been described and termed "metabolically obese, but normal weight" (MONW) (324;333). These individuals, characterized by cut-points for HOMA-IR as a marker of risk, accounted for 8–18% of the normal weight white population (237;326). When metabolic syndrome criteria (ATP III) (154) were applied to a large (N=3747) cross-sectional study, 11% of normal weight African American and Hispanic American women and 6% of white women were classified as MONW (332). In a longitudinal study, Meigs et al. (237) reported a similar prevalence in white individuals (8%). In South Africa in the 1980's, 18% of 50 urbanized normal weight black women were found to be 'at risk' as they presented with two or more adverse metabolic sequelae (171). In white populations, increased body fatness and centralisation of body fat distinguished MONW individuals from their insulin sensitive counterparts (268).

Factors underlying 'healthy' and 'at-risk' metabolic phenotypes in both normal weight and obese individuals have yet to be comprehensively described in black African women, who do not typically present with the same constellation of features of the metabolic syndrome as white women, as described in Chapter Three of this thesis. Since ethnic-specific cut-points for lipid levels indicative of risk have yet to be determined for black African women, this study aimed to identify factors distinguishing 'healthy' and 'at-risk' metabolic phenotypes in normal weight and obese urban black South African women, based on insulin resistance.

4.2 METHODS

4.2.1 Subjects

This cross-sectional study consisted of 103 normal weight (BMI ≤25 kg/m^2) and 122 obese (BMI ≥30 kg/m^2) premenopausal urban black South African women. Three of the obese subjects were classified as having type 2 diabetes mellitus based on a fasting glucose level of ≥7.0 mmol/L. As exclusion of these subjects did not change the findings of the study, they were included in the analysis. Further information on the subject recruitment strategy as well as inclusion and exclusion criteria are described in the methods section of Chapter Three. Permission to undertake the study was obtained from the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. Written informed consent was obtained from all subjects prior to participation.

4.2.2 Body composition assessment and measurement of the metabolic outcomes

Body composition (weight, height and DXA scan), body fat distribution (waist circumference, hip circumference and CT scan), BP plasma glucose, serum insulin, FFA, triglyceride, TC, HDL-C and LDL-C levels were measured. Insulin resistance was assessed using HOMA-IR (248). The measurement of these variables is described in detail in the methods section of Chapter Three of this thesis. Additionally, VAT/leg fat mass (cm^2/kg) was used to characterize body fat distribution pattern. This variable was utilized since increased VAT is associated with insulin resistance and other CVD risk factors (428) and increased leg fat mass has been shown to be metabolically protective (194;293).

4.2.3 Questionnaires

A demographic questionnaire was administered and included measures of socio-economic status (on the basis of factors such as asset index, education, housing density, and occupation), as well as questions relating to family medical history, personal health, reproductive history and life events. Women were classified as being either employed, unemployed or students. PAEE was characterized using the Global Physical Activity Questionnaire (GPAQ) using standard GPAQ criteria (429). These questionnaires are included in the Appendix of this thesis.

4.2.4 Measuring insulin resistance

Insulin resistance was selected as the determinant of the MHO and MONW phenotypes based upon its previous use in similar studies (267;268;270;327) and suggestions that lipid levels are a poor indicator for risk in an African American population (169;170). Additionally, HOMA-IR has been shown to be an indicator of type 2 diabetes risk in African individuals in a longitudinal study (275). Previous studies have identified MONW and MHO using HOMA-IR cut-points of 1.69 (270), 1.95 (267) and the upper and lower quartiles of HOMA-IR (237;271). When the data were analyzed according to these different identification criteria, although the proportion of MONW (29%, 22% and 8%, respectively) and MHO (33%, 38% and 59%, respectively) varied, the main determinants of the phenotypes remained the same. Therefore, a cut-point of 1.95 for HOMA-IR based on a glucose disposal cut-point of 8.0 mg/min/kg fat-free mass, derived from euglycaemic hyperinsulinemic clamp data from a large multi-centre study (269) was used to determine risk. As such, the groups were termed ‘insulin sensitive’ and ‘insulin resistant’. The same cut-point was used to identify insulin resistance in both the normal weight and obese subjects for purposes of comparison between the phenotypes.

4.2.5 Statistical analysis

The data was analyzed using the STATISTICA Version 7 statistical program (StatsSoft Inc., Tulsa, OK, USA) and expressed as unadjusted means ± SD. Data were normalised by log transformation if required. Differences between the four phenotypes were determined by one-way analysis of covariance (ANCOVA). The obese women were significantly older than the normal weight women; therefore all analyses including the obese phenotypes were age-adjusted. Comparisons between the normal weight and obese phenotypes were adjusted for fat mass (kg) where appropriate. Chi-Square analysis was used to determine categorical differences between the groups. PAEE data were analysed using suggested cut-points for total PAEE $\geq$150 minutes/week (430) and vigorous PAEE $\geq$60 minutes/week (431). Analysis was completed separately for the active (total PAEE $\geq$150 min/week) and sedentary (total PAEE $\leq$150 min/week) subjects, as well as for all of the subjects combined. Since there were no differences in the results, the combined data was used in the analysis and presented. Leisure MET/minutes/week (metabolic equivalents*minutes/week, moderate intensity and above, $\geq$3 METS), vigorous MET/minutes/week ($\geq$6 METS) and total MET/minutes/week of PAEE were log transformed and analyzed by one-way ANOVA. A forward stepwise linear regression model determined which variables explained unique variance in HOMA-IR in the normal weight and obese subjects. Variables that were significantly different between the metabolic phenotypes, and that were not co-linear were investigated. Statistical significance was accepted as $P<0.05$.

4.3 RESULTS

4.3.1 Basic subject characteristics

Twenty two percent of the normal weight women were classified as insulin resistant and 38% of the obese women were classified as insulin sensitive. The basic characteristics and body composition and of the subjects are presented below in Table 4.1.

Table 4.1. Basic characteristics of the insulin sensitive and insulin resistant normal weight and obese black South African women.

	Normal weight (BMI ≤25 kg/m^2)		Obese (BMI ≥30 kg/m^2)		Total model ANOVA
	Insulin sensitive (N=80)	Insulin resistant (N=23)	Insulin sensitive (N=46)	Insulin resistant (N=76)	P-value
Age (years)	25 ± 6§	23 ± 3‡	33 ± 8†§	28 ± 8†‡	*<0.001*
Height (cm)	160 ± 6	161 ± 6	160 ± 6	160 ± 6	0.904
Weight (kg)	57.3 ± 6.3§	59.0 ± 6.5‡	88.2 ± 11.3†§	95.9 ± 15.4†‡	*<0.001*
BMI (kg/m^2)	22.4 ± 2.4§	23.0 ± 2.5‡	34.5 ± 4.4†§	37.5 ± 6.0†‡	*<0.001*
Fat mass (kg)	16.8 ± 3.8§	18.1 ± 4.1‡	37.6 ± 6.4§	41.2 ± 9.9‡	*<0.001*
FFSTM (kg)	37.1 ± 3.9§	37.5 ± 3.4‡	44.0 ± 4.0†§	47.4 ± 4.6†‡	*<0.001*
Body fat (%)	29.8 ± 4.5§	31.2 ± 4.7‡	44.7 ± 3.5§	44.9 ± 4.6‡	*<0.001*

BMI, body mass index; FFSTM, fat free soft tissue mass. Values are expressed as unadjusted means ± SD. Total model P-values are adjusted for age. P-values for comparisons between the obese subjects are adjusted for age. †P<0.05 insulin sensitive obese vs. insulin resistant obese, ‡P<0.05 insulin resistant normal weight vs. insulin resistant obese, §P<0.05 insulin sensitive normal weight vs. insulin sensitive obese.

The obese women were significantly heavier, had greater fat mass and FFSTM and were older than the normal weight women (P<0.001). There were no differences in age, height or body composition between the insulin sensitive and insulin resistant normal weight women. In the obese subjects, the insulin resistant women were older and heavier than their insulin sensitive counterparts. However, after adjustment for age, there were no differences in fat mass or body fat percent between the obese phenotypes.

4.3.2 Body fat distribution

The body fat distribution patterns of the subjects are presented in Figure 4.1 and Table 4.2. Centralisation of body fat as measured by waist circumference, VAT and VAT/leg fat mass (cm^2/kg) distinguished between the insulin sensitive and insulin resistant normal weight women. Similarly, all measures of central fat mass including anthropometric (waist and hip circumferences), DXA-derived measures (central fat mass), CT-derived measures (VAT) and VAT/Leg fat mass (cm^2/kg) distinguished between the obese insulin sensitive and insulin resistant subjects ($P<0.001$).

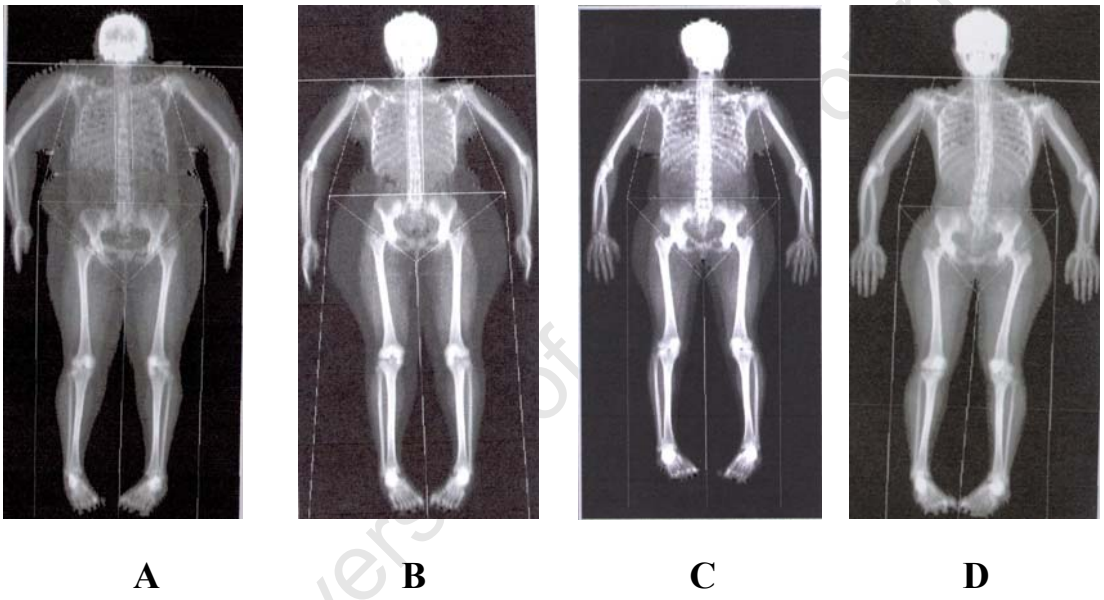

Figure 4.1. Examples of the body fat distribution patterns shown by DXA scan in black South African women typical of the; A) obese insulin resistant phenotype (49% body fat), B) obese insulin sensitive phenotype (47% body fat), C) normal weight insulin resistant phenotype (29% body fat), and D) normal weight insulin sensitive phenotype (26% body fat).

Notably, after adjustment for age and fat mass, VAT was not different between the normal weight and obese insulin resistant subjects (P=0.072), nor the normal weight and obese insulin sensitive subjects (P=0.474). In combined analysis of the normal weight and obese subjects, insulin sensitive subjects had increased leg fat mass compared to insulin resistant subjects, after adjusting for total adiposity (13.5 kg vs. 12.1 kg respectively, P<0.001). VAT/leg fat mass (cm^2/kg) was significantly higher in the insulin resistant vs. the insulin sensitive normal weight and obese women.

Table 4.2. Adipose tissue distribution of the insulin sensitive and insulin resistant normal weight and obese black South African women.

	Normal weight (BMI ≤25 kg/m^2)		Obese (BMI ≥30 kg/m^2)		Total model ANOVA
	Insulin sensitive (N=80)	Insulin resistant (N=23)	Insulin sensitive (N=46)	Insulin resistant (N=76)	P-value
Waist (cm)	82 ± 1*	86 ± 1*‡	89 ± 1†	94 ± 1†‡	*<0.001*
Hip (cm)	108 ± 1	108 ± 1‡	113 ± 1	113 ± 1.‡	*<0.001*
VAT (cm^2)	58.0 ± 5.9*	71.4 ± 7.4*	63.2 ± 6.6†	95.2 ± 5.8†	*<0.001*
SAT (cm^2)	350.5 ± 17.8	352.9 ± 22.5	351.2 ± 19.9	403.0 ± 17.5	*<0.001*
Waist/hip ratio	0.74 ± 0.07§	0.77 ± 0.05‡	0.80 ± 0.08†§	0.84 ± 0.08†‡	*<0.001*
Central fat mass (kg)	12.1 ± 0.3	12.6 ± 0.4‡	12.1 ± 0.3†	13.9 ± 0.3†‡	*<0.001*
Leg fat mass (kg)	13.8 ± 0.3	13.1 ±‡ 0.4‡	13.5 ± 0.3	11.5 ± 0.3‡	*<0.001*
VAT/leg fat mass (cm^2/kg)	5.3 ± 1.9*§	7.0 ± 3.8*	4.6 ± 2.0†§	6.8 ± 3.6†	*<0.001*

*VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; DXA, dual-energy x-ray absorptiometry. Values are expressed as unadjusted means ± SD. Total model P-values are adjusted for age. P-values for comparisons between the obese subjects are adjusted for age, and comparisons between the normal weight and obese subjects are adjusted for age and fat mass. *P<0.05 insulin sensitive normal weight vs. insulin resistant normal weight, †P<0.05 insulin sensitive obese vs. insulin resistant obese, ‡P<0.05 insulin resistant normal weight vs. insulin resistant obese, §P<0.05 insulin sensitive normal weight vs. insulin sensitive obese.*

4.3.3 Metabolic outcomes

By design, insulin resistance, as estimated by HOMA-IR and fasting insulin levels, was different between the insulin sensitive and insulin resistant women (P<0.001). HOMA-IR and fasting insulin levels also differed between the normal weight and obese women (P=0.001). Fasting plasma glucose levels were not different between the normal weight and obese women. Conversely, serum FFA levels were lower in the obese than the normal weight women (P<0.001).

Only 18% of all the women were hypertensive (BP >130/85 mmHg) with no differences between the normal weight and obese groups. Systolic BP was not altered by level of obesity, whereas diastolic BP was higher in the obese than in the normal weight women (P<0.05). Mean TC and LDL-C levels were within the normal range (154) for all of the phenotypes. In contrast, HDL-C levels, as well as the HDL-C/TC ratio, were lower in the obese than the normal weight women (P<0.001).

When the metabolic outcomes were investigated by phenotype (Table 4.3), the insulin resistant normal weight subjects had elevated BP and decreased HDL-C compared to their insulin sensitive counterparts, whereas triglyceride levels were not different. In the obese women, BP was also elevated, but triglyceride levels were higher and HDL-C was not different in the insulin resistant compared to the insulin sensitive subjects. Within the insulin resistant subjects there were no differences in BP or TC, while triglyceride level and HOMA-IR were greater in the obese than the normal weight women. There were no differences in BP, glucose, HDL-C, TC or insulin resistance between the insulin sensitive normal weight and obese women, although triglyceride levels were higher in the latter.

Table 4.3. Metabolic outcomes of the of the insulin sensitive and insulin resistant normal weight and obese black South African women

	Normal weight (BMI ≤25 kg/m^2)		Obese (BMI ≥30 kg/m^2)		Total model ANOVA
	Insulin sensitive (N=80)	Insulin resistant (N=23)	Insulin sensitive (N=46)	Insulin resistant (N=76)	P-value
Blood Pressure (BP) (mmHg)					
Systolic BP	106 ± 11*	113 ± 7*	111 ± 15†	116 ± 16†	*<0.001*
Diastolic BP	69 ± 9*	75 ± 9*	76 ± 10†	81 ± 10†	*<0.001*
Glucose tolerance and insulin sensitivity					
Glucose (mmol/L)	4.21 ± 0.39*	4.43 ± 0.38*	4.27 ± 0.62†	4.91 ± 1.25†	*<0.001*
Insulin (mU/L)	5.40 ± 2.00*	15.23 ± 4.21*‡	5.98 ± 2.06†	19.33 ± 7.32†‡	*<0.001*
HOMA-IR	1.0 ± 0.4*	2.9 ± 0.8*‡	1.1 ± 0.3†	4.3 ± 2.4†‡	*<0.001*
Lipid Profile (mmol/L)					
FFA	0.40 ± 0.21	0.49 ± 0.26‡	0.32 ± 0.16	0.33 ± 0.18‡	*0.002*
Cholesterol	4.0 ± 1.0*	3.6 ± 0.7*	4.0 ± 0.8	3.8 ± 1.0	0.151
HDL-C	1.6 ± 0.4*	1.3 ± 0.4*	1.3 ± 0.5	1.1 ± 0.3	*<0.001*
LDL-C	2.2 ± 0.9	2.0 ± 0.6	2.4 ± 0.6	2.3 ± 0.8	0.758
Triglycerides	0.6 ± 0.3§	0.6 ± 0.2‡	0.8 ± 0.4†§	0.9 ± 0.4†‡	*0.001*

*HOMA-IR, homeostasis model insulin resistance; FFA, free fatty acid; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. Values are expressed as unadjusted means ± SD. Total model P-values are adjusted for age. P-values for comparisons between the obese subjects are adjusted for age, and comparisons between the normal weight and obese subjects are adjusted for age and fat mass. *P<0.05 insulin sensitive normal weight vs. insulin resistant normal weight, †P<0.05 insulin sensitive obese vs. insulin resistant obese, ‡P<0.05 insulin resistant normal weight vs. insulin resistant obese, §P<0.05 insulin sensitive normal weight vs. insulin sensitive obese.*

4.3.4 Physical activity energy expenditure (PAEE)

Only 20% of the subjects met recommended levels of at least 150 minutes of moderate-to-vigorous PAEE each week (430), and there were no differences in the proportion of women that met this cut-point by phenotype (Table 4.4). Even fewer of the subjects (5-7%) completed the recommended level of vigorous PAEE (≥60 minutes/week) (431), and again there were no differences between the phenotypes. However, the insulin sensitive normal weight women performed more leisure time PAEE compared to the other phenotypes (P<0.01), more vigorous PAEE compared to the obese insulin resistant women (P=0.02) and more total PAEE compared to the obese subjects. There were no differences in total or vigorous PAEE between the insulin sensitive and insulin resistant normal weight women, but leisure time PAEE distinguished between these two phenotypes (P=0.02).

Table 4.4. PAEE of the insulin sensitive and insulin resistant, normal weight and obese black South Africa women

	Normal weight (BMI ≤25 kg/m^2)		Obese (BMI ≥30 kg/m^2)		Total model ANOVA
	Insulin sensitive (N=80)	Insulin resistant (N=23)	Insulin sensitive (N=46)	Insulin resistant (N=86)	P-value
Log total PAEE (MET/min/week)	2.40 ± 0.70§	2.04 ± 1.11	1.63 ± 1.20§	1.99 ± 0.90	*0.004*
Log vigorous PAEE (MET/min/week)	0.99 ± 1.18§	0.41 ± 0.97	0.37 ± 0.91§	0.29 ± 0.80	*<0.001*
Log leisure time PAEE (MET/min/week)	1.22 ± 1.15*§	0.34 ± 0.90*	0.43 ± 0.90§	0.35 ± 0.81	*<0.001*
Total PAEE ≥150 min/week (%)	21	26	20	17	0.802
Vigorous PAEE ≥60 min/week (%)	5	8	4	5	0.907

*PAEE, physical activity energy expenditure; BMI, body mass index. Cut-points of ≥150 minutes of moderate PAEE per week and ≥60 minutes of vigorous PAEE per week were used as suggested by Pate et al. (430) and Haskell et al. (431). Data are presented as percentages or as means ± SD of log transformed MET/minutes/week. P-values are adjusted for age. *P<0.05 insulin sensitive normal weight vs. insulin resistant normal weight, §P<0.05 insulin sensitive normal weight vs. insulin sensitive obese.*

4.3.5 Socio-economic and lifestyle variables

Socio-economic status defined by asset index and level of education was similar in the normal weight and the obese women, although more obese women were unemployed (P<0.001). In contrast, when the metabolic phenotypes were compared (Table 4.5), the normal weight insulin sensitive women were more educated and had lower unemployment (P<0.001) compared to their insulin resistant counterparts. These differences in education (P=0.002) and unemployment (P=0.003) were also seen in the obese women. Asset index was higher in the insulin sensitive normal weight women compared to the insulin resistant obese women (P<0.001), and there were no differences in housing density between any of the four phenotypes.

Table 4.5. Socio-demographic characteristics of the insulin sensitive and insulin resistant normal weight and obese black South African women

	Normal weight (BMI ≤25 kg/m^2)		Obese (BMI ≥30 kg/m^2)		Total model ANOVA
	Insulin sensitive (N=80)	Insulin resistant (N=23)	Insulin sensitive (N=46)	Insulin resistant (N=76)	P-value
Housing density (people/bedroom)	1.8 ± 1.1	2.4 ± 1.2	2.2 ± 1.2	2.2 ± 1.4	0.108
Asset index	8.7 ± 3.6	7.0 ± 3.6	7.6 ± 3.6	6.6 ± 2.8	*0.005*
Number of children	0.33 ± 0.58§	0.39 ± 0.78‡	1.6 ± 1.1†§	0.9 ± 1.2†‡	*0.004*
Education % matriculated	81%	39%	60%	44%	*<0.001*
Unemployed (%)	19%	48%	39%	55%	*<0.001*

Values are expressed as unadjusted means ± SD, or percentages. Total model P-values are adjusted for age. P-values for comparisons between the obese subjects are adjusted for age. †P<0.05 insulin sensitive obese vs. insulin resistant obese, ‡P<0.05 insulin resistant normal weight vs. insulin resistant obese, §P<0.05 insulin sensitive normal weight vs. insulin sensitive obese.

Oral and injected contraceptive use differed between the insulin sensitive and insulin resistant phenotypes (22% vs. 47% respectively, P<0.001) but was not different between the normal weight and obese women. Injected contraceptives were more commonly used than oral contraceptives in all groups. (Insulin resistant: 37% injected, 8% oral, Insulin sensitive: 13% injected, 9% oral). Only 6% (N=14) of the women were current smokers,

the majority (N=9) being insulin resistant. Self-reported family history of obesity, type 2 diabetes, hypertension, stroke or heart attack did not differ between the insulin resistant and insulin sensitive women.

4.3.6 Determinants of insulin resistance

Using forward stepwise regression, total adiposity, body fat distribution (VAT/leg fat mass), age, log leisure time PAEE, and use of injectable (but not oral) contraceptives explained 35% of the variance in HOMA-IR in the normal weight women (Table 4.6). When the normal weight subjects who completed ≥150 minutes of PAEE each week (active) were excluded from the regression analysis, these variables remained significant, but accounted for an additional 15% of the variance in HOMA-IR.

Table 4.6. Regression analysis for insulin sensitivity estimated by HOMA-IR in the normal weight women. R=0.63, r^2 =0.40, Adjusted r^2 =0.35, SEE=0.483, P<0.001

Independent Variable	β	B	SEE	P-value
Age (years)	-0.32	-0.036	0.099	*0.002*
Body fat (%)	0.25	0.032	0.101	*0.016*
VAT/leg fat mass (cm^2/kg)	0.38	0.084	0.104	*<0.001*
Log leisure time PAEE (MET/minutes/week)	-0.34	-0.183	0.105	*0.002*
Method of contraception	0.12	0.089	0.108	0.260

HOMA-IR, homeostasis model insulin resistance, β, partial correlation coefficient; B, parameter estimate; SEE, standard estimate of error; VAT, visceral adipose tissue; PAEE, physical activity.

In the obese women, 34% of the variance in HOMA-IR was explained by body fat distribution, total body fat percent, age and use of injected (but not oral) contraception. PAEE was not an important contributor to the model in the obese women (Table 4.7).

Table 4.7. Regression analysis for insulin sensitivity estimated by HOMA-IR in the obese women. R=0.61, r^2 =0.37, Adjusted r^2 =0.34, SEE=0.616, P<0.001

Independent Variable	β	B	SEE	P-value
Age (years)	-0.39	-0.034	0.103	*<0.001*
Body fat (%)	0.17	0.030	0.100	0.087
VAT/leg fat mass (cm^2/kg)	0.60	0.141	0.104	*<0.001*
Method of contraception	0.18	0.140	0.100	0.800

*HOMA-IR, homeostasis model insulin resistance, **β**, partial correlation coefficient; B, parameter estimate; SEE, standard estimate of error; VAT, visceral adipose tissue*

4.4 DISCUSSION

In this sample of 225 apparently healthy, normal weight and obese black South African women, 38% of the obese women were classified as insulin sensitive and 22% of normal weight women were regarded as insulin resistant, estimated by HOMA-IR. The prevalence of the MONW and MHO phenotypes are in agreement with studies that used similar criteria in both young (23-28 years) normal weight white women (268;270) and post-menopausal obese white women (327).

The proportion of 'healthy obese' black South African women in the present sample is considerably lower than that previously described by Walker et al. in the 1980's (171;172) in which 78% of obese women were considered "healthy". This discrepancy simply reflects the difference in 'healthy' criteria applied by Walker and colleagues compared to that used in the present study. Indeed, when Walker's criteria are applied to the present sample, 98% of obese and 100% of normal weight subjects would be considered 'healthy'. This apparent health can be explained by the use of Walker's criteria which are indicative of disease rather than cardiovascular disease risk, as well as the relative youth of the subjects in the present study compared to those in Walker's study.

Application of the more comprehensive Karelis criteria for MHO (HOMA-IR <1.95, triglyceride level ≤1.7 mmol/L, TC ≤5.2 mmol/L, HDL-C ≥1.3 mmol/L and LDL-C ≤2.6 mmol/L) (326) in the present study also did not distinguish between 'at-risk' and 'healthy' phenotypes in the normal weight or obese women (2 individuals identified as 'at-risk'). This is likely due to the dependency of the Karelis criteria on lipid values, which may not distinguish risk in black African populations who have favourable lipid profiles despite high levels of adiposity (174). Current ATP III cut-points for triglyceride and HDL-C levels may not be applicable in black African individuals (19;86;170;290). However, the relationship between lipid levels and CVD in black Africans has not been fully elucidated, and ethnic-specific cut-points have not been determined. Consequently insulin resistance may be a better indicator of risk in black Africans.

The low lipid levels in this and previous studies (169-172;432), may be related in part to the low levels of VAT in black South African women. Previous studies have shown that black South African women have less VAT (174) and a greater peripheral fat distribution (130) than white women. Indeed, on average, the insulin resistant and insulin sensitive obese black women had less VAT (106.6 and 79.3 cm^2, respectively) than reported in similar studies in white women (227-434 cm^2 and 141-179 cm^2 for insulin resistant and insulin sensitive obese women, respectively) (271;327).

Nonetheless, VAT area, or centralisation of body fat, was the single most important determinant of the insulin resistant phenotype, even after adjustment for age and fat mass (Table 4.2). Increased VAT has been suggested to increase metabolic risk via its high lipolytic activity and drainage of FFA into the hepatic system, thereby altering insulin signalling and impairing suppression of hepatic glucose production (433). Alternatively, increased expression of adipokines such as tumour necrosis factor-alpha in VAT compared to SAT may alter insulin sensitivity and increase metabolic risk (173). Conversely, when the normal weight and obese subjects were combined, after adjustment for total body adiposity, peripheral fat deposition (increased leg fat mass) was 'protective' as has been previously shown (197). Peripheral adipose tissue deposition may act as a 'reservoir' for circulating FFA, reducing ectopic fat deposition in muscle, liver and the visceral depot, thereby attenuating insulin resistance and reducing hepatic LDL-C production (195).

In agreement with previous research (327), differences in PAEE did not distinguish between the insulin sensitive and insulin resistant obese phenotypes. However, consistent with the findings of Dvorak et al. (268), lower levels of PAEE were a determinant of the insulin resistant normal weight phenotype. Specifically, leisure time PAEE distinguished between the insulin sensitive and insulin resistant normal weight phenotypes in this study. In addition, the insulin sensitive normal weight women performed more PAEE than the obese women. Indeed, physical inactivity has been implicated in the development of metabolic disease (434). PAEE positively impacts body composition and energy balance (435), and an association between high levels of PAEE and decreased VAT has been demonstrated (329). Despite this, the majority of the subjects did not meet recommended levels of PAEE ($\geq$150 minutes/week of moderate PAEE, or $\geq$60 minutes/week of vigorous

PAEE) (430;431) and PAEE did not explain any of the variance in HOMA-IR in the obese subjects. This may suggest that the role of PAEE in these subjects is one of primary prevention.

In the South African context, lifestyle factors such as contraceptive use are also influenced by socio-economic factors and impacted health. Thirty nine percent of the insulin resistant subjects used injectable contraceptives compared to 13% of the insulin sensitive subjects ($P<0.001$). In Khayelitsha, the informal settlement where most of the subjects in this study reside, 83% of women use injected contraceptives and in the majority (85%) the method of contraception was determined by the health care provider (36). Injected progestin-based contraceptives affect basal insulin levels, may compromise glucose tolerance (12) and result in increased adiposity and central to peripheral fat mass ratio (37). Although, in this study the use of injected contraceptives contributed to the variance in insulin resistance, it was not independent of VAT.

Despite suggestions that family history of metabolic disease and smoking status play an important role in the determination of metabolic risk (327), there was no association between either of these factors and metabolic phenotype in this study. This may be explained by issues such as the break down in family structures due to urbanization and poverty and a lack of access to medical services. The low prevalence of smoking limits its impact on metabolic health in this study. Parity was also not a significant contributor to metabolic risk in this study. Additionally the relative youth of the subjects in this study may impact the prevalence of metabolic disease.

In conclusion, metabolic risk criteria such as ATP III guidelines and the Karelis, and Walker criteria, which are primarily based on lipid levels, did not distinguish between insulin sensitive and insulin resistant women in a young urban black South African population. Thus, measures of insulin sensitivity may be a better indicator of risk in young black African populations. Using insulin resistance as a marker of risk, the major determinants of HOMA-IR included: i) increased centralisation of body fat and reduced peripheral fat distribution; ii) age; iii) decreased leisure time PAEE and iv) the use of progestin-based injected contraceptives, accounting for 35% of the variance HOMA-IR in

the normal weight women. The same factors, with the exclusion of leisure PAEE, accounted for 34% of the variance in HOMA-IR in the obese women. Some of these factors are modifiable and can be targeted to reduce the risk of developing metabolic disease in this population.

CHAPTER FIVE

GLUCOCORTICOID GENE POLYMORPHISMS ARE NOT ASSOCIATED WITH BODY COMPOSITION, BODY FAT DISTRIBUTION OR FEATURES OF THE METABOLIC SYNDROME IN URBANIZED BLACK SOUTH AFRICAN WOMEN: A PRELIMINARY INVESTIGATION

5.1 INTRODUCTION

The previous two chapters demonstrated that central obesity was closely associated with insulin resistance and a positive diagnosis of the metabolic syndrome in black South African women. One of the factors that may underlie the association between central obesity and the metabolic syndrome may be exposure to GC. A case in point is the pathological condition of cortisol excess, Cushing's syndrome, which is characterised by central obesity, hypertension, glucose intolerance and insulin resistance (347). However, circulating cortisol concentrations in obese individuals are normal or even reduced (348). Therefore, research has begun to focus on tissue-specific alterations in cortisol metabolism within adipose tissue.

Local adipose tissue GC metabolism is altered by 11β-HSD-1, a bi-directional enzyme that catalyzes the inter-conversion of active 11-hydroxycorticosteroids (cortisol) and inert 11-keto derivatives (cortisone) (350). *In vivo,* 11β-HSD-1 converts cortisone to cortisol in the presence of the coenzyme NADPH, which is produced in the adipocyte by H6PDH (340;436;437). Cortisol binds to the GR and regulates the transcription of GC responsive genes. Through the regulation of GC responsive genes, (including interleuken-6, tumour necrosis factor-alpha, glucose transporter-4, hormone sensitive lipase and leptin) GR influences adipocyte differentiation, inflammation and glucose homeostasis, thereby impacting obesity, body fat distribution and CVD risk factors (344), as illustrated in Figure 1.7 in Chapter One of this thesis.

Studies have shown that transgenic mice over-expressing 11β-HSD-1 were heavier, more centrally obese, and more insulin resistant compared to wild type mice (354;355). Conversely, 11β-HSD-1 knockout mice were protected from obesity, type 2 diabetes and dyslipidemia even when fed a high fat diet (356;357;438). Studies in humans are less consistent than murine models. However, increased 11β-HSD-1 mRNA levels and enzyme activity in VAT and/or SAT have been associated with obesity (61;349;360-362;439) and VAT accumulation (60;362), as well as features of the metabolic syndrome, including elevated BP, enlarged waist circumference and elevated triglyceride and fasting glucose

levels (60;360-362;364). In contrast, GR mRNA levels in SAT (60) and VAT (60) have been negatively associated with body fatness and VAT (60), suggesting a down-regulation of GR with obesity. However, Boullu-Cioca et al. (365) found that GR-alpha was not down-regulated in VAT with obesity, enabling VAT to retain full capacity to respond to increased local concentrations of cortisol. Although further research is needed, these studies suggest that differences in tissue-specific GC action influence obesity, body fat distribution and CVD risk factors.

Therefore, polymorphisms within genes involved in tissue-specific GC metabolism and their association with obesity and features of the metabolic syndrome have been investigated. Although findings have not always been consistent, polymorphisms within the *HSD11B1*, *H6PD* and *GR* genes have been associated with differences in sensitivity to GC (379;383;387;400), obesity (367;401), centralization of body fat (42) and CVD (42;367;381). For example, an adenine insertion at nucleotide 4436 within intron 3 (Ins4436A) of the *HSD11B1* gene has been associated with increased body mass index (BMI), waist/hip ratio and insulin resistance in overweight African American and white American children (367) as well as with type 2 diabetes in Pima Indians (368). Further, there is a nucleotide substitution in exon 5 of the *H6PD* gene which results in an amino acid substitution of R (arginine) to Q (glutamine). The A allele (Q amino acid) of this nonsynonymous polymorphism has been shown to decrease H6PDH enzyme activity (372;373) which is a crucial determinant of 11β-HSD-1 oxo-reductase activity (436). Finally, the G allele of the functional G>C substitution at nucleotide 646 within intron 2 (*Bcl*I) of the *GR* gene has been associated with hypersensitivity to GC (379;399;400), central obesity (391;401;403), insulin resistance (381) and CVD (404).

These polymorphisms may be of particular relevance in black South African population, since black women have a higher prevalence of obesity and central obesity, as defined by a waist circumference of >88 cm, and have been shown to be more insulin resistant than white women (4;174). However, the association of these polymorphisms with obesity has not been investigated in this population. Therefore, the aim of this preliminary study was to investigate the association of the *Bcl*I, Ins4436A and R453Q polymorphisms within the

GR, *HSD11B1* and *H6PD* genes, respectively, with obesity, body fat distribution and risk factors for CVD in a sample of black South African women.

5.2 METHODS

5.2.1 Subjects

This cross-sectional study consisted of 103 normal weight (BMI ≤25 kg/m^2) and 121 obese (BMI ≥30 kg/m^2) premenopausal urban black South African women. Further information on the subject recruitment strategy as well as inclusion and exclusion criteria are described in the methods section of Chapter Three. Only subjects that were successfully genotyped for the polmorphisms of interest were included in this chapter. Approval to undertake the study was obtained from the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. Written informed consent was obtained from all subjects prior to participation.

5.2.2 Body composition assessment and measurement of the metabolic outcomes

Body composition (weight, height and DXA scan), body fat distribution (waist circumference, hip circumference and CT scan), BP, plasma glucose, serum insulin, FFA, triglyceride, TC, HDL-C and LDL-C levels, as well as insulin resistance, as estimated by HOMA-IR, were measured. The measurement of these variables is described in detail in the methods section of Chapter Three.

5.2.3 DNA extraction

Approximately 5 ml of venous blood was collected from the antecubital vein of the subjects into an ethylenediaminetertaacetic acid (EDTA) vacutainer tube. Samples were stored at 4ºC for no longer than one week until DNA was extracted using a modification of the method of Lahiri and Nurnberger (440). Briefly, the blood samples were transferred into sterile 15 ml polypropylene tubes with 10 ml of TKM1 buffer (10 mM Tris-HCl, pH

7.6; 10 mM KCl; 10 mM $MgCl_2$; 2 mM EDTA) containing 2.5% NP40. After a 10 minute incubation period at room temperature, the samples were centrifuged at 1200X g for 10 minutes at room temperature. The supernatant was discarded and the white pellet was resuspended in 5 ml of TKM1 buffer without NP40 by vortex for 2 minutes. The samples were then centrifuged at 1200X g for 10 minutes at room temperature, the supernatant was discarded and the washed pellet was resuspended in 800 µl of TKM2 buffer (10 mM Tris-HCl, pH 7.6; 10 mM KCl; 10 mM $MgCl_2$; 2 mM EDTA; NaCl 0.4M) containing 0.6% sodium-dodecyl-sulphate by vortexing for 4 minutes. The samples were then incubated at 55ºC in a water bath for 1 hour or until the pellets had completely dissolved, after which 150 µl of 5 mM $NaClO_4$ and 500 µl of analytical grade chloroform were added to the dissolved pellets. After vortexing for 15 seconds, the solution was transferred into sterile 1.5 ml microfuge tubes and centrifuged at 600X g for 10 minutes at room temperature. The top aqueous phase (approximately 500 µl) was transferred to a new sterile microfuge tube, and 1 ml of absolute ethanol was added to precipitate the DNA. The samples were then centrifuged at 600X g for 10 minutes at room temperature, forming a pellet of DNA in the bottom of the tubes. The supernatant was removed and the DNA was left to air dry for up to 10 hours. The DNA was resuspended in 200 µl of Tris-EDTA buffer (10 mM Tris-HCl 10, pH 8.0; 1 mM EDTA). The samples were incubated for 15 minutes at 65ºC and stored at 4ºC until subsequent polymerase chain reaction (PCR) analysis. The detailed protocol and reagent recipes can be found in the Appendix of this thesis.

5.2.4 Genotype analysis

5.2.4.1 HSD11B1

The Ins4436A polymorphism within intron 3 of the *HSD11B1* gene was genotyped as previously described (367) using forward primer 5'- TTG GAG CAG CCT CAG CCC ACT AC-3' and reverse primer 5'-TGT CCC TGT CCC ACT TAC CAG CC-3'. The PCR reaction included 20 pmol of the forward and reverse primers, 20 mM Tris-HCl (pH 8.4), 10 mM KCl, 1.5 mM $MgCl_2$, 20 mM $MgSO_4$, 125 µm each of dATP, dCTP, dTTP and dGTP, and 5 U of *taq* DNA polymerase in a final volume of 40 µl and was amplified

using the BIOER XP Thermal Cycler PCR machine (Bioer technology CO. Ltd. Tokyo, Japan). The PCR conditions consisted of a 2 minute denaturing step at 94ºC, followed by 35 cycles of denaturing for 30 seconds at 94ºC, annealing for 30 seconds at 64ºC and extension for 45 seconds at 72ºC, followed by a final extension at 72ºC for 10 minutes. The 417 bp amplicon was digested with 5 U of *Xcm*1 for 6 hours at 36ºC. In the presence of the adenine insertion at 4436, an *Xcm*1 site was created resulting in fragments of 269 bp, 123 bp and 25 bp for the A allele and 392 bp and 25 bp for the wild type allele (W).

5.2.4.2 H6PD

The R453Q polymorphism within exon 5 of the *H6PD* gene was genotyped using amplification refractory mutation system (ARMS) PCR as previously described (441) with the following four primers (shown in Figure 5.1): outer forward primer (A) 5'-GCT ACG CTC GGA TCT TGT TCA AGA ACC A-3', inner forward primer (B) 5'-CCG ATT ACT ACG CCT ACA GCC CTG TGC A-3', outer reverse primer (C) 5'-TCC AGC AGA GGG GTC CAG AAG TTC CAG G-3' and inner reverse primer (D) 5'-AAG AAG ACG GAG TGG GCG TCC CGC TAC C-3'. The reaction consisted of 10 pmol of each primer, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 125 µm each of dATP, dCTP, dTTP and dGTP, and 5 U of *taq* DNA polymerase in a final volume of 40 µl and was amplified using the BIOER XP Thermal Cycler PCR machine (Bioer technology CO. Ltd. Tokyo, Japan). The PCR conditions consisted of a 5 minute denaturing step at 95ºC, followed by 5 cycles of denaturing for 25 seconds at 95ºC, annealing for 45 seconds at 70ºC and extension for 45 seconds at 72ºC, followed by 27 cycles of denaturing for 25 seconds at 95ºC, annealing for 45 seconds at 65ºC and extension for 30 seconds at 72ºC, followed by a final extension at 72ºC for 10 minutes. Amplicons of 373 bp and 286 bp indicated the presence of the G allele, and amplicons of 373 bp and 142 bp indicated the presence of the A allele.

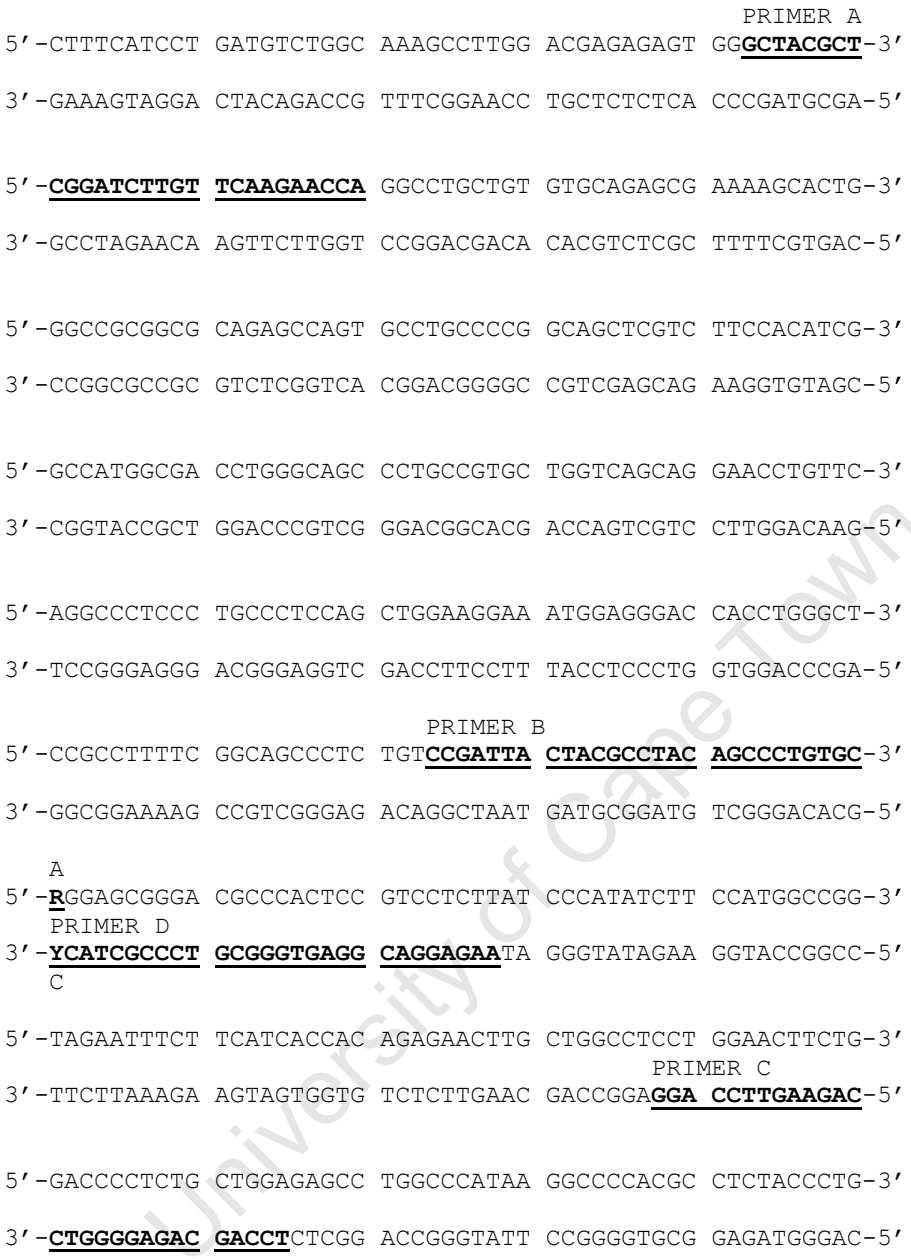

Figure 5.1. The double-stranded nucleotide sequence, obtained from the NCBI database (www.ncbi.nlm.nih.gov), containing the R453Q polymorphism (nucleotide 301 in the sequence). The ambiguous nucleotide R at position 301 is an A or G, while nucleotide Y is a C or T. The four primers in the sequence are in bold and underlined. Specific sequence mismatches introduced into the primers are shown above the sequence.

5.2.4.3 GR

The subjects were genotyped for the *Bcl*I restriction fragment length polymorphism (RFLP) located within intron 2 of the *GR* gene by polymerase chain reaction (PCR) in a final volume of 50 µl as previously described (407) using the PCR express thermo cycler (Hybaid Ltd., Middlesex, UK). Briefly, the DNA sequence was amplified using the following: forward, 5'- AAA TTG AAG CTT AAC AAT TTT GGC-3' and reverse 5'- GCA GTG AAC AGT GTA CCA GAC C-3' primers. The PCR reaction included 20 pmol of the forward and reverse primers, 20 mM Tris-HCl (pH 8.4), 10 mM KCl, 2 mM $MgCl_2$, 20 mM $MgSO_4$, 125 µm each of dATP, dCTP, dTTP and dGTP, and 5 U of *taq* DNA polymerase and was amplified using the BIOER XP Thermal Cycler PCR machine (Bioer technology CO. Ltd. Tokyo, Japan). The PCR conditions consisted of a 5 minute denaturing step at 94ºC, followed by 40 cycles of denaturing for 30 seconds at 94ºC, annealing for 30 seconds at 59ºC, and extension for 45 seconds at 72ºC, followed by a final extension at 72ºC for 10 minutes. The 206 base pair (bp) amplicon (10 µl) was digested with 4 U of *Bcl*I for 6 hours at 50ºC. The G to C transition at nucleotide 646 in intron 2 abolished the *Bcl*I restriction site, resulting in fragments of 116 bp and 90 bp for the G allele and 206 bp for the C allele.

5.2.5 Gel electrophoresis

The PCR products for the *HSD11B1* and *GR* polymorphisms were resolved by electrophoresis on 8% polyacrylamide gels using a 40% Bis-acrylamide/acrylamide mix. The PCR products for the *H6PD* polymorphism were resolved on a 2% agarose gel. All gels were stained for 15 minutes with ethidium bromide (2 µg/ml) for visualisation under ultra-violet light. A 100 bp DNA ladder (Promega G210A) was used as a size marker. Examples of a genotyping gel for the *Bcl*I polymorphism within the *GR* gene, the Ins4436A polymorphism within the *HSD11B1* gene, and the R453Q polymorphism within the *H6PD* gene are shown in Figure 5.2.

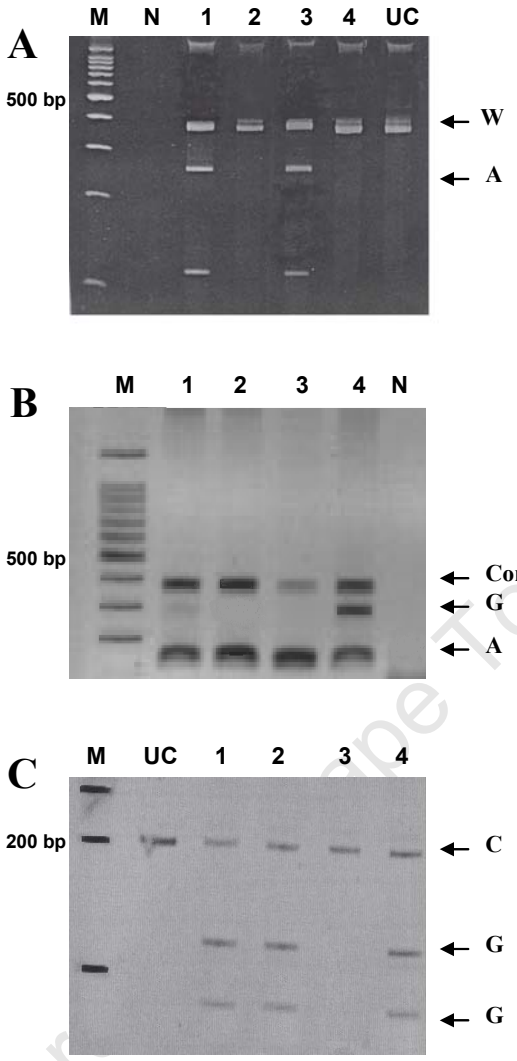

Figure 5.2: Typical genotyping gels demonstrating the sizes of the alleles indicated with arrows obtained for the; (A) Ins4436A polymorphism within the *HSD11B1* gene (392, 269, 123 and 25 bp), (B) R453Q polymorphism within the *H6PD* gene (373, 286 and 142 bp) and (C) *Bcl*I polymorphism within the *GR* gene (206, 116 and 90 bp). M, molecular weight marker with the 500 bp band indicated in A and B and the 200 bp band indicated in C; Con, control band; UC, uncut; N, negative control.

5.2.6 Statistical analysis

The data was analyzed using the STATISTICA Version 7 statistical program (StatsSoft Inc., Tulsa, OK, USA) and GraphPad InStat version 2.05a (GraphPad Software, San Diego CA, USA) statistical programs. Data were expressed as means ± standard deviations (SD) and log transformed when necessary. Chi-square analysis was used to determine differences between the genotype and allele distributions. An independent T-test was used to determine statistical differences in the basic characteristics and metabolic outcomes of normal weight and obese subjects, and the subjects categorized by genotype. Noteably, not all of the subjects were succsefully genotyped for all three of the polymorphisms. Therefore the number of subjects included in the separate genotype analyses differs. Statistical significance was accepted as $P<0.05$. Hardy-Weinberg equilibrium was established using the genepop web version 3.1c, available from http://genepop.curtin.edu.au/.

Initially the data for the normal weight and obese groups were compared. To my knowledge, no studies that have compared the genotype effects of the Ins4436A, R453Q and *Bcl*I polymorphisms within the *HSD11B1*, *H6PD* and *GR* genes respectively between normal weight and obese subjects have reported odds ratios for obesity. Therefore, in order to demonstrate a major genotype effect, an odds ratio of 2.25 for obesity was assumed when estimating the required sample size. Based on this odds ratio and an obesity prevalence of 31.8% (4), the sample size required to show a 5% significance level and 80% power to detect a difference in all three genes between the normal weight and obese women was approximately 110 normal weight and 110 obese subjects.

Subsequently, the data for the normal weight and obese women were combined in order to explore the metabolic effects of extreme ranges in BMI while maintaining a unimodal distribution for all other parameters (body fat percent, waist circumference, VAT, SAT, BP, HOMA-IR and lipid profile). Data were analysed by genotype groups for the Ins4436A, R453Q and *Bcl*I polymorphisms in the *HSD11B1*, *H6PD* and *GR* genes respectively. The sample size required to show a 5% significance level and 80% power to detect a difference was calculated to be approximately 50 subjects per group. This power

calculation was based on the difference in the means and the SD of BMI, waist circumference and VAT by genotype groups reported in previous studies (42;367;401).

5.3 RESULTS

5.3.1 Basic subject characteristics

The majority of the subjects reported being of Xhosa ancestry (76%), with the other 24% being either of Zulu, Tswana, Sotho or of mixed black South African tribal ancestry. There were significantly more women of Xhosa ancestry in the obese compared to the normal weight group (P<0.001). However, neither the exclusion of non-Xhosa subjects nor adjustment for ethnicity altered the results of the study. The obese subjects were older than the normal weight subjects and subsequent analyses were adjusted for age (Table 5.1). By design, the obese subjects were heavier and had higher levels of adiposity compared to the normal weight subjects. Height was not different between the normal weight and obese subjects.

Table 5.1. Descriptive characteristics of the normal weight and obese black South African women.

	Normal weight (BMI ≤25 kg/m^2)	Obese (BMI ≥30 kg/m^2)	P-value
Xhosa ancestry (%)	57%	90%	*<0.001*
Age (years)	24 ± 6 (103)	30 ± 8 (121)	*<0.001*
Height (cm)	160 ± 6 (103)	160 ± 6 (121)	0.323
Weight (kg)	57.5 ± 6.3 (103)	92.8 ± 14.5 (121)	*<0.001*
BMI (kg/m²)	22.5 ± 2.5 (103)	36.3 ± 5.7 (121)	*<0.001*
Fat (kg)	17.1 ± 3.8 (99)	39.7 ± 8.9 (103)	*<0.001*
Body fat (%)	30.2 ± 4.5 (99)	44.8 ± 4.2 (103)	*<0.001*
Waist (cm)	73 ± 6 (103)	104 ± 12 (121)	*<0.001*
Hip (cm)	98 ± 7 (103)	125 ± 10 (121)	*<0.001*
WHR	0.75 ± 0.06 (103)	0.83 ± 0.08 (121)	*<0.001*
VAT (cm^2)	47.5 ± 20.0 (75)	96.5 ± 41.7 (100)	*<0.001*
SAT (cm^2)	194.7 ± 99.5 (75)	557.4 ± 156.3 (100)	*<0.001*

Values are expressed as means ± SD. N is shown in parenthesis. Data are unadjusted and P-values are adjusted for age. BMI, body mass index; WHR, waist/hip ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

5.3.2 Metabolic outcomes

The metabolic outcomes of the normal weight and obese subjects are presented in Table 5.2. After adjustment for age, diastolic BP was elevated in the obese women compared to their normal weight counterparts, while systolic BP was not significantly different. The obese women had higher fasting glucose and insulin levels and greater insulin resistance, as estimated by HOMA-IR, compared to the normal weight women. TC and LDL-C were not different between the groups, while triglyceride levels were higher and HDL-C was lower in the obese compared to the normal weight women.

Table 5.2. Metabolic outcomes of the normal weight and obese black South African women.

	Normal weight (BMI ≤25 kg/m^2)	**Obese (BMI ≥30 kg/m^2)**	**P-value**
BP			
Systolic BP (mmHg)	108 ± 11 (103)	114 ± 16 (121)	0.209
Diastolic BP (mmHg)	71 ± 9 (103)	79 ± 10 (121)	*<0.001*
Insulin Sensitivity			
Glucose (mmol/L)	4.3 ± 0.4 (101)	4.7 ± 1.1 (121)	*0.017*
Insulin (mU/L)	7.7 ± 4.9 (103)	14.1 ± 8.8 (121)	*<0.001*
HOMA-IR	1.5 ± 0.9 (101)	3.1 ± 2.5 (121)	*<0.001*
Lipid Profile			
TG (mmol/L)	0.61 ± 0.23 (103)	0.85 ± 0.43 (121)	*<0.001*
TC (mmol/L)	4.0 ± 1.0 (103)	4.0 ± 1.0 (121)	0.192
HDL-C (mmol/L)	1.52 ± 0.44 (103)	1.17 ± 0.40 (121)	*<0.001*
LDL-C (mmol/L)	2.2 ± 0.8 (102)	2.4 ± 0.8 (121)	0.708

Values are expressed as means ± SD. N is shown in parenthesis. Data are unadjusted and P-values are adjusted for age.
BP, blood pressure; HOMA-IR, homeostasis assessment model of insulin resistance (248); TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

5.3.3 Genotype and allele frequencies

The genotype and allele frequencies of the Ins4436A, *Bcl*I and R453Q polymorphisms within the *HSD11B1*, *GR* and *H6PD* genes respectively in the normal weight and obese women are presented in Tables 5.3. and 5.4. There were no significant differences in the genotype or allele frequencies of these polymorphisms between the normal weight and obese subjects. Additionally, there were no differences in the genotype or allele frequencies of these polymorphisms between the insulin resistant and insulin sensitive subjects as characterized in Chapter Three (data not shown).

Table 5.3. The relative genotype distributions of the Ins4436A, *Bcl*I and R453Q polymorphisms within the *HSD11B1*, *GR* and *H6PD* genes, respectively, in the normal weight and obese black South African women.

Genotype	Normal weight (BMI ≤25 kg/m^2)	Obese (BMI ≥30 kg/m^2)	X^2 P-value
R453Q (H6PD)			
A/G	75.3 (67)	65.2 (73)	0.121
G/G	24.7 (22)	34.8 (39)	
BclI (GR)			
G/G	68.0 (70)	64.2 (77)	0.551
C allele*	32.0 (33)	35.8 (43)	
Ins4436A (HSD11B1)			
W/W	66.3 (67)	66.1 (80)	0.972
A allele†	33.7 (34)	33.9 (41)	

*Values are expressed as frequency (%) with N in parenthesis. *Due to the small number of individuals homozygous for the C allele of the BclI polymorphism within the GR gene, all subjects with the C allele were combined (normal weight: 29=G/C, 4=C/C and obese: 40=G/C, 3=C/C). †Due to the small number of subjects homozygous for the A allele of the Ins4436A polymorphism within the HSD11B1 gene, all subjects with the A allele were combined (normal weight: 30=W/A, 4=A/A and obese: 4=W/A, 0=A/A).*

Table 5.4. The relative allele distributions of the Ins4436A, *Bcl*I and R453Q polymorphisms within the *HSD11B1*, *GR* and *H6PD* genes, respectively, in the normal weight and obese black South African women.

Allele	Normal weight (BMI ≤25 kg/m^2)	Obese (BMI ≥30 kg/m^2)	X^2 P-value
R453Q (H6PD)			
A	38 (67)	33 (73)	0.171
G	62 (111)	67 (151)	
BclI (GR)			
G	82 (169)	81 (194)	0.420
C	18 (37)	19 (46)	
Ins4436A (HSD11B1)			
W	81 (164)	83 (201)	0.348
A	19 (38)	17 (41)	

Values are expressed as frequency (%) with N in parenthesis.

5.3.4 Hardy-Weinberg equilibrium

The *Bcl*I polymorphism within the *GR* gene was in Hardy-Weinberg equilibrium in both the normal weight (P=0.735) and obese groups (P=0.556). The Ins4436A polymorphism within the *HSD11B1* gene was in Hardy-Weinberg equilibrium in the normal weight group (P=0.748), but not in the obese group (P=0.022). The R453Q polymorphism within the *H6PD* gene was not in Hardy-Weinberg equilibrium in either the normal weight or obese groups (P<0.001).

5.3.5 Ins4436A polymorphism (HSD11B1 gene)

The combined normal weight and obese subjects were divided into two groups based on their *HSD11B1* genotype in order to investigate the associations between genotype and phenotype. Those who were homozygous for the wild type allele (W/W) were included in one group, while those who were homozygous or heterozygous for the A allele (A/A and W/A) were included in a second group. The basic characteristics and metabolic outcomes of the subjects by Ins4436A genotype are presented in Tables 5.5 and 5.6, respectively. There were no significant differences in age, height, body fat percent, body fat distribution, lipid profile, HOMA-IR or BP between the genotypes.

Table 5.5. Subject characteristics for genotype groups of the Ins4436A polymorphism within the *HSD11B1* gene in black South African women.

	W/W	W/A & A/A	P-value
Age (yrs)	27 ± 7 (148)	27 ± 8 (74)	0.695
Height (cm)	160 ± 5 (148)	160 ± 6 (74)	0.529
Weight (kg)	76.3 ± 20.4 (148)	77.7 ± 22.8 (74)	0.653
BMI (kg/m²)	29.8 ± 8.0 (148)	30.3 ± 8.9 (74)	0.653
Fat (kg)	28.6 ± 13.6 (130)	28.6 ± 13.1 (65)	0.992
Body fat (%)	37.5 ± 8.6 (130)	37.7 ± 8.6 (65)	0.881
Waist (cm)	89 ± 18 (148)	91 ± 19 (74)	0.505
Hip (cm)	112 ± 16 (148)	113 ± 16 (74)	0.990
WHR	0.79 ± 0.08 (148)	0.80 ± 0.09 (74)	0.574
VAT (cm^2)	75.8 ± 40.6 (114)	74.3 ± 44.3 (54)	0.819
SAT (cm^2)	405.4 ± 224.9 (114)	406.6 ± 228.8 (54)	0.975

Values expressed as means ± SD. N is shown in parenthesis.
BMI, body mass index; WHR, waist/hip ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; W, wild type.

Table 5.6. Metabolic outcomes for genotype groups of the Ins4436A polymorphism within the *B11HSD1* gene in black South African women.

	W/W	W/A & A/A	P-value
BP			
Systolic BP (mmHg)	109 ± 14 (148)	112 ± 10 (74)	0.431
Diastolic BP (mmHg)	74 ± 14 (148)	75 ± 10 (74)	0.431
Insulin Sensitivity			
Glucose (mmol/L)	4.5 ± 1.0 (145)	4.4 ± 0.5 (74)	0.318
Insulin (mU/L)	10.6 ± 7.4 (148)	12.0 ± 9.0 (74)	0.227
HOMA-IR	2.3 ± 2.2 (145)	2.4 ± 2.0 (74)	0.668
Lipid Profile			
TG (mmol/L)	0.72 ± 0.37 (148)	0.77 ± 0.37 (74)	0.340
TC (mmol/L)	3.9 ± 1.0 (148)	4.0 ± 0.6 (74)	0.570
HDL-C (mmol/L)	1.3 ± 0.5 (148)	1.4 ± 0.4 (74)	0.758
LDL-C (mmol/L)	2.3 ± 0.8 (148)	2.3 ± 0.7 (74)	0.966

Values are expressed as means ± SD. N is shown in parenthesis.
W, wild type; BP, blood pressure; HOMA-IR, homeostasis assessment model of insulin resistance (248); TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

5.3.6 R453Q polymorphism (H6PD gene)

The combined normal weight and obese subjects were divided into two groups based on their *H6PD* genotype (A/A and A/G). The basic characteristics and metabolic outcomes of the subjects by R453Q genotype are presented in Tables 5.7 and 5.8, respectively. There were no significant differences in age, height, total adiposity, body fat distribution, lipid profile, HOMA-IR or BP between the genotypes.

Table 5.7. Subject characteristics for genotype groups of the R453Q polymorphism within the *H6PD* gene in black South African women.

	A/G	A/A	P-value
Age (yrs)	27 ± 8 (140)	27 ± 8 (61)	0.808
Height (cm)	161 ± 6 (140)	160 ± 6 (61)	0.603
Weight (kg)	76.9 ± 21.5 (140)	79.7 ± 21.5 (61)	0.401
BMI (kg/m²)	30.0 ± 8.4 (140)	31.1 ± 8.4 (61)	0.401
Fat (kg)	28.2 ± 13.5 (117)	30.7 ± 13.8 (56)	0.267
Body fat (%)	37.3 ± 8.4 (117)	38.6 ± 9.1 (56)	0.339
Waist (cm)	89 ± 19 (140)	92 ± 18 (61)	0.366
Hip (cm)	112 ± 17 (140)	115 ± 16 (61)	0.512
WHR	0.79 ± 0.09 (140)	0.80 ± 0.08 (61)	0.814
VAT (cm^2)	75.0 ± 40.5 (105)	78.1 ± 41.2 (46)	0.668
SAT (cm^2)	410.2 ± 227.8 (105)	407.4 ± 237.4 (46)	0.944

Values are expressed as means ± SD. N is shown in parenthesis.
BMI, body mass index; WHR, waist/hip ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

Table 5.8. Metabolic outcomes for genotype groups of the R453Q polymorphism within the *H6PD* gene in black South African women.

	A/G	A/A	P-value
BP			
Systolic BP (mmHg)	112 ± 114 (140)	108 ± 25 (61)	0.175
Diastolic BP (mmHg)	76 ± 10 (140)	73 ± 18 (61)	0.196
Insulin Sensitivity			
Glucose (mmol/L)	4.5 ± 0.8 (137)	4.6 ± 1.1 (60)	0.501
Insulin (mU/L)	10.5 ± 7.3 (139)	12.0 ± 8.8 (60)	0.199
HOMA-IR	2.2 ± 2.0 (137)	2.7 ± 2.4 (60)	0.151
Lipid Profile			
TG (mmol/L)	0.75 ± 0.39 (139)	0.75 ± 0.30 (61)	0.996
TC (mmol/L)	4.0 ± 1.0 (139)	3.8 ± 0.8 (61)	0.389
HDL-C (mmol/L)	1.3 ± 0.5 (139)	1.3 ± 0.4 (61)	0.621
LDL-C (mmol/L)	2.3 ± 0.8 (139)	2.2 ± 0.8 (61)	0.489

Values are expressed as means ± SD. N is shown in parenthesis.
BP, blood pressure; HOMA-IR, homeostasis assessment model of insulin resistance (248); TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

5.3.7 BclI polymorphism (GR gene)

The pooled normal weight and obese subjects were divided into two groups based on their *GR* genotype. Those who were homozygous for the G allele (G/G) were included in one group, while those who were homozygous or heterozygous for the C allele (C/C and G/C) were included in a second group. The basic characteristics and metabolic outcomes of the subjects by *Bcl*I genotype are presented in Tables 5.9 and 5.10, respectively. There were no significant differences in age, height, total adiposity, body fat distribution, lipid profile, HOMA-IR or BP between the genotypes.

Table 5.9. Subject characteristics for genotype groups of the *Bcl*I polymorphism within the *GR* gene in black South African women.

	G/G	G/C & C/C	P-value
Age (yrs)	27 ± 8 (147)	28 ± 8 (76)	0.447
Height (cm)	160 ± 5 (147)	160 ± 6 (76)	0.843
Weight (kg)	75.1 ± 20.2 (147)	78.8 ± 22.9 (76)	0.215
BMI (kg/m²)	29.3 ± 7.9 (147)	30.8 ± 8.9 (76)	0.215
Fat (kg)	28.1 ± 13.1 (130)	28.6 ± 13.7 (66)	0.814
Body fat (%)	37.3 ± 8.5 (130)	37.6 ± 8.7 (66)	0.785
Waist (cm)	89 ± 18 (147)	90 ± 19 (76)	0.500
Hip (cm)	111 ± 16 (147)	113 ± 17 (76)	0.314
WHR	0.79 ± 0.08 (147)	0.79 ± 0.09 (76)	0.860
VAT (cm^2)	77.5 ± 42.7 (112)	69.1 ± 39.9 (66)	0.225
SAT (cm^2)	397.7 ± 225.1 (112)	389.0 ± 222.1 (66)	0.813

Values expressed as means ± SD. N is shown in parenthesis.
BMI, body mass index; WHR, waist/hip ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

Table 5.10. Metabolic outcomes for genotype groups of the *Bcl*I polymorphism within the *GR* gene in black South African women.

	G/G	G/C & C/C	P-value
BP			
Systolic BP (mmHg)	110 ± 17 (147)	110 ± 14 (76)	0.807
Diastolic BP (mmHg)	75 ± 12 (147)	74 ± 10 (76)	0.889
Insulin Sensitivity			
Glucose (mmol/L)	4.4 ± 0.5 (144)	4.6 ± 1.3 (76)	0.125
Insulin (mU/L)	11.0 ± 7.3 (147)	11.3 ± 8.8 (76)	0.692
HOMA-IR	2.2 ± 1.6 (144)	2.6 ± 2.8 (76)	0.257
Lipid Profile			
TG (mmol/L)	0.71 ± 0.35 (147)	0.79 ± 0.42 (74)	0.134
TC (mmol/L)	3.9 ± 9.2 (147)	4.1 ± 1.0 (74)	0.194
HDL-C (mmol/L)	1.4 ± 0.4 (147)	1.3 ± 0.5 (73)	0.271
LDL-C (mmol/L)	2.2 ± 0.8 (147)	2.4 ± 0.8 (73)	0.080

Values are expressed as means ± SD. N is shown in parenthesis.
BP, blood pressure; HOMA-IR, homeostasis assessment model of insulin resistance (248); TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

5.3.8 Combined genotype analysis

There were no significant differences in any of the investigated combined genotype or allele distributions between the normal weight and obese subjects (data not shown). Previous studies have shown that the G allele of the *Bcl*I polymorphism within the *GR* gene and the A allele of the Ins4436A polymorphism within the *HSD11B1* gene are associated with risk for CVD and centralisation of body fat (42;367). In the present study 30.3% of the normal weight subjects (30 of 99) and 32.2% of the obese subjects (37 of 115) had either the A allele of the *HSD11BI* polymorphism and/or the G allele of the *GR* polymorphism which was not significantly different (P=0.883).

5.4 DISCUSSION

Although differences in the expression of 11β-HSD-1, H6PDH and GR in VAT (61;363) and SAT (61;349;360;361;439) have been associated with obesity (61;349;360-362;439), VAT accumulation (60;362), and features of the metabolic syndrome (60;362), studies investigating polymorphisms in the *HSD11B1*, *H6PD* and *GR* genes have reported inconsistent findings. Some studies have highlighted associations between specific alleles in these genes and obesity, body fat distribution and CVD (42;367;368;373), while other studies have not found associations (370;371;375;376). Although there is a paucity of data in Sub-Saharan African populations, based on evidence from other populations, it was hypothesized that the high levels of obesity, central obesity (4) and insulin resistance (22) in black South African women may be the result of altered GC action in adipose tissue, due to variants within these genes. However, this preliminary study found that the *Bcl*I, Ins4436A and R453Q polymorphisms within the *GR*, *HSD11B1* and *H6PD* genes, respectively, were not associated with differences in obesity, body fat distribution or metabolic outcomes in black South African women.

Since obesity, centralisation of body fat and insulin resistance have been associated with up-regulation of 11β HSD-1 expression in SAT and VAT (60;362), the first gene investigated in this thesis was *HSD11B1*. Nair et al. (368) recently reported that the Ins4436A and the G4478T polymorphisms within intron 3 of this gene were associated with type 2 diabetes in Pima Indians. I am unaware of any studies that have investigated polymorphisms within this gene with regard to obesity, body fat distribution or CVD in Sub-Saharan Africans. Data on African Americans is also limited. A single study including black and white American children (N=106 and 136, respectively) reported an association between the A/A genotype of the Ins4436A polymorphism within the *HSD11B1* gene and obesity, increased waist/hip ratio and insulin resistance as estimated by HOMA-IR (367). Notably, there were only 11 children with the A/A genotype and these results should therefore be interpreted with caution. In the present study, due to low frequency of the A/A genotype in the black South African women (N=4), there was insufficient statistical power to explore an A/A genotype effect. Therefore, the subjects with the A/A and W/A genotypes were combined for the analysis and no genotype

associations with obesity, central obesity of the metabolic outcomes were found. Similarly, when middle-aged French Canadians (N=217), with the A/A and W/A genotypes of the Ins4436A polymorphism were combined, there were no genotype associations with BMI, waist circumference, VAT, lipid profile, BP, fasting glucose, fasting insulin or HOMA-IR (370).

To my knowledge, there is no published genotype frequency data available on the *HSD11B1* Ins4436A polymorphism in black Africans. A larger sample size is needed for further investigation of the A/A genotype of this polymorphism in black South African women. However, since the A/A genotype is rare, it is not likely to play a major role in the high prevalence of obesity or insulin resistance (4;174) in this population.

The second gene investigated in this study was the *H6PD* gene. H6PDH produces NADPH that drives 11β HSD-1's conversion of cortisone to cortisol within adipose tissue (436). In cultured cells from Indo-Asian and European cortisone reductase deficiency (CRD) patients and control subjects, the A allele (Glutamine, Q amino acid) of the R453Q polymorphism within this gene was associated with a 50% reduction in H6PDH enzymatic activity (372). Based on this finding, it was hypothesized that a high frequency of A allele (Q amino acid) of the R453Q polymorphism within the *H6PD* gene could decrease H6PDH enzyme activity, down-regulate GC action in adipose tissue and explain, in part, the relatively low levels of VAT in black South African women. In support of this hypothesis, White et al. (374) reported that African Americans had a higher frequency of the A allele (Q amino acid) (48%) of the R453Q polymorphism within the *H6PD* gene compared to white Americans (31%) in a large population from the Dallas Heart study (N=1241 white and 1776 African American). However, additional information from the NCBI database suggests that the frequency of the A allele of the R453Q polymorphism ranges widely within black African subjects (35-50%) (www.ncbi.nlm.nih.gov). The frequency of the A allele (Q amino acid) of the R453Q polymorphism in the present study is at the lower end of this range (35%) and is the first reported frequency in the black South African population.

Further, White et al. (374) did not find any associations between the A allele of the R453Q polymorphism in the *H6PD* gene and obesity, body fat distribution or CVD in African or white Americans. The lack of association between the A allele of the R453Q polymorphism and obesity, body fat distribution and CVD was corroborated in relatively young Spanish (N=192) and British (N=1018) PCOS patients and controls (373;375), elderly white men (N=6152) (376), European and Indo-Asian CRD patients and controls (N=157) (372), as well as in the results of the present study. It is therefore unlikely that a higher frequency of the A allele (Q amino acid) of the R453Q polymorphism, which was previously associated with decreased H6PDH enzyme activity (372), explains the relatively low levels of VAT seen in black compared to white South African women (216).

The final gene investigated in this study was the *GR* gene. In adipose tissue, GR activation is amplified by increased 11β-HSD-1 activity in the tissue (442). However, to my knowledge, the *Bcl*I polymorphism within the *GR* gene has not been investigated in a black African population with regard to obesity, body fat distribution and CVD. In the present study, the frequency of the G allele of the *Bcl*I polymorphism in both the normal weight (82%) and obese (81%) black South African women was similar to that reported previously in black Africans (80%) (407). Although some studies reported associations between the G allele of the *Bcl*I polymorphism in the *GR* gene and obesity (379), body fat distribution (42;403) and CVD (381;404), other studies (399;406) including our own, have failed to corroborate these findings.

The association of the G allele of the *Bcl*I polymorphism within the *GR* gene with central obesity as measured by anthropometry is also questionable. In middle-aged Swedish men, Rosmond et al. (401) reported that the G allele of this polymorphism was associated with increased waist/hip ratio, although this was not corroborated by other studies in obese Italians (406) or elderly white men and women (379). However, the findings related to the direct measure of VAT, by CT scan, are more conclusive. In a large sample of men and women from the Quebec family study (N=742), Ukkola et al. (403) found that the G allele of the *Bcl*I polymorphism within the *GR* gene was associated with increased VAT ($P<0.001$) independently of total adiposity. Further, in middle-aged Canadian men and women (N=152), Buemann et al. (42) reported similar findings in men and also found that

in the lowest tertile of body fat percent, the G allele explained 41% and 35% of the variance in VAT in men and women, respectively.

Based on these studies, it is possible that the G allele of the *Bcl*I polymorphism affects VAT, but not SAT. This might explain why findings are different for anthropometric vs. CT-derived measures of central obesity. Therefore, the relatively low levels of VAT seen in black South African women (174), despite high levels of central obesity as measured by waist circumference (4), might confound the association between the G allele of the *Bcl*I polymorphism and VAT. Investigation of the mutant allele of the ER22/23EK polymorphism within the *GR* gene which down-regulates GC activity in adipose tissue (377;380;443), may be of greater relevance in black South African women. In support of this, it has recently been shown that despite similar levels of 11β-HSD-1 and H6PDH mRNA in SAT, GR mRNA levels were decreased in normal weight and obese, black compared to the white South African women (Goedecke et al., personal communication).

The two sample populations (normal weight and obese women) investigated for the *Bcl*I polymorphism in *GR* gene were in Hardy-Weinberg equilibrium, but this was not the case for the R453Q polymorphism within the *H6PD* gene. Additionally, the obese sample was not in Hardy-Weinberg equilibrium for the Ins4436A polymorphism within the *HSD11B1* gene. This therefore suggests that these two populations do not represent a randomly mating population. The selection criteria for the normal weight and obese women included a BMI cut-point of $\leq$25 kg/m^2 and $\geq$30 kg/m^2 respectively, a specific gender (females) and ancestry (South African black population) and therefore it is conceivable that the sample populations were selective. For this reason, it would be interesting to determine the frequency distribution of all three polymorphisms (Ins4436A, R453Q and *Bcl*I within the *HSD11B1*, *H6PD* and *GR* genes respectively) investigated in this study in a randomly sampled group representing the South African black female population, irrespective of BMI.

The inclusion of relatively young subjects without known disease and an atypical presentation of CVD risk factors may in part explain the negative findings of this study. Using the IDF metabolic syndrome diagnostic criteria (155), very few of the subjects had

elevated triglyceride levels (3.9%), impaired fasting glucose (3.9%) or the metabolic syndrome (13.9%), despite high levels of central obesity (61%) and insulin resistance (estimated by HOMA-IR) (as described in Chapter Three of this thesis). In contrast, other studies that have explored the association between polymorphisms in the *HSD11B1*, *H6PD* and *GR* genes and risk factors for CVD included older subjects (42;376;379;401) or subjects presenting with CVD disease (404;406). These differences complicate comparison of the studies, as it is not known if the black South African women in this study will develop disease as they age or remain apparently healthy. Additionally, the studies that have shown genotype differences were not undertaken in black African populations. Therefore, differences in the presentation of CVD risk factors between black and white women, as described in Chapter Three of this thesis, may have also contributed to the negative findings of this study.

Additionally, the negative findings of this study could be related to the study methodology in which candidate genes and then SNPS were identified based on published association studies that used the biological basis of disease mechanism to identify candidate genes. A more powerful research methodology would be a genome wide association study (GWAS) (444). This type of research methodology involves the analysis of thousands of DNA samples from individuals with a particular disease and control individuals (445). GWAS identify loci with statistically significant differences in allele or genotype frequency (444). GWAS are a powerful method for identifying disease susceptibility genes for common complex diseases such as type 2 diabetes (444;445), obesity (446), coronary artery disease (444) and the metabolic syndrome (447). This methodology is particularly relevant in ethnic-specific studies due to the genetic variation between ethnic groups (448). For example, in a multiethnic population including black, Caucasian, Mexican and Japanese Americans, Edwards et al. (447) recently determined that there was a large amount of heterogeneity in the loci involved in genetic susceptibility to the metabolic syndrome.

Although there is a paucity of data investigating genetic contributors to obesity, insulin resistance, type 2 diabetes and CVD in Sub-Saharan black Africans, a GWAS could potentially identify novel susceptibility factors. However, the large sample sizes, the resources and technology were not available to complete a GWAS.

In conclusion, there is strong biological evidence that tissue-specific GC action influences obesity, body fat distribution and CVD risk factors. However, the Ins4436A, R453Q and *Bcl*I polymorphisms within the *HSD11B1*, *H6PD* and *GR* genes respectively were not associated with obesity, body fat distribution, lipid level, BP or insulin resistance in the present study. Despite the negative findings of this preliminary study, further investigation of the association between alleles of other polymorphisms that down-regulate tissue-specific GC action and obesity, central obesity and insulin resistance may aid in explaining the unique metabolic phenotype of black South African women.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

The main findings of this thesis are summarized below in Table 6.1 and further discussed in this Chapter.

Table 6.1. Summary of main findings by chapter.

Chapter	Summary of main findings
Two	NIR and DXA body fat percent were significantly correlated (r=0.55-0.69, P<0.001). NIR under predicted body fat percent in the normal weight and obese women. This under prediction was greater in the black compared to white women (P<0.001). Body fat percent measured by NIR leveled off at between 45-47% resulting in bias in the limits of agreement between NIR and DXA with increasing levels of adiposity. Single-site NIR should not be used to quantify total adiposity in black African women and women with high levels of adiposity. Skin colour, body fat distribution pattern and the most appropriate NIR measurement site should be taken into account and population specific equations derived. This was the largest study to date undertaken on the validity of NIR as a measure of body fat % in black Africans and the first study in sub-Saharan Africans.
Three	Agreement was high between the IDF and ATP III metabolic syndrome diagnostic criteria (K=0.88). Neither the IDF nor ATP III metabolic syndrome criteria accurately identified insulin resistance (K=0.15 and 0.14 respectively). Waist circumference had a 20% higher specificity and 66% higher positive predictive value for identifying insulin resistance compared to the IDF and ATP III metabolic syndrome criteria. VAT was the largest contributor to a positive diagnosis of the metabolic syndrome while DSAT and tSAT were less significant. Insulin resistant subjects presented with lipid levels below the IDF and ATP III metabolic syndrome criteria cut points, highlighting their unique metabolic phenotype. This study has important public health implications in South Africa since waist circumference (a non blood based measure) indicated insulin resistance. This was the first study to investigate the ability of the IDF and ATP III metabolic syndrome criteria to identify insulin resistance in black South African women. This was the first study to investigate the influence of the different abdominal tissue compartments on the presentation of the metabolic syndrome in black South African women.

Table 6.1 continued

Chapter	Summary of main findings
Four	22% of the normal weight women were insulin resistant. 38% of the obese women were insulin sensitive. Increased VAT, independent of total adiposity, distinguished between the insulin sensitive and insulin resistant phenotypes. Insulin sensitive women were of higher socioeconomic status, did more leisure time PAEE and were less likely to use injected contraceptives compared to their insulin resistant counterparts. Body fat distribution, body fat percent, age, leisure PAEE and use of injected contraceptives accounted for 35% of the variance in HOMA-IR in the normal weight women. When physically active women were excluded, 50% of the variance was explained by the same variables. Body fat distribution, body fat percent, age and use of injected contraceptives accounted for 34% of the variance in HOMA-IR in the obese women. No differences in family history of metabolic diseases or smoking status were found between the insulin sensitive and insulin resistant phenotypes. This investigation of the determinants of insulin resistance in black South African women was the first of its kind and identified modifiable contributors to HOMA-IR.
Five	Within the normal weight and obese women, there were no significant differences in the genotype or allele frequencies of the *BCl*I, INS4436A and R453Q polymorphisms within the *HSD11B1*, *H6PD* and *GR* genes. There were no genotype effects on body composition, body fat distribution, insulin sensitivity, lipid profile or blood pressure. This was the first study to investigate the *BCl*I, INS4436A and R453Q polymorphisms within the *HSD11B1*, *H6PD* and *GR* genes in a black South African population.

In South Africa, a country currently undergoing epidemiological transition, urban black women have a higher prevalence of obesity compared to other demographic groups. Despite high levels of adiposity, black South African women have an atypical presentation of CVD risk factors, presenting with relatively low levels of VAT and a favourable lipid profile compared to white South African women. The objective of this thesis was therefore to investigate the impact of body fat and its distribution on the presentation and identification of CVD in a cohort of 103 normal weight (BMI ≤25 kg/m^2) and 121 obese (BMI ≥30 kg/m^2) black South African women. In order to investigate CVD risk factors prior to the onset of disease, relatively young women with no known disease were recruited for the study.

Since obesity is associated with increased risk of CVD, accurate quantification of body fatness is particularly important in health risk appraisal. However, in developing countries, "gold standard" measures of body fat percent such as underwater weighing and DXA are not always practical, as access to facilities and resources are limited. Further, field methods for measuring body fat percent such as BMI, sum of skin folds and BIA have limitations. NIR is another possible field measure of body fat percent; however, its validity in obese individuals and black African individuals has been questioned. Therefore, the first study in this thesis examined the validity of single-site NIR (Futrex-6100 A/ZL) as a measure of body fat percent compared to the criterion method of DXA in normal weight and obese, black and white South African women. Although NIR and DXA body fat percent were significantly correlated (r=0.55-0.69, P<0.001), NIR under-predicted body fat percent compared to DXA in the normal weight and obese women. This under-prediction was significantly greater in the black compared to the white women (P<0.001), independent of adiposity. Moreover, body fat percent measured using NIR appeared to level off at between 45-47%, resulting in bias in the limits of agreement between NIR and DXA with increasing levels of adiposity. Therefore, single-site NIR should be used with caution as a measure of body fat percent, particularly in black African women and women with very high levels of adiposity. To my knowledge, this is the largest study undertaken on the validity of NIR as a measure of body fat percent in black African subjects, and the first study in Sub-Saharan Africans.

The ability of the IDF and ATP III metabolic syndrome criteria to identify black African individuals at increased risk of CVD has been questioned. The first aim of the second study of this thesis was to examine the level of agreement between these two metabolic syndrome criteria, which differ in their emphasis on central obesity. Further, the degree to which these criteria predicted insulin resistance in black South African women was also determined. Agreement was high between the IDF and ATP III metabolic syndrome criteria (kappa=0.88) in this population; however, neither criteria accurately identified insulin resistance, as estimated by HOMA-IR (kappa=0.15 and 0.14 respectively). In fact, waist circumference (>80 cm) had a 20% higher specificity and 66% higher positive predictive value for identifying insulin resistance compared to either of the metabolic syndrome criteria. Therefore, the second aim of this study was to investigate the extent to which a diagnosis of the metabolic syndrome could be explained by body fat and its distribution. Regression analysis indicated that VAT was the largest contributor to diagnosis of the metabolic syndrome (IDF and ATP III criteria) while tSSAT was less important and abdominal DSAT was not a significant contributor. Additionally, this study highlighted that even the most insulin resistant subjects presented with lipid levels below the suggested IDF and ATP III metabolic syndrome cut-points. In conclusion, waist circumference was a better indicator of insulin resistance than the IDF or ATP III metabolic syndrome criteria. This has major implications for public health policy, since waist circumference is a non-blood based indicator of insulin resistance in young black African women without known disease. Additionally, to my knowledge this was the first South African study to investigate the agreement between the IDF and ATP III metabolic syndrome, and their ability to identify insulin resistance in black women. Further, this study was the first to investigate the influence of the different abdominal adipose tissue compartments on the presentation of the metabolic syndrome in black South African women.

Using the ATP III and IDF criteria, only 13% of the subjects were classified as having the metabolic syndrome. As a result of these and other similar criteria being used to identify CVD risk in black South African women, a high prevalence of “healthy obesity” has been described. Therefore, in the third study of this thesis, the determinants of the “healthy obese” and conversely, the “at-risk normal weight” metabolic phenotypes were

investigated. As the results of Chapter Three suggested that insulin resistance may be a better indicator of CVD risk in young black South African women than criteria including lipid cut-points, the "at-risk" phenotype was based on insulin resistance, as estimated by HOMA-IR. Twenty-two percent of the normal weight women were insulin resistant and 38% of the obese women were insulin sensitive. Increased VAT (P=0.001) and decreased VAT/leg fat mass (cm^2/kg) (P<0.001), independent of total body fatness, distinguished between the phenotypes. Moreover, the insulin sensitive women were of higher socio-economic status, did more leisure PAEE, and were less likely to use injectable contraceptives compared to their insulin resistant counterparts. Using a regression model, body fat distribution, body fat %, age, leisure PAEE and use of injected contraception were found to account for 35% of the variance in HOMA-IR in the normal weight women. When physically active normal weight women (≥150 minutes of physical activity per week) were excluded from the analysis, 50% of the variance in HOMA-IR was explained by the same variables. In the obese women, 34% of the variance in HOMA-IR was explained by the same variables, excluding leisure PAEE. No differences in smoking status or family history of metabolic disease were found between the phenotypes. In conclusion, central body fat distribution, total adiposity, socio-economic status, leisure PAEE and injectable contraceptive use distinguished between insulin sensitive and insulin resistant black South African women. This investigation of the determinants of "healthy obesity" was the first of its kind in a black South African population. Further, this study identified method of contraception and leisure time PAEE as determinants of insulin resistance, which are modifiable and were not previously identified in the literature investigating the metabolically obese normal weight (MONW) and metabolically healthy obese (MHO) phenotypes.

This thesis has identified central obesity as a major determinant of CVD risk in black South African women. Further, black South African women have a higher prevalence of obesity, central obesity, as determined by waist circumference, and insulin resistance compared to white South African women. This could be related to altered tissue-specific GC action within adipose tissue, which may affect body fat distribution and CVD risk factors. Therefore, the final study of this thesis was a preliminary investigation of the influence of *Bcl*I, Ins4436A and R453Q polymorphisms within the *HSD11B1*, *H6PD* and

GR genes, respectively, involved in tissue-specific GC action, on body composition, body fat distribution and risk factors for CVD in black South African women. The results indicated that there were no significant differences in genotype or allele frequencies within the normal weight and obese subjects. Further, there were no genotype effects on body composition, body fat distribution, insulin sensitivity, lipid profile or blood pressure. In conclusion, in this preliminary investigation, the *Bcl*I, Ins4436A and R453Q polymorphisms within the *GR*, *HSD11B1* and *H6PD* genes, respectively, were not associated with obesity, body fat distribution or CVD risk factors in this cohort of normal weight and obese black South African women. Prior to this study, these polymorphisms had not been investigated in black South Africans. The results of this study provide information on the frequencies of the *Bcl*I, Ins4436A and R453Q polymorphisms within the *GR*, *HSD11B1* and *H6PD* genes in this population that were previously unknown.

Together the studies that comprise this thesis demonstrate how body fat and its distribution influence the presentation and identification of CVD risk factors in black South African women. In terms of the presentation of CVD risk factors, increased levels of VAT and decreased peripheral fat were associated with insulin resistance, independently of total adiposity, while SSAT and DSAT were less important contributors. Despite high levels of central obesity and insulin resistance, black South African women had low lipid levels, possibly due to their relatively low levels of VAT. As a consequence of this unique phenotype, commonly used diagnostic criteria for the identification of CVD such as the IDF and ATP III metabolic syndrome were poor indicators of insulin resistance in these young black women. Further, waist circumference was a better indicator of insulin resistance than a positive diagnosis of the metabolic syndrome.

The studies in this thesis have some limitations that should be noted. The subjects were not randomly sampled, but were recruited through local newspapers, church groups and universities. Therefore, the data may not be representative of the black South African population. The sampling procedures also resulted in an age difference between the normal weight and obese women, which had to be adjusted for in some of the analyses.

In Chapter Three, the ability of the IDF and ATP III metabolic syndrome criteria to predict HOMA-IR was investigated. It should be noted that the IDF and ATP III metabolic syndrome criteria were developed to identify individuals at risk of CVD and type 2 diabetes, and not insulin resistant individuals. However, these criteria were developed without inclusion of the African Diaspora, in whom insulin resistance may be of greater clinical relevance given their peripheral fat deposition and favorable lipid profile. Further, it should be noted that the Smith equations (189) used for the conversion of VAT and DSAT cross sectional areas to volumes in Chapter Three are based on 18 men and women of undefined race. These equations were used for the reasons stated in Chapter Three, but were not derived specifically for black African women, which is a limitation to their use in this thesis. In Chapter Four of this thesis, although the analyses were adjusted for smoking, exposure to passive smoke was not measured. Exposure to passive smoke is a health risk and could have affected the results of the study.

In Chapter Five of this thesis, although the sample size was large enough based on the sample size determination, the small number of subjects that were homozygous for the A allele of the Ins4436A polymorphism within the *HSD11B1* may be a limitation. Chapter Five of this thesis was therefore presented as a preliminary investigation. Further, it should be noted that the negative findings of Chapter Five could be related in part to the methodology employed. Ideally, the research questions would have been investigated in a GWAS. This methodology is of particular importance in the investigation of genetic associations with complex disease in specific ethnic populations (444). For example, although there is a paucity of data investigating genetic contributors to obesity, insulin resistance, type 2 diabetes and CVD in Sub-Saharan black Africans, a GWAS could potentially identify novel susceptibility factors. This methodology would be less biased and not rely on previous candidate gene studies in ethnically different populations (449). Further, the inclusion of the necessary large sample sizes for a GWAS would allow detection of genes associated with a modest increase in disease risk (450). However, the large sample size, resources and technology needed for a GWAS make it an inappropriate method for the purposes of this thesis.

A further limitation of this thesis was that insulin resistance was estimated by HOMA-IR rather than measured by an oral glucose tolerance test or clamp methodology due to limited resources. Although HOMA-IR is an established CVD risk factor and has been used in similar studies, use of a more dynamic measure of insulin resistance may have altered the study results.

Despite these limitations, this thesis has important and novel practical implications regarding the presentation and identification of CVD risk in young black South African women. The data showed that single-site NIR (Futrex-6100 A/ZL) was not a valid measure of body fat percent in black South African women and should be used with caution in health risk appraisal. Further, the results of this thesis highlighted that waist circumference; an inexpensive and practical field method for quantifying central obesity was a better indicator of insulin resistance in young black women without known disease than a positive diagnosis of the metabolic syndrome using either the IDF or ATP III criteria. This thesis also identified modifiable determinants of insulin resistance, such as PAEE and method of birth control, in normal weight and obese black South African women. This thesis has major implications for public health practices in South Africa in relation to the identification of CVD risk at a national level. These findings may assist in cost-effective early identification of CVD risk and expedite early intervention in this high risk population.

Based on the findings of this thesis, it is recommended that future research determine ethnic-specific cut-points for lipid levels and waist circumference which would aid in identification of at-risk individuals in this population. Ideally, a longitudinal study investigating risk factors of CVD in black South African women should be undertaken. Further, as the HIV/AIDS epidemic progresses in South Africa, investigation of the affects of anti-retroviral medications on body fat distribution and CVD is also needed. Finally, the role of genetics in body fat distribution in black South African women requires further investigation. Future genetic research should ideally be undertaken in a Sub-Saharan black African population to further investigate complex diseases such as obesity and CVD using the GWAS methodology.

CHAPTER SEVEN

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CHAPTER EIGHT

APPENDIX

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17 February 2004

8.1 SUBJECT QUESTIONNAIRE-ENGLISH VERSION

GENOTYPE AND PHENOTYPE INTERACTIONS IN LEAN AND OBESE SOUTH AFRICAN WOMEN

IDENTIFICATION AND CONTACT DETAILS	
Name _____ ID number: _____ Date of Birth _____ Age: _____ Ethnicity: _____ Physical Address: _____ _____ Postal Address: _____ _____ Tel No's: _____ (h) _____ (w) _____ (Cel) Alternative contact Person: _____ Tel No: _____ **TO BE KEPT SEPARATE FROM QUESTIONNAIRE DATA**	**SUBJECT CODE:**

GENOTYPE AND PHENOTYPE INTERACTIONS IN LEAN AND OBESE SOUTH AFRICAN WOMEN

SUBJECT CODE:

ANCESTRY

Ethnic Group (only required for and used for research purposes) Black/African ___ White ___ Indian ___ Asian ___ Mixed Ancestry (Colored) ___ Other ___

Ancestry: Tribal or national background (e.g. Xhosa, Dutch, Zulu) Father _____ Unknown ____ Mother _____ Unknown ____

PRELIMINARY TESTING

Date of testing: _____.. Time of testing: _____

Date of blood sample : _____ Date of DEXA scan: _____

Time of last meal/drink: _____ Hours fasted: _____

Contraception: None: ☐
Pills ☐ Name: _____
Injection ☐ Name: _____
IUD ☐ Date inserted: _____
Sterilization (tubes tied) ☐ Date: _____
Other ☐ Details: _____

How would you describe your health TODAY? (How are you feeling?):

Good ☐ Fair ☐ Poor ☐

If POOR, explain why: _____

_____

SECTION 1: DEMOGRAPHIC AND SOCIOECONOMIC DETAILS: **SUBJECT CODE: _____**

How many people living in your household, including you? ☐

1. How many rooms do you have in your house (including kitchen, lounge, dining room, bedrooms)? ☐ rooms
2. In your home, how many rooms are there just for sleeping? ☐ rooms
3. How would you describe your home (tick the one that best describes it)?

Room/garage attached /not attached to house		House		Shared house	
Flat		Hostel		Other:	

4. What type of household water do you have access to?

Indoor water		Only outside tap water		Other water source	

5. What type of toilet do you have?

Flush inside		Only flush outside	
Outside toilet		Other:	

6. Which of the following do you have in your household at the present time?

	YES	NO		YES	NO
Electricity			Telephone		
Television			Video machine		
Radio			Microwave		
Motor vehicle			Computer		
Fridge			Cellular telephone		
Stove and oven			Mnet		
Washing machine			DSTV		

7. Marital status:

Single		Divorced/separated	
Married		Widowed	
Living with partner, not married			

8. How many children do you have? ☐ How many pregnancies have you had? ☐
9. What are the ages of the children? _____
10. Education (last standard passed):

No formal education		Std 8 (Grade 10)	
Sub A/B (Grade 1-2)		Std 9 (Grade 11)	
Std 1-3 (Grade 3-5)		Matric (Grade 12)	
Std 4-5 (Grade 6-7)		College or Technician	
Std 6-7 (Grade 8-9)		University	

Are you:

Employed		A student	
Self employed		Informal	
Unemployed		Other	

11. If employed, What work do you do?: _____
12. Name of employer: _____ Period of employment: _____
13. How many people do you support with this income? ☐ : Adults: ☐ Children: ☐
14. Do you have any other sources of income or financial assistance?

None		Support from husband/partner	
Informal income		Support from parents	
Support from children		Support from other family member	
Other:		Disability grant	

15. Are you covered by a Medical Aid or a Medical Benefit Scheme, or any scheme that helps you pay for health care or drug services?

Yes ☐ No ☐ Brief description: _____

16. Which language do you speak at home? _____

SECTION 2: FAMILY MEDICAL HISTORY **SUBJECT CODE: _____**

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	SKIP
9	Now I would like to ask you about your family. Do you have a **close blood relative** (grandparents, father, mother, brother, sister or child) who has ever had any of the following conditions:		
9A	High Blood Pressure?	YES..... NO..... DON'T KNOW..... If yes, who? …… …… …… …… ……	
9B	Heart attack or angina or chest pain when exerting himself/herself?	YES..... NO..... DON'T KNOW..... If yes, who…… . …… …… …… ……	1 ➤ 9D
9C	Was this relative younger or older than 50 years old when they first had a heart attack, angina or chest pain?	YOUNGER THAN 50 YEARS..... …… …….. OLDER THAN 50 YEARS ..... …… …….. DON'T KNOW..... …… ……	
9D	Stroke?	YES..... NO..... DON'T KNOW..... If yes, who? …….. …… …… …… ……	
9E	Diabetes?	YES..... NO..... DON'T KNOW..... If yes, who? …… ……	

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	SKIP
		…… …… …….. Adult/ child onset? …… …… …… ……	
9F	Obesity? (Were they abnormally large? Or have difficulty moving?)	YES ..... NO ..... DON'T KNOW ..... If yes, who? …… …… …… …… …… …… ……	

SECTION 3 : QUALITY OF LIFE AND CLINICAL CONDITIONS **SUBJECT CODE: _____**

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
10A	Would you say your health is poor, average, good, or very good/excellent ?	POOR .....1 AVERAGE .....2 GOOD.....3 VERY GOOD/EXCELLENT.....4
10B	Do you personally think that you are underweight, normal weight or overweight?	UNDERWEIGHT.....1 NORMAL WEIGHT .....2 OVERWEIGHT.. .....3 DON'T KNOW.....8
11	Has a **doctor** or **nurse** or **health worker** at a **clinic** or at **hospital told you** that you had or have any of the following conditions:	
11A	High Blood Pressure?	YES.....1 NO .....2 DON'T KNOW.....8
11B	Heart attack or angina (chest pains)?	YES.....1 NO .....2 DON'T KNOW.....8
11C	Stroke?	YES.....1 NO .....2 DON'T KNOW.....8
11D	High blood cholesterol or fats in the blood?	YES.....1 NO .....2 DON'T KNOW.....8
11E	Diabetes or Blood Sugar?	YES.....1 NO .....2 DON'T KNOW.....8
11F	Emphysema/Bronchitis?	YES.....1 NO .....2 DON'T KNOW.....8
11G	Asthma?	YES.....1 NO .....2 DON'T KNOW.....8
11H	Sore joints, e.g. Arthritis, gout?	YES.....1 NO .....2 DON'T KNOW.....8
11I	Osteoporosis?	YES.....1 NO .....2 DON'T KNOW.....8
11J	Epilepsy / fits?	YES.....1 NO .....2 DON'T KNOW.....8
11K	TB?	YES.....1 NO .....2 DON'T KNOW.....8
11L	How many episodes of TB have you ever been treated for?	NUMBER OF TB EPISODES……. |▒▒|▒▒| Are you currently on TB medications? …… When was your last TB episode: ……

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
11M	Cancer?	YES ..... 1 NO ..... 2 DON'T KNOW ..... 8 If yes, what? ……..

SECTION 4 : HEALTH KNOWLEDGE **SUBJECT CODE: _____**

6	Do you know your HIV status?	YES ..... 1 NO 2
6	If yes, are you:	Positive ..... 1 Negative ..... 2 Unwilling to disclose ……..
6	Have you had your blood pressure measured in the past 12 months?	YES ..... 1 NO 2
7	Do you know what your blood pressure is?	YES ..... 1 NO 2
8	Is it high, normal or low?	HIGH ..... 1 NORMAL ..... 2 LOW ..... 3 DON'T KNOW 8

SECTION 5: MEDICATION

	Now I want to ask you about any medication you take		
17a	Do you use any medicine regularly or daily that a doctor or nurse has prescribed?	YES ..... NO ..... DON'T KNOW	☐ ☐ ➤18
17b	How many different medicines do you use regularly (more than once a month)?	NUMBER_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ ☐☐	
17c	Who pays for most of the medication, prescribed by a doctor or nurse, that you use? **(READ THE OPTIONS)**	RESPONDENT ..... ☐ FAMILY ..... ☐ MEDICAL AID ..... ☐ PROVIDED AT CLINIC OR PUBLIC HOSPITAL ..... ☐ EMPLOYER ..... ☐ OTHER _ ☐ (SPECIFY)	

SECTION 8: HABITS AND LIFESTYLE *SUBJECT CODE:* _____

	SECTION 8A: PHYSICAL ACTIVITY (Modified STEPs Core data set) *The next questions are about the time you spend doing different types of physical activities. This includes activities you do at home, at work, traveling from place to place and during your spare time. You are requested to answer the questions even if you don't consider yourself to be an active person.*		
	Occupation-related Physical Activity (paid or unpaid work): *When answering the following questions, think back over the **past 12 months** and consider (think of) **a usual week**:*		
18	Does your work involve mostly sitting or standing still, **OR** walking for very short periods (less than 10 minutes)?	YES ..... NO	—➢21
19a	Does your work involve vigorous activities, (like heavy lifting, digging, or heavy construction) for **at least 10 minutes** at a time?	YES ..... NO	➢20a
19b	In **a usual week**, how many days do you do vigorous activities as part of your work?	DAYS_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	
19c	On a usual day on which you do vigorous activities, how much time do you spend doing such work?	_____ HOURS _____ MINS	
20a	Does your work involve moderate-intensity activities (like brisk walking or carrying light loads) for **at least 10 minutes** at a time?	YES ..... NO	—➢21
20b	In **a usual week**, how many days do you do moderate-intensity activities as part of your work?	DAYS_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	
20c	On a usual day on which you do moderate-intensity activities, how much time do you spend doing such work?	_____ HOURS _____ MINS	
21	How long is your usual workday?	_____ HOURS _____ MINS	
	Travel-related Physical Activity: *Other than activities that you've already mentioned; I would like to ask you about the way you travel to and from places (to work, to shopping, to market, to church, etc).*		
22a	Do you walk or use a bicycle (pedal cycle) for **at least 10 minutes** at a time to get to and from places?	YES ..... NO	—➢23
22b	In **a usual week**, how many days do you walk or cycle for at least 10 minutes to get to and from places?	DAYS_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	
22c	On a usual day, how much time do you spend walking and cycling for travel	_____ HOURS _____ MINS	
	Non-work related and leisure time Physical Activity: *The next questions ask about activities you do in your leisure or spare time, for recreation or fitness. Do not include the physical activities you do at work or for travel already mentioned*		
23	In your leisure or spare time do you do any vigorous or moderate-intensity physical activity lasting **more than 10 minutes** at a time?	YES ..... NO	—➢26
24a	In your leisure or spare time, do you do any vigorous activities (like running or strenuous sports, weightlifting) for **at least 10 minutes** at a time?	YES ..... NO	-➢25a
24b	IF YES, in **a usual week**, how many days do you do vigorous activities as part of your leisure or spare time?	DAYS_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	
24c	How much time do you spend doing this on a usual day?	_____ HOURS _____ MINS	

25a	In your leisure or spare time, do you do any moderate-intensity activities (like brisk walking, cycling or swimming) for **at least 10 minutes** at a time?	YES..... NO	—➢26
25b	IF YES, in **a usual week**, how many days do you do moderate-intensity activities as part of your leisure or spare time?	DAYS_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	
25c	How much time do you spend doing this on a usual day?	_____ HOURS _____ MINS	
	Sitting / Resting Activity: Now I would like to ask you about the time spent *sitting or resting, not including sleeping, **in the past 7 days**. This may include time sitting at a desk, visiting friends, reading, or sitting down to watch television **during working hours and leisure or spare time**.*		
26.	Over the **past 7 days**, how much time did you spend sitting or reclining (lying) on a **usual day (exclude sleeping)**?	_____ HOURS _____ MINS	

	SECTION 8C: TOBACCO USE (WHO STEPwise Questionnaire)		
33a	Do you **currently smoke** any tobacco products, such as cigarettes, cigars, or pipes?	YES ..... NO	—➢36
33b	Do you currently smoke tobacco products **daily**?	YES ..... NO	—➢36
34a	**How old** were you when you first **started** smoking daily?	YEARS OLD _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ IF "YOU DON'T REMEMBER", ...	—➢35
34b	If you do not remember how old you were, do you remember how long ago it was?	WEEKS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ **1**	
		MONTHS AGO _ _ _ _ _ _ _ _ _ _ _ _ _ **2**	
		YEARS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ **3**	
35	On average, how many of the following items do you smoke each day? (CHECK EACH ITEM, IF NOT SMOKING AN ITEM, CODE 00)	MANUFACTURED CIGARETTES _ _ _ _	
		HAND-ROLLED CIGARETTES _ _ _ _ _	
		PIPES FULL OF TOBACCO _ _ _ _ _ _ _	
		CIGARS/CHEROOTS/CIGARILLOS _ _ _	
		OTHER_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	
36	**In the past,** did you ever smoke daily?	YES ..... NO	—➢38a
37a	**How old** were you when you **stopped** smoking daily?	YEARS OLD:_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ IF "YOU DON'T REMEMBER",	—➢38a
37b	If you do not remember how old you were, do you remember how long ago it was?	WEEKS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ **1**	
		MONTHS AGO _ _ _ _ _ _ _ _ _ _ _ _ _ **2**	
		YEARS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ **3**	

ANTHROPOMETRIC DATA SHEET **SUBJECT CODE: _____**

49	DATE	2 0 0 (d d m m y y y y)
51	WEIGHT (KG)	
52	HEIGHT (CM)	
53	MID-UPPER-ARM CIRCUMFERENCE (CM)	Arm
54	WAIST CIRCUMFERENCE (CM)	
55	HIP CIRCUMFERENCE (CM)	
56	SYSTOLIC BLOOD PRESSURE 1	Arm
57	DIASTOLIC BLOOD PRESSURE 1	
58	PULSE 1	
59	SYSTOLIC BLOOD PRESSURE 2	
60	DIASTOLIC BLOOD PRESSURE 2	
61	PULSE 2	
62	SYSTOLIC BLOOD PRESSURE 3	
63	DIASTOLIC BLOOD PRESSURE 3	
64	PULSE 3	

8.2. USE OF THE NEAR INFRARED INTERACTANCE LIGHT SHIELD AND LIGHT WAND

Performing The Body Fat Evaluation

Place the Light Shield on the Light Wand as shown in Illustration 3, aligning the white stripe on the Light Shield with the silver stripe on the Light Wand. Be sure that the Light Wand protrudes approximately 1/8" (3 mm) past the bottom of the Light Shield.

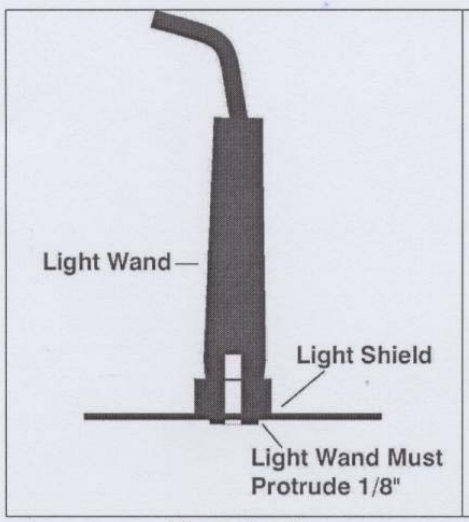

Illustration 3

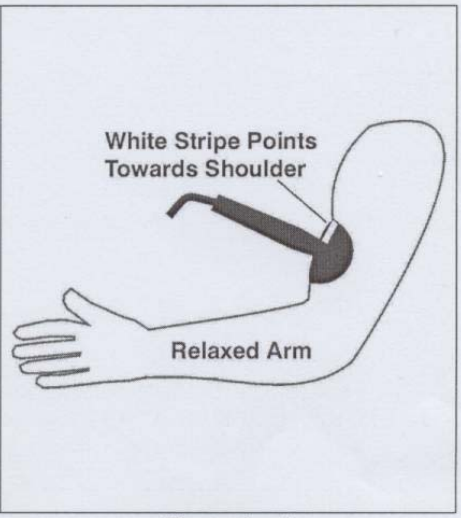

Illustration 4

8.3. DETERMINATION OF THE NEAR INFRARED INTERACTANCE BICEPS MEASUREMENT SITE

Selecting the Measurement Site

Prior to conducting the actual body composition analysis, you should locate the proper measurement site at the biceps of the dominant arm.

1. Have the test subject hold his/her arm straight out, with the elbow locked, and the palm of the hand facing up towards the ceiling.

2. Using the Biceps Locator (shown in Illustration 1), find the "halfway point" between the armpit and the inside of the elbow, where the elbow bends (i.e. the acromion). As shown in Illustration 2, place the end marked "To Underarm" at the point marked "A." The end marked "To Elbow" should extend past the bending point of the elbow towards point marked "B" in Illustration 3.

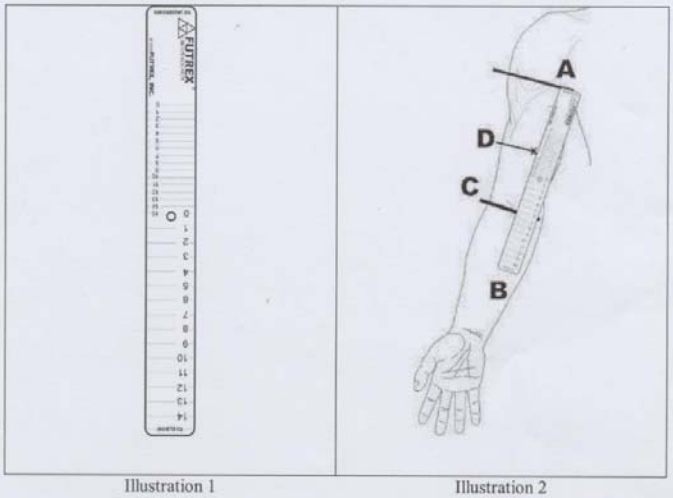

Illustration 1
Biceps Locator

Illustration 2
Locating The Measurement Point

3. Once the Biceps locator is in place as described in step 2, identify the number on the Biceps Locator that crosses the point "C" on the arm. Point "C" is the place where the elbow bends.

8.4 REAGENTS FOR ISOLATION OF DNA FROM WHOLE BLOOD

Last modified March 11 2005

1. TKM1 Buffer (pH 7.6)

	Final concentration	Molecular weight	For 500 ml	Supplier
Tris-HCl	10 mM	121.00	0.6056	Sigma
KCl	10 mM	74.56	0.3728	Sigma
$MgCl_2.6H_2O$	10 mM	203.20	1.016	Merck
EDTA	2 mM	372.24	0.372	Sigma
dH_2O			to 500 ml	

- Autoclave
- Make up 1 volume which includes 2.5% NP40 and 1 volume without NP40

2. TKM2 Buffer (pH 7.6)

	Final Concentration	Molecular weight	For 200 ml	Supplier
Tris-HCl	10 mM	121.00	0.242	Sigma
KCl	10 mM	74.56	0.149	Sigma
$MgCl_2.6H_2O$	10 mM	203.20	0.406	Merck
EDTA	2 mM	372.24	0.1488	Sigma
NaCl	0.4 M	58.44	4.675	Merck
dH_2O			to 200 ml	

- Autoclave

3. 10% SDS

	Final Concentration	Molecular weight	For 200 ml	Supplier
SDS	10%		20	Sigma
dH_2O			to 200 ml	

- Autoclave

4.1X TE buffer (pH 8.0)

	Final Concentration	Molecular weight	For 100 ml	Supplier
Tris-HCl	10 mM	121.00	0.121	Sigma
EDTA	1 mM	372.24	0.037	Sigma
dH_2O			to 100 ml	

- Autoclave

5. 5M $NaClO_4$

	Final Concentration	Molecular Weight	For 100 ml	Supplier
$NaClO_4$	5 M	122.4	61.2	Sigma
dH_2O			to 100 ml	

- Autoclave

5. Other chemicals and reagents

- Chloroform (analytic grade) –Merck Saarchem
- NP40–Sigma
- Absolute ethanol–Kimix

8.5. PROTOCOL FOR THE ISOLATION OF DNA FROM WHOLE BLOOD

Last modified March 11 2005

1. Draw 5 ml of blood into an EDTA vacutainer tube (Purple top).
2. Blood can be stored at 4ºC up to 1 week before the DNA is extracted.
3. Transfer the blood to a sterile 15ml polypropylene tube.
4. Add 2 volumes (10 ml) of TKM1 buffer containing 2.5% NP40.
5. Mix by inverting several times and incubate at room temperature for 10 minutes in order to enhance the haemolysis of red blood cells.
6. Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
7. Decant off the supernatant containing leaving the white pellet at the bottom of the tube.
8. Add 1 volume (5 ml) of TKM1 buffer (without NP40).
9. Invert and vortex the solution.
10. Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
11. Decant the supernatant leaving the white pellet in the bottom of the tube.
12. Repeat steps 7-10 until the pellet in the bottom of the tube is clean and white.
13. Add 800ul of TKM2 buffer and 50ul of the 10% SDS solution.
14. Vortex and then mix using a blue pipette tip in order to assist in the lyses of the white blood cells.
15. Incubate for 60 minutes at 55ºC in a water bath. Make sure the pellet is totally dissolved before moving on.
16. Add 150ul of 5M $NaClO_4$.
17. Add 500ul of molecular biology grade chloroform.
18. Vortex the solution.
19. Transfer the solution to sterile 1.5ml microfuge tubes.
20. Centrifuge at 1300rpm at room temperature for 5 minutes.
21. Carefully transfer 500ul of the top aqueous phase to a new sterile microfuge tube.
22. Add 1ml of absolute ethanol.
23. Invert until DNA precipitates.

24. Centrifuge at 1300rpm at room temperature for 5-10 minutes.
25. Carefully tip off supernatant leaving the pellet in the bottom of the tube.
26. Allow pellet to air dry completely.
27. Add 200ul of 1XTE buffer.
28. Incubate the tubes at 65ºC for 15 minutes in a heating block.
29. Store DNA at 4ºC.

Reference: Lahiri and Nurnberger (440)

8.6. RELATIVE CONTRIBUTION TO DIFFERENT ASPECTS OF THIS THESIS

1. Ethics approval and funding
    a. I assisted with the application for ethics approval and with the subsequent request for more information from the ethics committee.
    b. The main source of funding for my thesis was a South African National Research Foundation grant. I assisted with the application for this grant.
2. Concept and ideas
    a. Chapter Two in this thesis was a result of several other NIR projects that I worked on with Professor Vicki Lambert which were then applied to my own study sample.
    b. The ideas presented in Chapters Three and Four came about through analysis of the data, literature review and many discussions with my supervisors.
    c. Dr. Malcolm Collins and Dr. Julia Goedecke both helped me define my research question and construct the study design for Chapter Five.
3. Operational Details
    a. I recruited the subjects via church groups, universities and other community groups by providing and organizing health checks. I maintained the records and filed all data sheets and informed consent documents. I contacted the subjects after the health checks.
    b. I managed subject payment, organization and preparation of subject food, subject reimbursement for travel and maintained study account records.
    c. I managed scheduling of subject testing (including scheduling of blood work, meeting with dietician, DXA and CT scans) and the necessary booking of drivers and vehicles to transport subjects, booking of translators (first half of study, prior to hire of field workers) and testing preparation and cleanup. I completed the majority of the anthropometric testing. I assisted with the recruitment and hiring of fieldworkers for the study and managed the field workers.
    d. I assisted with the pricing for lab analysis of insulin and lipid profile.
4. Subject feedback
    a. I created and mailed study results and personal results to the subjects of the study on all but the dietary aspects of the project. I arranged a subject feedback and appreciation evening as well as a trip to the community where the majority of our subjects were recruited from for the students and staff in the department.
5. Lab work
    a. I completed the DNA isolation and genotyping
6. Data entry and analysis
    a. Data entry was completed mainly by myself
    b. I completed the data analysis under the supervision of Dr. Julia Goedecke.
7. Compilation of thesis
    a. I completed the compilation of my thesis.