DETERMINANTS OF BODY COMPOSITION CHANGES OVER 5.5-YEARS AND THE ASSOCIATED CARDIO-METABOLIC RISK FACTORS IN FREE-LIVING BLACK SOUTH AFRICAN WOMEN

By
Miss Sarah Anne Chantler
Student Number: CHNSAR004

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN
In fulfilment of the requirements for the degree

MASTER OF SCIENCE (MED) EXERCISE SCIENCE

Faculty of Health Sciences
UNIVERSITY OF CAPE TOWN

University of Cape Town

Date of submission
FEBUARY 2014

Supervisors

Dr. J.H. Goedecke

Dr. L.K. Micklesfield

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

University of Cape Town

TABLE OF CONTENTS

DECLARATION **3**

ABREVIATIONS **4**

ABSTRACT **6**

CHAPTER 1: LITERATURE REVIEW **8**

1.1 INTRODUCTION 9

1.2 INCREASING BURDEN OF OBESITY AND ASSOCIATED METABOLIC DISEASES 10

1.3.1 CONTRIBUTION OF BODY FAT DISTRIBUTION TO METABOLIC DISEASE 12

1.3.2 CHANGES IN BODY COMPOSITION OVER TIME 18

1.4 NON-MODIFIABLE FACTORS ASSOCIATED WITH FUTURE WEIGHT GAIN 19

1.4.1 Effect of age, BMI, ethnicity, and sex on weight gain *19*

1.5 POTENTIALLY MODIFIABLE DETERMINANTS OF OBESITY AND WEIGHT GAIN 21

1.5.1 Socio-economic status *21*

1.5.2 Physical activity *23*

1.5.3 Dietary intake *23*

1.5.4 Contraceptive use and parity *25*

1.6 IN SUMMARY 26

1.7 AIMS AND OBJECTIVES 27

CHAPTER 2: LONGITUDINAL CHANGES IN BODY FAT AND ITS DISTRIBUTION IN RELATION TO CARDIO-METABOLIC RISK IN BLACK SOUTH AFRICAN WOMEN **28**

2.1 INTRODUCTION 29

2.2. MATERIALS AND METHODS 30

2.2.3 Participants *30*

2.2.3 Body composition assessment *31*

2.2.4 Metabolic risk factors for cardiovascular disease and type 2 diabetes *32*

2.2.3 Statistical analysis *33*

2.3 RESULTS 33

2.3.1 Changes in body composition *33*

2.3.2 Changes in metabolic outcomes *36*

2.3.3 Associations between changes in body composition and changes in metabolic outcomes *36*

2.4 DISCUSSION 40

CHAPTER 3: DETERMINANTS OF CHANGES IN BODY WEIGHT AND BODY FAT DISTRIBUTION OVER 5.5-YEARS IN A SAMPLE OF FREE-LIVING BLACK SOUTH AFRICAN WOMEN **45**

3.1 INTRODUCTION: 46

3.2 METHODS: 47

3.2.1 Participants *47*

3.2.2 Socio-economic status and behaviour/lifestyle factors *47*

3.2.3 Physical activity *48*

3.2.4 Dietary intake *48*

3.2.5 Statistical analysis *48*

3.3 RESULTS: 49

3.3.1 Assessment of socio-demographic and lifestyle factors at baseline and follow-up *49*
3.3.2 Summary of weight and body composition changes over 5-5 years follow-up period *50*
3.3.3 Effect of baseline age and BMI on changes in body composition over the 5.5-year follow-up period *50*
3.3.4 Relationships between baseline and changes in SES and lifestyle factors and changes in body composition over the 5.5-year follow-up period *57*
3.3.5 Multiple regression analyses *59*
3.4 DISCUSSION: 60

SUMMARY AND CONCLUSIONS 66

REFERENCES 70

APPENDICES 81

APPENDIX 1: SUBJECT QUESTIONNAIRE-ENGLISH VERSION 82
FAMILY MEDICAL HISTORY *90*
QUALITY OF LIFE AND CLINICAL CONDITIONS *93*
BODY IMAGE ASSESSMENT *95*
SEDENTARY BEHAVIOUR QUESTIONNAIRE *99*
TOBACCO USE (WHO STEPwise Questionnaire) *100*
ALCOHOL INTAKE *102*
PSYCHOLOGICAL QUESTIONNAIRE (Kessler 10) *103*
TESTING SESSIONS 104
TESTING SESSION 1 – Fasting bloods, OGTT, Questionnaires, Anthropometry *104*
TESTING SESSION 2 – DEXA, CT Scan *105*
ANTHROPOMETRIC DATA SHEET *106*
CHECKLIST 107

APPENDIX 2: CHANGES IN BODY FAT DISTRIBUTION, EXPRESSED RELATIVE TO TOTAL FAT MASS, OVER THE 5.5-YEAR FOLLOW-UP PERIOD IN THE BASELINE BMI GROUPS. **108**

DECLARATION

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

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature:……

Date:……

ABREVIATIONS

ACSM - American College of Sports Medicine
App FM - Appendicular Fat Mass
AIDS - Acquired Immunodeficiency Syndrome
ARV - Anti-retroviral
BMI - Body Mass Index
BP - Blood Pressure
CFM - Central Fat Mass
cm - centimetres
cm^2 - centimetres squared
CHO – carbohydrate
CHO/kg – carbohydrate per kilogram body weight
CRF - Cardiorespiratory Fitness
CT - Computerised Tomography
CVD - Cardiovascular Disease
CV% - Coefficient of Variance
DBP - Diastolic Blood Pressure
DDS - Dietary diversity score
DMPA - Depot-medroxyprogesterone acetate
DQI-I - Dietary Quality Index-International
DSAT - Deep Subcutaneous Adipose Tissue
DSTV - Digital Satellite Television
DXA - Dual-energy X-ray Absorptiometry
FFA - Free Fatty Acid
FFSTM - Fat-free Soft Tissue Mass
FFQ - Food Frequency Questionnaire
FM - Fat Mass
GPAQ - Global Physical Activity Questionnaire
HDL-C - High-density Lipoprotein Cholesterol
HICs - High-Income Countries
HIV - Human Immunodeficiency Virus
HOMA-IR - Homeostatic Model of Insulin Resistance
hr/day - hours per day
IR - Insulin Resistance
IPAQ - International Physical Activity Questionnaire
kcal - kilocalorie
kg - kilograms
kcal/kg – kilocalorie per kilogram body weight
kg/m^2 - kilograms per metre squared
kg/yr - kilograms per year
LDL-C - Low-density Lipoprotein Cholesterol
LPL - Lipoprotein Lipase
LMICs - Low- to Middle-Income Countries
m - metres
m^2 - metres squared

mins/day – minutes per day
mmol/L - millimoles per Litre
MNET - Electronic Media Network
MVPA - Moderate- to Vigorous-intensity Physical Activity
mU/L - milliunits per Litre
NCDs - Non-communicable Diseases
OGTT - Oral Glucose Tolerance Test
PA - Physical Activity
SA - South Africa
SANHANES-1 - South African National Health and Nurition Examination Survey-1
SAT - Subcutaneous Adipose Tissue
SBP - Systolic Blood Pressure
SES - Socio-economic Status
SSAT- Superficial Subcutaneous Adipose Tissue
T2D - Type 2 diabetes
TC - Total Cholesterol
TC/HDL-C - Total Cholesterol to High-density Lipoprotein Cholesterol ratio
TG/HDL-C - Triglyceride to High-density Lipoprotein Cholesterol ratio
USA - United States of America
VIF – varience inflation factor
VO_{2max} - Maximum oxygen consumption
yrs - years

ABSTRACT

Introduction: Research from both developed and developing countries, including South Africa (SA), has shown that black women experience a disproportionately high burden of obesity and associated non-communicable diseases compared to their white counterparts. To our knowledge, there are no longitudinal studies that have examined the determinants of obesity and the associated cardio-metabolic risk factors in black SA women.

Aims: The overall aim of this thesis was to examine the determinants of the changes in body composition and the associations with cardio-metabolic risk factors over a 5.5-year follow-up period in a cohort of free-living black SA women. These aims were addressed in two separate chapters with the following specific aims: Chapter 1: To examine changes in body weight, body composition and body fat distribution over 5.5-years in relation to changes in cardio-metabolic risk factors; Chapter 2: To examine the modifiable and non-modifiable determinants of the changes in body weight and body fat distribution over the follow-up period.

Methods: A convenience sample of 64 apparently healthy black SA women (26±7 years) were tested at baseline and again after approximately 5.5-years. Testing included measures of body composition and body fat distribution (dual-energy x-ray absorptiometry and computerised tomography), blood pressure, fasting glucose, insulin and lipid concentrations. An oral glucose tolerance test was performed at follow-up only, from which insulin sensitivity (Matsuda index) and secretion (insulinogenic index) were calculated. In addition, participants completed questionnaires to assess socio-economic status (SES) and lifestyle factors including reproductive health at baseline and follow-up. Physical activity (Global Physical Activity Questionnaire) and dietary intake (Quantified Food Frequency questionnaire) were reported at baseline only.

Results: Chapter 1: There was a significant increase in body weight (8.8±12.5%) and total fat mass (16.4±26.9%), and all other measures of body fat distribution (range: 1.4–35.1%). When expressed relative to total fat mass (%FM), there was an increase in trunk fat mass with a concomitant decrease in peripheral fat mass, which was associated with increased fasting glucose concentrations and reduced insulin sensitivity, and a compensatory increase in insulin secretion at follow-up.

Chapter 2: The increase in body weight was associated with a lower baseline body mass index (BMI), being nulliparous at baseline, and not having children over the follow-up period. Sanitation at baseline and change in sanitation over time had opposing effects on body weight, with smaller increases in weight in those women who had access to sanitation at baseline and larger increases in weight seen in those who improved sanitation over the follow-up period. Centralisation of body fat was associated with a lower BMI and being nulliparous at baseline only. Being younger at baseline was also associated with larger increases in body weight and centralisation of fat mass, but was not independent of the other variables.

Conclusion: In a sample of free-living black SA women, body weight (~9%) and fat mass (~17%) increased significantly over a 5.5-year follow-up period. These increases, and specifically the increase in central fat mass, were associated with increased fasting glucose concentrations and reduced insulin sensitivity. When examining the determinants of weight gain and increasing centralisation of fat mass, younger women without children, with a lower BMI, were at greatest risk of increased weight gain, and should be the target of future interventions in this population. The risk of weight gain was further mediated by changes in SES, which should be examined further.

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Obesity has been described as one of the world's most pressing health concerns due to its association with adverse disease outcomes. Further, obesity rates worldwide continue to increase in spite of public health promotions and policy changes to the contrary [1,2]. Previously thought to be isolated to high-income countries (HIC), increasing focus has now moved towards low-to-middle income populations, such as in South Africa (SA), where the impact of increasing body weight/obesity has been shown to contribute to the significant increase in non-communicable diseases (NCD) such as type 2 diabetes (T2D) and cardiovascular disease (CVD) [3].

Of growing concern is that results from consecutive population surveys in SA and worldwide show increases in the prevalence of obesity, T2D and CVD over the past decade [2,4,5]. Further, research has shown that black women, including black SA women, suffer from a disproportionately high burden of obesity and the associated NCD's compared to their white counterparts [6-8]. Body fat distribution has also been found to differ by ethnicity, and could explain some of the variation in disease risk found in these groups [9,10]. In addition, several socio-cultural and behavioural factors (socio-economic status (SES), diet, physical activity, contraceptive use) may contribute to the apparent ethnic differences in obesity and disease risk. There is a paucity of longitudinal data on changes in body weight, body composition, and SES/behavioural factors in SA. Studies in developed countries have pointed to the importance of diet, physical activity, in addition to other behavioural and lifestyle factors in future weight gain. Hence, it is essential to understand their role in the emerging obesity epidemic in the a developing country context.

Therefore, this review focuses on studies investigating the association between changes in body weight and body composition in relation to changes in metabolic outcomes. Further, the review considers and the upstream factors (SES, diet, physical activity) that may contribute to increased weight gain. Particular focus is on black SA women, using white women as a comparison. Further we also compare this to research in African American women, who may have mixed ancestry, but who present with a similar phenotype to black South African women. Further, there has been extensive research undertaken to characterise their specific phenotype of African American women compared to white American women.

1.2 Increasing burden of obesity and associated metabolic diseases

The burden of obesity in SA is higher than in other low- to middle-income countries (LMICs) in Southern Africa. Overweight/obesity rates have been reported to range from 22.4% in Zimbabwe to 50.6 and 54.9% in Swaziland and SA respectively [11]. Recent data from the SA National Health And Nutrition Examination Survey (SANHANES-1) suggests the secular increases in obesity, highlighting a growing public health concern. Data from the South African Demographic Health Survey (SADHS) in 1998 and 2003 showed the rate of obesity/overweight in black women increased from 57.1% and 56.2% [12,13], with a further increase to 64.8% in the 2012 survey [6] (See Figure 1). Results from SANHANES-1 also highlight the disproportionate burden of obesity in black women, which was more than four times higher than black men (39.9% vs. 9.4%, respectively) and higher than females from other ethnic groups (39.9% vs. 32.4% and 34.9% for black vs. Asian/Indian and Coloured women respectively (data for white women was not reported in SANHANES-1 due to sample size) [6].

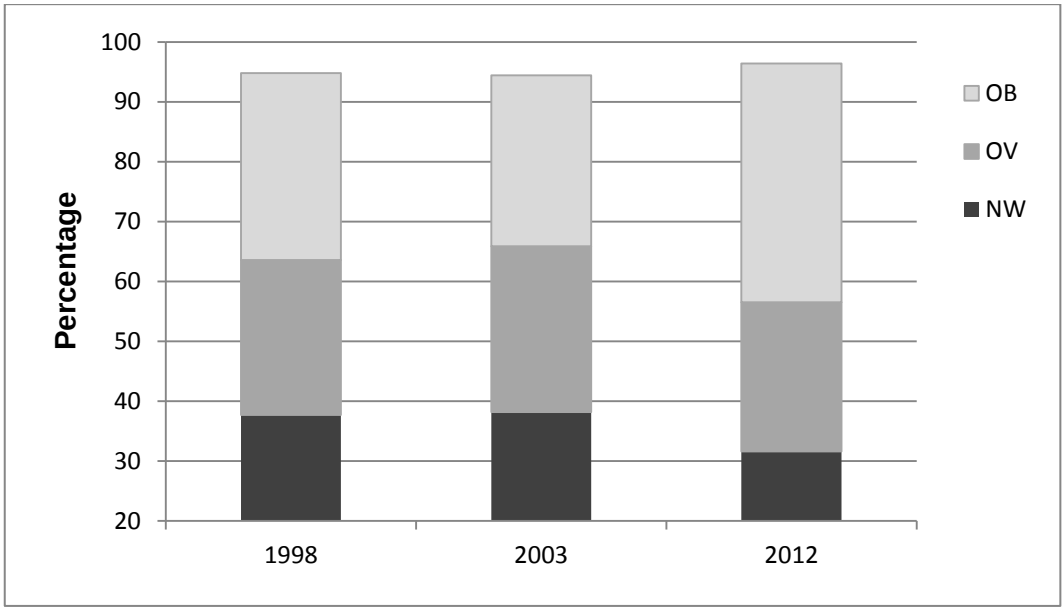


Figure 1: Prevalence of normal weight, overweight and obesity in black South African women from 3 national surveys, 1998 and 2003, South African Demographic Health Survey (SADHS), 2012, South African National health and nutrition examination survey (SANHANES-1), NW, normal weight (BMI18.5-24.9kg/m^2), OV, overweight (BMI 25-29.9kg/m^2), OB, obese (BMI ≥ 30kg/m^2) [6 12 13]

The finding of increasing obesity rates in SA is in accordance with international longitudinal studies that have shown steady increases in weight and body mass index (BMI) over time, particularly in women [14,15,16]. Although data from LMICs is sparse, similar trends have been found in the Philippines [17] and Brazil [18], with reported increases of 0.45 and 0.72 kg/year

over time, respectively. These findings of consistent weight gain and associated increases in body weight/BMI over time have been related to an increase in cardio-metabolic risk. The CARDIA study (3095 non-diabetic black and white men and women) showed an association between 7-year weight gain and increases in fasting blood glucose concentrations were reported [19]. Further, increased CVD risk was related to weight gain at 15 years in 3276 black and white American adults [20], and after a 20 year period, increased waist circumference (WC) was associated with a decrease in insulin sensitivity in the same population [21]. Similarly, Siervogel et al. [22] showed increases in lipid profile with increasing adiposity over time in 423 white adults. These longitudinal studies are in accordance with the large body of cross-sectional studies in HICs that have reported the positive relationship between obesity and increased risk of adverse metabolic outcomes [23].

From the repeated SA national surveys, using the mean weight of black women from the SADHS (1998, n=6178) and SANHANES-1 (2012, n=3367) of 68.2 and 72.6 kg respectively, the annual mean weight gain was 0.31 kg/year [6,13]. Although there is no longitudinal data available on weight gain and associated metabolic risk in SA, cross-sectionally, a higher BMI (>21kg/m^2) has been found to contribute to 87% and 68% of deaths from T2D and hypertension, respectively, with the risk of overall mortality being double in women compared to men [24]. As with increasing rates of obesity, the incidence of T2D and dyslipideamia in SA may also be increasing, specifically in black women. In a peri-urban sample of 1071 black men and women in Cape Town, Peer et al. [25] reported an increase in diabetes prevalence from 8% to 12.2% from 1990 to 2008/09. See figure 2 for the distribution and increase in T2D in the age and gender groups from the 1990 and 2008 samples. Of those found to be diabetic (T2D) by the 1985 WHO definition, 80% were overweight/obese (BMI>25kg/m^2). Further, within the same sample, the prevalence of elevated total cholesterol (TC) or low-density lipoprotein (LDL-C) was 23.1 and 47% respectively [26], which was significantly greater than that shown in the same population in 1990. Results from the Heart of Soweto study, examined risk factors for CVD outcomes in patients referred to the cardiovascular clinic. In the sample of black men and women, the prevalence of elevated TC and LDL-C was 39 and 44%[27]. A higher BMI contributed significantly to elevated serum lipids in this sample. Another study of 1040 black SA women (15-70 years) from the North West Province of SA, reported that BMI and WC were significantly correlated to markers of diastolic blood pressure, serum triglyceride concentrations (TG) and measures of glucose control and were predictors of metabolic risk[28]. Even with these reported increases in rates of elevated blood lipids, research has shown that black women have a more favourable lipid profile than white women, with lower TGs and high-density lipo-protein (HDL-C) [29,30].

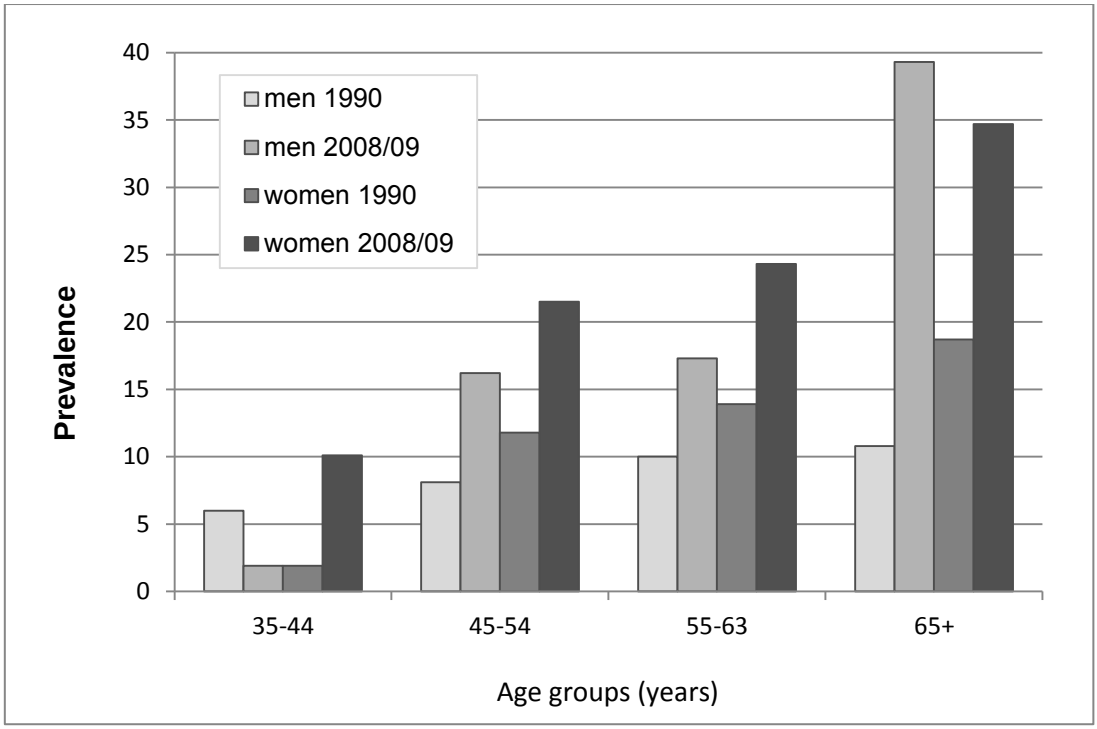


Figure 2: Illustrating the prevelance of T2D within age and gender groups in 1990 and 2008/09 samples [25]

1.3.1 Contribution of body fat distribution to metabolic disease

Body fat distribution, rather than body fatness alone, has been found to be an important factor when considering the impact of obesity on the risk of metabolic disease [31]. Initial observations showed that centralised (trunk and android) obesity posed a higher cardio-metabolic risk compared to peripheral (gluteofemoral, leg and arm) obesity. With the development of more sophisticated radiological imaging techniques, several studies have shown that increased abdominal visceral adipose tissue (VAT) is associated with a higher metabolic risk, specifically increased rates of insulin resistance (IR), dyslipidaemia and T2D [31,32], compared to the associations found with increased abdominal subcutaneous adipose tissue (SAT) [32]. In contrast, increased leg or gluteofemoral fat mass (FM) may have protective benefits against poorer metabolic outcomes [33]. However, these results are based on studies in predominantly white/European populations, and more recent studies in African American and black SA women have shown that ethnicity may alter the relationship between VAT and metabolic outcomes in black compared to white women [10,34]. This may be due to the ethnic differences in body fat distribution, as studies have shown that black women have less VAT, more SAT and more gluteofemoral and leg FM compared to their white counterparts [9,34,35].

However, even with this more favourable body fat distribution, black women have been found to have lower insulin sensitivity and higher rates of T2D compared to white women[10,34]. This apparent paradox has lead to an interest in SAT, and its sub-depots, for its possible contribution to IR in black women. Specifically, Tulloch-Ried et al. found that total SAT was a stronger correlate of IR than VAT in black women [36]. Further, Goedecke et al. [34] showed that deep SAT (DSAT) was the strongest correlate to IR compared to superficial SAT (SSAT) and VAT within a group of black SA women, supporting the argument that the sub compartments of SAT may also have different metabolic effects compared to VAT [37]. The ethnic-specific associations between adipose depots and insulin sensitivity in black and white SA women [34] are presented in Figure 3. Table 1 provides a summary of selected studies that examined the ethnic differences in the association between adipose depots and metabolic outcomes. Studies show that with trunk adiposity, white women have a higher risk of CVD, and a more prevelant association with blood lipids compared to black women [38,39]. Further, black women show consistent associations between VAT, SAT and its subdepots and glucose/insulin measures and HDL. While white women show similar relationships, the relationship between SSAT and insulin is oppostite to that found in black women [34].

Several possible mechanisms have been proposed to explain the ethnic variation between adipose depots in relation to metabolic risk factors. In a comprehensive review by Tchernof et al. [32] the metabolic effects of VAT are partially attributed to its contribution to systemic free fatty acids (FFA) and unique inflammatory profile [40]. It is hypothesised that when the body is faced with excess FFA, a 'tipping point' is reached as these excess FFA result in triglyceride deposition in ectopic tissues causing an inflammatory cascade and eventually IR (Figure 4). VAT has been found to more lipolytic in nature, being more resistant to the antilypolytic effects of insulin and with greater adrenoreceptor sensitivity, therefore adding to the systemic supply of FFA [32,41]. However, when considering the role of SAT, Patel et al. [42] has proposed that due its larger size, total SAT has a greater influence on systemic FFA, potentially contributing up to 80% of the FFA in circulation. In line with this hypothesis, black SA women have more SAT than their white counterparts [34], which may in turn have a greater impact on IR.

Black women have been found to have a higher inflammatory SAT gene expression than white women, including increased expression of chemokines, macrophage markers, and cytokines such as tumour necrosis factor-α [43]. However, Evans et al. [43] found that despite higher inflammatory SAT profile in black SA women, that this did not account for a significant amount of variance in IR. Although continued research is required to explain the ethnic

variation described above, it is probable that the FFA tipping point may be reached at different points on a continuum of adiposity depending on genetic, ethnic and environmental interactions [42]. Besides body fat distribution and obesity-associated inflammation, other factors that may contribute to the ethnic disparity in IR. These have been described in a review by Goedecke et al. [44] and are will not be discussed further, as they are beyond the scope of this thesis.

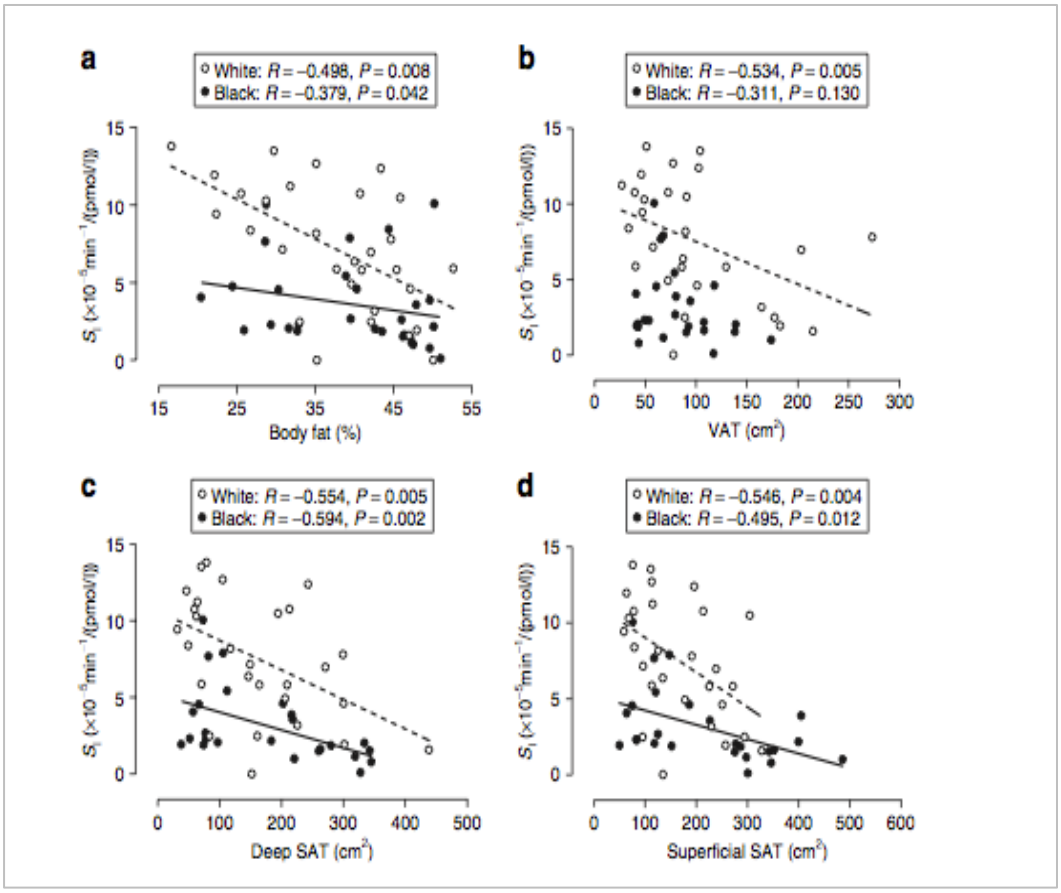


Figure 3: The ethnic-specific relationships between insulin sensitivity index (S_i) and a) body fat, b) visceral adipose tissue, C) deep subcutaneous adipose tissue (SAT) and d) superficial SAT depots in black and white SA women. Regression lines are solid for black women and dashed for white women. When a relationship is not significant, no regression line is shown [34]

Higher VAT levels have also been associated with higher blood lipids, with this relationship first being established in white women [45,46]. As mentioned earlier, black women in SA and the USA have a more favourable blood lipid profile and have lower rates of dyslipidaemia compared to white women [27,29]. This was initially attributed to lower levels of VAT, but more recently, other mechanisms have also been proposed. Sumner et al. [47] recently found that low apolipoprotein C-III levels in conjunction with VAT contributed to the ethnic differences in

serum TG levels. In addition, ethnic differences in serum lipid levels may also be attributed, in part to differences in lipid metabolism, specifically differences in lipoprotein lipase (LPL) activity [7] and genetic variations in the Proprotein convertase subtilisin/kexin type 9 (*PCSK9)* gene [48]. It is still worthwhile to note that in spite of these lower rates of dyslipidaemia, there are still significant increases in the prevalence of dyslipideamia in recent different SA studies [26,49] in relation to increasing obesity (as mentioned previously) .

In contrast to central obesity, peripheral fat mass has been shown to play an independent protective role against IR/T2D [50]. The Hoorn study that includes Dutch obese post-menopausal women, found that increasing leg fat mass (via leg to trunk ratio) and high thigh circumference were protective against elevated blood glucose levels, independent of trunk

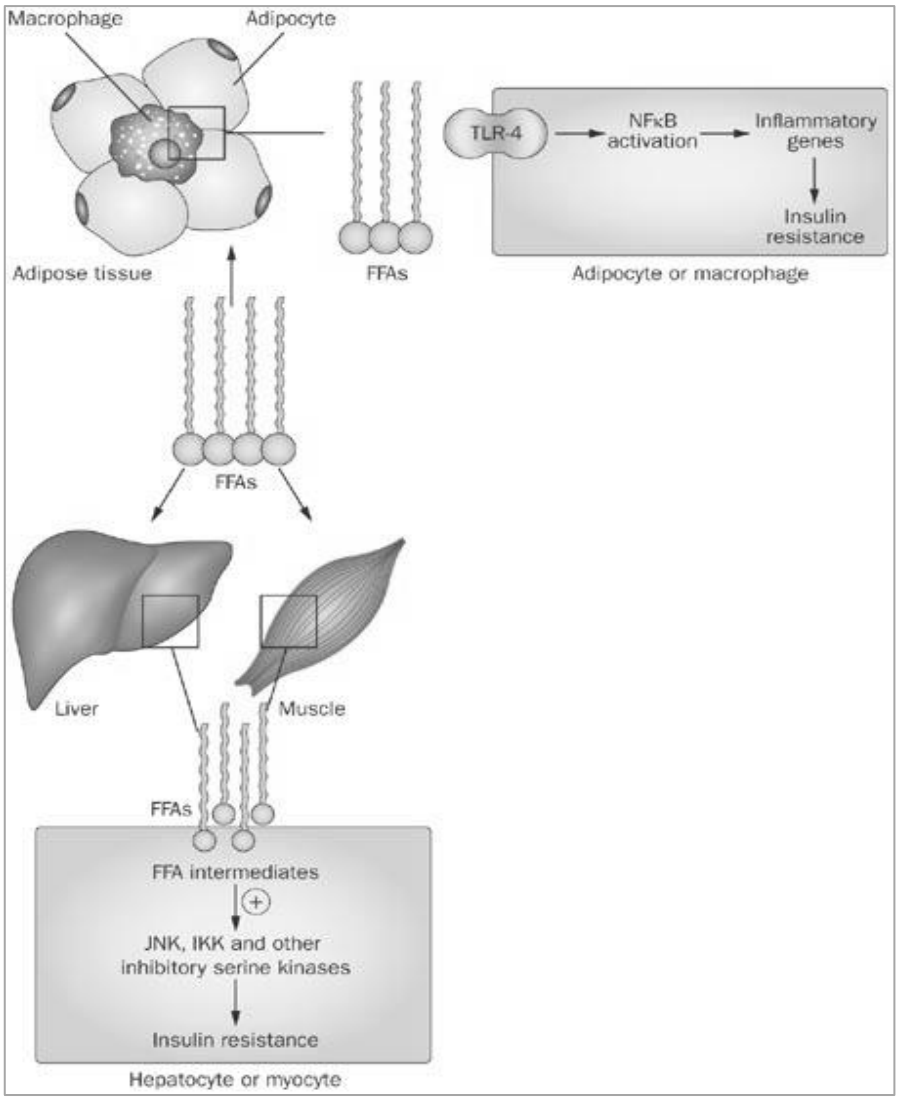


Figure 4: Hypothesised mechanism for the development of insulin resistance due to excess free fatty acids. Credited to Rocha V. and Libby P, Obesity, inflammation and atherosclerosis in Nature Reviews Cardiology 6, 399-409 (June 2009). FFA, free fatty acids; IKK, IκB kinase; JNK, Jun N-terminal kinase; NFκB, nuclear factor κB; TLR-4, toll-like receptor 4 [51]

Table 1: A summary of relevant research examining the ethnic-specific relationships between adipose tissue depots and cardio-metabolic outcomes

	Characteristics	Trunk			
		Black women		White women	
The Pennington Center Longitudinal Study (PCLS) [38]	779 black women 1129 white women BMI<40, 18-64 years Age: 18-64 years Trunk adiposity stratified into tertiles	2nd and 3rd tertile OR = 1.91 and 2.87, respectively for 2 or more CVD risk factors (P for trend = 0.010) 38% diagnosed with 2 or more CVD criteria		2nd and 3rd tertile: OR = 3.47 and 7.60, respectively for 2 or more CVD risk factors (P for trend <0.001) 32.5% diagnosed with 2 or more CVD criteria	
Center for interdisciplinary research in Women's Health, University of Texas [39]	204 black, 247 white, and 257 Hispanic women Age NS between groups Age: 16-33 years B>W weight, body fat	TG: β=1.13 HDL: β=-0.38 VLDL: β=0.23 TC: HDL β=0.04		TG: β = 3.53* HDL: β=-0.41* VLDL: β=0.71 * TC:HDL: β=0.07* * P<0.05 for B vs.W β: adjusted regression coefficients	
		VAT		SAT	
		Black women	White women	Black women	White women
Cape Town study group [30, 34]	15 normal weight and 13 obese black women, 15 normal weight and 13 obese white women VAT B<W (P=0.051) SAT B>W (P=0.003)	S_I: R= -0.311, NS Not correlated to blood lipids	S_I. R = −0.534, P = 0.005 Not correlated to blood lipids	DSAT vs. S_I R = −0.594, P = 0.002 SSAT: vs. S_I R = 0.495, P = 0.012	DSAT with S_I R = −0.554, P = 0.005 SSAT with S_I R = −0.546, P = 0.004

The Healthy Transitions Study [10]	55 black women, 103 white women Age: >43 years but premenopausal B>W for BMI, body fat% VAT B<W but NS SAT and SSAT B>W	Glu R=0.41, Insulin R=0.41 HDL R=-0.35	Insulin R=0.57 HDL R=-0.33, LDL R=0.34	HDL R=-0.23 Insulin R=0.49 Glu R= 0.31	TC R=0.36 HDL R=-0.32 LDL R=0.37 Insulin R=0.56 Glu R=0.23
		P<0.05 for associations between depots and metabolic outcomes			
The Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study [7]	143 black women: 31.7±11.1 years 240 white women, 34.4±14.0 years B>W for BMI, body fat%, WC VAT B<W but NS SAT B>W	HDL R=-0.30 (P<0.0005), VAT explained most of variance for lower HDL, TG, TC and ApoB	HDL R= 0.15 (P<0.05)	Not shown	

NS; not significant, OR; odds ratio, VAT; Visceral adipose tissue, SAT; Subcutaneous adipose tissue, DSAT; deep subcutaneous adipose tissue, SSAT; superficial subcutaneous adipose tissue, B, Black, W, white, CVD, cardiovascular disease; S_I, insulin sensitivity, TC, total cholesterol, TG, triglyceride, HDL, high density lipoprotein, LDL, low density lipoprotein, ApoB, apiloprotein B

fat mass [33,52]. Similarly, McLAughlin et al. [53] showed that SAT thigh fat was protective against IR after adjusting for VAT and BMI, while Wiklund et al. [54] showed that gynoid to trunk ratio was inversely associated with CVD outcomes in women. The positive relationship between peripheral fat mass and lower blood lipid concentrations has also been found [55].

These studies hypothesised that the peripheral depots have an ability to take up more of the systemic FFA, therefore protecting against the ectopic deposition in certain tissues (muscle, liver, pancreas). McCarty et al. [56] proposed a further mechanism that involved the effect of preferential post-prandial uptake of FFAs by glutoefemoral SAT due to its greater insulin sensitivity compared to the insulin resistant VAT. Similarly, these findings were predominantly in white women. While black women have been found to have more gluteofemoral fat then their white counterparts [57], initial research from Goedecke et al. [57] found decreased adipogenic gene expression in the gluteal depot of obese black SA women, and this was associated with a decrease in insulin sensitivity. This study questioned the idea that the gluteal SAT depot could continue to increase *ad libitum* with increasing obesity and therefore remain a protective FFA 'sink', or, alternatively, if the reduction in adipogenic potential with increasing obesity leads to a decrease in peripheral FM and redistribution of fat centrally. However, longitudinal studies examining the changes in gene expression in relation to adipose depots over time are required to answer these questions.

1.3.2 Changes in body composition over time

Whilst secular trends in weight gain and subsequent obesity have been relatively well described, there is a paucity of data on the longitudinal changes in body fat distribution. One study comparing increases in VAT and SAT in 1233 black and Hispanic American adults (20-69 years) over 5 years, showed a larger increase in VAT in black compared to Hispanic women [58]. A second study including 65 black and white women (22-47 years) showed that over the same time period, black women had lower VAT at baseline and follow-up, but increased their VAT at a similar rate to their white counterparts, and in proportion to their increases in fat mass [59]. Unfortunately, neither of these studies measured the impact of these changes in VAT on metabolic risk. However, some assumptions might be drawn from various weight loss studies that have shown improved insulin sensitivity with greater losses of VAT [60,61]. In a 24-month weight loss and lifestyle intervention (n=112), a reduction in VAT was predictive of the relative increase in insulin sensitivity, after adjusting for sex, age, baseline VAT, baseline insulin sensitivity and time of follow-up [61]. This in accordance with results from a Korean study, where loss of VAT over 12 weeks was associated with greater improvements in metabolic outcomes than the comparative losses in SAT or body weight [60].

Further clinical controlled trials using diet alone vs. diet and exercise also showed significant simultaneous decreases in VAT and IR [62]. However, to our knowledge, there are no longitudinal studies that have examined changes in peripheral FM in relation to metabolic consequences.

1.4 Non-modifiable factors associated with future weight gain

1.4.1 Effect of age, BMI, ethnicity, and sex on weight gain

While this review has highlighted the ethnic variation in weight gain, body fat distribution and its relationship to metabolic risk, there may be other non-modifiable factors that also influence this relationship rather than ethnicity alone. In particular, there is evidence to suggest that those who are younger tend to increase their body weight more than their older counterparts over the same time period (Figure 5). In a study including Scottish women of two different age groups (n=582) it was shown that 39-year old women (at baseline) increased their body weight over 9 years significantly more than their 59 year old counterparts (of 5.5 kg vs. 1.6 kg, respectively) [63]. A similar effect was found in the Black Women's Health Study (BWHS), which tracked weight changes in a large cohort of 41351 African American women over 14 years. In this study, participants who were less than 35 years of age at baseline had a mean increase of 13.1 kg compared to 9.1 kg in those who were over 45 years [64]. Within LMICs, a similar effect was found in Filipino women, as women who were 20 years old at baseline had a 12.4 kg increase compared to 4.9 kg in those who were 35 years at baseline [17]. Unfortunately, these studies did not propose a mechanism for the larger weight gain in the younger age groups. Possible reasons may include transition from adolescence to adulthood, starting working life, or increased reproductive capacity, which may involve child bearing or use of hormonal contraceptives; however data supporting/corroborating these factors is sparse. Interestingly one study has shown that the transition period from school to tertiary education (generally persons 17-20 years) is more likely to be weight promoting [65]. However in the Western Cape, the percentage of students who complete secondary school is currently 24.6% and those who go on to complete tertiary level education is 16.7% [66], and this may be lower in females, which may be considered too small for this to be the same effect. Further research is required to consider the reasons for this possible increased risk of weight gain in younger age groups.

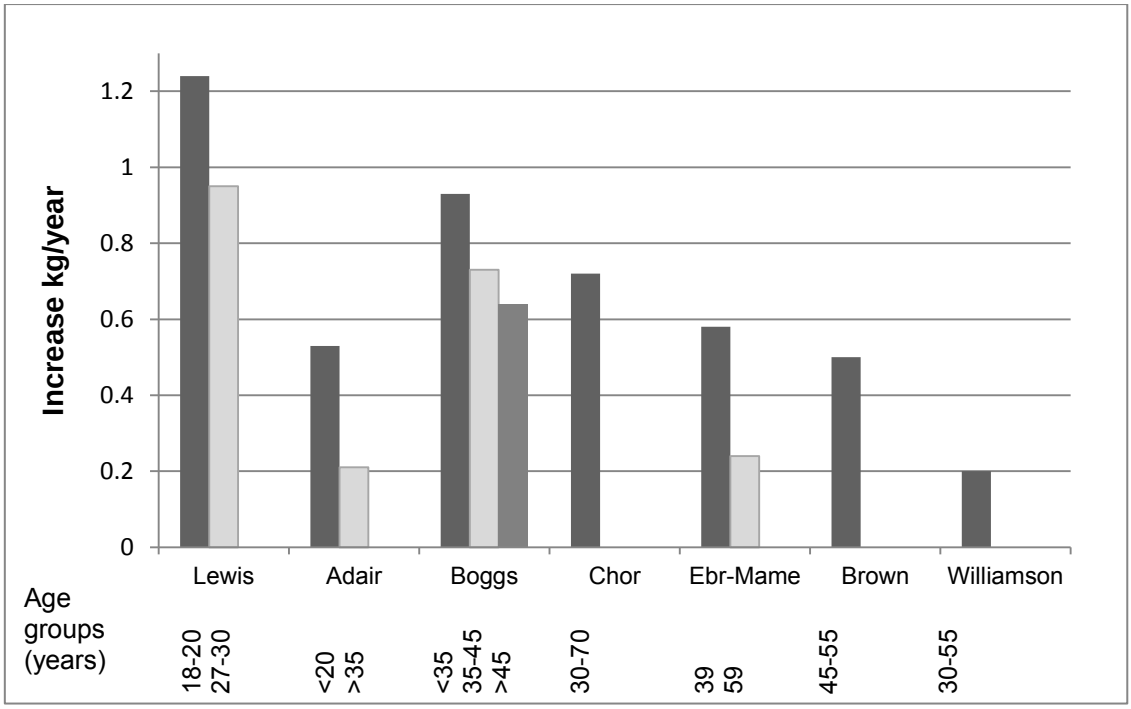


Figure 5: A graphical representation of annual weight gain in different age groups from various studies, age groups from left to right in years: 18-20 years, 27-30 years [67], <20 and >35 years [17], <35, 35-45, >45 [64], 30-70 [18], 39 and 59 [63], 45-55 [16], 30-55 [68] years, respectively

In addition to the effect of starting age of participants, baseline BMI may impact the propensity for weight gain, though this effect has only been described in two studies. Brown et al. found that baseline BMI in conjunction with higher energy intake, was a determinant of 5 year weight gain in women (45-55 years) [16], while the BWHS also showed that those women who had a normal BMI (<25 kg/m^2) at baseline had larger increases in weight over 14 years compared to those who started the study at a BMI of >30 kg/m^2 (10.58 vs. 9.93 kg respectively) [64]. Again, due to the changes in lifestyle over time, it is difficult to determine a direct cause for this effect, but it can be hypothesised that the capacity for weight gain may increase the risk in this BMI group, or amplify other risk factors.

Notably, the rate of weight gain has been found to impact of the development of metabolic consequences. An increase of 5% or more of body weight over 5 years was found to increase the risk of metabolic syndrome [69]. In addition, the progression of CVD was found to be higher in relation to the years spent obese [70], adding to the evidence that the progression of weight gain should be delayed as long as possible, and that if younger women are at higher risk, that this will further magnify the burden of NCDs once they are older.

When comparing the rate of weight gain between different ethnicities and sexes, data from various longitudinal studies in the USA show that in various sample populations black women tend to increase their weight more over time compared their white female counterparts [14,67,71]. At a 7 year follow-up, black women (18-30 years at baseline) had a mean increase of 8.6±9.8 kg compared to 5.2±8.4 kg in white women [14]. This trend was also observed at the 10 year follow-up in the same study [67]. Considering differences in sex, SANHANES-1 reports that black women have a higher rate of obesity, dyslipideamia, and elevated HbA1c compared to black men [6]. Interestingly Case et al. [72] proposed that it was not due to sex itself, but more closely related to the interaction of childhood food security, SES and sex that may lead to increased risk of obesity. Given the socio-political history of SA, it is important to consider that differences found between ethnicities and sexes may be due to differences in SES, which has also been shown to impact on weight gain and associated health outcomes.

1.5 Potentially modifiable determinants of obesity and weight gain

1.5.1 Socio-economic status

The predominant finding in HICs shows that SES is inversely associated with the prevalence of obesity, with the relationship being stronger in women than in men [73,74]. Conversely, initial data in LMICs revealed the opposite effect with higher SES being associated to higher rates of obesity [75,76]. More recently, however, some studies in LMICs have found a shift towards increasing obesity in lower SES groups, particularly in resource limited populations, mimicking the effect found in developed nations [77,78]. These conflicting results may illustrate the economic transition or effects of urbanisation, as urbanisation may alter the lifestyles of the populations in these countries, and show that the relationship between SES and obesity may change over time. SA has a high GINI co-efficient, representing a large ditribution of wealth/income, adding to the relationship between SES and obesity, and that it is not linear in nature [79].

Of note, many of the studies examining the effects of SES use level of education or employment/income as proxy indices for SES. Often this may render international studies non-comparable, as certain LMICs may not find these variables the best proxy for household wealth/SES [80]. Recently, more studies have used composite scores of SES, including occupational status, education and household wealth (excluding income) [81], or used household appliances and/or housing density as proxy measures for SES [30]. Other demographic surveys, such as the ones mentioned above have used gross domestic

product, which may consider overall national economic status, but miss pockets or localised populations.

In addition to the multiple proxies used, SES is usually measured as a static variable and used as a predictor of weight gain, rather than a factor with the capacity to change. Currently, to my knowledge, there are currently no studies considering the dynamic effects of changing SES on obesity risk and weight gain in individuals (rather than the effect of urbanisation or economic improvement on a population). In a population that migrates with seasonal work, has informal employment, monthly variation in household food security and/or household numbers, overcrowding, poor sanitation and other similar factors, this may be pertinent to consider in relation to risk of weight gain. Currently, 13.6% of SA lives in informal housing [82]. Further, due to the interaction between obesity and SES, there are several cultural and behavioural factors, including physical activity (PA) and diet, which may contribute to these associations (See Figure 6).

Figure 6: A schematic representation of the overlapping relationship between socio-cultural, environmental and behavioural factors that may contribute to obesity and weight gain. The centre represents the non-modifiable factors (age, gender etc.), which in turn are influenced by the traditional modifiable factors (SES, socio-economic status, Physical activity, diet etc.), which may in turn overlap with other society specific variables (access to health care etc.)

1.5.2 Physical activity

Low levels of PA or high levels of inactivity are positively associated with higher BMIs and thought to contribute to obesity risk due to low energy expenditure and its effect on energy balance [83]. In LMICs, cross-sectional data from African countries has shown a large variance in activity levels between countries [84]. Further, national data from the World Health Survey in 2003, showed that according to the International Physical Activity Questionnaire (IPAQ), 49% of SA is considered insufficiently active. Using the Global Physical Activity Questionnaire (GPAQ), the SADHS (2003) reported that black urban women accumulated more PA than their white counterparts[12], which is in agreement with smaller SA studies [34,85] . It is important to note that findings from studies that use subjective questionnaires for measuring PA need to be interpreted with caution due to the possibility of misreporting the amount of time spent being physically active. More appropriate are studies using objective measures (pedometry and accelerometry), though studies of this nature are limited, particularly in LMICs. Cook et al. [86] showed that steps per day, measured using pedometers, were associated with a dose-dependent reduction in risk of obesity in black rural women from the Limpopo Province (*n*=121). Results from the study showed that measures of obesity (BMI and WC), were significantly lower (52%) in those who reported >10 000 steps/day compared to those who were classified as sedentary (<5 000 steps/day). Recently, higher cardiorespiratory fitness (predicted VO_{2max}) has been shown to be inversely associated with BMI and fat mass in a sample of black women (Dickie, unpublished data). To my knowledge, there are currently no longitudinal data describing changes in PA in relation to adiposity over time in SA. However, international longitudinal studies have shown that daily walking time or high levels of PA can diminish weight gain over time [87,88]. In summary, in spite of different methodologies, physical activity is seen as a contributing factor towards diminishing weight gain over time and improved metabolic outcomes [87].

1.5.3 Dietary intake

Dietary intake also impacts energy balance due to its affect on energy balance [89]. Studies from SA have shown that black women have a habitual fat intake above the prudent guideline of 30% of dietary energy, and that it contributes more to overall energy intake than white women [11,34,90]. Notably, as with subjective PA reporting, there are challenges with dietary recall, such as under-reporting, which have been shown in multiple studies [91], posing additional limitations to interpreting traditional dietary data. This has lead to the development of various alternative ways of analysing dietary intake.

Different international studies have used measures of dietary quality rather than micronutrient or macronutrient breakdown in relation to weight gain and metabolic outcomes [92]. Although the golden standard is considered a 7-day weighed food record, the burden to participants is often considered too high. Therefore, these measures are often based on food-frequency questionnaires, which are then grouped into food groups or dietary patterns. These measures include the Dietary Quality Index (DQI) [93], which has been adapted to be internationally comparable (DQI-I) [94]. Similarly, food cluster analysis [95], or food pattern analysis [96] were introduced as an alternative methodology to consider the whole diet as a construct rather than individual foods. In SA, food frequency questionnaires have been validated in local populations [97,98], introducing food photos and pictoral portion sizes to assist with understanding. Other studies have used dietary diversity scores (DDS) to assess dietary adequacy[99]. One study assessing DDS in peri-urban women found that household food insecurity reduced DDS and therfore dietary adequacy[100]. Recently the SANHANES-1 found that 28.2% of the Western Cape sample had a very poor DDS score (DDS <4). In addition, when considering urban and rural groups, Labadarious et al. found that those in informal urban areas were more likely to have poorer DDS, with 55.7% reporting a score of under 4[101]. Although this data on dietary quality in SA populations has not been associated to weight gain, various other studies have shown that dietary quality or certain food patterns can predict weight gain [64,93,102,103] Specifically, a more westernised eating pattern is associated with higher weight gain over time [92,104] compared to a prudent intake. In the THUSA study in SA women, increasing urbanisation was linked to increasing BMI and higher dietary fat intakes and higher micronutrient intakes [90], but the longitudinal effects of urbanisation on dietary quality have yet to be determined.

In addition, in SA, it is also important to consider the contribution of street or ready-made food. A recent review indicated that in developing countries, street food could contribute 13 to 50% of total dietary energy intake in adults [105]. SES is likely to have a further impact on dietary quality [106,107], as cheaper foods are often higher in energy density, but lower in nutrient quality [108] and healthy diets are considered more expensive [107,109,110].

Lastly, household food insecurity, an extention of SES, has been found to promote obesity [111], and recent data from SANHANES-1 has shown that 28.3% of the households were at risk of hunger while 26% experienced hunger, with greater food insecurity in the urban informal group (32.4%) [6]. Although this is still high, it is worth noting that this is an improvement from the original National Food consumption surveys of 1999 and 2005, which reported household hunger at 52% [112]. In the current SA context, it is difficult to separate

these factors when considering dietary intake, and should be considered in terms of their impact on dietary intake and therefore possible energy imbalance. In summary, due to the variation in dietary measures used, and the evolution of improved tools, direct comparison can be difficult. However, multiple studies have shown a consistent link between higher energy intakes and weight gain over time [92,104]. This has further been linked to increased risk of poorer metabolic outcomes[19] . This effect may be further mediated by household food security, SES and urbanisation. However, these factors have not been studied simultaneously in the SA context.

1.5.4 Contraceptive use and parity

Reproductive health, in terms of hormonal contraceptive use and childbearing, has also been investigated with regards to weight gain in women. Injectable hormonal contraceptive use has been found to increase weight and central to peripheral fat mass ratio over 3 years compared to oral hormonal contraceptive or no hormonal contraceptive use [113]. Similarly, in a sample of 323 women (18-35 years), Clark et al. [114] reported a mean weight increase of 6.1 kg in association with 30 month use of progesterone only depot-medroxyprogesterone acetate (DMPA), compared to no weight changes in non-contraceptive controls. Although the mechanisms behind this weight gain is not fully understood, animal and human studies have indicated that DMPA induces hypoestrogenaemia, which has been linked to increases in VAT [114]. In SA, contraceptive use (vs. none) has been associated to higher education and employment [115], and more women use injectable contraceptive compared to oral forms [116] which may be due to due to limitated resources within the public health system. While there is no longitudinal data examining weight gain and contraceptive in SA women, Goedecke et al. [30] found that injectable contraceptive use was associated with a higher lipid profile in black SA women. Berenson et al. [117] found a similar effect, where hormonal contraceptive (both oral and hormonal) increased LDL-C and decreased HDL, but was tansient for those on DMAP (injectable), and returned to baseline after 6 months.

Weight gain related to childbearing continues to be reported by some, but not all studies [118] [119] [120]. Lower post-partum weight loss increases the risk of obesity at an older age, but there is a dearth of research in SA women examining this effect. Further, there is little research regarding changes in body composition and body fat distribution in women during and post-partum. Risk of lower post-partum weight loss has been strongly linked to the gestational weight gain itself [121], which may be further influenced by other factors, such as ethnicity [122,123], SES [124] [125], and dietary intake [126].A Chilean study found that although BMI increased marginally with childbearing, there were no increases in WC or central measures of

obesity[127].

Post-partum weight loss may also be affected by feeding methods for the infant. In a study including 427 American women, Rothberg et al[124]. showed that post partum weight loss over 12 months improved with breastfeeding. In contrast, in a smaller (n=20) study, Chou et al[128]. found no differences in weight loss at 12 weeks post partum between women who were breastfeeding or formula feeding. In a recent review, Neville et al. [129] highlighted that most of the current research regarding post-partum weight loss and feeding methods has been observational, and that the associations are difficult to isolate in these circumstances, and that more research is required. Therefore, it may be plausible that feeding choices combined with gestational weight gain, and other factors such as SES and diet may influence post-partum weight loss women.

1.6 In summary

Obesity is increasing in SA, with the highest burden being experienced by black women. Obesity has been related to the increasing prevalence of NCD's, and has been shown to be increasing in proportion to the increase found in obesity [130]. Recently, in research published by Peer et al., it was found that the rate of T2D and dyslipidaemia has increased over the last 20 years, specifically in the resource limited population within Cape Town. Further, there is little to no data on the longitudinal effects of weight gain/increasing BMI, changes in body fat distribution and its associated cardio-metabolic consequences in black SA women. As reviewed here, there is limited understanding regarding the contribution of various socio-cultural and behavioural determinants of risk of weight gain or obesty in this group. Due to the differences in cardio-metabolic risk and disease prevalence within ethnic groups, studies have found that factors such as age and sex, under the influence of nutrition, physical activity, contraceptive use and reproductive outcomes, as well as SES, may have a unique influence on this population, and be relevant to consider in black SA women. In spite of the trends described, black SA women are traditionally an understudied population.

Therefore, due to the disproportionately high prevalence of obesity in black SA women and the associated increased risk of NCD's described, this group should be considered a priority. It is vital to assess and describe the longitudinal changes in weight and body fat distribution in relation to cardio-metabolic risk in order to establish better understanding of the ethnic specific relationships between the two. In addition, examining the determinants of body composition changes will allow for increased ability to plan interventions or create health

policy to target certain areas that will reduce the increasing weight gain/BMI and its consequences in this group.

1.7 Aims and objectives

These aims will be addressed in two separate chapters in this thesis:

1) To assess the changes in body composition and body fat distribution women in relation to changes in cardio-metabolic risk factors over a 5.5-year period in a sample of black South African free-living; and
2) To examine the relationship between socio-cultural and behavioural factors at baseline and their changes over 5.5 years, in relation to changes in body composition and body fat distribution free-living black SA women.

CHAPTER 2: LONGITUDINAL CHANGES IN BODY FAT AND ITS DISTRIBUTION IN RELATION TO CARDIO-METABOLIC RISK IN BLACK SOUTH AFRICAN WOMEN

2.1 Introduction

Studies from both high income countries (HICs) and low to middle income countries (LMICs), including South Africa (SA), have found that black women are at a higher risk of developing T2D, IR and hypertension compared to white women [10,131-133]. Further, a recent study in SA suggests that the prevalence of T2D in the black population has increased significantly over the past 20 years from 8.0 to 12.2% [25]. Despite the high prevalence of T2D, IR and hypertension in black communities, the risk of CVD is lower compared to other ethnic groups [13] which is likely related to their more favourable lipid profile [27].

The exact reasons for the ethnic disparities in T2D and CVD are not known. However, this may be explained, in part, by the higher prevalence of obesity in black women compared to other ethnic groups in SA (36.1% in urban black vs. 28.5% in mixed ancestry, 21.3% in Asian and 25.5% in white women) [13,134]. Indeed, increasing BMI has been linked to increasing risk for T2D, dyslipidemia and hypertension [23]. Within SA, excess body weight (BMI>21 kg/m^2) contributes to 87.0% of deaths from T2D and 68.0% from hypertensive disease, with the risk being double in women compared to men [24].

Further, it is well known that the distribution of body fat, rather than total body fatness alone, is associated with increased risk of CVD or metabolic disease [31,135]. Compared to white women, black women have less VAT, but more SAT, including abdominal SAT and peripheral gluteofemoral SAT [9,34,35]. Low levels of VAT in black women may explain their more favourable lipid profile but not their increased level of IR [136]. Rather, studies done in black women from SA and the USA have shown that abdominal SAT, specifically deep SAT (DSAT), is more closely associated with IR than VAT in this population [31,34]. When examining the abdominal depot-specific changes over time, two studies have shown that black women increased VAT to a lesser extent than their white counterparts [59], but more than Hispanic women [58]. However, neither of these studies examined changes in VAT, or any other fat depot, in relation to changes in metabolic outcomes. Further, in a small SA study, Goedecke et al. [57] showed that gluteal fat was inversely associated with insulin sensitivity in black women. This study is in contrast to other published studies, predominantly in white populations, that have shown that greater peripheral fat mass is associated with reduced IR in women [33,53,137]. However, these findings are based on cross-sectional studies, from which one cannot infer causality.

To our knowledge, there are few studies that have monitored longitudinal changes in body composition in relation to metabolic outcomes, with none that have examined this in a black SA population. A previous longitudinal study (CARDIA, USA) in a mixed population showed that increasing WC over a period of 20 years was associated with increased IR [21]. In addition, data from weight loss intervention studies in predominantly white populations have shown that a decrease in VAT is associated with improved insulin sensitivity [60,61]. Longitudinal studies in black populations are required to understand the relative contribution (either positive or negative) of central and peripheral adipose tissue depots to the development of CVD and T2D disease risk.

Thus, the aim of this study is to examine changes in body composition and body fat distribution over a 5.5-year period in a sample of free-living black SA women, and to examine their association with changes in metabolic risk factors for T2D and CVD.

2.2. Materials and Methods

2.2.1 Participants

Participants included a group of 64 women who were recruited from an original convenient sample of 240 apparently healthy premenopausal black SA women that were tested in 2005/06, as previously described [85], and were followed up approximately 5.5-years later. The original cohort of women were recruited at baseline from church groups, community centres, and 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 T2D, hypertension, HIV/AIDS, or any other metabolic diseases; iii) were not pregnant, lactating or postmenopausal (self-reported); and iv) of SA ancestry (self-reported).

At follow-up, the original cohort of 240 women were contacted and invited to participate in the longitudinal follow-up study in 2010/11. In addition, at follow-up voluntary HIV screening was performed and participants were excluded on the basis of a confirmed positive test (Sanitests Home Test Kits, SA). Those who declined HIV screening were not excluded from the sample. The same Xhosa speaking fieldworker involved in the original baseline study assisted with the participant recruitment and follow-up testing.

Of the 240 women, only 64 completed the follow-up testing. The remaining 176 women were not included in the follow-up study for the following reasons: i) a positive diagnosis of diabetes at baseline (n=2); ii) currently lactating (n=1); iii) known HIV positive at follow-up (n=8); iv) deceased (n=1); v) altered contact details/moving residence or city (n=126); or vi) unwilling to be retested (n=38). Testing procedures were identical to those at baseline as described by Jennings et al. [85], but also included an oral glucose tolerance test (OGTT), and are described in detail below.

2.2.2 Body composition assessment

2.2.2.1 Anthropometry

Weight and height, in light-weight clothing without shoes, were measured using a standard scale and stadiometer, respectively (Detecto, Model UWE BW-150, Cardinal Scale Manufacturers, Webb City, Missouri, USA). Waist circumference, at the level of umbilicus, and hip circumference, at the largest protruberance of the buttocks, were measured with a tape measure.

2.2.2.2 Dual-energy x-ray absorptiometry

Whole body composition, including FM and fat-free soft tissue mass (FFSTM), was measured by dual-energy x-ray absorptiometry (DXA) (Discovery-W®, software version 12.7.3.7; Hologic, Bedford, MA) according to standard procedures [138]. Percentage FM for the whole body was obtained and FM for the various regions of interest, including the trunk, limbs, android and gynoid regions, were derived by using DXA cut-off lines positioned at anatomical markers as previously described [138,139]. In vivo precision (CV) was 0.7% and 1.67% for FFSTM and FM, respectively.

2.2.2.3 Computerised tomography

A single-slice computerised tomography (CT) (Toshiba X-press Helical Scanner®; Toshiba Medical Systems, Tokyo, Japan) scan was taken at the level of the L4-L5 lumbar vertebrae to determine VAT and SAT areas [16,138]. Due to logistical issues associated with obtaining CT measurements in this study (women exceeding the scanning region and failure to report for scan, which was performed off-site), data was obtained from 43 women (67% of sample) for VAT and 47 (73% of sample) women for SAT.

2.2.3 Metabolic risk factors for cardiovascular disease and type 2 diabetes

2.2.3.1 Blood pressure

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

2.2.3.2 Blood sampling and analysis

Blood samples were drawn after an overnight fast (10-12 hours) for the measurement of plasma glucose and serum insulin, TC, TG, HDL-C and LDL-C concentrations.

At follow-up testing only, the participants underwent a standard 2-hour (2-hr) oral glucose tolerance test (OGTT). After an overnight fast, participants ingested 75 g glucose diluted in 250ml of water. Blood samples were taken at 30-minute intervals (0, 30, 60, 90 and 120 minutes) for the subsequent measurement of plasma glucose and serum insulin concentrations.

Fasting plasma glucose levels at baseline and follow-up were measured using the glucose oxidase method [(Baseline: Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA with intra-assay CV: 0.67%)] [Follow-up: YSI 2300 STAT PLUS, YSI Life Sciences, Yellow Springs, OH with intra-assay CV: 1.17% and inter-assay CV: 2.29%)]. An inter-method comparison was performed and yielded an inter-assay CV of 1.53%. Serum insulin levels were measured by a Micro-particle Enzyme Immunoassay (MEIA) (AxSym Insulin Kit, Abbot, IL, USA) [Baseline: intra-assay CV: 2.3% and inter-assay CV: 3.2%)] [Follow-up: intra-assay CV: 3.28% and inter-assay CV: 5.45%)]. Serum TC (intra-assay %CV: 0.4%), TG (intra-assay %CV: 0.6%), and HDL-C (intra-assay %CV: 0.55%) concentrations were measured on the Roche Modular Auto Analyzer (Roche/ Hitachi Cobas C System from Roche Diagnostics GmbH, D-68298, Mannheim) using enzymatic colorimetric assays. LDL-C was calculated using the Friedewald equation [140].

Insulin sensitivity was estimated using the homeostasis model of insulin resistance (HOMA-IR) at baseline and follow-up [141]. The Matsuda and insulinogenic index (IGI) were calculated from the OGTT at follow-up only [142,143] Matsuda index is defined as (10,000/square root of [fasting glucose x fasting insulin] x [mean glucose x mean insulin during OGTT]), and IGI as ($insulin_0$ – $insulin_{30}$) / ($glucose_0$ – $glucose_{30}$). These are validated measures for insulin sensitivity and insulin secretion in relation to glucose, and are based on the results from the OGTT.

2.2.4 Statistical analysis

Normally distributed continuous data and the % change from baseline are presented as mean ± standard deviation. Non-parametric variables are presented as median and inter-quartile ranges (denoted by an asterisk in the tables). While the baseline and follow-up values were predominantly parametric, the changes in metabolic outcomes were consistently non-parametric in nature, and were normalised by adding a constant and then log transformed or by inversion (1/variable). Body composition and metabolic outcomes, in diffferent groups, over the 5.5-years were compared using repeated measures ANOVA and ANCOVA, adjusting for age, while non-parametric variables were compared using the Wilcoxon-rank test. The unadjusted values and the age-adjusted *P* values are presented in the tables. Spearman's rank correlation coefficients were used to assess the relationships between changes in body composition and changes in metabolic outcomes. Based on the results of these associations, multiple linear regression was used to assess the independent effects of central vs. peripheral body fat distribution on the metabolic outcomes, while adjusting for baseline age and FM (kg). Multico-linearity was tested using the variance inflation factor (VIF) calculation for each model. This provides as index to estimate how much of the variance is effected by colinnearity. Statistical significance was based on a $P<0.05$. All data were analysed using STATISTICA version 10 (StatsoftInc. Tulsa, OK), and Stata (Version 12.1, Statcorp, College Station, TX).

2.3 Results

Participants had a mean baseline age of 27±8 years. Follow-up testing was performed after approximately 5.5±0.6 years (range: 4.25-6.75 years), resulting in a mean follow-up age of 32±8 years.

2.3.1 Changes in body composition

Over the 5.5-year follow-up period, mean body weight and BMI increased by 8.8% (Table 1). As FFSTM did not change significantly, the increase in body weight was attributed to an increase in FM ($P<0.001$). There was a significant increase in all measures of body fatness and regional body fat distribution. In addition, when expressed relative to total FM (%FM), there was a significant increase in trunk FM, and a significant decrease in leg, but not arm FM. While the absolute mass of both gynoid and android depots increased over the 5.5-year follow-up period, when expressed relative to total FM, there was an increase in android FM and a decrease in gynoid FM. Abdominal VAT and SAT increased by 23% and 8%, respectively.

Table 1: Body composition at baseline and 5.5-year follow-up

	N	Baseline	Follow-up	Absolute change	Mean % change	P value
Body Composition						
Weight (kg)	64	86.9 ± 19.6	92.8 ± 18.9	6.9 ± 9.9	8.8 ± 12.5	<0.001
Height (m)	64	1.6 ± 0.1	-	-	-	-
BMI (kg/m^2)	64	33.8 ± 7.5	36.4 ± 7.7	2.8 ± 3.9	8.8 ± 12.5	<0.001
FFSTM (kg)	64	45.6 ± 6.8	46.2 ± 6.3	0.8 ± 3.5	2.0 ± 8.5	0.234
Fat mass (kg)	64	37.1 ± 13.8	41.3 ± 13.2	4.3 ± 6.9	16.4 ± 26.9	<0.001
Body fat (%)	64	42.3 ± 7.8	44.9 ± 6.4	2.6 ± 4.3	-	<0.001
Body fat distribution						
Waist (cm)	63	99.8 ± 16.8	107.7 ± 16.3	8.3 ± 11.4	9.1 ± 12.0	<0.001
Hip (cm)	63	119.3 ± 15.3	120.7 ± 14.7	2.1 ± 7.8	1.4 ± 7.5	0.086
Trunk FM (kg)	61	16.5 ± 7.1	19.2 ± 6.9	2.7 ± 3.7	25.8 ± 40.7	<0.001
Trunk FM (% FM)	61	43.6 ± 5.8	46.2 ± 5.3	2.5 ± 3.4	-	<0.001
Appendicular FM (kg)	61	19.6 ± 7.2	21.2 ± 7.5	1.7 ± 3.3	11.1 ± 22.2	0.001
Appendicular FM (% FM)	61	53.6 ± 5.2	51.4 ± 5.1	-2.1 ± 2.9	-	<0.001
Arm FM (kg)	61	4.0 ± 1.5	4.6 ± 1.7	0.6 ± 1.0	20.5 ± 32.9	<0.001
Arm FM (% FM)	61	10.9 ± 2.1	11.2 ± 2.0	0.2 ± 1.2	-	0.176

Leg FM (kg)	61	15.5 ± 6.2	16.6 ± 6.3	1.1 ± 2.6	9.0 ± 21.2	0.007
Leg FM (% FM)	61	42.6 ± 6.3	40.1 ± 6.1	-2.4 ± 2.9	-	<0.001
Android FM (kg)	61	2.9 ± 1.4	3.5 ± 1.4	0.5 ± 0.7	31.5 ± 51.7	<0.001
Android FM (% FM)	61	7.7 ± 1.6	8.4 ± 1.6	0.5 ± 1.0	-	<0.001
Gynoid FM (kg)	61	6.8 ± 2.5	7.5 ± 2.6	0.6 ± 1.2	12.7 ± 23.1	<0.001
Gynoid FM (% FM)	61	19.3 ± 2.7	18.5 ± 2.4	-0.7 ± 1.2	-	<0.001
VAT (cm^2)*	43	59 (37-93)	75 (49-110)	13 (-17-29)	23 (-15-74)	0.038
SAT (cm^2)*	47	508 (324-611)	499 (352-604)	43 (-43-103)	8 (-8-26)	0.013

Data presented as means ± standard deviations or *median (inter-quartile range). BMI, body mass index; FFSTM, fat-free soft tissue mass; FM, fat mass; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue. P value adjusted for age.

2.3.2 Changes in metabolic outcomes

The changes in metabolic outcomes over the 5.5-year follow-up period are presented in Table 2. Apart from serum HDL-C and TC:HDL ratio, all serum lipids increased significantly over the 5.5-year period ($P<0.001$). Neither systolic nor diastolic BP changed significantly over the follow-up period. Fasting plasma glucose concentrations increased, while fasting serum insulin concentrations and HOMA-IR did not change. Insulin sensitivity and secretion, estimated from the OGTT using the Matsuda index and IGI, yielded median (IQR) values of 3.1 (2.2 - 4.8) and 34.9 (24.1 - 58.5), respectively.

2.3.3 Associations between changes in body composition and changes in metabolic outcomes

The absolute change in FM (kg) was associated with a lower Matsuda index and higher IGI at follow-up (Table 3). All other measures of change in absolute FM, including VAT and SAT areas, were not associated with changes in metabolic outcomes (data not shown). However, when expressed as a % of total FM (%FM), the increase in trunk FM was associated with an increase in fasting glucose, TC and TG concentrations, and HOMA-IR, as well as an increase in Matsuda index and decrease in IGI at follow-up. Conversely, the relative decrease in peripheral FM (appendicular, leg and/or gynoid FM as %FM) was associated with an increase in fasting glucose and insulin concentrations, HOMA-IR, TG and LDL-C concentrations, and an increase in Matsuda index and decrease in IGI at follow-up (Table 3). The same relationships were reflected in the ratios of central-to-peripheral FM, with trunk:gynoid ratio showing the strongest associations with cardio-metabolic risk factors.

Multiple regression was then used to determine the contribution of trunk:gynoid ratio to the changes in plasma glucose and serum lipid concentrations over the 5.5-year follow-up period, as well as the OGTT-derived measures of insulin sensitivity and secretion at follow-up, adjusting for baseline age and FM (Table 4). The models were significant for changes in plasma glucose, and measures of insulin sensitivity and secretion at follow-up, while the changes in serum lipids were no longer significant after adjusting for baseline age and FM.

We further investigated the independent contribution of central and peripheral FM to these metabolic changes. The beta values for changes in relative trunk and gynoid FM (%FM) in relation to change in fasting glucose concentration, and Matsuda and IGI indices at follow-up are presented in Figure 1. Although the model for the change in plasma glucose

Table 2: Metabolic outcomes at baseline and 5.5-year follow-up

	N	Baseline	Follow-up	Absolute change	Mean % change	P value
Serum lipids						
Total cholesterol (mmol/L)	58	3.8 ± 0.8	4.1 ± 0.9	0.3 ± 0.7	9.5 ± 21.7	<0.001
Triglycerides (mmol/L)*	58	0.6 (0.5-1.0)	0.8 (0.7-1.2)	0.2 (-0.1-0.5)	28.8 (-4.2-77.1)	<0.001
HDL-C (mmol/L)	58	1.2 ± 0.3	1.2 ± 0.3	0.0 ± 0.3	6.5 ± 26.7	0.063
LDL-C (mmol/L)	58	2.2 ± 0.8	2.5 ± 0.9	0.2 ± 0.6	13.5 ± 32.0	0.002
TC:HDL	58	3.4 ± 1.2	3.6 ± 1.1	0.1 ± 1.1	8.0 ± 30.3	0.234
TG:HDL	58	0.7 ± 0.5	0.8 ± 0.5	0.1 ± 0.5	43.3 ± 89.9	0.003
Blood pressure						
Systolic BP (mmHg)	61	112.1 ± 13.6	111.4 ± 18.6	-0.7 ± 13.5	-0.5 ± 11.9	0.118
Diastolic BP (mmHg)	61	78.6 ± 7.2	76.7 ± 12.8	-1.9 ± 11.2	-2.5 ± 13.9	0.112
Glucose tolerance and insulin resistance						
Glucose (mmol/L)	63	4.5 ± 0.6	5.1 ± 1.3	0.6 ± 1.4	15.7 ± 31.0	<0.001
Insulin (mU/L)*	60	14.4 (7.1-20.6)	12.3 (7.7-16.7)	-0.2 (-6.7-2.9)	-1.4 (-41-37)	0.546
HOMA-IR	63	3.1 ± 2.1	3.1 ± 1.7	-0.0 ± 1.9	35.8 ± 105.1	0.969
Matsuda index	64		3.1 (2.2 - 4.8)			
IGI	64		34.9 (24.1 - 58.5)			

Data presented as mean ± standard deviation or *median (inter-quartile range). HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC:HDL, total cholesterol to high density lipoprotein ratio; TG:HDL, triglyceride to high density lipoprotein ratio, BP, blood pressure; HOMA-IR, Homeostasis model assessment of insulin resistance [141]. Matsuda index [142;] IGI, insulinogenic index [143], P values adjusted for age.

Table 3: Univariate associations between the changes in body composition and changes in metabolic outcomes over the 5.5-year follow-up period

	Δglu	Δins	Δ HOMA-IR	Δ TC	Δ TG	Δ HDL	Δ LDL	Matsuda	IGI
Δ FM (kg)	0.10	-0.08	-0.01	0.19	0.05	0.20	0.05	-0.28*	0.27*
Central depots									
Δ Trunk FM (%FM)	0.31*	0.16	0.26*	0.25*	0.29*	0.10	0.20	-0.28*	0.31*
Δ And FM (%FM)	0.22	0.07	0.18	0.18	0.28*	0.07	0.11	-0.25	0.24
Peripheral depots									
Δ App FM (%FM)	-0.30*	-0.11	-0.22	-0.20	-0.26*	-0.06	-0.20	-0.26	-0.26*
Δ Arm FM (%FM)	-0.08	-0.06	-0.03	0.04	0.10	-0.14	-0.06	-0.15	-0.00
Δ Leg FM (%FM)	-0.29*	-0.12	-0.27*	-0.23	-0.36**	-0.10	-0.16	0.32*	-0.29*
Δ Gyn FM (%FM)	-0.27*	-0.31*	-0.45**	-0.25	-0.37**	0.07	-0.30*	0.35**	-0.38**
Ratios									
Δ Trunk:Leg (kg)	0.23	0.16	0.26*	0.25*	0.33**	0.05	0.19	-0.39**	0.33*
Δ Trunk:Gynoid (kg)	0.28*	0.30*	0.43**	0.26*	0.37**	-0.01	0.28*	-0.44**	0.39**
Δ And:Gyn (kg)	0.22	0.21	0.33**	0.21	0.35**	-0.02	0.19	-0.42**	0.30*

Data are Spearman's correlation coefficients, R, FM; fat mass; And FM, android fat mass; App FM, appendicular fat mass; Gyn FM, gynoid fat mass; And:Gyn, android to gynoid ratio, glu, glucose; ins, insulin; HOMA-IR, Homeostatic model assessment (29); TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; Matsuda, Matsuda index measured only at follow-up [142]; IGI, insulinogenic index measured only at follow-up[143], * P<0.05, ** P<0.01

Table 4: Regression models with metabolic outcomes in relation to body fat distribution

	Δ glu		Matsuda		IGI		ΔTC		ΔTG		ΔLDL	
R^2	0.13		0.24		0.16		0.01		0.11		0.07	
P value model	**0.046**		**0.002**		**0.023**		0.791		0.078		0.207	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*
Δ Trunk:gynoid	0.17	0.211	-0.52	<0.001	0.30	0.033	-0.03	0.819	0.34	0.015	0.28	0.050
Age	0.11	0.384	0.10	0.410	-0.23	0.063	012	0.354	-0.05	0.638	0.10	0.424
FM	-0.24	0.082	-0.16	0.220	-0.03	0.811	0.02	0.885	0.05	0.672	0.14	0.298

Glu, glucose; Matsuda, Matsuda index; IGI, Insulinogenic index; TC, total cholesterol; TG, triglycerides; LDL, low density lipoprotein, FM, fat mass, regression used age and fat mass at baseline. Change in glucose, Matsuda index, IGI, and change in TC were normalised for analysis

concentrations was significant (R^2=0.17, *P*=0.035), the changes in relative trunk and gynoid FM were not independent of each other. However, in the model for Matsuda and IGI, the change in relative gynoid FM was independent of changes in relative trunk FM, after adjusting for baseline age and FM.

Figure 1: Contribution of changes in adipose depots relative to total fat mass in regression models. Beta values for changes in relative trunk and gynoid fat mass in relation to changes in fasting plasma glucose concentrations over the 5.5-year follow-up period, and insulin sensitivity (Matsuda Index) and secretion (insulinogenic index) at follow-up, after adjusting for baseline age and FM. Change in glucose: R^2 = 0.17, *P*=0.035; Matsuda at follow-up: R^2 = 0.17, *P*=0.050; IGI at follow-up; R^2 = 0.21, *P*=0.012. Glucose, Matsuda index and IGI were logged for analysis. *P<0.05

2.4 Discussion

In this longitudinal study of free-living apparently healthy black SA women, participants increased their body weight by approximately 9% over a 5.5-year period. The increase in body weight was attributed to an increase in FM rather than FFSTM, and when expressed relative to total FM (%FM), was associated with an increase in central FM and a concomitant decrease in peripheral FM. This relative increase in central FM and decrease in peripheral FM were associated with increases in plasma glucose, HOMA-IR and serum lipid concentrations, however these changes in central and peripheral fat mass were not

independent of each other. In contrast, the relative change in gynoid FM was independently associated with insulin sensitivity and secretion at follow-up.

The women in this study had a mean body weight increase of 1.2 kg/year, which is higher than the average annual gains reported in other large longitudinal studies in HICs [67]. The Black Women's' Health Study in the USA reported a mean increase of 0.9 and 0.7 kg/year over 14 years for women aged <35 and >45 years, respectively [64]. Similarly, the Australian Longitudinal Study on Women's Health reported an average increase of 0.5 kg/year for women aged 45-55 years [16], and a study from Brazil reported an increase of 0.7 kg/year in black women aged 35-54 years [18]. The high rate of weight gain in this study may be explained, in part, by their relatively young baseline age (27±8 years) compared to the above-mentioned studies. Indeed, weight gain has been shown to be greater in younger compared to older age groups [63,64]. In addition, the effect of baseline BMI has been shown to be a significant contributor to weight gain in women when accompanied by a higher energy intake [16]. This may indicate a greater capacity for weight gain, but may also be an artefact of an age effect, or a regression towards the mean, as those who are younger are also more likely to have a lower BMI. Irrespective of baseline age or BMI, the high rate of weight gain in the cohort of this study is cause for concern as it has been shown that while obesity is associated with increased metabolic risk, the rate of weight gain may confer increased risk over and above the weight gain itself [69].

The weight gain in the present study sample was due to an increase in FM, with a relative change in distribution of fat from the periphery (gynoid area and leg) to the central depots (trunk and android area). Several cross-sectional studies have shown that black women have greater peripheral (gluteofemoral), and less central FM compared to white women [9,34]. In these studies [9,34], although the initial comparisons were age adjusted, they compared black and white women of significantly different ages. One may therefore hypothesise that with increasing age and FM, central FM may increase in black women to a level similar to that of white women. To my knowledge, the relative decrease in peripheral FM (leg and gynoid %FM), with increasing weight gain over time has not been previously reported. It may however be explained, in part, by the findings of Goedecke et al. [57], who showed that with obesity, the expression of adipogenic (e.g. peroxisome proliferator-activated receptor-λ) and lipogenic genes (e.g. lipoprotein lipase) are down-regulated in the gluteal depot of black, but not white women. However, longitudinal studies examining changes in adipose tissue gene and protein expression in relation to changes in body fat distribution are required to confirm this.

The increase in central FM was accompanied by significant increases in both abdominal VAT and SAT areas. Studies from both the USA and SA have shown that for the same waist circumference, black women have less VAT, but more SAT than their white counterparts [9,144,145]. In this study, for every one kilogram increase in FM, VAT increased by an average of 2.8 cm^2 compared to 9.3 cm^2 for SAT. Although the absolute increase in SAT was greater than VAT, the increase in VAT as a percentage of baseline VAT exceeded that for SAT (median change of 23% vs. 8%, respectively). In comparison, in a group of normal-weight white and black American women (22-44 yrs), Lara-Castro et al. [59] showed that for each kg increase in FM over a 5-year period, VAT increased by 2.4 cm^2 , independent of ethnicity This may highlight that although black women have lower VAT compared to other ethnicities [9,145] they are at similar risk of rapid gains in VAT with increasing central FM.

Centralisation of body fat, in particular an increase in VAT, has been associated with poorer metabolic outcomes [31]. While results from this study showed significant associations between the increase in trunk FM (%FM), and fasting glucose concentrations and HOMA-IR, changes in VAT and SAT were not associated with any metabolic outcomes, which may be due to the smaller sample size for these measures. Mechanistically, VAT is thought to contribute more to IR than other adipose depots due to its higher lipolytic activity, metabolic turnover and inflammatory profile [146]. However, studies in black women from USA and SA have shown that SAT may be a stronger correlate of IR than VAT [34,36]. Patel et al. [42] proposed that this may be explained by the sheer volume of SAT, which may contribute more free fatty acids (FFA) and inflammatory cytokines than VAT to the circulation. Indeed, Evans et al. [43] showed that black women have a higher abdominal SAT inflammatory profile than white SA women, although this was not found to explain large portions of the ethnic variance in insulin sensitivity. Nonetheless, we showed that relative increases in trunk FM (%FM), which encompasses both VAT and SAT, were also associated with a decrease in insulin sensitivity and a compensatory increase in insulin secretion at the 5.5-year follow-up. These findings are in agreement with the results of a 20-year longitudinal study in the USA, in which an increase in waist circumference over time were associated with an increase in IR [21].

Notably, the study showed that independent of the relative increase in trunk FM (%FM), the relative decrease in peripheral FM (%FM), was associated with lower insulin sensitivity and a greater insulin secretion at follow-up. Similarly, Snijder et al. [137] showed that higher leg FM was protective against elevated plasma glucose concentrations after controlling for trunk FM,

while Wiklund et al. [54] found that trunk:gynoid ratio was the strongest predictor of CVD risk in women. In our study, the relative decrease in gynoid FM (%FM) was independently (of trunk FM) associated with a decrease in insulin sensitivity and increase in insulin secretion at follow-up. The proposed mechanisms for this protective effect includes the ability of peripheral SAT to take up excess circulating FFA, thereby protecting against ectopic (muscle, liver, pancreas) fat deposition [137]. It has been shown previously that black women have greater peripheral adipose tissue stores [57], but it is not known if these greater stores confer reduced risk with increasing obesity in this population. It seems unlikely, as studies in USA and SA have shown that despite greater peripheral FM in black compared to white women, black women have higher levels of IR [10].

Although this study showed significant univariate associations between changes in central and peripheral FM (%FM) and changes in TG and TC concentrations, when entered into a regression model with baseline age and FM, these associations were no longer significant. Higher TG concentrations have been associated with lower insulin sensitivity in white women [46], but not in black women from the USA or SA [147,148]. Lower lipid levels in black women may be explained by genetic differences in lipid metabolism [7,48]. The ethnic-specific relationship between centralisation of body fat and serum lipids has also raised the question regarding the applicability of current metabolic syndrome criteria in black populations [29]. However, it is not known whether with increasing age and centralisation of FM, serum lipids will increase in black women. Age related changes in TC have been found in SA urban populations [26], with higher TC concentrations being reported with increasing BMI in black SA women [49].

A limitation of this study includes the use of a convenience sample that was constrained by the poor response to follow-up, and selection bias cannot be excluded, as there is no information on those who could not be reached, or chose not to participate. There were further limitations with regards to baseline CT data not being performed on all the participants, reducing the ability to generalise the results found with VAT and SAT changes. In addition, measurements were only taken at two time points, limiting the interpretation of changes during the intervening period. However, to my knowledge, this is the first longitudinal study that has examined changes in body composition and body fat distribution, using state-of-the-art techniques, in relation to changes in cardio-metabolic risk in a free-living SA population. This information is essential to our understanding of the ethnic-specific role of changes in body composition in relation to changes in metabolic health.

In conclusion, this study showed a significant increase in body weight and FM in a sample of apparently healthy free-living black SA women over a 5.5-year follow-up period. The increase in FM was characterised by a change in proportion in the distribution of body fat from the peripheral to the central depots, which was associated with a deterioration in glucose tolerance, insulin sensitivity and serum lipid profile. Future research should focus on the determinants of this weight gain over time, examining the influence of environmental and behavioural factors, in order to highlight areas for intervention to reduce future weight gain and associated increases in cardio-metabolic risk in this population.

CHAPTER 3: DETERMINANTS OF CHANGES IN BODY WEIGHT AND BODY FAT DISTRIBUTION OVER 5.5-YEARS IN A SAMPLE OF FREE-LIVING BLACK SOUTH AFRICAN WOMEN

3.1 Introduction:

Chapter one of this thesis showed that over a 5.5-year period, a group of free-living peri-urban black SA women increased their body weight by almost 9%, equivalent to an average increase of 1.2 kg/year. The weight gain was attributed predominantly to an increase in FM, which was distributed towards the central rather than peripheral depots. The relative redistribution of body fat was associated with increases in fasting plasma glucose and TG concentrations over the 5.5-year period, with reduced insulin sensitivity and a compensatory increased insulin secretion at follow-up.

The rate of weight gain of the women in that study was shown to be higher than reported in other longitudinal studies in which weight gain ranged from 0.5-0.9 kg/year [16,64 93 14]. However, these studies were undertaken in HICs, with limited longitudinal data being available from populations living in LMICs. Determinants of weight gain in these studies include non-modifiable factors such as age, sex and ethnicity, and modifiable factors such as baseline BMI [16,64], dietary intake [64,93,104,149], SES [77,73], parity [121,150,16,64,151,104,152] and PA [87,153]. Associations between these factors are often complex in nature, and it is difficult to make conclusions regarding the relative contribution of these determinants to increasing body weight in different populations. While the possible determinants of weight gain have been considered, many of these studies failed to assess the impact of these determinants on changes in body composition or body fat distribution. Due to the risk of cardio-metabolic disease associated with increasing central FM [10,154,155], and possible protective benefit associated to peripheral FM [52], a greater understanding of the determinants of these changes are important to inform future intervention studies.

In addition, within LMICs, other factors such as urbanisation and the “transition” from a traditional to a more westernised lifestyle, have been associated with the incidence of obesity and other NCDs [11,156,157,158]. Moreover, SES impacts obesity risk, but the relationship between SES and BMI in LMICs differs to that in HICs. Studies have shown that in LMICs, higher SES was associated with higher BMI, with the inverse association being reported in HICs [78]. Recently, a review by Dinsa et al. [79] illustrated that studies in LMICs find both positive and negative associations with SES and BMI depending on sex and which marker of SES is used. While the relationship between SES and body composition has been assessed in SA children [159], there is limted research in adults. In addition, to my knowledge, most of the current research is cross-sectional in nature and have not assessed the impact of

changes in SES on body composition and body fat distribution; hence further longitudinal research is required.

Therefore, the aim of this study was to assess changes in physiological, lifestyle and SES variables over a 5.5-year follow-up period, and examine their association with changes in body weight and body fat distribution in a sample of peri-urban free-living black SA women.

3.2 Methods:

3.2.1 Participants

As described in detail in Chapter 1, participants included a sample of 64 women from the original convenience sample of 240 apparently healthy premenopausal black SA women who were tested in 2005/06 as previously described [85], and were followed up approximately 5.5-years later.

3.2.2 Body composition

Body composition was assessed using basic anthropometry (weight, height and circumferences), and DXA and CT-scans, as described in Chapter 1. Whole body composition, including FM and FFSTM as well as VAT and SAT were used as markers for body compostion and body fat distribution. Relative body fat distribution was determined in relation to total body fat (%FM). Trunk:leg FM ratio was also used as a measure of body fat distribution.

3.2.3 Socio-economic status and behaviour/lifestyle factors

The same Xhosa speaking field worker administered the socio-demographic questionnaire at baseline and follow-up (See Appendix 1). The questionnaire included measures of SES such as housing density, asset index, education level, current employment, and household sanitation. Housing density was defined as the number of persons in the household divided by the number of rooms. Asset index was based on 14 appliances/items reflecting the individual and household wealth and resources, including electricity in the home, ownership of a television, radio, motor vehicle, fridge, stove/oven, washing machine, telephone, video machine, microwave, computer, cellular telephone and paid television channels (MNET or DSTV). Level of education was described as those who had completed grade 12 (secondary school) or lower. Participants were categorised as employed (including students) or

unemployed. Sanitation was described as access to running water or a flush toilet inside or outside the house.

Behaviour and lifestyle factors included self-reported indicators of smoking status (present smoker or non-smoker), alcohol consumption (non-drinker or drinker of any alcohol), and hormonal contraceptive use (none, oral or injectable). Parity was defined as those who had children at baseline or follow-up, and those who had children over the 5.5-years.

3.2.4 Physical activity

PA was assessed at baseline using the GPAQ [160]. Total PA time was reported as minutes of activity per day (min/day), quantified as total moderate to vigorous-intensity (MVPA) activity. GPAQ data was not available at follow-up. Due to the majority of black women using walking for transport as at least a third of their daily PA [12] and the availability of this data at baseline and follow-up, walking for transport was used as a simple proxy for PA at both time points.

3.2.5 Dietary intake

Dietary intake was determined using a quantitative Food Frequency Questionnaire (FFQ) [161] which has been validated in black South African women by De Villiers et al. [98]. The questionnaire uses 100 photographs of food items with pictures of common utensils to determine portion size. Two different registered dieticians administered the FFQ questionnaire at baseline and follow-up. The data were analysed using Food Finder III [162] (South African Medical research Council, Parow, South Africa). Due to the large inter-dietician variation in dietary assessment, the baseline and follow-up dietary intake could not be compared. The baseline dietary intake data was chosen for the analysis, as a possible predictor of weight gain over the 5.5-year follow-up period. The dietary quality index international (DQI-I) was calculated to provide a measure of the quality of dietary intake [94]. The total score of 100 is a composite of points for variety (20), adequacy (40), moderation (30) and dietary balance (10). A higher DQI-I score represents a higher quality of dietary intake. In addition, due to the range of BMI values in this sample, macronutrient intakes at baseline were quantified relative to body weight (kcal/kg or g/kg), to take into account body size.

3.2.6 Statistical analysis

Parametric data are presented as means and standard deviations and non-parametric data

are presented as medians and IQR. Socio-demographic or categorical data are presented as percentages and were compared over the follow-up period using McNemar Chi-squared tests. For univariate analysis, Spearman's rank correlations were used to assess non-parametric associations between continuous variables (housing density, asset index) and the changes in body composition. When exploring the effect of the categorical variables of parity, access to sanitation, smoking, alcohol and walking for travel on changes in body composition, one-way analysis of variance (ANOVA) was used. Due to the possible effect of age and BMI at baseline, *P* values are presented as unadjusted, and adjusted. To further analyse the effect of baseline age and BMI, age (<25 and ≥ 25 year) and BMI (<25, 25-30 and >30 kg/m^2) groups were created and Mann-Whitney and Kruskal-Wallis tests were used to assess changes in non-parametric continuous data within these groups (dietary and physical activity variables). In order to determine whether the changes in body composition over time differed between the BMI groups, a two-way analysis of covariance (ANCOVA), adjusting for age, was performed. When assessing the changes in categorical variables over time, dummy variables were used. Due to their significance within the univariate analysis, multiple stepwise linear regression was used to determine the independent contribution of baseline age and BMI, access to sanitation, parity, level of education and employment status and changes in these SES and lifestyle variables over time towards weight gain and changes in body fat distribution over the 5.5-year follow-up period. Statistical significance was set at $P<0.05$. Data were analysed using STATISTICA version 10 (Statsoft Inc. Tulsa, OK) and STATA 12.1 (StataCorp, College Station TX).

3.3 Results:

3.3.1 Assessment of socio-demographic and lifestyle factors at baseline and follow-up

Subject characteristics, including socio-economic and lifestyle variables, at baseline and follow-up are presented in Table 1. Overall, SES of the participants increased over the 5.5-year follow-up period, as characterised by increases in asset index ($P<0.001$), access to flush toilets and running water inside the house ($P<0.001$), and an increase in the number of participants who were employed. At baseline, 58% of the women had at least one child, and these women were significantly older than their nulliparous counterparts. At the 5.5-year follow-up, the proportion of women who had at least one child had increased to 86% ($P=0.001$). During the 5.5-year follow-up period, 24 women (38%) had one child, and three women (5%) had two children. The majority of women reported using injectable contaceptive and not oral contraceptive (n=1), therefore hormonal contraceptive use includes both types. Hormonal contraception use did not change significantly over time ($P=0.134$). Those who

smoked at baseline had a significantly higher BMI than those who did not smoke. The proportion of women who smoked increased over the follow-up period, but the proportion of women who consumed any alcohol did not change. At baseline, the median MVPA of the women was 60 mins/day, with 70% of women using walking as a mode of travel, which increased to 85.9% at follow-up (P<0.001). At baseline, the majority of dietary energy (kcal) was derived from carbohydrate (52.2%), followed by fat (34.8%) and protein (12.4%). The median total DQI score was 54 (IQR: 47-60), with a median (IQR) variety score of 17 (15-20), adequacy score of 25 (20-32), moderation score of 6 (3-12) and balance score of 3 (2-4).

3.3.2 Summary of weight and body composition changes over 5-5 years follow-up period

The changes in body composition over the 5.5-year follow-up period are presented in Table 1 of Chapter 1. In summary, the mean weight gain of the participants was 8.8%, with an average increase of 1.2 kg/year. There was a significant increase in FM (16.4±26.9%, P<0.001) over the 5.5-years, with no significant increase in fat-free soft tissue mass (P=0.234). The increase in FM was largely attributed to an increase in central FM, characterised by increases in trunk and android FM, as well as both VAT and SAT areas. Conversely, there was a decrease in relative peripheral FM (gynoid and leg FM).

3.3.3 Effect of baseline age and BMI on changes in body composition over the 5.5-year follow-up period

Baseline age and BMI were inversely associated with change in body weight over the 5.5-year follow-up period (Figure 1a and 1b). However, there was no association between age and BMI at baseline (Figure 1c). To further investigate the effect of age on changes in body composition, participants were divided into two categories, those above and below the median age of 25 years (Figure 2). The younger participants (<25 years) gained significantly more body weight, total FM, appendicular FM, and trunk FM than the older participants (>25 years), (P<0.05), with a 3-fold greater increase in FM in the younger compared to the older group (6.3±6.9 kg vs. 2.1±6.5 kg, P=0.016). The increase in FM in the younger vs. the older group occurred mainly in the central (trunk) region (3.9±3.7 kg vs. 1.2±3.4 kg; P=0.005) rather than the appendicular region (2.4±3.4 kg vs. 0.8±3.2 kg; P=0.066).

Table 1: Socio-economic and lifestyle variables at baseline and after 5.5-years follow-up

Indicator	Baseline	Follow-up	P value
Age (years)	27 ± 7.5	32 ± 7.6	-
BMI (kg/m^2)	33.8 ± 7.5	36.4 ± 7.7	<0.001
Housing			
Housing density (people/room)	1.33 ± 0.9	1.38 ± 1.19	0.630
Running water inside house (%)	26.5	37.5	<0.001
Flush toilet inside house (%)	26.5	40.6	0.001
Appliances in house (%14)	42.1 ± 19.4	55.8 ± 17.3	<0.001
Education and employment			
Obtained grade 12 (%)	32.8	42.1	0.134
Employed/Students (%)	32.8	45.3	0.042
Reproductive health			
Hormonal contraceptive use (%)	46.8*#	34.3	0.201
Parity (≥1 child) (%)	57.8*	85.9	0.001
Lifestyle			
Current smoker (%)	12.5#	15.6	<0.001
Consume alcohol (%)	37.5	48.4	0.291
Physical activity			
Walk for travel (%)	70.3	85.9	<0.001
MVPA (mins/day)	60 (18.2–150.0)	-	-
Dietary intake			
Total Energy (Kcal)	3570 ± 1393	-	-
Carbohydrate (g)	422.7 ± 176.9	-	-
Carbohydrate (%TE)	52.2 ± 7.0	-	-
Protein (g)	109.9 ± 48.9	-	-
Protein (%TE)	12.4 ± 2.1	-	-
Fat (g)	138.8 ± 63.1	-	-
Fat (%TE)	34.8 ± 6.0	-	-

Data are represented as either mean ± standard deviations or medians (interquartile-range), Continuous data were compared using Wilcoxon rank test or dependent t-test, frequencies were compared using McNemar Chi-square test, significance P<0.05, MVPA, moderate to vigorous physical activity, %TE, percentage of total dietary energy, *significant difference in age between groups at baseline, #significant difference in BMI between groups at baseline.

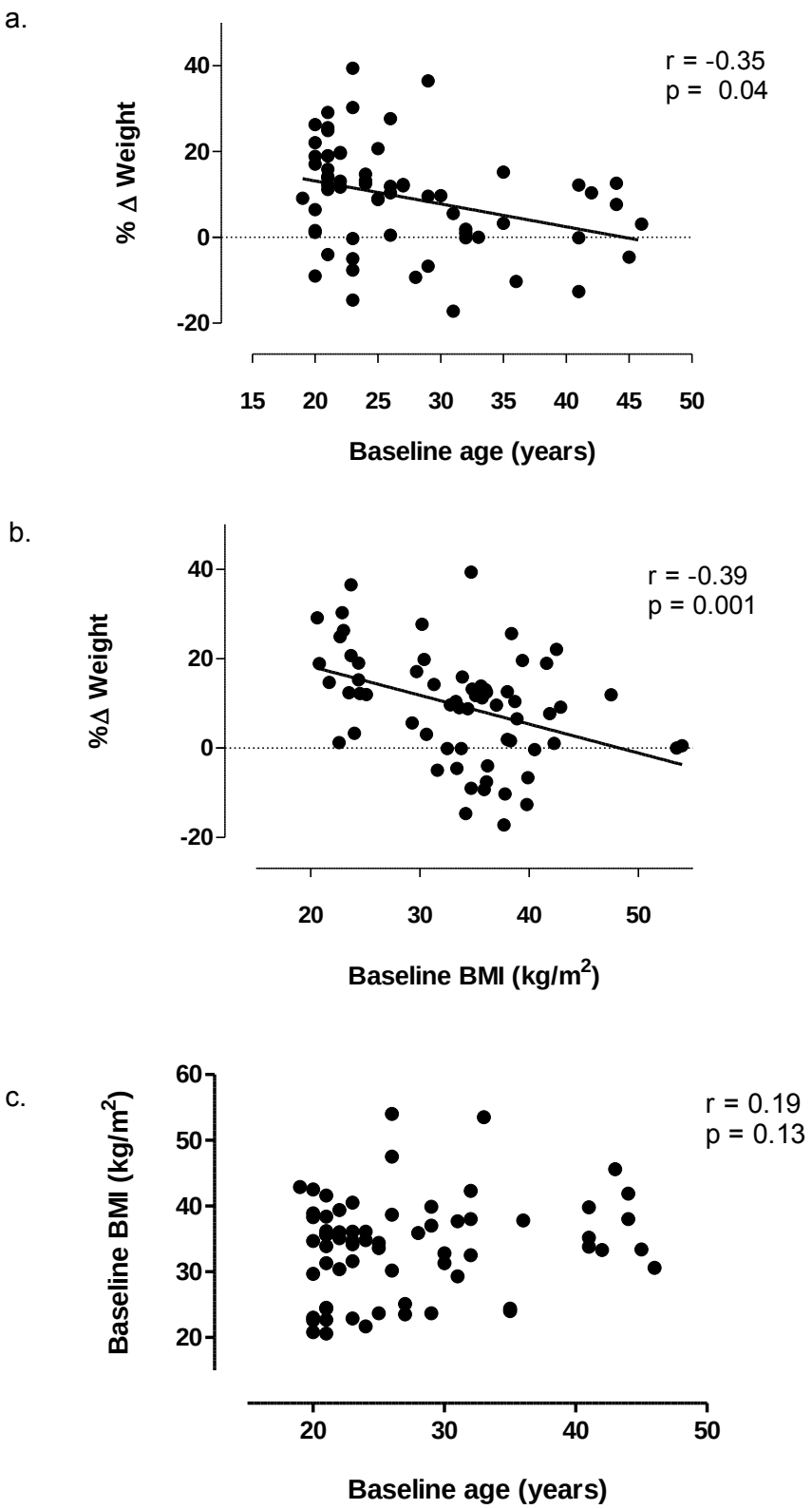


Figure 1: Relationship between baseline age, baseline BMI and relative change in body weight (%)

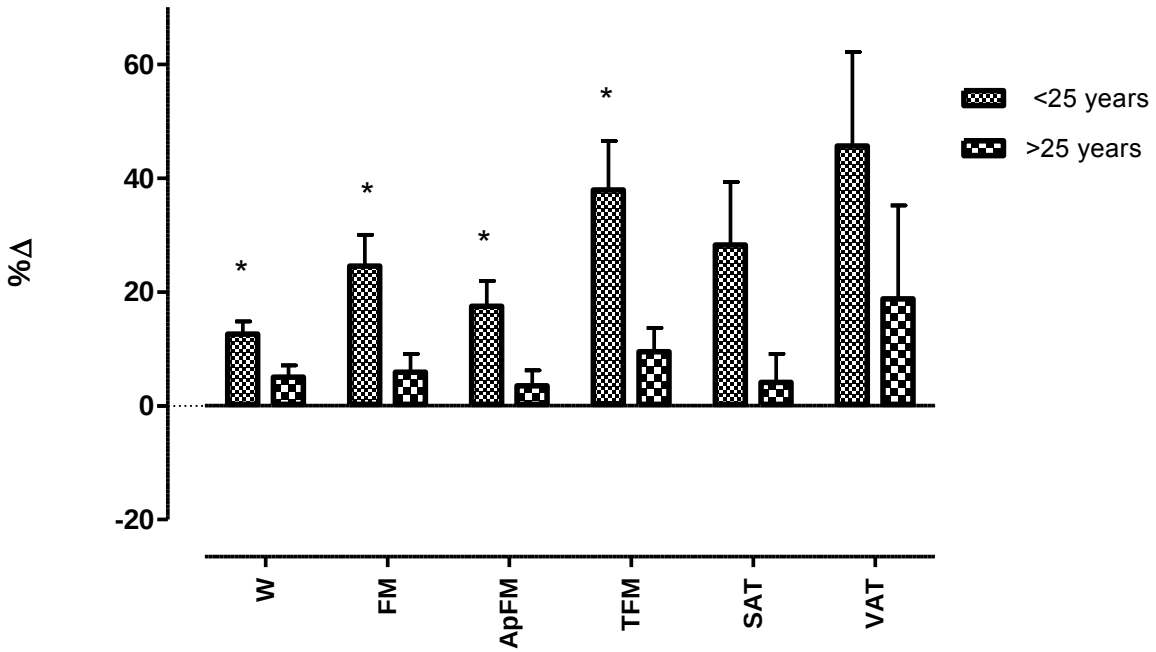


Figure 2: Relative changes in body composition variables (%) in younger (<25 years) and older age groups (>25 years). Data are means ± standard error. %Δ, relative change in each body composition variable, W, weight; FM, fat mass; ApFM, appendicular fat mass; TFM, trunk fat mass; SAT, superficial adipose tissue; VAT, visceral adipose tissue; * *P*<0.01

To further explore the effect of baseline BMI on changes in body composition, the participants were separated into three BMI categories, non-obese (NO: BMI<29.9 kg/m^2, n=17), obese class 1 (OBc1: BMI: 30-34.9 kg/m^2, n=17) and obese class 2 (OBc2: BMI>35 kg/m^2, n=35), based on their baseline BMI. The individual changes in BMI for the 3 BMI groups over the follow-up period are represented graphically in Figure 3. The annual average weight gain was 1.8±0.9, 1.2±2.1 and 0.9±1.9 kg in the NO, OBc1 and OBc2 groups, respectively.

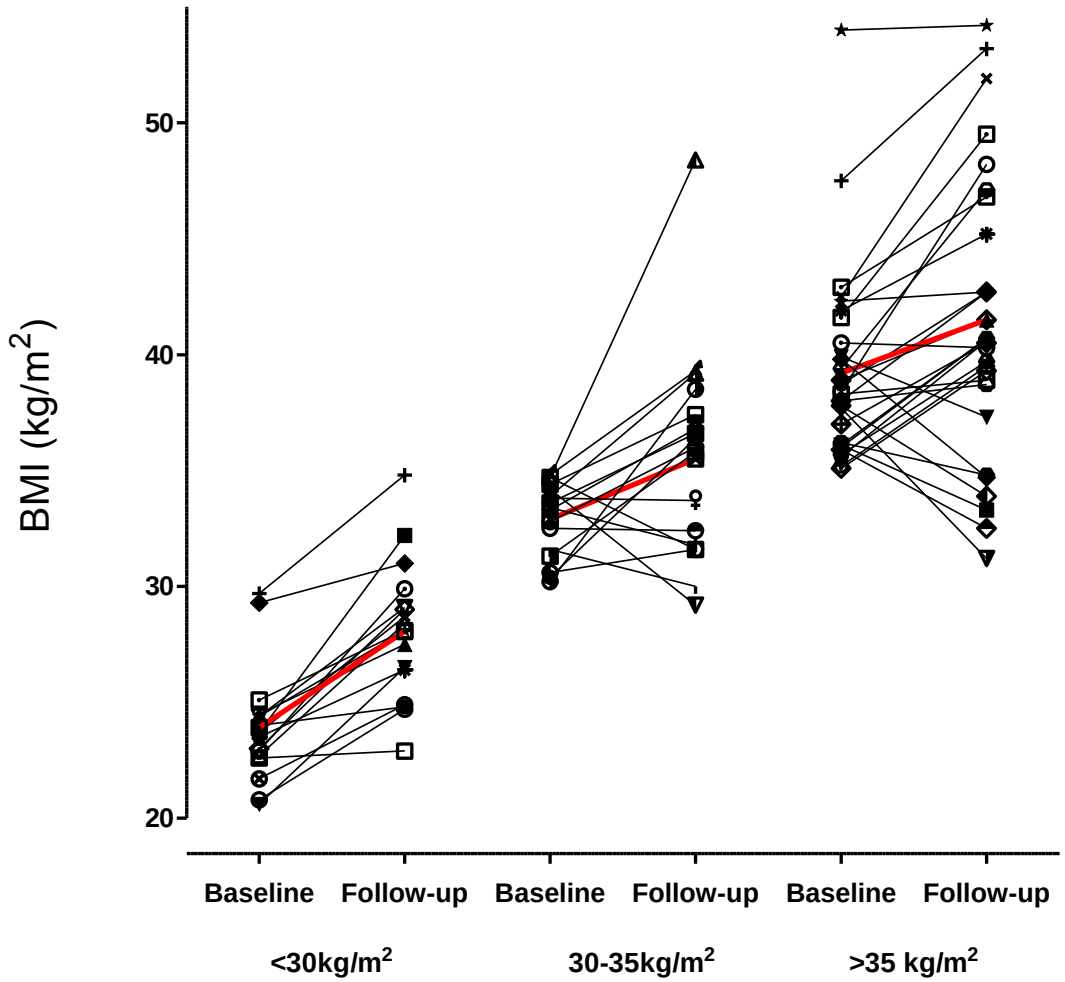


Figure 3: Individual changes in BMI over the follow-up period in three BMI groups, defined at baseline. BMI groups at baseline, NO: BMI<30 kg/m^2, OBc1; BMI 30-34.9kg/m^2, OBc2; BMI>35kg/m^2; mean increase in BMI per group (in red) ± standard deviation: NO: 24.0±2.5 to 28.1±3.0 kg/m^2, OBc1: 32±1.6 to 35.6±4.6 kg/m^2, OBc2; 39±4.7 to 41±6.4kg/m^2 (group x time: *P*=0.28)

Absolute and relative (as % of baseline value) changes in body composition over the 5.5-year follow-up period in the three BMI groups are presented in Table 2. There was no significant difference in age between the BMI groups (NO: 24.7±1.8 vs. OBc1: 28.7±1.8 vs. OBc2: 27.2±1.3 years, *P*=0.283). All body composition measure in all group increased over time (*P*<0.05, see Appendix 2). While the absolute changes in body composition between groups were not significantly different, the relative (to baseline values) changes in body composition were significantly greater in the NO group compared to the other two groups. In addition, there were significant group x time interaction effects for the relative (as %FM) changes in body fat distribution such that there was a significantly greater increase in central (trunk and android) FM and decrease in peripheral (leg and gynoid) FM in the NO group, compared to the other two obese groups (Figure 4, Appendix 2).

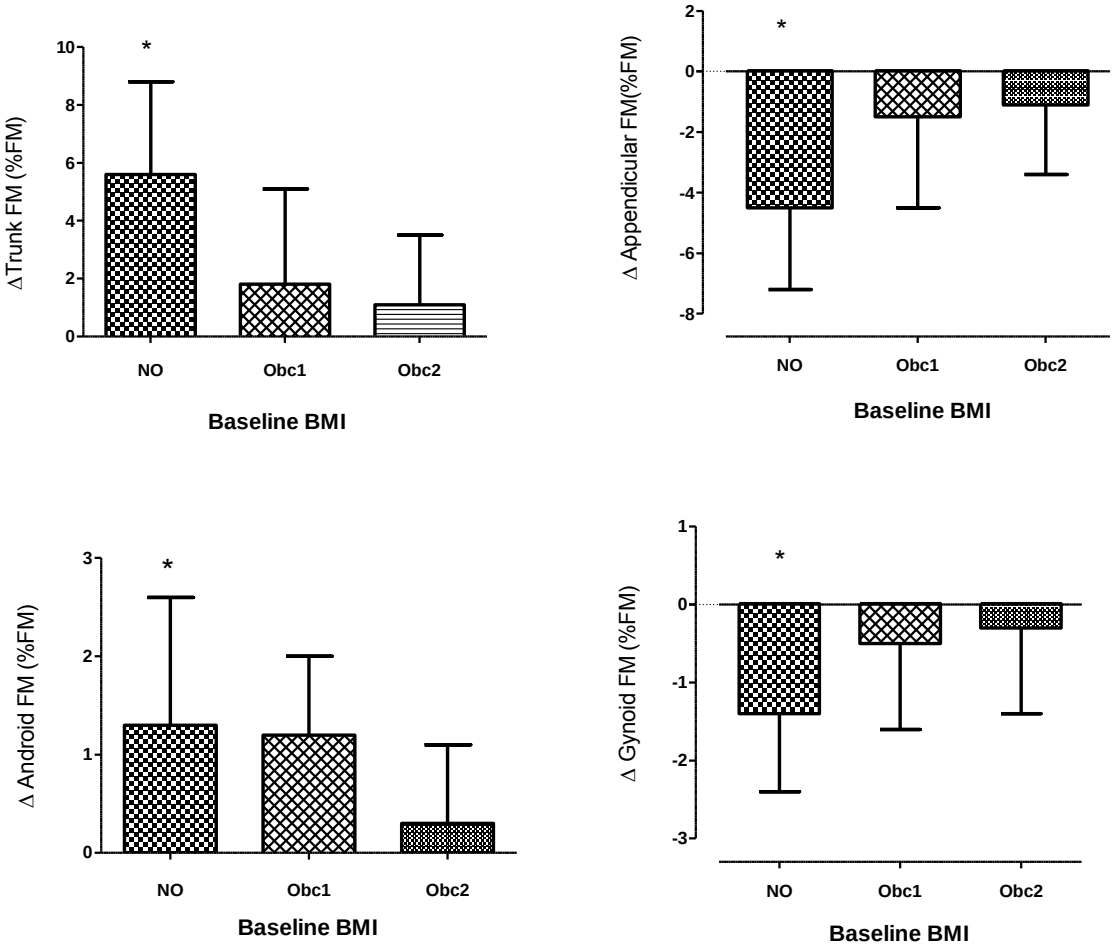


Figure 4: Relative changes in body composition, expressed as a percentage of total fat mass (kg) in 3 BMI groups determined at baseline. Data are represented as means ± standard deviation, FM; fat mass. Participants are in three BMI groups defined at baseline, NO: <30kg/m^2, OBc1: 30-34.9kg/m^2, and OBc2: >35kg/m^2. * Change in NO group significantly different to both other BMI groups *P*<0.01 via Tukey post-hoc analysis

As baseline BMI was such a strong determinant of weight gain over the follow-up period, differences in baseline SES and lifestyle variables between BMI groups (NO, OBc1 and OBc2) were examined, and significant correlates with baseline BMI were explored. There were no differences in SES, reproductive health, MVPA, walking for travel, absolute dietary intake or DQI between BMI groups (data not shown). However, the NO group had significantly higher relative (to total body weight) intakes of energy compared to the OBc2 group (52.8 kcal/kg vs. 34.5 kcal/kg, *P*=0.007), and both the NO and Obc1 groups had higher relative (to total body weight) carbohydrate intakes compared to the Obc2 group (6.0 g/kg and 5.9 g/kg vs. 4.2 g/kg, *P*=0.005).

Table 2: Changes in body composition over the 5.5-year follow-up period by baseline BMI groups

	BMI (kg/m^2)	Baseline	Follow-up	Absolute change	Relative change	P value*
Weight (kg)	NO	61.2 ± 9.2	71.7 ± 9.6	10.4 ± 5.4	17.6 ± 9.7#	0.009
	OBc1	85.9 ± 6.4	91.6 ± 12.2	5.6 ±12.1	6.8 ± 14.2	
	OBc2	100.4 ± 14.5	105.1 ± 15.2	4.7 ±10.5	5.2 ± 10.8	
FM (kg)	NO	19.6 ± 5.8	26.3 ± 5.8	6.7 ± 4.1	39.6 ± 31.8#	<0.001
	OBc1	37.5 ± 5.5	40.7 ± 9.4	2.5 ± 8.5	7.6 ± 23.8	
	OBc2	46.7 ± 10.2	50.0 ± 11.5	3.6 ± 7.2	8.0 ± 16.4	
TFM (kg)	NO	7.6 ± 2.7	11.6 ± 2.9	4.0 ± 2.1	63.2 ± 51.9#	<0.001
	OBc1	16.5 ± 3.6	18.7 ± 5.3	1.8 ± 4.7	13.7 ± 32.9	
	OBc2	21.6 ± 4.9	23.7 ± 5.5	2.2 ± 3.9	11.0 ± 20.1	
ApFM (kg)	NO	11.1 ± 3.4	13.8 ± 3.2	2.6 ± 2.1	27.9 ± 26.1#	0.003
	OBc1	20.2 ± 3.2	21.2 ± 5.4	0.8 ± 4.0	3.3 ± 20.6	
	OBc2	24.1 ± 6.2	25.4 ± 7.0	1.4 ± 3.6	5.8 ± 15.3	
SAT (cm^2)	NO	227.1 ± 100.5	300.4 ± 78.3	90.5 ± 68.4	58.9 ± 62.6#	0.033
	OBc1	487.7 ± 107.7	495.7 ± 107.7	15.6 ± 99.3	2.3 ± 27.2	
	OBc2	637.1 ± 118.9	651.6 ± 131.4	18.8 ± 84.1	2.2 ± 13.5	
VAT (cm^2)	NO	31.9 ± 12.5	54.9 ± 29.4	24.8 ± 23.1	94.1 ± 101.1#	0.003
	OBc1	77.3 ± 44.3	81.4 ± 38.2	2.9 ± 38.9	15.4± 67.8	
	OBc2	73.4 ± 36.0	99.9 ± 38.6	1.5 ± 23.5	7.7± 30.5	

Data are represented as means ± standard deviations. BMI, body mass index, FM, fat mass, TFM, trunk fat mass, ApFM, appendicular fat mass, LFM, SAT, subcutaneous adipose tissue, VAT, visceral adipose tissue. Groups are divided into BMI at baseline as NO: <30 kg/m^2 (n=17), OBc1: 30-34.9kg/m^2 (n=17), and OBc2:>35kg/m^2 (n=30), *P value refers to the differences in the relative changes in body composition between BMI groups; #Change in NO group significantly greater than both OB groups, Kruskal-Wallis used for the relative change in SAT and VAT

When expressed as a continuum, the relative (to total body weight) energy (Figure 5) and macronutrient intakes were all negatively associated with baseline BMI (carbohydrate (g/kg): R=-0.44, *P*<0.01; protein (g/kg): *R*=-0.34, *P*=0.007; fat (g/kg): *R*=-0.29, *P*=0.019).

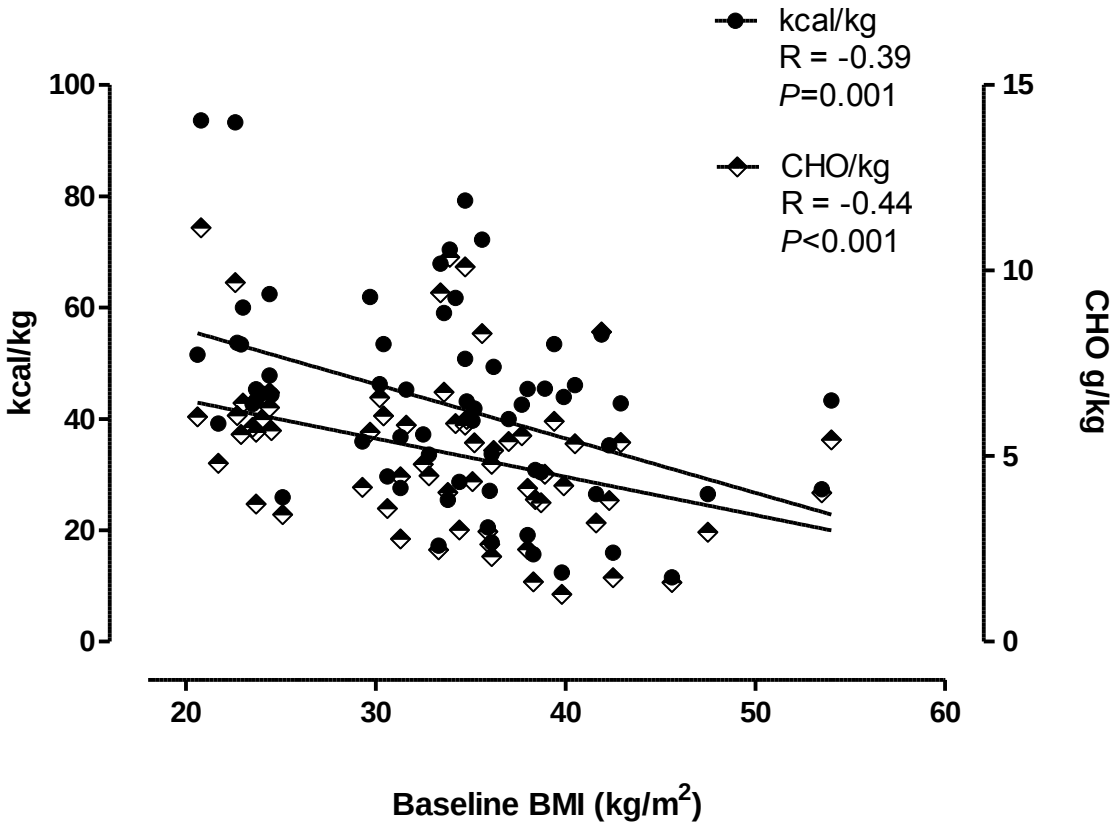


Figure 5: Relationship between baseline body mass index (BMI) and dietary energy and carbohydrate intake relative to total body weight. Left axis; kcal/kg, energy intake (relative to total body weight), right axis, CHO g/kg, carbohydrate intake grams per kilogram body weight, Associations performed using Spearman's correlations

3.3.4 Relationships between baseline and changes in SES and lifestyle factors and changes in body composition over the 5.5-year follow-up period

Baseline housing density and asset index were not associated with changes in body weight or body composition/fat distribution. In contrast, access to household sanitation at baseline was significantly associated with changes in body weight and body composition. Those who had inside running water and a flush toilet at baseline had significantly smaller increases in body weight than those who did not (inside running water: 1.7±11.2 vs. 8.8±8.8 kg,

unadjusted *P*=0.012, adjusted for baseline BMI and age *P*=0.008; inside flush toilet: 2.3±12.1 vs. 8.5±8.8 kg, unadjusted *P*= 0.032, adjusted for baseline BMI and age *P*=0.024). Conversely, those women who improved their access to sanitation (flush toilet or running water, n=14) over the 5.5-year follow-up period had significantly larger increases in body weight over the same time period compared to those who had no changes in sanitation (n=44, adjusted for baseline BMI and age *P*=0.002).

Education level at baseline was not related to changes in body weight (*P*=0.803). However, those who increased their education level (n=11) had a greater increase in relative trunk FM (%FM), compared to those who did not (n=53) (*P*=0.035). The women who were employed at baseline (n=20) had significantly larger increases in weight over the 5.5-year follow-up period than those who were unemployed (10.2±10.9 vs. 5.1±8.7 kg, unadjusted *P*=0.050, adjusted for baseline age and BMI, *P*=0.025). From baseline to follow-up, eight women lost employment, 19 women gained employment, and the employment status of the remaining 35 women did not change. Those who lost employment increased their body weight more than those who did not lose employment (weight: 11.7±6.4 kg vs. 5.8±11.6 kg unadjusted *P*=0.043, adjusted for baseline age and BMI *P*=0.024).

The use of hormonal contraceptive use at baseline or at follow-up did not influence weight gain (*P*=0.982). However, those women who were nulliparous at baseline gained significantly more body weight over time compared to those who already had children at baseline (10.7±9.5 vs. 3.8±8.9 kg unadjusted *P*=0.005, adjusted for baseline age and BMI *P*=0.019). The relative distribution of FM was also affected by parity with larger decreases in relative appendicular (%FM) (-3.1±2.9% vs. -1.5±2.7%, unadjusted *P*=0.040, adjusted for baseline age and BMI *P*=0.006) and gynoid depots (%FM) (-1.1±1.0% vs. -0.5±1.2% unadjusted *P*=0.088 adjusted for baseline age and BMI *P*=0.009), and larger increases in relative trunk FM (%FM) (3.69±3.5% vs. 1.9 ± 3.1% unadjusted *P*=0.044, adjusted for baseline age and BMI *P*=0.003) and trunk:leg ratio (0.19±0.2% vs. 0.08±0.1%, unadjusted *P*=0.004, adjusted for baseline age and BMI *P*<0.001) in the nulliparous women compared to the women with children. Further, those women who were still nulliparous at follow-up (n=9) increased their body weight significantly more than their childbearing counterparts over the 5.5-year follow-up period (16.6±7.2 vs. 5.4±9.3 kg, unadjusted *P*=0.001, adjusted for baseline age and BMI *P*=0.011). There was a trend for those who had children over the 5.5-year period to increase their body weight less than those who already had children, but it was not significant after adjusting for baseline age and BMI.

Baseline PA (walking for travel) and daily MVPA (mins/day) was not associated with changes in body composition. In spite of the inverse relationship between baseline BMI and baseline relative dietary energy intake (kcal/kg), there were no significant associations between absolute or relative dietary energy or macronutrient intake and changes in body weight or body composition over time. Further, there were no associations with baseline DQI and changes in body composition (data not shown). Changes in smoking and alcohol intake had no significant effects on changes in body composition.

3.3.5 Multiple regression analyses

Multiple regression analysis was used to explore the independent determinants of the changes in body weight or body fat distribution over the 5.5-year follow-up period, based on the significant univariate analyses described above (Table 4). Based on the regression model, increasing body weight over time was associated to a lower baseline BMI, being nulliparous at baseline and not having children during the follow-up period. In addition, lack of household sanitation at baseline and improved sanitation at follow-up were independently associated with weight gain. This model explained 51% of the variance in the change in body weight (P<0.001). The model that explained the greatest variance in the change in relative trunk FM (%FM), change in trunk:leg ratio and change in relative gynoid FM (%FM) (Model B) included only BMI and being nulliparous at baseline.

Table 4: Multivariate models for changes in body composition over 5.5-year period

A: Change in body weight (kg)

Variable	β	SEE	P value
Baseline BMI	-0.24	0.13	0.016
Presence of running water and a flush toilet	-0.28	2.66	0.023
Improvement in sanitation (toilet and water)	0.30	2.41	0.005
Child/children at baseline	-0.42	2.07	0.000
Children over follow-up period	-0.25	2.12	0.025

R^2= 0.51, P<0.001 VIF: 1.25

B: Change in body fat distribution

Variable	**Δ Trunk FM (%FM)**			**Δ Trunk:leg**			**Δ Gynoid FM (%FM)**		
	R^2=0.43		**P<0.001**	**R^2= 0.35**		**P < 0.001**	**R^2= 0.20**		**P=0.001**
VIF	**1.02**			**1.02**			**1.02**		
	B	**SEE**	**P value**	**β**	**SEE**	**P value**	**B**	**SEE**	**P value**
Baseline BMI	-0.61	0.04	<0.001	-0.47	0.00	<0.001	0.40	0.02	0.001
Child/children at baseline	-0.34	0.69	0.001	-0.42	0.03	<0.001	0.22	0.27	0.022

3.4 Discussion:

The results of this study showed that lower BMI and nulliparity, together with sanitation as a proxy for SES, were significant determinants of weight gain and change in body fat distribution over a 5.5-year period in a sample of free-living peri-urban black SA women. In addition, younger women increased their body weight more than their older counterparts, but this association was not independent of the other determinants.

A major finding of this study was the strong inverse relationship between baseline BMI and weight gain. Few researchers have highlighted the effect of baseline BMI as a predictor of future weight gain. Brown et al. [16] showed that baseline BMI (25-30 kg/m^2), in conjunction with high energy intake, was a significant determinant of weight gain over 5 years in middle-aged Australian women. Another study in the USA reported greater weight gain in those who were both younger and who had a lower baseline BMI [64]. However, in my study, BMI and age were not correlated, suggesting that the BMI effects were independent of age. In addition, this study showed that a lower baseline BMI was associated with a greater redistribution of fat from the periphery to the central depots. The women with a lower BMI may have a higher capacity for increasing body weight and increased centralisation of FM over time. This highlights a group that is at increased risk and should be targeted for future interventions aimed at preventing increased in body weight and centralisation of FM due to the associated negative cardio-metabolic outcomes [10,31,32].

A younger age at baseline was also associated with increased weight gain and centralisation of FM over the 5.5-year follow-up period. Women under 25 years of age increased their weight by an average of 1.2 kg/yr, compared to 0.3 kg/yr in those who were older than 25 years. These marked age-related differences in weight gain have been reported previously, with increases in body weight being more pronounced in the younger compared to the older age groups over the same time period [63,64]. These studies reported increases in body weight of 0.93, 0.73, 0.61 and 0.17 kg/yr in participants from 21-35, 35-45, 39 and 59 years of age, respectively [63,64]. The CARDIA study, which included black women of similar age to our study, showed that the black women between the ages of 18-20 yrs at baseline increased their weight by an average of 1.2 kg/yr, compared to 0.9 kg/yr over 10 years in those who were 27-30 years at baseline [67]. Although, illustrating the possible inverse effect of age on body weight gain, none of these studies measured changes in body composition or body fat distribution over time. This study also showed a greater increase in trunk FM, and abdominal VAT and SAT areas in the younger age group, reflecting an increased centralisation of FM. However, when entered into the multiple regression analysis, baseline age was replaced by parity in the model to predict weight gain and centralisation of body fat over the follow-up period. Notably, those who did not have children at baseline were significantly younger than those who already had children, illustrating the possible colinearity between parity and age. The younger women, who were also nulliparous, were also significantly less active, further confounding the effect of parity on weight gain.

The regression model also showed that having a child over the follow-up period was associated with less weight gain over the 5.5-year follow-up period. This finding contrasts to previous research that has reported that child bearing is weight-promoting [119]. Rosenberg et al. [150] have shown in a group of black women that the first child was more weight promoting. In other studies from the USA, a higher energy intake [126], and lower SES [122] increased the risk of poor post-partum weight losses, while a study from Brazil found that both high pre-pregnancy weight and higher gestational weight gain [121] increased obesity risk. Although the women in my study were of a very low SES (Table 1), I found weight gain to be lower in women who gave birth during the follow-up period compared to those who did not have further children, or remained nulliparous. It has been shown that with exclusive breastfeeding, post-partum weight loss may be improved [124], and even though exclusive breastfeeding rates in SA are considered low (1.4% of infants at 6 months), breastfeeding as part of mixed feeding is still popular [13] and may contribute to lower weight gain post-partum in these women. Further, recent data from SANHANES-1 reported that that one in three women (32.4%) experience hunger in the urban informal (peri-urban) environment [6]. In

addition, although I excluded those who were confirmed HIV positive, 57% of the sample agreed to test and those who refused to test were still included in the study. The current HIV prevalence in the Western Cape is 5.3% (18-45 years) [163], which although lower than the national average, will not take into account the higher risk in certain groups of women. Unfortunately, since my study did not have mandatory HIV testing, assess breastfeeding rates, gestational weight gain, or household food insecurity, it is difficult to draw further conclusions as to the effect of these factors on postpartum weight loss. Nonetheless, given the poor SES of the study population, it is likely that more children introduced into the house may promote greater food insecurity, facilitating higher post-partum weight loss.

Longitudinal studies to determine risk of future weight gain in high-income populations often use more static variables of education, employment or income as proxies for SES. To my knowledge, there are no longitudinal studies examining the impact of changes in SES on body weight or obesity risk, which may be an important factor to consider in this population. Although, in this study there was a significant improvement in socio-economic indicators over the follow-up period, specifically sanitation, household assets, level of education and rate of employment, it is still worth noting that less than 50% of the women had completed secondary level education, were formally employed, or came from households with running water and a toilet inside. In spite of the improvements noted in SES, within this population, the traditional markers of SES including level of education and employment, and the changes in these markers over time, were not independently associated with changes in body weight or body fat distribution. However women who had access to sanitation at baseline, representing a higher SES, had smaller weight gains over time. Conversely, those who improved their sanitation (and hence SES) over the 5.5-years had larger gains in body weight. This may suggest that with improving SES, women may increase their body weight, whereas if SES is stable, body weight might remain stable. In high-income populations, it has been shown that SES is inversely associated with obesity, with a stronger relationship in women than men [73]. Conversely, in LMICs, studies have shown a positive association between SES and BMI [75,164], while others have shown an increasing obesity prevalence in the lower SES groups [77,103] . Therefore, sanitation rather than the traditional measures of SES may better reflect the poor SES in this study.

Although this study did not find any associations with baseline dietary intake or DQI and changes in body composition, other large longitudinal studies have shown that different dietary patterns or specific aspects of the diet have been associated with weight gain over time [64,93,102,104,149,165,166]. Poor dietary quality or the change from a traditional towards a more

westernised diet, often referred to as the nutrition transition [90], have been associated with increased risk of obesity. This transition may be linked to urbanisation, which is associated with a higher dietary fat intake (% of total energy) and a proportional increase in BMI [90 11]. The most recent data from SANHANES-1 and other studies also highlight poorer dietary diversity in the urban informal (peri-urban) environment compared to the urban formal areas [6,100], which may illustrate the interaction between SES and dietary quality. Therefore, even though dietary intake did not directly influence changes in body composition in this study, it is likely to be mediated by SES. Further, although the FFQ used in this study has been validated in black SA women, the lack of findings in relation to changes in body composition may be due to limitations of dietary reporting. The study was unable to examine possible changes in dietary intake over the follow-up period due to the limitations of this methodology. Considering the body of evidence from other studies showing the impact of dietary intake on increases in body weight, this would be a priority to assess further. Lastly, this study was unable to assess household food security. Due to its interaction with both SES and dietary quality, this would be vital to include in future research.

Although most women met the physical activity guidelines (≥30mins MVPA/day, American College of Sports Medicine (ACSM)), baseline physical activity was not associated with weight gain. Most of the physical activity was reported to be for transport, which is performed at a low-intensity and may not confer any reasonable effect on energy balance [167]. As with dietary intake, the use of subjective measures of PA (GPAQ) may be a limitation due to the misinterpretation of light- and moderate-intensity activity, leading to falsely elevated levels of reported daily physical activity [168].

While the change in parity independently influenced weight gain, the use of hormonal contraceptives was not associated with a change in body weight or body fat distribution in this study. While previous studies have found significant increases in body weight and central FM with the use of hormonal contraceptives [169,170], due to the lack of data regarding length of usage, it is difficult to correctly estimate the effect of hormonal contraceptive use in these women. In addition, SES and access to health care may influence the choice, as well as the consistency of contraceptive use, in this group and dilute the possible impact on weight change.

The limitation of using two time points rather than measurements on a more continuous basis within the 5.5-years may possibly limit our interpretation of subtle changes within the

time period. Thus, acute, or short-term changes could not be measured. Even though the sample size was limited, the longitudinal nature of this study contributes to knowledge regarding the determinants of weight gain and their impact over time in an under-studied population at high risk of obesity and metabolic disease.

In summary, this study showed that lower BMI and nulliparity in the younger women at baseline were significant determinants of weight gain and centralisation of body fat over 5.5-years. In addition, although higher SES at baseline was associated with a smaller change in body weight, improvement in SES over the follow-up period were associated with greater weight gain. Future interventions should focus on younger, normal weight women before they have children as to the importance of maintaining body weight, thereby aiming to prevent any rapid increase in body weight. Further investigations should examine the effect of SES and more short term changes in the behavioural and lifestyle factors that may influence weight gain.

CHAPTER FOUR

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Black women in SA suffer from a disproportionately high rate of obesity and its associated cardio-metabolic risk factors [6,10]. Cross-sectional surveys in SA have reported a higher prevalence of obesity and T2D in black compared to white women [6,13]. In addition, recent local SA studies have shown an increase in the prevalence of obesity, T2D and dyslipideamia in black women [25,26]. Although the reasons for increasing prevelance of obesity or the ethnic disparity in T2D has not been well established, studies have reported marked differences in body fat distribution between black and white women. Black women have less VAT, but more abdominal and peripheral SAT than their white counterparts [9,10,34], which may influence the relationship with cardio-metabolic risk. Contributors towards increasing adiposity may include diet, physical activity, SES and other lifestyle factors [77,64,87,126]. Currently, there is no longitudinal data in black SA women examining changes in body composition and body fat distribution, its determinants, and how these changes influence cardio-metabolic outcomes.

Therefore, the main aim of this thesis was to examine the determinants of changes in body composition and body fat distribution and the associated metabolic outcomes over a period of 5.5-years in a cohort of 64 free-living black SA women. This aim was addressed in two chapters of this thesis with the following objectives: Chapter 2: To examine the changes in body weight, body composition and body fat distribution in relation to the changes in cardio-metabolic risk factors; Chapter 3: To assess the determinants of these changes in body composition and body fat distribution. These two objectives were addressed and the findings are summarised below.

To the authors' knowledge this is the first study in SA that has examined longitudinal changes in body composition, body fat distribution and their determinants, and the relationship with changes in cardio-metabolic risk factors in a population of free-living black SA women. A major strength of the study is the use of state-of-the-art measurements for body composition including DXA and CT scans, as well as laboratory based biochemical markers. The main findings of this thesis were that body weight (~9%), BMI (33.8±7.5 to 36.4±7.7 kg/m^2, P<0.001) and FM (16.4±26.9%) increased significantly over the 5.5-year follow-up period. This increase was attributed to an increase in FM rather than FFSTM. When expressed relative to total body fat, there was an increase in trunk FM with a concomitant decrease in peripheral FM. Previous research has shown that black women

have lower central FM, in particular VAT, compared to their white counterparts, which was thought to be metabolically favourable [9]. However, a novel finding of this study was that over time, with increasing body fat, black women significantly increased their central FM, which in turn, was related to an increase in cardio-metabolic risk factors. This is in accordance with longitudinal research from developed countries showing poorer glucose outcomes with increased WC over 20 years [21]. Further, the findings highlight the protective effect of peripheral SAT (gynoid depot), which was associated with improved insulin sensitivity at follow-up, independent of baseline age, FM and changes in trunk FM. Although we characterised CT-derived measures of abdominal VAT and SAT, these were not associated with changes in cardio-metabolic risk factors. These findings contrast to previous studies from our laboratory and abroad [7,34,36], but may be the result of the smaller sample of women that completed CT scans at baseline and follow-up (n=43). However, due to the associations found with total trunk adiposity, which includes VAT and SAT, it may be appropriate to use waist circumference in a resource limited setting such as SA, as it has been shown to be a good measure of risk in black women [171]. Notably, this study was undertaken in pre-menopausal women, who were tested before the menopausal transition, which is typically characterised by increased centralisation of FM. Future studies in black SA women should examine the changes in body fat distribution and associated cardio-metabolic risk over this period.

Chapter 3 of this thesis examined the determinants of weight gain in these free-living black SA women, and found that a lower BMI and nulliparity at baseline, and household sanitation were associated with greater weight gain over time. These findings are somewhat surprising as bearing children is typically associated with weight gain due to poor post-partum weight loss [119]. The effect of nulliparity may have been confounded by age, as those who had not had children were also significantly younger than those who did have children at baseline. A younger age was associated with larger increases in body weight in the univariate associations, as those who were younger than 25 years at baseline increased their body weight at 1.2 kg/year compared to 0.3kg/year in those who were older than 25 years. It is possible that those who were younger, with a lower BMI, and who have not yet had children, may be in a period of transition and may be at increased risk of weight gain due to changing lifestyle patterns. Further research is required to understand the reasons for the rapid weight gain in this group.

Surprisingly, the traditional markers of SES, including education or employment, were not associated with weight gain in these women. Rather, the lack of sanitation at baseline and its

improvement (gaining sanitation) over the follow-up period were significantly correlated to increasing body weight. Access to sanitation was used as a proxy for household SES, and highlights the low overall SES in this group. To my knowledge this is the first study that has shown a relationship between sanitation and risk of weight gain. Considering that 13.6% of South Africa still live in informal housing [66], the effect of SES/sanitation may have a profound influence in this group. Due to the inability to generalise to those with higher SES, or more stable housing, further research needs to examine the effect of sanitation and other SES markers on weight gain in a more representative sample of black women. Further, although black men may be at lower risk of obesity than women [72], research is required to examine changes in body composition and the determinants of these changes in a representative sample of black men.

Contrary to other studies, dietary intake and physical activity were not associated with weight gain or changes in body fat distribution. It is possible that the failure to show an association may be confounded by the effect that SES has on these lifestyle factors. SES has been shown to affect household food security and therefore dietary quality [172], and may be linked to physical activity patterns through employment and its effect on transport habits. However, both dietary intake and physical activity were examined using subjective reporting tools, which may have influenced the findings. Indeed, due to the limitations of reporting and poor reproducibility of follow-up data, the change in these outcome measures were not included in this thesis. Future studies should include objective measures of physical activity, and alternative methods to capture dietary intake to provide more insight into the effect of these factors and their impact on weight changes over time.

Other limitations of the study not already mentioned above include the small convenience sample of black women, which are not fully representative of the black SA population. Further, only a third of the baseline sample (n=240) were recruited for the follow-up study, further reducing the generalizability of the findings. The retention rate was poor as most women in this study live in informal housing, which is relatively transient. Selection bias in this sample can not be ruled out, as we canot account for the movements of those who could not be contacted, or the reason behind those who declined to participate. Those who refused to participate may have been due to employment, which would also change the socio-economic profile of the group as it was easier to partipate if they were employed. With regads to the analysis of the blood samples, they were not performed at the same time, but the intra- and inter-assay CV was low. The measurements at only two time points and not repeated measures at regular time points during the 5.5-years limits the interpretation of the

longitudinal changes. This is particularly relevant when considering that changes in SES had such a profound effect on weight change in this sample of women. It would be important to consider how acute or short-term changes in SES may affect household food security and therefore dietary intake, physical activity patterns and consequently weight changes over time. In addition, while this study showed an increase in certain cardio-metabolic risk factors over the 5.5-year follow-up period, progression to metabolic disease was not found. This is likely due to the relatively young age of the sample. In future studies, it will be important to examine whether with increasing age, these risk factors result in T2D and CVD.

In conclusion, the main findings of this thesis were that body weight (~9%), and body fat increased over the 5.5-year follow-up period. This increase in body weight was associated with a lower BMI, nulliparity and lack of sanitation at baseline and improvement in sanitation and not having a child over the follow-up period. The relative centralisation of FM in relation to total body fat was only associated with BMI and nulliparity at baseline. This increase in centralisation of body fat was associated with an increase in serum triglyceride and fasting blood glucose concentrations, as well as a decrease in insulin sensitivity with a compensatory increase in insulin secretion at follow-up. A larger study with a more representative population is required to confirm the findings of this study to determine if the increase in central FM with increasing obesity is consistent across ethnicites and sexes. Further, this study highlights the need for interventions to prevent early weight gain in young normal-weight women, rather than trying to reduce weight in obese women, which has proven difficult. Lastly, it is vital to further examine the impact of changes in SES in this population on behaviour and lifestyle facotrs in relation to the future risk of obesity.

REFERENCES

1. Wild, S., Roglic, G., Green, A., Sicree, R. & King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* **27,** 1047–1053 (2004).
2. World Health organistion, *World Health statistics*. 1–172 (2013).
3. Wagner, K.-H. & Brath, H. A global view on the developement of non communicable diseases. *Prev Med* **54,** S38–S41 (2012).
4. Mayosi, B. M. *et al.* The burden of non-communicable diseases in South Africa. *The Lancet* **374,** 934–947 (2009).
5. Mokdad, A. H. *et al.* Diabetes trends in the U.S.: 1990-1998. *Diabetes Care* **23,** 1278–1283 (2000).
6. Shisana, O. *et al.* SANHANES-1. 1–423 (2013).
7. Despres, J. P. *et al.* Race, Visceral Adipose Tissue, Plasma Lipids, and Lipoprotein Lipase Activity in Men and Women : The Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study. *Arteriosclerosis, Thrombosis, and Vascular Biology* **20,** 1932–1938 (2000).
8. Ellis, A. C., Alvarez, J. A., Granger, W. M., Ovalle, F. & Gower, B. A. Ethnic differences in glucose disposal, hepatic insulin sensitivity, and endogenous glucose production among African American and European American women. *Metabolism: clinical and experimental* **61,** 634–640 (2012).
9. Rush, E. C. *et al.* BMI, fat and muscle differences in urban women of five ethnicities from two countries. *Int J Obes Relat Metab Disord* **31,** 1232–1239 (2007).
10. Lovejoy, J. C., Smith, S. R. & Rood, J. C. Comparison of regional fat distribution and health risk factors in middle-aged white and African American women: The Healthy Transitions Study. *Obesity research* **9,** 10–16 (2001).
11. Abrahams, Z., Mchiza, Z. & Steyn, N. P. Diet and mortality rates in Sub-Saharan Africa: Stages in the nutrition transition. *BMC Public Health* **11,** 801 (2011).
12. Department of Health, S. A. South African Demographic Health Survey 2003. 1–201 (2008).
13. Department of Health, S. A. South African Demographic and Health Survey (1998). 1–418 (2003).
14. Lewis, C. E. *et al.* Seven-year trends in body weight and associations with lifestyle and behavioral characteristics in black and white young adults: the CARDIA study. *Am J Public Health* **87,** 635–642 (1997).
15. Hopman, W. M. *et al.* Changes in body mass index in Canadians over a five-year period: results of a prospective, population-based study. *BMC Public Health* **7,** 150 (2007).
16. Brown, W. J., Williams, L., Ford, J. H., Ball, K. & Dobson, A. J. Identifying the energy gap: magnitude and determinants of 5-year weight gain in midage women. *Obesity research* **13,** 1431–1441 (2005).
17. Adair, L. S., Gultiano, S. & Suchindran, C. 20-Year Trends in Filipino Women's Weight Reflect Substantial Secular and Age Effects. *Journal of Nutrition* **141,** 667–673 (2011).
18. Chor, D., Faerstein, E., Kaplan, G. A., Lynch, J. W. & Lopes, C. S. Association of weight change with ethnicity and life course socioeconomic position among Brazilian civil servants. *International Journal of Epidemiology* **33,** 100–106 (2004).
19. Folsom, A. R. *et al.* Increase in fasting insulin and glucose over seven years with increasing weight and inactivity of young adults. The CARDIA Study. Coronary Artery Risk Development in Young Adults. *Am. J. Epidemiol.* **144,** 235–246 (1996).
20. Truesdale, K. P. *et al.* Changes in risk factors for cardiovascular disease by

baseline weight status in young adults who maintain or gain weight over 15 years: the CARDIA study. *Int J Obes Relat Metab Disord* **30,** 1397–1407 (2006).
21. Park, K. *et al.* Association of long-term change in waist circumference with insulin resistance. *Obesity (Silver Spring)* **18,** 370–376 (2010).
22. Siervogel, R. M. *et al.* Serial Changes in Body Composition Throughout Adulthood and Their Relationships to Changes in Lipid and Lipoprotein Levels : The Fels Longitudinal Study. *Arteriosclerosis, Thrombosis, and Vascular Biology* **18,** 1759–1764 (1998).
23. Bays, H. E., Chapman, R. H., Grandy, S.the SHIELD Investigators' Group. The relationship of body mass index to diabetes mellitus, hypertension and dyslipidaemia: comparison of data from two national surveys. *International Journal of Clinical Practice* **61,** 737–747 (2007).
24. Joubert, J. *et al.* Estimating the burden of disease attributable to excess body weight in South Africa in 2000. *S. Afr. Med. J.* **97,** 683–690 (2007).
25. Peer, N. *et al.* Rising diabetes prevalence among urban-dwelling black South Africans. *PLoS ONE* **7,** e43336 (2012).
26. Peer, N., Steyn, K., Lombard, C., Gaziano, T. & Levitt, N. Alarming rise in prevalence of atherogenic dyslipidaemia in the black population of Cape Town: the Cardiovascular Risk in Black South Africans (CRIBSA) study. *Eur J Prev Cardiol* (2013)
27. Sliwa, K. *et al.* Different lipid profiles according to ethnicity in the Heart of Soweto study cohort of de novo presentations of heart disease : cardiovascular topics. *Cardiovascular Journal Of Africa* **23,** 389–395 (2012).
28. Kruger, H. S., Venter, C. S. & Vorster, H. H. Obesity in African women in the North West Province, South Africa is associated with an increased risk of non-communicable diseases: the THUSA study. *Br. J. Nutr.* **86,** 733 (2007).
29. Sumner, A. E. & Cowie, C. C. Ethnic differences in the ability of triglyceride levels to identify insulin resistance. *Atherosclerosis* **196,** 696–703 (2008).
30. Goedecke, J. H. *et al.* Ethnic differences in serum lipoproteins and their determinants in South African women. *Metabolism: clinical and experimental* **59,** 1341–1350 (2010).
31. Smith, S. R. *et al.* Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. *Metabolism: clinical and experimental* **50,** 425–435 (2001).
32. Tchernof, A. & Despres, J. P. Pathophysiology of Human Visceral Obesity: An Update. *Physiol. Rev.* **93,** 359–404 (2013).
33. Aasen, G., Fagertun, H. & Halse, J. Regional fat mass by DXA: high leg fat mass attenuates the relative risk of insulin resistance and dyslipidaemia in obese but not in overweight postmenopausal women. *Scand. J. Clin. Lab. Invest.* **68,** 204–211 (2008).
34. Goedecke, J. H. *et al.* Differential Effects of abdominal adipose tissue Distribution on Insulin sensitivity in Black and White south african Women. *Obesity* **17,** 1506–1512 (2009).
35. Hill, J. O. *et al.* Racial differences in amounts of visceral adipose tissue in young adults: the CARDIA (Coronary Artery Risk Development in Young Adults) study. *The American Journal of Clinical Nutrition* **69,** 381–387 (1999).
36. Tulloch-Reid, M. K. *et al.* Both subcutaneous and visceral adipose tissue correlate highly with insulin resistance in african americans. *Obesity research* **12,** 1352–1359 (2004).
37. Walker, G. E. *et al.* Deep subcutaneous adipose tissue: a distinct abdominal adipose depot. *Obesity (Silver Spring)* **15,** 1933–1943 (2007).
38. Hu, G. *et al.* Trunk Versus Extremity Adiposity and Cardiometabolic Risk Factors

in White and African American Adults. *Diabetes Care* **34,** 1415–1418 (2011).
39. Hosain, G. M. M., Rahman, M., Williams, K. J. & Berenson, A. B. Racial differences in the association between body fat distribution and lipid profiles among reproductive-age women. *Diabetes & Metabolism* **36,** 278–285 (2010).
40. Alvehus, M., Burén, J., Sjöström, M., Goedecke, J. & Olsson, T. The Human Visceral Fat Depot Has a Unique Inflammatory Profile. *Obesity* **18,** 879–883 (2009).
41. Hoffstedt, J., Arner, P., Hellers, G. & Lönnqvist, F. Variation in adrenergic regulation of lipolysis between omental and subcutaneous adipocytes from obese and non-obese men. *The Journal of Lipid Research* **38,** 795–804 (1997).
42. Patel, P. & Abate, N. Role of Subcutaneous Adipose Tissue in the Pathogenesis of Insulin Resistance. *J Obes* **2013,** 1–5 (2013).
43. Evans, J. *et al.* Depot- and ethnic-specific differences in the relationship between adipose tissue inflammation and insulin sensitivity. *Clinical Endocrinology* **74,** 51–59 (2010).
44. Goedecke, J. H. *et al.* The role of adipose tissue in insulin resistance in women of african ancestry. *J Obes* **2013,** 952916 (2013).
45. Veilleux, A., Caron-Jobin, M., Noel, S., Laberge, P. Y. & Tchernof, A. Visceral Adipocyte Hypertrophy is Associated With Dyslipidemia Independent of Body Composition and Fat Distribution in Women. *Diabetes* **60,** 1504–1511 (2011).
46. Berings, M. *et al.* Glucose Intolerance and the Amount of Visceral Adipose Tissue Contribute to an Increase in Circulating Triglyceride Concentrations in Caucasian Obese Females. *PLoS ONE* **7,** e45145 (2012).
47. Sumner, A. E. *et al.* ApoC-III and visceral adipose tissue contribute to paradoxically normal triglyceride levels in insulin-resistant African-American women. *Nutr Metab (Lond)* **10,** 73–73 (2012).
48. Hooper, A. J., Marais, A. D., Tanyanyiwa, D. M. & Burnett, J. R. The C679X mutation in PCSK9 is present and lowers blood cholesterol in a Southern African population. *Atherosclerosis* **193,** 445–448 (2007).
49. Oosthuizen, W. *et al.* Impact of urbanisation on serum lipid profiles--the THUSA survey. *S. Afr. Med. J.* **92,** 723–728 (2002).
50. Manolopoulos, K. N., Karpe, F. & Frayn, K. N. Gluteofemoral body fat as a determinant of metabolic health. *Int J Obes (Lond)* **34,** 949–959 (2010).
51. Rocha, V. Z. & Libby, P. Obesity, inflammation, and atherosclerosis. *Nature Publishing Group* **6,** 399–409 (2009).
52. Snijder, M. B. *et al.* Larger thigh and hip circumferences are associated with better glucose tolerance: the Hoorn study. *Obesity research* **11,** 104–111 (2003).
53. McLaughlin, T., Lamendola, C., Liu, A. & Abbasi, F. Preferential Fat Deposition in Subcutaneous Versus Visceral Depots Is Associated with Insulin Sensitivity. *J Clin Endocrinol Metab* **96,** 1756–1760 (2011).
54. Wiklund, P. *et al.* Abdominal and Gynoid Fat Mass Are Associated with Cardiovascular Risk Factors in Men and Women. *Journal of Clinical Endocrinology & Metabolism* **93,** 4360–4366 (2008).
55. Sánchez-López, M. *et al.* Leg fat might be more protective than arm fat in relation to lipid profile. *European Journal of Nutrition* **52,** 489–495 (2012).
56. McCarty, M. F. A paradox resolved: the postprandial model of insulin resistance explains why gynoid adiposity appears to be protective. *Medical Hypotheses* **61,** 173–176 (2003).
57. Goedecke, J. H. *et al.* Reduced Gluteal Expression of Adipogenic and Lipogenic Genes in Black South African Women Is Associated with Obesity-Related Insulin Resistance. *Journal of Clinical Endocrinology & Metabolism* **96,** E2029–E2033 (2011).
58. Hairston, K. G. *et al.* Five-Year Change in Visceral Adipose Tissue Quantity in a

Minority Cohort: The Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes Care* **32,** 1553–1555 (2009).
59. Lara-Castro, C., Weinsier, R. L., Hunter, G. R. & Desmond, R. Visceral adipose tissue in women: longitudinal study of the effects of fat gain, time, and race. *Obesity (Silver Spring)* **10,** 868–874 (2002).
60. Park, H. S. & Lee, K. Greater beneficial effects of visceral fat reduction compared with subcutaneous fat reduction on parameters of the metabolic syndrome: a study of weight reduction programmes in subjects with visceral and subcutaneous obesity. *Diabetic medicine* **22,** 266–272 (2005).
61. Thamer, C. *et al.* High visceral fat mass and high liver fat are associated with resistance to lifestyle intervention. *Obesity (Silver Spring)* **15,** 531–538 (2007).
62. Trussardi Fayh, A. P., Lopes, A. L., Fernandes, P. R., Reischak-Oliveira, A. & Friedman, R. Impact of weight loss with or without exercise on abdominal fat and insulin resistance in obese individuals: a randomised clinical trial. *Br. J. Nutr.* **110,** 486–492 (2013).
63. Ebrahimi-Mameghani, M., Scott, J. A., Der, G., Lean, M. E. J. & Burns, C. M. Changes in weight and waist circumference over 9 years in a Scottish population. *European Journal of Clinical Nutrition* **62,** 1208–1214 (2008).
64. Boggs, D. A. *et al.* Dietary patterns and 14-y weight gain in African American women. *The American Journal of Clinical Nutrition* **94,** 86–94 (2011).
65. Lloyd-Richardson, E. E., Bailey, S., Fava, J. L. & Wing, R. A prospective study of weight gain during the college freshman and sophomore years. *Prev Med* **48,** 256–261 (2009).
66. Africa, S. S. Census 2011 Statistical report. 1–88 (2013).
67. Lewis, C. E. C. *et al.* Weight gain continues in the 1990s: 10-year trends in weight and overweight from the CARDIA study. Coronary Artery Risk Development in Young Adults. *Am. J. Epidemiol.* **151,** 1172–1181 (2000).
68. Williamson, D. F., Kahn, H. S. & Byers, T. The 10-y incidence of obesity and major weight gain in black and white US women aged 30-55 y. *The american journal of clinical nutrition* **53,** 1515S–1518S (1991).
69. Lin, Y.-C., Chen, J.-D. & Chen, P.-C. Excessive 5-year weight gain predicts metabolic syndrome development in healthy middle-aged adults. *World J Diabetes* **2,** 8–15 (2011).
70. Reis, J. P. *et al.* Association between duration of overall and abdominal obesity beginning in young adulthood and coronary artery calcification in middle age. *JAMA* **310,** 280–288 (2013).
71. Truong, K. D. & Sturm, R. Weight Gain Trends Across Sociodemographic Groups in the United States. *Am J Public Health* **95,** 1602–1606 (2005).
72. Case, A. & Menendez, A. Sex differences in obesity rates in poor countries: evidence from South Africa. *Econ Hum Biol* **7,** 271–282 (2009).
73. Coogan, P. F. *et al.* Neighborhood socioeconomic status in relation to 10-year weight gain in the Black Women's Health Study. *Obesity (Silver Spring)* **18,** 2064–2065 (2010).
74. Berry, T. R. *et al.* Changes in BMI over 6 years: the role of demographic and neighborhood characteristics. *Int J Obes Relat Metab Disord* **34,** 1275–1283 (2010).
75. Subramanian, S., Perkins, J. M., Ozaltin, E. & Davey Smith, G. Weight of nations: a socioeconomic analysis of women in low- to middle-income countries. *American Journal of Clinical Nutrition* **93,** 413–421 (2011).
76. Jones-Smith, J. C., Gordon-Larsen, P., Siddiqi, A. & Popkin, B. M. Cross-National Comparisons of Time Trends in Overweight Inequality by Socioeconomic Status Among Women Using Repeated Cross-Sectional Surveys From 37 Developing Countries, 1989-2007. *Am. J. Epidemiol.* **173,** 667–675

(2011).
77. Monteiro, C. A., Moura, E. C., Conde, W. L. & Popkin, B. M. Socioeconomic status and obesity in adult populations of developing countries: a review. *Bull World Health Org* **82,** 940–946 (2004).
78. Dinsa, G. D., Goryakin, Y., Fumagalli, E. & Suhrcke, M. Obesity and socioeconomic status in developing countries: a systematic review. *Obesity Reviews* **13,** 1067–1079 (2012).
79. Micklesfield, L. K. *et al.* Socio-cultural, environmental and behavioural determinants of obesity in black South African women. *Cardiovascular Journal Of Africa* **24,** 1–7 (2013).
80. Ball, K. K., Mishra, G. G. & Crawford, D. D. Which aspects of socioeconomic status are related to obesity among men and women? *Int J Obes (Lond)* **26,** 559–565 (2002).
81. Fillol, F. *et al.* Accounting for the multidimensional nature of the relationship between adult obesity and socio-economic status: the French second National Individual Survey on Food Consumption (INCA 2) dietary survey (2006–07). *Br. J. Nutr.* **106,** 1602–1608 (2011).
82. Housing development agency. South Africa: Informal settlements status. 1–64 (2012).
83. Fogelholm, M. & Kukkonen-Harjula, K. Does physical activity prevent weight gain--a systematic review. *Obesity Reviews* **1,** 95–111 (2000).
84. Guthold, R. *et al.* Physical Activity in 22 African Countries. *American Journal of Preventive medicine* **41,** 52–60 (2011).
85. Jennings, C. L. *et al.* Determinants of insulin-resistant phenotypes in normal-weight and obese Black African women. *Obesity (Silver Spring)* **16,** 1602–1609 (2008).
86. Cook, I., Alberts, M. & Lambert, E. V. Relationship between adiposity and pedometer-assessed ambulatory activity in adult, rural African women. *Int J Obes Relat Metab Disord* **32,** 1327–1330 (2008).
87. Hankinson, A. L. *et al.* Maintaining a high physical activity level over 20 years and weight gain. *JAMA* **304,** 2603–2610 (2010).
88. Gordon-Larsen, P. *et al.* Fifteen-year longitudinal trends in walking patterns and their impact on weight change. *American Journal of Clinical Nutrition* **89,** 19–26 (2008).
89. Hill, J. O. & Peters, J. C. Environmental contributions to the obesity epidemic. *Science* **280,** 1371–1374 (1998).
90. Vorster, H. H., Venter, C. S., Wissing, M. P. & Margetts, B. M. The nutrition and health transition in the North West Province of South Africa: a review of the THUSA (Transition and Health during Urbanisation of South Africans) study. *Public Health Nutr* **8,** (2007).
91. Macdiarmid, J. & Blundell, J. Assessing dietary intake: Who, what and why of under-reporting. *Nutrition research reviews* 231–253 (1998).
92. Fung, T. T., Willett, W. C., Stampfer, M. J., Manson, J. E. & Hu, F. B. Dietary patterns and the risk of coronary heart disease in women. *Arch. Intern. Med.* **161,** 1857–1862 (2001).
93. Zamora, D., Gordon-Larsen, P., Jacobs, D. R. & Popkin, B. M. Diet quality and weight gain among black and white young adults: the Coronary Artery Risk Development in Young Adults (CARDIA) Study (1985-2005). *The american journal of clinical nutrition* **92,** 784–793 (2010).
94. Kim, S., Haines, P. S., Siega Riz, A. M. & Popkin, B. M. The Diet Quality Index-International (DQI-I) provides an effective tool for cross-national comparison of diet quality as illustrated by China and the United States. *J. Nutr.* **133,** 3476–3484 (2003).

95. Devlin, U. M., McNulty, B. A., Nugent, A. P. & Gibney, M. J. The use of cluster analysis to derive dietary patterns: methodological considerations, reproducibility, validity and the effect of energy mis-reporting. *Proc Nutr Soc* **71,** 599–609 (2012).
96. Hu, F. B. Dietary pattern analysis: a new direction in nutritional epidemiology. 1–7 (2002).
97. MacIntyre, U. E., Venter, C. S. & Vorster, H. H. A culture-sensitive quantitative food frequency questionnaire used in an African population: 2. Relative validation by 7-day weighed records and biomarkers. *Public Health Nutr* **4,** (2007).
98. De Villiers A, L, D., EV, L. & M, S. *Relative validity of a quantified food frequency questionnaire in Black South African women [thesis]. (2006)*
99. Ruel, M. T. Is Dietary Diversity an Indicator of Food Security or Dietary Quality? A Review of Measurement Issues and Research Needs. 1–58 (2002).
100. Oldewage-Theron, W. & Kruger, R. Dietary diversity and adequacy of women caregivers in a peri-urban informal settlement in South Africa. *Nutrition* **27,** 420–427 (2011).
101. Labadarios, D., Steyn, N. P. & Nel, J. How diverse is the diet of adult South Africans? *Nutrition Journal* **10,** 33 (2011).
102. Quatromoni, P. A., Pencina, M., Cobain, M. R., Jacques, P. F. & D'Agostino, R. B. Dietary quality predicts adult weight gain: findings from the Framingham Offspring Study. *Obesity (Silver Spring)* **14,** 1383–1391 (2006).
103. Popkin, B. M. & Gordon-Larsen, P. The nutrition transition: worldwide obesity dynamics and their determinants. *Int J Obes Relat Metab Disord* **28,** S2–S9 (2004).
104. Schulze, M. B., Fung, T. T., Manson, J. A. E., Willett, W. C. & Hu, F. B. Dietary patterns and changes in body weight in women. *Obesity (Silver Spring)* **14,** 1444–1453 (2012).
105. Steyn, N. P. *et al.* Nutritional contribution of street foods to the diet of people in developing countries: a systematic review. *Public Health Nutr* 1–12 (2013).
106. Drewnowski, A. & Specter, S. E. Poverty and obesity: the role of energy density and energy costs. *The american journal of clinical nutrition* **79,** 6–16 (2004).
107. Temple, N. J. & Steyn, N. P. The cost of a healthy diet: A South African perspective. *Nutrition* **27,** 505–508 (2011).
108. Feeley, A., Pettifor, J. M. & Norris, S. A. Fast-food consumption among 17-year-olds in the Birth to Twenty cohort. *South African journal of Clinical Nutrition* **22,** (2009).
109. Fulp, R. S., McManus, K. D. & Johnson, P. A. Barriers to purchasing foods for a high-quality, healthy diet in a low-income African American community. *Fam Community Health* **32,** 206–217 (2009).
110. Drewnowski, A. & Darmon, N. The economics of obesity: dietary energy density and energy cost. *The American Journal of Clinical Nutrition* **82,** 265S–273S (2005).
111. Pan, L., Sherry, B., Njai, R. & Blanck, H. M. Food Insecurity Is Associated with Obesity among US Adults in 12 States. *JAND* **112,** 1403–1409 (2012).
112. Labadarios, D. *et al.* Executive summary of the National Food Consumption Survey Fortification Baseline (NFCS-FB-I) South Africa, 2005. 1–60 (2008).
113. Berenson, A. B. & Rahman, M. Changes in weight, total fat, percent body fat, and central-to-peripheral fat ratio with injectable and oral contraceptive use. *Am. J. Obstet. Gynecol.* **200,** 329.e1–329.e8 (2009).
114. Clark, M. K., Dillon, J. S., Sowers, M. & Nichols, S. Weight, fat mass, and central distribution of fat increase when women use depot-medroxyprogesterone acetate for contraception. *Int J Obes Relat Metab Disord* **29,** 1252–1258 (2005).
115. MacPhail, C., Pettifor, A. E., Pascoe, S. & Rees, H. V. Contraception use and

pregnancy among 15-24 year old South African women: a nationally representative cross-sectional survey. *BMC Med* **5,** 31 (2007).
116. Seutlwadi, L., Peltzer, K., Mchunu, G. & Tutshana, B. Contraceptive use and associated factors among South African youth (18 - 24 years): A population-based survey. *South African Journal of Obstetrics and Gynaecology* **18,** 43–47 (2012).
117. Berenson, A. B., Rahman, M. & Wilkinson, G. Effect of Injectable and Oral Contraceptives on Serum Lipids. *Obstet Gynecol* **114,** 786–794 (2009).
118. Abrams, B., Altman, S. L. & Pickett, K. E. Pregnancy weight gain: still controversial. *The American Journal of Clinical Nutrition* **71,** 1233s–1241s (2000).
119. Gore, S. A., Brown, D. M. & West, D. S. The role of postpartum weight retention in obesity among women: a review of the evidence. *ann. behav. med.* **26,** 149–159 (2003).
120. Hernandez, D. C. Gestational Weight Gain as a Predictor of Longitudinal Body Mass Index Transitions Among Socioeconomically Disadvantaged Women. *Journal of Women's Health* **21,** 1082–1090 (2012).
121. Kac, G., Benício, M. H. D. A., Velásquez-Meléndez, G., Valente, J. G. & Struchiner, C. J. Gestational weight gain and prepregnancy weight influence postpartum weight retention in a cohort of brazilian women. *J. Nutr.* **134,** 661–666 (2004).
122. Parker, J. D. & Abrams, B. Differences in postpartum weight retention between black and white mothers. *Obstet Gynecol* **81,** 768–774 (1993).
123. Pawlak, M. T., Alvarez, B. T., Jones, D. M. & Lezotte, D. C. The Effect of Race/Ethnicity on Gestational Weight Gain. *J Immigr Minor Health* (2013).
124. Rothberg, B. E. G., Magriples, U., Kershaw, T. S., Rising, S. S. & Ickovics, J. R. Gestational weight gain and subsequent postpartum weight loss among young, low-income, ethnic minority women. *YMOB* **204,** 52.e1–52.e11 (2011).
125. Davies, H. R. *et al.* An Investigation into the Influence of Socioeconomic Variables on Gestational Body Mass Index in Pregnant Women Living in a Peri-Urban Settlement, South Africa. *Matern Child Health J* **16,** 1732–1741 (2011).
126. Boardley, D. J., Sargent, R. G., Coker, A. L., Hussey, J. R. & Sharpe, P. A. The relationship between diet, activity, and other factors, and postpartum weight change by race. *Obstet Gynecol* **86,** 834–838 (1995).
127. Koch, E. *et al.* Impact of parity on anthropometric measures of obesity controlling by multiple confounders: a cross-sectional study in Chilean women. *J Epidemiol Community Health* **62,** 461–470 (2008).
128. Chou, T. W., Chan, G. M. & Moyer-Mileur, L. Postpartum body composition changes in lactating and non-lactating primiparas. *Nutrition* **15,** 481–484 (1999).
129. Neville, C. E., McKinley, M. C., Holmes, V. A., Spence, D. & Woodside, J. V. The relationship between breastfeeding and postpartum weightchange—a systematic review and critical evaluation. *Int J Obes Relat Metab Disord* **38,** 577–590 (2013).
130. Hall, V., Thomsen, R. W., Henriksen, O. & Lohse, N. Diabetes in Sub Saharan Africa 1999-2011:Epidemiology and public health implications. asystematic review. *BMC Public Health* **11,** 564 (2011).
131. van der Merwe, M.-T. *et al.* Evidence for insulin resistance in black women from South Africa. *Int J Obes (Lond)* 1340–1346 (2000).
132. Hasumi, T. & Jacobsen, K. H. Hypertension in South African adults. *Journal of Hypertension* **30,** 2098–2104 (2012).
133. Haffner, S. M. *et al.* Increased insulin resistance and insulin secretion in nondiabetic African-Americans and Hispanics compared with non-Hispanic whites. The Insulin Resistance Atherosclerosis Study. *Diabetes* **45,** 742–748

(1996).
134. Puoane, T. *et al.* Obesity in South Africa: the South African demographic and health survey. *Obesity research* **10,** 1038–1048 (2002).
135. Nieves, D. J. *et al.* The Atherogenic Lipoprotein Profile Associated With Obesity and Insulin Resistance Is Largely Attributable to Intra-Abdominal Fat. *Diabetes* **52,** 172–179 (2002).
136. Deschênes, D., Couture, P., Dupont, P. & Tchernof, A. Subdivision of the subcutaneous adipose tissue compartment and lipid-lipoprotein levels in women. *Obesity research* **11,** 469–476 (2003).
137. Snijder, M. B. *et al.* Trunk fat and leg fat have independent and opposite associations with fasting and postload glucose levels: the Hoorn study. *Diabetes Care* **27,** 372–377 (2004).
138. Micklesfield, L. K. *et al.* Dual-energy X-ray Absorptiometry and Anthropometric Estimates of Visceral Fat in Black and White South African Women. *Obesity* **18,** 619–624 (2009).
139. Goedecke, J. H. *et al.* Effect of Different Antiretroviral Drug Regimens on Body Fat Distribution of HIV-Infected South African Women. *AIDS Research and Human Retroviruses* **29,** 557–563 (2013).
140. Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. Estimationof the Concentrationof Low-Density LipoproteinCholesteroiln Plasma,Without Useof the PreparativeUltracentrifuge. *Clinical Chemistry* **18,** 499–502 (1972).
141. Matthews, D. R. *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28,** 412–419 (1985).
142. Matsuda, M. & DeFronzo, R. A. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* **22,** 1462–1470 (1999).
143. Muniyappa, R., Lee, S., Chen, H. & Quon, M. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am. J. Physiol. Endocrinol. Metab.* **294:E15 - E26,** 1–13 (2007).
144. Goedecke, J. H. *et al.* Insulin Response in Relation to Insulin Sensitivity: An appropriate -cell response in black South African women. *Diabetes Care* **32,** 860–865 (2009).
145. Sumner, A. E. *et al.* Waist Circumference, BMI, and Visceral Adipose Tissue in White Women and Women of African Descent. *Obesity* **19,** 671–674 (2010).
146. Wilcox, G. Insulin and insulin resistance. *Clinical Biochemist Reviews* **26,** 19 (2005).
147. Sumner, A. E. *et al.* Low HDL-cholesterol with normal triglyceride levels is the most common lipid pattern in West Africans and African Americans with Metabolic Syndrome: Implications for cardiovascular disease prevention. *CVD Prevention and Control* **5,** 75–80 (2010).
148. Knight, M. G. *et al.* The TG/HDL-C ratio does not predict insulin resistance in overweight women of African descent: a study of South African, African American and West African women. *Ethn Dis* **21,** 490–494 (2011).
149. Rosell, M., Appleby, P., Spencer, E. & Key, T. Weight gain over 5 years in 21,966 meat-eating, fish-eating, vegetarian, and vegan men and women in EPIC-Oxford. *Int J Obes (Lond)* **30,** 1389–1396 (2006).
150. Rosenberg, L. *et al.* A prospective study of the effect of childbearing on weight gain in African-American women. *Obesity research* **11,** 1526–1535 (2003).
151. Luoto, R., Männistö, S. & Raitanen, J. Ten-Year Change in the Association Between Obesity and Parity: Results From the National FINRISK Population Study. *GENM* **8,** 399–406 (2011).
152. Gunderson, E. P. *et al.* Excess gains in weight and waist circumference

associated with childbreaing: The Coronary Artery risk developement in you adults study (CARDIA). 1–20 (2011).
153. Littman, A. J., Kristal, A. R. & White, E. Effects of physical activity intensity, frequency, and activity type on 10-y weight change in middle-aged men and women. *Int J Obes Relat Metab Disord* (2005).
154. Wagenknecht, L. E. *et al.* Insulin Sensitivity, Insulin Secretion, and Abdominal Fat The Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes* **52,** 2490–2496 (2003).
155. Liu, J. *et al.* Impact of Abdominal Visceral and Subcutaneous Adipose Tissue on Cardiometabolic Risk Factors: The Jackson Heart Study. *Journal of Clinical Endocrinology & Metabolism* **95,** 5419–5426 (2010).
156. Sodjinou, R., Agueh, V., Fayomi, B. & Delisle, H. Obesity and cardio-metabolic risk factors in urban adults of Benin: Relationship with socio-economic status, urbanisation, and lifestyle patterns. *BMC Public Health* **8,** 84 (2008).
157. Feeley, A. B., Musenge, E., Pettifor, J. M. & Norris, S. A. Investigation into longitudinal dietary behaviours and household socio-economic indicators and their association with BMI Z-score and fat mass in South African adolescents: the Birth to Twenty (Bt20) cohort. *Public Health Nutr* **16,** 693–703 (2013).
158. Vorster, H. H. The emergence of cardiovascular disease during urbanisation of Africans. *Public Health Nutr* **5,** 239–244 (2002).
159. Griffiths, P. L., Rousham, E. K., Norris, S. A., Pettifor, J. M. & Cameron, N. Socio-economic status and body composition outcomes in urban South African children. *Arch. Dis. Child.* **93,** 862–867 (2008).
160. Bull, F. C., Maslin, T. S. & Armstrong, T. Global physical activity questionnaire (GPAQ): nine country reliability and validity study. *J Phys Act Health* **6,** 790–804 (2009).
161. Steyn, N. P. & Senekal, M. *A guide for the use of the dietary assessment and education kit (DAEK)*.
162. Grant, K. I., Langenhoven, M. L. & Stockton, M. A. Foodfinder dietary analysis software. Release 1.10. Medical Research Council: Cape Town, 1992.
163. Shisana, O. *et al.* South African National HIV Prevalence, Incidence, Behaviour and Communication Survey, 2008. 1–120 (2009).
164. Fernald, L. C. H. Socio-economic status and body mass index in low-income Mexican adults. *Social Science & Medicine* **64,** 2030–2042 (2007).
165. Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C. & Hu, F. B. Changes in diet and lifestyle and long-term weight gain in women and men. *N. Engl. J. Med.* **364,** 2392–2404 (2011).
166. Liu, S. *et al.* Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *The american journal of clinical nutrition* **78,** 920–927 (2003).
167. Luke, A. & Cooper, R. S. Physical activity does not influence obesity risk: time to clarify the public health message. *International Journal of Epidemiology* **42,** 1831–1836 (2014).
168. Nicaise, V., Marshall, S. & Ainsworth, B. E. Domain-specific physical activity and self-report bias among low-income Latinas living in San Diego County. *J Phys Act Health* **8,** 881–890 (2011).
169. Berenson, A. B. & Rahman, M. Changes in weight, total fat, percent body fat, and central-to-peripheral fat ratio associated with injectable and oral contraceptive use. *Am. J. Obstet. Gynecol.* **200,** 329.e1–8 (2009).
170. Clark, M. K., Dillon, J. S., Sowers, M. & Nichols, S. Weight, fat mass, and central distribution of fat increase when women use depot-medroxyprogesterone acetate for contraception. *Int J Obes Relat Metab Disord* **29,** 1252–1258 (2005).
171. Evans, J. *et al.* Diagnostic ability of obesity measures to identify metabolic risk

factors in South African women. *Metabolic Syndrome and Related Disorders* **9,** 353–360 (2011).
172. Ivers, L. C. & Cullen, K. A. Food insecurity: special considerations for women. *American Journal of Clinical Nutrition* **94,** 1740S–1744S (2011).

APPENDICES

APPENDIX 1: SUBJECT QUESTIONNAIRE-ENGLISH VERSION

Subject Number: _____ Date: _____

SOCIO-DEMOGRAPHIC QUESTIONNAIRE
Longitudinal follow-up study
Genotype and Phenotype Interactions in Black South African Women

Contact Information:
Miss Sarah Chantler
082 2965838
chnsar004@myuct.ac.za

SUBJECT CODE:

ANCESTRY

Ancestry: Tribal or national background (e.g. Xhosa, Zulu, Dutch, English):

Father _____

Grandfather _____

Grandmother _____

Mother _____

Grandfather _____

Grandmother _____

Self: _____

CONTRACEPTION

Contraception:
None: ☐
Pills ☐
Name: _____Duration: _____

☐
Injection
Name: _____Duration:_____

IUD ☐
Date inserted: _____

Sterilization (tubes tied) ☐
Date: _____

Other ☐
Details: _____

Menstrual cycle: Regular Yes ☐ No ☐
Start date of last menstrual cycle: _____

HIV STATUS

1	Do you know your HIV status?	YES..... NO.....
2	If yes, are you:	Positive..... Negative..... Unwilling to disclose ……
3	When was your last test?	.....
4	Are you receiving treatment for HIV/AIDS	YES..... NO.....

MEDICATION		
1a	Do you use any medicine regularly or daily that a doctor or nurse has prescribed?	YES..... NO.....
1b	How many different medicines do you use regularly (more than once a month)?	NUMBER: .....
1 c	Name of medication and dosage	
1 d	Do you use nutritional or other supplements?	YES NO
1 e	Name the supplement and dosage:	

DEMOGRAPHIC AND SOCIOECONOMIC DETAILS:
SUBJECT CODE: _____

How many people living in your household, including you?
..... people
How many rooms do you have in your house (including kitchen, lounge, dining room, bedrooms)?
.....rooms
In your home, how many rooms are there just for sleeping?
.....rooms
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:	

How would you describe the ownership of your home (tick the one that best describes it)?

Own with no bond/mortgage		Own with bond/mortgage		Rent the house	
Rent a room		Pay board and lodging (e.g. residence)		Free accommodation	
Subsidised housing		Other			

What type of household water do you have access to?

Indoor water		Only outside tap water		Other water source	

What type of toilet do you have?

Flush inside		Only flush outside	
Outside toilet		Other:	

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		

Marital status:

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

How many children do you have? ..... children
How many pregnancies have you had? .....pregnancies

What are the ages of the children? .....

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 Technicon	
Std 6-7 (Grade 8-9)		University	

Are you currently doing work for which you get payment?
Yes ..... No .....

14. For how long have you being doing this? .....

15. How many days a week do you work? .....

16. How do you receive payment?

Monthly salary		Daily wage	
Weekly wage		Informal trading or vendor	
Casual hourly rate			

Describe your occupation .....

Period of current employment: .....
19. Household income from all sources (tick the appropriate boxes)

Salary (monthly)		Wage (weekly)	
Casual work		No of days working/week	
Income from informal work		No of days working/week	
Grants		Specify:	
Support from partner		Support from family	

Monthly Household Income:
R 0 to R 2 500 ☐

R 2 500 to R5 000 ☐

R 5 000 to R 7500 ☐

R 7 500 to R 10 000 ☐

R 10 000 to R 15 000 ☐

R 15 000 to R 20 000 ☐

R 20 000 to R 30 000 ☐

Other give range .....- .....

Not willing to disclose: .....

How many people do you support with this income?

Adults: .....
Children:.....

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 ☐

Name of medical insurance? .....
Full medical cover ☐
Savings plan ☐
Hospital plan ☐
Other: .....

We would like to know more about the community in which you live. Which of the facilities listed below are within about 2km from your house that you can walk to within 20 min? Do you use these facilities?

	Tick if facility present	Tick if you use facility	Do you walk there on most occasions		Tick if facility present	Tick if you use facility	Do you walk there on most occasions
Primary school				Internet café			
Secondary school				Bar/tavern			
Hospital				Nightclub			
Primary healthcare clinic (day hospital)				Cinema			
Private doctor				Restaurant (sit-down)			
Pharmacy/chemist				Community/recreation al centre			
Police station				Place of worship (church)			
Shopping mall/centre				Library			
Fast food outlet				Sports fields/courts			
Convenience store				Gym			
Park (green area)				Petrol station			
Car dealership				Bus stop			
Train station				Taxi rank			
Street vendor				Hair salon			
Cell phone vendor				Bank			

Which of the following are in your neighbourhood? (tick if yes)

	Yes		Yes
Postal service		Gravel roads	
Street lighting in working condition		Tar roads	
Piped water supply		Garbage removal	

Which language do you speak at home? .....

27. How would you self-identify your ethnicity? .....

FAMILY MEDICAL HISTORY

SUBJECT CODE: _____

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	SKIP
1	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:		
1a	High Blood Pressure?	YES.....1 NO.....2 DON'T KNOW.....8 If yes, who? …… …… …….....	
1b	Heart attack or angina or chest pain when exerting himself/herself?	YES.....[1] NO.....[2] DON'T KNOW.....[8] If yes, who…… …… …. …… …… …… …… …… …… ……	

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	SKIP
1c	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 …… …… ……..	
1d	Stroke?	YES.....[1] NO.....[2] DON'T KNOW.....[8] If yes, who? …… …… …….. …… …… …… …… …… …… ……	
1e	Diabetes?	YES.....[1] NO.....[2] DON'T KNOW.....[8] If yes, who? …… …… ……	

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	SKIP
		…… Adult/ child onset? …… …… ……	
1f	Obesity? (Were they abnormally large? Or have difficulty moving?)	YES.....[1] NO.....[2] DON'T KNOW.....[8] If yes, who? …… …… …….. …… …… …… ……	

QUALITY OF LIFE AND CLINICAL CONDITIONS

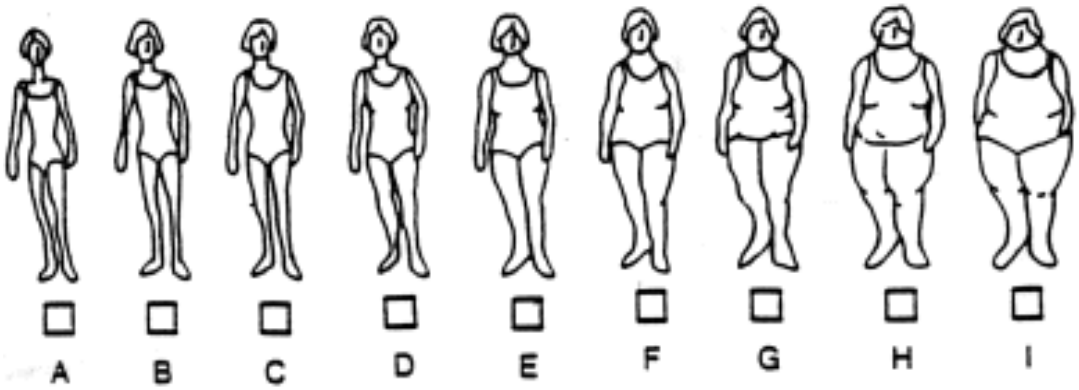
SUBJECT CODE: _____

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	SKIP
1a	Would you say your health is : 'poor, average, good, or very good/excellent'?	POOR.....[1] AVERAGE.....[2] GOOD.....[3] VERY OOD/EXCELLENT.....[4]	
1b	Do you personally think that you are underweight, normal weight or overweight?	UNDERWEIGHT.....[1] NORMAL WEIGHT.....[2] OVERWEIGHT.....[3] DON'T KNOW.....[8]	
2	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:		
2a	High Blood Pressure?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2b	Heart attack or angina (chest pains)?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2c	Stroke?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2d	High blood cholesterol or fats in the blood?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2e	Diabetes or Blood Sugar?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2f	Emphysema/Bronchitis?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	SKIP
2g	Asthma?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2h	Sore joints, e.g. Arthritis, gout?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2i	Osteoporosis?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2j	Epilepsy / fits?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2k	TB?	YES.....[1] NO.....[2] DON'T KNOW.....[8]	
2l	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: ……	
2m	Cancer?	YES.....[1] NO.....[2] DON'T KNOW.....[8] If yes, what? …… ……..	

BODY IMAGE ASSESSMENT

SUBJECT CODE: _____



Choose the picture of a woman that you think is

Thin		Normal weight		Fat	

Choose the picture of the woman you think will:

Look best		Be the strongest	
Be clumsy		Be the weakest	
Have more respect than the others		Be the happiest	
Have less respect than the others		Be the most unhappy	

Choose the woman that:

You would want to look like		Your friend will want you to look like	
Your husband / partner will want you to look like		Your children will want you to look like	

Which of the pictures do you think you look the most like?

.....

How happy are you with your present weight?

Happy		Somewhat happy		Unhappy	

Do you think you are:

Underweight		Normal weight		Overweight	

No.	Question:	YES	NO	Amount
7	Have you ever thought that you are thin?			N/A
8	Have you ever thought that you are fat?			N/A
9	Has your husband or partner ever told you that you are thin?			N/A
10	Have your friends ever told you that you are thin?			N/A

11	Have your children ever told you that you are thin?			N/A
12	Has your husband or partner ever told you that you are fat?			N/A
13	Have your friends ever told you that you are fat?			N/A
14	Have your children ever told you that you are fat?			N/A
15	Do you worry about being thin?			N/A
16	Do you worry about being fat?			N/A
17	Have you lost weight in the last 3-6 months without trying?			N/A
18	Have you gained weight in the last 3-6 months without trying?			N/A
19	Have you ever tried to lose / are currently trying to lose weight?			N/A
20	Have you ever tried to gain / are currently trying to gain weight?			N/A

Choose the method that you used / are using to lose weight:

Reduced the amount of food I eat		Starve myself	
Exercise more		Use weight-reducing medications	
Skip more meals		Other	
Use a specific diet:		N/A	

Choose the method that you think caused you to gain weight

Increase the amount of food I eat		Eat more meals than I usually eat each day	
Exercise more		Take supplements to increase energy intake:	
Use a specific diet:		Other or N/A	

23. If a woman your age is thin, she would:

Have more friends	yes	no	Be beautiful	yes	no
Feel better about herself	yes	no	Feel more like a woman	yes	no
Be healthier	yes	no			

24. If a woman your age is fat, she would:

Have more friends	yes	no	Be beautiful	yes	no
Feel better about herself	yes	no	Feel more like a woman	yes	no
Be healthier	yes	no			

HABITS AND LIFESTYLE

	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, travelling 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:		Go to:
1	Does your work involve mostly sitting or standing still, OR walking for very short periods (less than 10 minutes)?	YES.....[1] NO.....[2]	Q.4
2a	Does your work involve vigorous activities, (like heavy lifting, digging, or heavy construction) for at least 10 minutes at a time?	YES.....[1] NO.....[2]	Q.3a
2b	In a usual week, how many days do you do vigorous activities as part of your work?	.....DAYS	
2c	On a usual day on which you do vigorous activities, how much time do you spend doing such work?	.....HOURS .....MINUTES	
3a	Does your work involve moderate-intensity activities (like brisk walking or carrying light loads) for at least 10 minutes at a time?	YES.....[1] NO.....[2]	Q.4
3b	In a usual week, how many days do you do moderate-intensity activities as part of your work?	.....DAYS	
3c	On a usual day on which you do moderate-intensity activities, how much time do you spend doing such work?	.....HOURS .....MINUTES	
4	How long is your usual workday?	.....HOURS .....MINUTES	
	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).		
5a	Do you walk or use a bicycle (pedal cycle) for at least 10 minutes at a time to get to and from places?	YES.....[1] NO.....[2]	Q.6
5b	In a usual week, how many days do you walk or cycle for at least 10 minutes to get to and from places?	.....DAYS	
5c	On a usual day, how much time do you spend walking and cycling for travel	.....HOURS .....MINUTES	

	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		
6	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.....[1] NO.....[2]	Q.9
7a	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.....[1] NO.....[2]	Q.8a
7b	IF YES, in a usual week, how many days do you do vigorous activities as part of your leisure or spare time?	.....DAYS	
7c	How much time do you spend doing this on a usual day?	.....HOURS .....MINUTES	
8a	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.....1 NO.....2	Q.9
8b	IF YES, in a usual week, how many days do you do moderate-intensity activities as part of your leisure or spare time?	.....DAYS.	
8c	How much time do you spend doing this on a usual day?	.....HOURS .....MINUTES	
	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.		
9.	Over the past 7 days, how much time did you spend sitting or reclining (lying) on a usual day (exclude sleeping)?	.....HOURS .....MINUTES	

SEDENTARY BEHAVIOUR QUESTIONNAIRE

SUBJECT CODE: _____Date: _____

Interviewer: Please ask the respondent the following questions which relates to the time they spend sitting during various activities.

Ask them to think about a usual day or a normal day for each of the questions.
All answers are per day.

We are very interested in the amount of time people spend sitting. I am going to ask you a few questions about your sitting behaviour. These questions all refer to a usual day, and please answer in hours and minutes per day.

	Question	Week day (hours: minutes)	Week end day (hours: minutes)
1a	How much time do you spend sitting while you are at work? (per day)	Hrs: Min:	Hrs: Min:
1b	How many days per week do you work?		..... days
1c	Do you work on the week-end?		Yes ☐ No ☐
2	How much time do you spend sitting while watching TV per day?	Hrs: Min:	Hrs: Min:
3	How much time do you spend sitting while using the computer per day, excluding your normal working hours	Hrs: Min:	Hrs: Min:
	Question	Week day (hours: minutes)	Week end day (hours: minutes)
4	How much time do you spend sitting while travelling from place to place (e.g. in car, bus, train)	Hrs: Min:	Hrs: Min:

5	How much time do you spend sitting while eating and socialising and relaxing on a week day (Monday – Friday)?	Hrs: Min:	Hrs: Min:
Sleep The following question relates to the amount of time you spend sleeping.			
6	How many hours per day do you sleep?	Hrs: Min:	Hrs: Min:

TOBACCO USE (WHO STEPwise Questionnaire)

			Go to:
1a	Do you currently smoke any tobacco products, such as cigarettes, cigars, or pipes?	YES.....1 NO.....2	Q.4a
1b	Do you currently smoke tobacco products daily?	YES.....1 NO.....2	Q.4a
2a	How old were you when you first started smoking daily?	.....YEARS OLD IF "YOU DON'T REMEMBER", 98	Q.3
2b	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]	
3	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 [96] (SPECIFY)	
4a	In the past, did you ever smoke daily?	YES[1] NO[2]	
4b	How old were you when you stopped smoking daily?	.....YEARS OLD. .....IF "YOU DON'T REMEMBER", [98]	
4c	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]	

ALCOHOL INTAKE

1 standard drink is equal to 10 g of pure alcohol: 200 ml of beer 1 glass of wine 1 tot (25 ml) spirits 1 small glass (50ml) of sherry/port

How often do you have a drink containing alcohol?

Never		2-3 times per week	
Monthly or less		4 or more times per week	
2-4 times per month			

On a typical WEEK day, how many drinks containing alcohol do you drink? (how many standard drinks)

1 or 2		7, 8 or 9	
3 or 4		10 or more	
5 or 6		0	

On a typical WEEKEND day, how many drinks containing alcohol do you drink? (how many standard drinks)

1 or 2		7, 8 or 9	
3 or 4		10 or more	
5 or 6		0	

PSYCHOLOGICAL QUESTIONNAIRE (Kessler 10)

The next ten questions are about how you have been feeling in the past 4 weeks: (Tick the appropriate box)					
	None of the time 1	A little of the time 2	Some of the time 3	Most of the time 4	All of the time 5
In the past four weeks, about how often did you feel worn out for no good reasons?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel nervous?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel so nervous that nothing could calm you down?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel hopeless?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel restless or fidgety?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel so restless you could not sit still?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel depressed?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel that everything was an effort?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel so sad that nothing could cheer you up?	☐	☐	☐	☐	☐
In the past four weeks, about how often did you feel worthless?	☐	☐	☐	☐	☐

END OF QUESTIONNAIRE

FOR OFFICE USE
SUBJECT CODE: _____

TESTING SESSIONS

TESTING SESSION 1 – Fasting bloods, OGTT, Questionnaires, Anthropometry

Date of testing: ..... Time of testing:.....
Time of last meal/drink: ..... Hours fasted:.....
How would you describe your health TODAY? (How are you feeling?):

☐ Good ☐ Fair ☐ Poor

If POOR, explain why: .....

OGTT:
Fasting blood sample: Tubes: purple ☐ , grey ☐ , 3 x 9.5 ml yellow ☐
Drink: ☐ Time: .....Comments: .....

30 min: ☐ Time: .....Comments: .....

60 min ☐Time: ..... Comments:.....

90 min: ☐Time: .....Comments:.....

120 min:☐ Time: .....Comments:.....

TESTING SESSION 2 – DEXA, CT Scan

DXA-scan: Full Body ☐ Half Body ☐
Comments: _____

CT-scan: Full Body ☐ Half Body ☐
Comments: _____

ANTHROPOMETRIC DATA SHEET

SUBJECT CODE: _____

DATE					2	0	_	_
	d	d	m	m	y	y	y	y

WEIGHT		kg
HEIGHT (CM)		cm
MID-UPPER-ARM CIRCUMFERENCE		cm
WAIST CIRCUMFERENCE		cm
HIP CIRCUMFERENCE		cm
SYSTOLIC BLOOD PRESSURE		mm Hg
DIASTOLIC BLOOD PRESSURE		mmHg
HEART RATE		bpm

Measurement:	1			2			3		
BICEP SKINFOLD									
TRICEP SKINFOLD									
SUBSCAPULAR SKINFOLD									
SUPRAILIAC SKINFOLD									
ABDOMINAL SKINFOLD									
FRONT THIGH SKINFOLD									
MEDIAL CALF SKINFOLD									

CHECKLIST

	Tick box if done	Comments
Date screened		
Informed consent:		
Blood pressure:		
Fasting bloods:		
Glucose:		
Basic Anthropometry:		
Socio-Dem Questionnaire:		
OGTT:		
D-XA-scan:		
CT-scan:		
Food Frequency Questionnaire		
Dietary attitudes & Beliefs Questionnaire:		
Feedback		

Appendix 2: Changes in body fat distribution, expressed relative to total fat mass, over the 5.5-year follow-up period in the baseline BMI groups.

	BMI (kg/m^2)	Baseline measure	Follow-up measure	Absolute change	Group	Time	Group x time
TFM (% FM)	NO	38.5 ± 4.5	44.1 ± 4.6	5.6 ± 3.2*	0.001	0.002	<0.001
	OBc1	43.2 ± 5.6	45.2 ± 6.0	1.7 ± 3.4			
	OBc2	46.2 ± 4.3	47.4 ± 4.3	1.2 ± 2.5			
ApFm (% FM)	NO	57.0 ± 4.0	52.5 ± 4.5	-4.5 ± 2.7*	0.050	0.006	<0.001
	OBc1	54.5 ± 5.4	52.6 ± 5.9	-1.6 ± 3.0			
	OBc2	51.7 ± 4.3	50.7 ± 4.6	-1.0 ± 2.3			
LFM (%FM)	NO	46.5 ± 5.2	41.8 ± 5.1	-4.7 ±2.7*	0.092	0.001	<0.001
	OBc1	43.2 ± 6.0	41.3 ±6.5	-1.4 ± 2.9			
	OBc2	40.8 ± 5.7	39.4 ± 5.8	-1.4 ± 2.2			
AFM (%FM)	NO	10.5 ± 1.9	10.7 ± 1.5	0.2 ± 1.1	0.827	0.206	0.213
	OBc1	11.3 ± 2.0	11.2 ± 1.7	-0.2 ± 0.8			
	OBc2	10.8 ± 2.3	11.3 ± 2.2	0.5 ± 1.4			
Android FM (%FM)	NO	6.0 ±1.0	7.3± 1.4	1.3±1.3*	0.041	<0.001	0.006
	OBc1	7.9 ± 1.4	8.2± 1.5	0.2±0.8			
	OBc2	8.7± 1.1	9.1±1.3	0.4±0.8			
Gynoid FM (%FM)	NO	20.9± 2.3	19.5±2.1	-1.4±1.0#	0.276	0.052	0.010
	OBc1	18.7±3.0	17.8±3.3	-0.7±1.2			

OBc2	18.4±2.4	18.0±2.3	-0.4±1.1

Group x time interaction via two way ANCOVA adjusting for age, TFM, trunk fat mass; ApFM, Appendicular fat mass; LFM, leg fat mass; AFM, arm fat mass FM, fat mass, * NO group significantly different to both OB groups, # NO group significantly different to OBc2 group. *P*<0.05 via Tukey post-hoc analysis