INSULIN SENSITIVITY AND RESPONSE IN MIDDLE-AGED BLACK SOUTH AFRICAN MEN AND WOMEN: ASSOCIATIONS WITH BODY FAT DISTRIBUTION, MENOPAUSE AND OBJECTIVELY MEASURED PHYSICAL BEHAVIOURS

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DECLARATION

I, NYUYKI CLEMENT KUFE (Student Number: 395736), declare that the thesis entitled: *"Insulin Sensitivity and Response in Middle-Aged Black South African Men and Women: Associations with Body Fat Distribution, Menopause And Objectively Measured Physical Behaviours"* submitted for the degree of Doctor of Philosophy (PhD) to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg is the result of my own original work.

All references made to the work of others and any assistance received has been fully acknowledged.

No part of this work has been submitted before for any degree or examination purposes to this or any other university or institution.

Signed:  this 31st of August 2022

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DEDICATION

This thesis is first of all dedicated to God the Father Almighty for His unfailing love for me. “God has unexpected and curious ways”.

Secondly, special dedication to my grandparents. The dust and mud of years past is thick on your graves. You never left me to the fortunes of adversary. You gave me the best from the depths of your hearts. Your memory alone spurs me to hope for a better tomorrow. Thank you!

Last but not the least this work is dedicated with love and gratitude to my family, friends, and well–wishers who toiled unassumingly, selflessly and sometimes unknowingly for me to realise my academic dreams.

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STUDENT'S CONTRIBUTION TO THE STUDY

Under the guidance of my supervisors, I was involved in the development of the study protocol, standard operating procedures (SOPs), data collection tools, participants' information sheet, informed consent and obtained independent ethical clearance for my PhD work. I completed Basic, Intermediate and Advanced training in Research Electronic Data Capture (REDCap) and was responsible for managing and maintaining the REDCap database for the whole study. I was also actively involved in data collection, entry and quality control. I carried out statistical analysis and interpretation of data and prepared the manuscripts for publication and chapters herein.

I attended the 9th Physical Activity Measurement Seminar (PAMS) at the Møller Centre, Cambridge from Monday 10th September to Friday 14th September 2018 organised by the Medical Research Council (MRC) Epidemiology Unit of the University of Cambridge, United Kingdom (UK) exploring various methods of measuring physical activity, transcription, laboratory analysis, statistical analysis and interpretation of physical activity data. I spent an additional two months at the MRC Epidemiology Unit of the University of Cambridge to process and develop an algorithm for the physical activity data collected and learn statistical methods used, analysis and interpretation of objectively measured physical activity data. The results are part of chapter 4 of this thesis entitled "*Physical behaviours and their association with type 2 diabetes mellitus risk markers in urban South African middle-aged adults: An isotemporal substitution approach*" and the methods paper currently in second review in Medicine and Science in Sports and Exercise entitled "*Physical activity and posture profile of a South African cohort of middle-aged men and women as determined by integrated hip and thigh accelerometry*".

I also attended the Berzelius Symposium on Obesity and Type 2 Diabetes – Understanding the role of ethnicity from 11–12th September at the Vaven conference centre, Storgatan 46A, Umea, Sweden where I presented a poster on *"Differences in body composition, insulin sensitivity and beta-cell function between Black South Africa men and women, with and without HIV"*. I attended the 54th conference of Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) and presented an abstract on *"Sex Differences in Type 2 Diabetes Risk and the Association with Total and Regional Adiposity in Middle-Aged Black South African Men and Women"*.

Other training I received during the course of my PhD includes Good Clinical Practice, basic course for non-clinical support staff and Comprehensive Systematic Review Training Programme from 8–14 November 2017 after which a wrote I wrote the *"Protocol for systematic review and meta-analysis of sex hormones and diabetes risk in ageing men and women of African ancestry"* published in BMJ Open.

SCIENTIFIC CONTRIBUTIONS TO THE STUDY AND THESIS

Publications arising from the PhD thesis and funding sources

During the process of completion of this PhD thesis, various components of the study were published in peer-reviewed journals and two publications (one accepted and one in second review) are part of this PhD thesis.

Publications that are part of the PhD thesis:

1. **Kufe CN,** Micklesfield LK, Masemola M, Chikowore T, Kengne AP, Karpe F, Norris SA, Crowther NJ, Olsson T, Goedecke JH. Increased Risk for Type 2 Diabetes in Relation to Adiposity in Middle-Aged Black South African Men compared to Women. *Eur J Endocrinol*. 2022; 186(5):523-533. doi: https://doi.org/10.1530/EJE-21-0527.
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4. Ratshikombo T, Goedecke JH, Soboyisi M, **Kufe C**, Makura-Kankwende CBT, Masemola M, Micklesfield LK, Chikowore T. Sex Differences in the Associations of Nutrient Patterns

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5. Goedecke JH, Nguyen K, **Kufe C**, Masemola M, Chikowore T, Mendham AE, Norris SA, Crowther NJ, Karpe F, Olsson T, Kengne AP, Micklesfield LK. Waist circumference thresholds predicting incident dysglycaemia and type 2 diabetes in Black African men and women. *Diabetes, Obes Metab*. 2022;1-10. doi: 10.1111/dom.14655.
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7. Micklesfield, L., Westgate, K., Smith, A., **Kufe, C.,** Mendham, A., Lindsay, T., Wijndaele, K., Goedecke, J., Brage, B. Physical Activity Behaviours of a Middle-aged South African Cohort as Determined by Integrated Hip and Thigh Accelerometry. *Medicine & Science in Sports & Exercise*: April 22, 2022 - Volume - Issue. doi: 10.1249/MSS.0000000000002940
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LIST OF ABBREVIATINS, ACRONYMS AND GLOSSARY

ADA	American Diabetes Association
AIDS	Acquired Immune Deficiency Syndrome
ART	Anti-Retroviral Therapy
ARV	Anti–retroviral
BAI	Body Adiposity Index
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CAGE	Concern/Cut-down, Anger, Guilt, and Eye-Opener
CHD	Coronary Heart Disease
CI	Confidence Interval
CRP	C-reactive protein
CT	Computerized Tomography
CV	Coefficient of variation
CVD	Cardiovascular Disease
DALYs	Disability–Adjusted Life–Years
DBP	Diastolic Blood Pressure
DI	Disposition Index
DPHRU	Developmental Pathways for Health Research Unit
DXA	Dual Energy X–Ray Absorptiometry
E2	Oestrogen
FFA	Free Fatty Acids
FFM	Fat Free Mass
FFSTM	Free Fat Soft Tissue Mass
FIRI	Fasting Insulin Resistance Index
FM	Fat Mass
FPG	Fasting Plasma Glucose
FSIVGTT	Frequently Sampled Intravenous Glucose Tolerance Test
FSH	Follicle stimulating hormone
GBD	Global Burden of Disease
GI	Glucose Insulin
GPAQ	Global Physical Activity Questionnaire
HbA1c	Glycated haemoglobin
HBP	High Blood Pressure
HC	Hip Circumference
HDL	High Density Lipoproteins
HGP	Hepatic Glucose Production
HIV	Human Immunodeficiency Virus
HOMA–IR	Homeostasis Model Assessment–Insulin Resistance
HR	Hazard Ratio
IAPP	Islet Amyloid Polypeptide
IFG	Impaired Fasting Glucose
IGFBP-1	Insulin Growth Factor Binding Protein-1
IGI	Insulinogenic Index
IGT	Impaired Glucose Tolerance
IQR	Interquartile Range

IR	Insulin Resistance
IS	Insulin Sensitivity
ISI	Insulin Sensitivity Index
LDL	Low Density Lipoproteins
LH	Luteinising hormone
LPA	Light Intensity Physical Activity
LMIC	Low-and middle-income countries
MODY	Maturity–Onset Diabetes of the Young
MRC	Medical Research Council
MVPA	Moderate-to-Vigorous intensity Physical Activity
NCD	Non–Communicable Disease
NGT	Normal Glucose Tolerance
oDI	Oral Disposition Index
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PAMS	Physical Activity Measurement Seminar
PA	Physical Activity
PG	Post Glucose
PKC	Protein kinase C
QUICKI	Quantitative Insulin Sensitivity Check Index
REDCap	Research Electronic Data Capture
SA	South Africa
SAT	Subcutaneous Adipose Tissue
SBP	Systolic Blood Pressure
SEMDSA	Society for Endocrinology, Metabolism and Diabetes South Africa
SES	Socio–Economic Status
SHBG	Sex Hormone Binding Globulin
SOP	Standard Operating Procedure
SSA	Sub–Saharan Africa
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TG	Triglycerides
TNF	Tumour Necrosis Factor
VAT	Visceral Adipose Tissue
WBFM	Whole Body Fat Mass
WC	Waist Circumference
WHO	World Health Organisation
WHR	Waist-to-Hip Circumference Ratio
WHtR	Waist Height Ratio
YLD	Years Lived with Disability

Abstract

Aims: To determine whether type 2 diabetes mellitus (T2DM) risk markers are different between middle-aged Black South African men and women, and between women at different stages of the menopause transition, and to determine whether there are sex-specific associations between these markers, and body composition and physical behaviours.

Methods: This cross-sectional study included 804 Black South African men (n=388) and women (n=416) with a mean age of 54.6±6.0 years from the Middle-aged Soweto Cohort (MASC) from Soweto, Johannesburg. Dual-energy x-ray absorptiometry was used to measure total and regional adiposity. Glycated haemoglobin (HbA1c), and fasting plasma glucose, serum insulin and C–peptide concentrations were measured, and insulin resistance was calculated using the homeostasis model assessment (HOMA-IR). All participants completed an oral glucose tolerance test, from which measures of glycaemic and insulin dynamics were determined which included insulin sensitivity (Matsuda index), secretion (C-peptide index), and clearance (C-peptide/insulin ratio), beta cell function (oral disposition index), and integrated area under the curve (iAUC) for glucose, insulin and C-peptide were calculated. Total movement volume (average movement in milli-g) and time (minutes/day) spent in different physical behaviours, namely awake sitting/lying, standing, light intensity physical activity (LPA) and moderate-to-vigorous intensity physical activity (MVPA) were determined by combining the signals from two triaxial accelerometers worn simultaneously on the hip and thigh. Menopausal status was determined by self-reported final menstrual period (FMP) and women were categorised as pre-menopausal, early post-menopausal and late postmenopausal. Sex-specific associations between adiposity and markers of T2DM risk, and between physical behaviours and markers of T2DM risk, were examined by robust regressions and isotemporal substitution, respectively.

Results: Men (mean± standard deviation) (54.2±6.2) were younger than women (55.0±5.8) and significantly more men currently smoked (46.1% vs. 7.2%) and consumed alcohol (30.4% vs. 4.6%) than women. Mean BMI was higher in women than men ($p<0.001$), and accordingly a larger proportion of the women presented with obesity (70.2% vs. 26.6%) and had higher whole body fat mass (FM) (kg and %) and fat mass index (FMI). Women also had significantly greater leg FM, while men had more central FM (trunk) and a higher VAT/SAT ratio.

Unadjusted HbA1C and 2-h glucose, as well as fasting insulin and C-peptide, and iAUC for insulin were higher in women than men. HOMA-IR was higher and insulin sensitivity (Matsuda index) was lower in women compared to men, which was accompanied by a higher insulin response (IGI) characterised by higher insulin secretion (C-peptide index) and lower insulin clearance (basal and postprandial). The oral disposition index did not differ by sex. After adjusting for sex differences in fat mass index, men were less insulin sensitive and had lower beta cell function than women ($p<0.001$), with the strength of the associations with measures of total and central adiposity being greater in men than women ($p<0.001$ for interactions). When exploring the relationship between adiposity and impaired glucose metabolism (IGM) and T2DM, using normal glucose tolerance as the reference, there was a significant sex*FMI interaction such that the relative risk ratio's for IGM and T2D were greater for men than women [relative risk ratio (95% confidence interval), IGM: 1.70 (1.27– 2.29) vs 1.23 (0.95–1.60); T2DM: 2.05 (1.42–2.96) vs. 1.38 (1.03–1.85)]. The prevalences of NGT, IGT and T2DM were similar in men and women.

When exploring differences in total and regional adiposity, and glycaemic and insulin dynamics across the menopausal transition in women, BMI and all the DXA-derived measures of total and regional adiposity were similar between the menopausal groups. Although HbA1c and iAUC for glucose were significantly higher in the late post-menopausal than the pre-menopausal group (HbA1c: 6.4±1.4% vs. 5.8±0.9%, p=0.019 and iAUC for glucose: 169.4 mmol/L vs 137.9 mmol/L, p=0.026) this was no longer significant after adjusting for age. Fasting insulin and C–peptide, iAUC for insulin, and insulin sensitivity and clearance were not different between the groups. However, iAUC for C-peptide was higher in the early post-menopausal women compared to the pre-menopausal and the late post-menopausal women before and after adjusting for age (762.9 ng/ml vs. 552.6 ng/ml and 637.5 ng/ml, all $p<0.05$). Insulin secretion (4.2 ng/mmol vs. 2.3 ng/mmol, p=0.001) and beta cell function (20.5 mIU/mmol vs. 11.8 mIU/mmol, p=0.008) were higher in early than late post-menopause before and after adjusting for age.

Although time spent in LPA (mins/day) was not different between men and women, total movement volume, time spent in MVPA (mins/day) and time spent sitting/lying were higher in men, while time spent standing was higher in women. Total movement volume was inversely associated with measures of fasting and 2-h glucose, and positively associated with insulin sensitivity, basal insulin clearance, and beta-cell function, but these associations were not independent of fat mass, except for basal insulin clearance in women. Using isotemporal substitution, in men replacing 30 minutes of sitting/lying, standing or LPA with the same amount of MVPA time was associated with 1.2–1.4 mmol/L lower fasting glucose and 12.3–13.4 mgl^2/mUmin higher insulin sensitivity. In women, substituting sitting/lying with the same amount

of standing time or LPA was associated with 0.5–0.8 mmol/L lower fasting glucose. Substituting 30 minutes sitting/lying with the same amount of standing time was also associated with 3.2 mgl^2/mUmin higher insulin sensitivity, and substituting 30 minutes of sitting/lying, standing or LPA with the same amount of MVPA time was associated with 0.25-0.29 ng/mIU higher basal insulin clearance in women.

Conclusion: Although the prevalence of obesity is lower in men compared to women they may be at higher risk for T2DM due to higher central body fat which is more strongly associated with T2DM risk markers than in women. I have also shown that despite similar adiposity between menopausal groups, which may be due to the high prevalence of obesity in this sample, late menopause is associated with higher risk of T2DM. Further, I have shown that physical activity recommendations to reduce risk markers for T2DM may need to be different for men and women as men need to participate in higher intensity physical activity in order to reduce their risk, however these findings need to be confirmed in intervention studies.

Key words: Body fat distribution, T2DM risk, physical behaviours, isotemporal substitution, pre-menopause, early post-menopause, late post-menopause, men and women.

CHAPTER 1

LITERATURE REVIEW

This chapter presents a brief overview of the global picture of diabetes, followed by the pathophysiology of Type 2 Diabetes Mellitus (T2DM) with specific reference to Black African populations, and more specifically the South African population. Factors that influence insulin sensitivity and response include sex and age (non-modifiable), as well as lifestyle factors such as physical activity (PA) that are modifiable, with the relationship between these factors and T2DM risk potentially being mediated by body composition. This chapter includes a brief overview of this literature, identifying knowledge gaps and concludes with a section on the various methods used to assess insulin sensitivity and response before presenting the objectives and aims of this thesis.

1.1 INTRODUCTION

The prevalence of diabetes worldwide has witnessed a four–fold increase since 1980 (1,2). T2DM is on the rise globally with 537 million (10.5%) adults aged 20–79 years affected in 2021, 80% of whom are living in low and middle–income countries (LMIC) (3). The burden of T2DM is exacerbated by late diagnosis accompanied by preventable complications such as retinopathy, nephropathy, neuropathy, and cardiovascular diseases (CVDs) increasing health care expenses (4). By 2012, diabetes was responsible for as many deaths as HIV/AIDS in South Africa (5). While the HIV epidemic was being stabilised with a global prevalence cases of HIV/AIDS resulting in mycobacterial infection of 724 700 and HIV/AIDS resulting in other diseases of 28 506 600 in 2013 with a percentage change in prevalence of 275.7 and 234.1 corresponding to 268 300 and 3 795 300 years lived with disability (YLD) respectively, the prevalence cases of diabetes was 409

967 000 in 2013 giving a percentage change in prevalence of 132.9 from 1990 to 2013 corresponding to 29 518 100 YLDs in 2013 (5).

According to the latest data from the International Diabetes Federation (IDF), it is estimated that the number of people with diabetes in the African region will increase by 129% to 55 million by 2045, representing the highest predicted increase of all the IDF Regions (3). Globally in 2019, one in two (50.1%) of the 463 million adults living with diabetes were unaware of their condition (4) and in 2021, the highest proportion of undiagnosed diabetes (60%) in adults globally were in the Africa region, with diabetes responsible for 416 000 deaths in the African region (3). The health systems in Africa suffer from acute shortages of qualified health workers, infrastructure, dependency on foreign aid and is under pressure from infectious diseases such as malaria, HIV/AIDS and tuberculosis which have exhausted health care resources (4). Notably, in 2021 the African region had the second lowest diabetes-related expenditure of USD 13 billion representing 1% of global expenditure (3).

South Africa (SA) had the highest number of people with diabetes in the African region with an increase from 1.9 million in 2011 to 4.2 million people in 2021 (3). In SA, T2DM was the second leading underlying cause of death in 2016 representing 5.5% of total deaths, and the leading (7.1%) underlying cause of death among women and adults ≥ 65 years (8.9%) (6). A recent study reported that T2DM accounted for 12% of SA's national budget in 2018 (7). The prevalence of T2DM in SA, particularly in Black African urban–dwelling populations increased significantly over the past 20 years (8), most likely driven by the increasing prevalence of obesity, affecting the majority of

Black SA women (39.9%) (9). SA also has an additional challenge of a high burden of infectious diseases, with a national HIV prevalence of 12.2% (10,11).

Data from SA has reported that Black South African women are more susceptible to developing insulin resistance (IR) and T2DM than their white counterparts (8,12), with international data reporting similarly (13,14). Accordingly, race/ethnicity is preponderant in the development of T2DM (15). Although most studies have been done in Europe and United States of America, there is an increase in the number of studies being done in Africa as the pandemic of diabetes unfolds. This thesis is based on data collected from Black South African men and women residing in Soweto, Johannesburg. According to the 2011 census, the population of Soweto was approximately 1.3 million living in an area of appropriately 200 km^2. Soweto forms part of greater Johannesburg area which is home to more than five million people. Soweto is a heterogeneous township with accessible health facilities and the majority of the residents are Blacks from across different housing, language, occupation and living standards representing urban groups in South Africa (16).

1.2 PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus accounts for approximately 90% of all diabetes cases globally (4). T2DM is a heterogeneous disorder due to β–cell dysfunction resulting in insufficient insulin production, and/or insulin resistance, which is reduced sensitivity or responsiveness of target tissues to the metabolic actions of normal circulating levels of insulin, resulting in hyperglycaemia.

Insulin is produced in the beta-cells of the pancreas and it travels through the portal circulation to the liver where over 50% is cleared by the hepatocytes. The remaining insulin leaves the liver by the hepatic vein, continues through venous circulation to the heart, and is distributed to the rest of the body through arterial circulation. During the second passage through the liver it is further cleared. Insulin exerts its metabolic effects in the muscle and fat cells where it stimulates GLUT4 translocation and glucose uptake. The remaining circulating insulin is delivered to and degraded by the kidney.

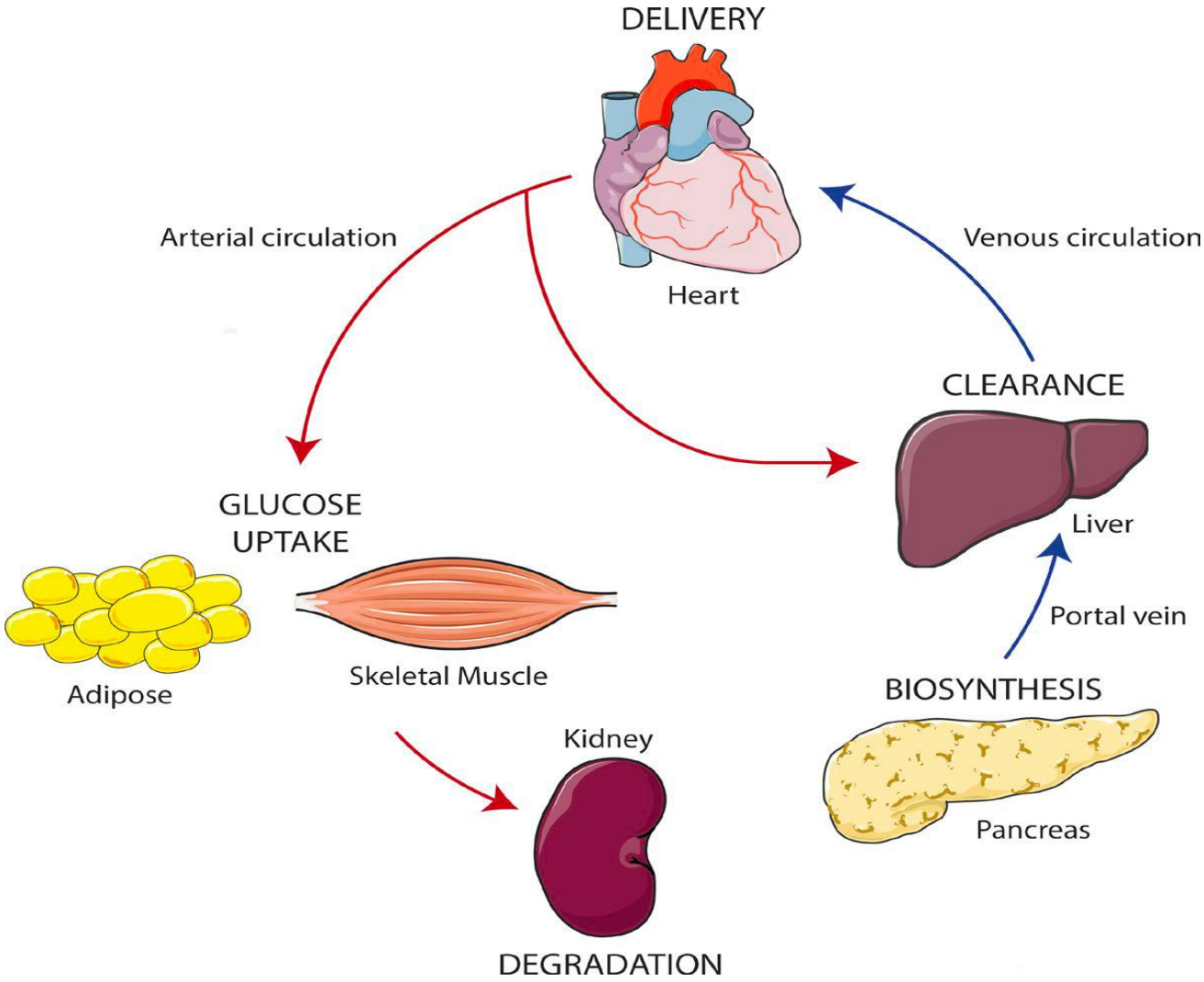

Figure 1.1: Journey of insulin in the body. Adapted from Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. J Cell Biol. 2018;217(7):1–17 (17).

The main function of insulin is regulation of glucose homeostasis, which it does by performing two key complementary roles in the body: (1) stimulation of glucose uptake from the systemic circulation into skeletal muscles and adipocytes, enhancing glycogen synthesis in skeletal muscles, and (2) suppressing hepatic glucose production (HGP) in the liver and inhibiting lipolysis in adipocytes (Figure 1.1) (18,19). However, the action of insulin is controlled by a cascade of physiologic events which when disordered may lead to physiological processes associated with disease.

Insulin resistance refers to the impairment in glucose disposal and lipid metabolism in tissues sensitive to insulin particularly muscle, liver and adipose tissue (20–22). Majority of patients with T2DM are insulin resistant but T2DM can occur without insulin resistance (23–25). At the same time, many obese individuals that are insulin resistant do not develop T2DM (20) because they increase their insulin secretory levels as a compensation to reduced insulin sensitivity. Insulin resistance is therefore not a sufficient cause for T2DM (26,27). Accordingly, the development of T2DM necessitates insulin-secretory defects or dysfunctional beta-cell that impairs the compensatory response of reduced insulin sensitivity (27,28).

Ethnic differences in insulin sensitivity, secretion and clearance account for the higher prevalence of T2DM in Black Africans as compared to European counterparts, with Black Africans having a phenotype of low insulin sensitivity, increased insulin secretion and reduced hepatic insulin clearance compared to their White Europeans counterparts (29,30).

The relationship between insulin secretion and sensitivity can be described as hyperbolic with the product of the two variables a constant for individuals with the same degree of glucose tolerance. The product of insulin sensitivity and section, the disposition index, is an estimate of beta-cell function (31–33). A hyperbolic relationship between insulin sensitivity and insulin secretion presupposes that changes in insulin sensitivity are matched by appropriate changes in insulin secretion to maintain the same degree of glucose tolerance in nondiabetic individuals (32). This curvilinear relationship has been reported in response to the frequently sampled intravenous glucose tolerance test (FSIGT) (32,34,35), as well as the oral glucose tolerance test (OGTT) (36). A shift in the curve to the bottom left is associated with reduced glucose tolerance whereas a shift in the curve to the upper right is associated with improved glucose tolerance (Figure 1.2).

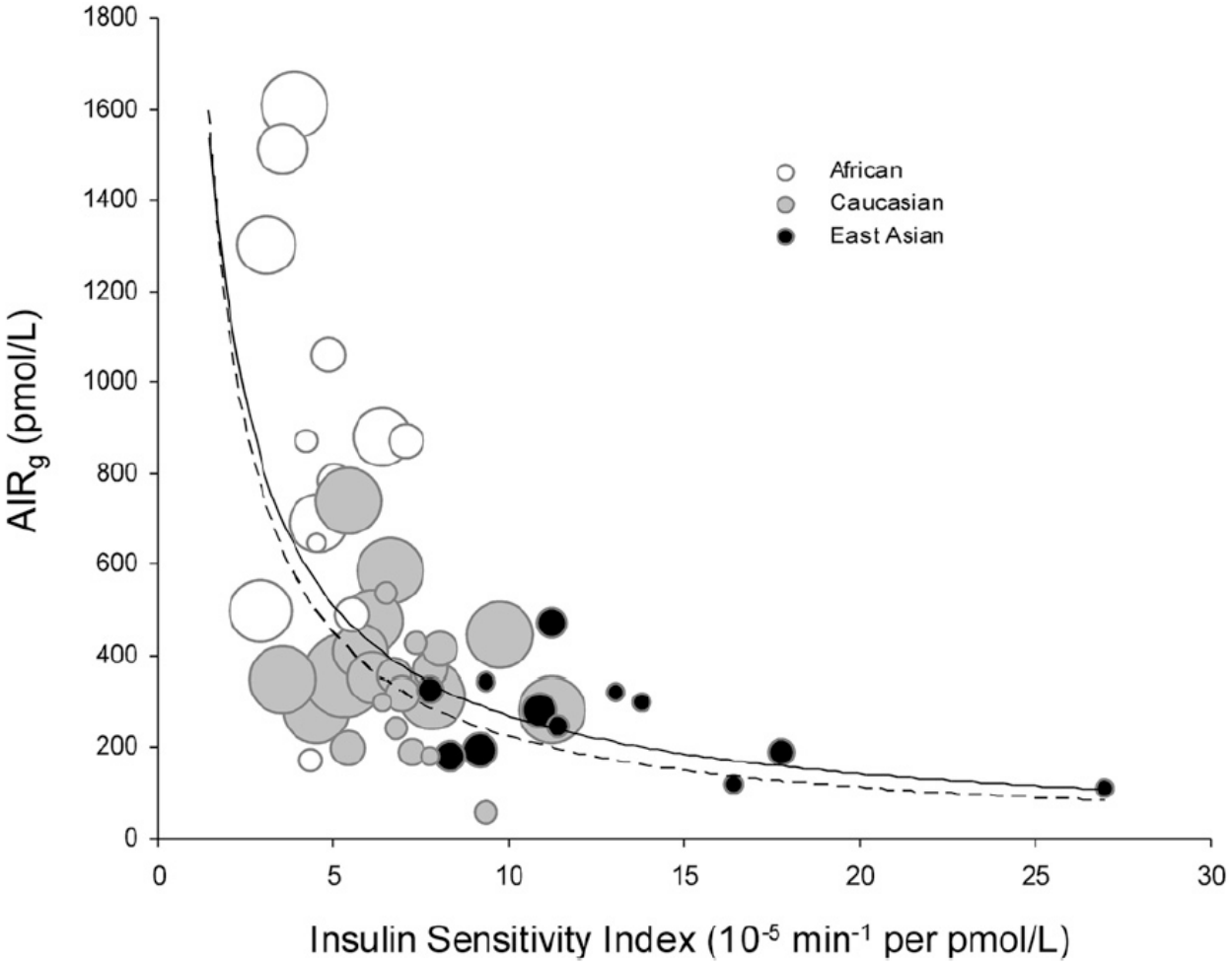

Figure 1.2: The hyperbolic relationship between insulin sensitivity and insulin secretion. Ethnic differences in the relationship between insulin sensitivity and insulin response in NGT cohorts. Scatter plot of S_1 vs. AIR_g measured in NGT (healthy) African, Caucasian, and East Asian cohorts. Each circle represents one study cohort. Circle area is proportional to cohort sample size. The solid line is the curve calculated in the meta-analysis [$\ln(AIR_g)=-0.915 \times \ln(S_1)-2.82$]. The dashed line is the curve of Kahn et al. (32) describing healthy individual who were primarily Caucasian [$\ln(AIR_g) = -1.0 \times \ln(S_1) - 3.80$]. Adapted from Kodama K, Tojjar D, Yamada S, Toda K, Patel CJ BA. Ethnic Differences in the Relationship Between Insulin Sensitivity and Insulin Response. A systematic review and meta-analysis. Diabetes Care. 2013;36(6):1789–96 (37).

1.3 Methods used in assessing insulin sensitivity and response

The need to quantify insulin sensitivity/resistance is of great importance in epidemiologic and clinical studies in order to understand the pathophysiology of T2DM. Hyperinsulinemic euglycemic glucose clamp quantitatively assesses insulin-mediated whole body glucose disposal (38) and is considered the "Gold Standard" method to measure insulin sensitivity (39–41). The hyperinsulinemic euglycemic glucose clamp technique estimates insulin sensitivity/resistance by measuring directly whole body glucose disposal at a given level of insulinemia under steady-state conditions *in vivo*. It is straight forward and has a limited number of defined assumptions. The glucose disposal rate at steady state (M) has a coefficient of variation of 0.10 and a discriminant ratio of ≈6 which is a measure of reproducibility and ability to distinguish individual results which are both excellent (42). The gold standard is therefore appropriate where the primary interest is to measure insulin sensitivity/resistance and feasibility is not an issue but it is time consuming, labour intensive, expensive and needs an experienced specialist to manage the technical difficulties. It is not appropriate for large epidemiological studies, clinical investigations and may not accurately reflect insulin action and glucose dynamics during physiological conditions as obtained during an oral glucose load. Indices calculated from fasting plasma and from the oral glucose tolerance test (OGTT) are more widely used to calculate insulin sensitivity and response.

The OGTT is a simple test widely applicable in large epidemiologic studies and in clinical practice to diagnose glucose intolerance and T2DM. The Homeostasis Model Assessment of insulin resistance (HOMA-IR) relies on the glucose and insulin dynamics interaction to predict fasting steady-state glucose and insulin concentrations for a range of combinations of insulin resistance

and beta-cell function by assuming a feedback loop between the liver and beta-cell (43). The HOMA-IR is routinely used in research settings where insulin sensitivity/resistance is of secondary interest or feasibility issues preclude the use of direct measurements by the gold standard (44,45). HOMA-IR has been shown to have reasonable linear correlation with the glucose clamp of insulin sensitivity/resistance in several studies and populations. The HOMA-IR is widely used in epidemiological, research and clinical trials depending on the interaction between glucose and insulin dynamics presupposes a feedback loop between the liver and beta-cell (45). The HOMA-IR is cheap, do not need extensive technical input, requires only one fasting blood draw, practical, evaluated and validated for epidemiologic and clinical studies. However, HOMA-IR does not provide information on the peripheral glucose uptake that is relevant in predicting insulin action/resistance but indicate only what is occurring with homeostatic mechanisms in the fasting state (42,45).

Plasma concentrations of insulin and glucose are be used to calculate the Matsuda index to estimate insulin sensitivity for participants using OGTT values at 0, 30, 60, 90 and 120 minutes (46) and the composite measure using OGTT values at 0 and 120 minutes (47). The Matsuda index is simple to compute, can be used in large epidemiologic studies and approximates well with whole–body insulin sensitivity, combining both hepatic and peripheral tissue insulin sensitivity, thus portraying insulin secretion at the same time as insulin action. The fasting component of the Matsuda index reflects hepatic insulin sensitivity while the mean of the dynamic component represents skeletal muscle insulin sensitivity and this has been validated using the glucose clamp studies (48). The insulin secretion or disposition index calculated as the product of insulin secretion measured as $\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$ or $\Delta I_{0\text{-}120}/\Delta G_{0\text{-}120}$ and the Matsuda index or the composite using plasma glucose and

insulin concentration at 30 minutes during the OGTT had been reported to have excellent power to predict onset of T2DM (49). The disposition index which reflects insulin secretion adjusted for the level of insulin sensitivity can be estimated as the product of insulinogenic index and Matsuda index has been shown to have a hyperbolic relationship and is used as measure of beta-cell function (32,33,50,51). It has been reported that the physiological relationship between insulin sensitivity and insulin secretion may not be hyperbolic but can vary depending on the method(s) employed (52). The Matsuda Index from OGTT as compared to other dynamic methods has been shown to be more reliable in African American males and superior to the HOMA–IR (46,53,54). Further, the insulin sensitivity estimated using the Matsuda index correlates well with the Gold Standard (46). Insulin secretion can be calculated using the C-peptide index, the ratio of the increment in C-peptide relative to glucose in the first 30 minutes of OGTT (50). C-peptide is produced in equi-molar quantities to endogenous insulin, and unlike insulin, there is negligible hepatic extraction of C-peptide, and hence the C-peptide index and the C-peptide to insulin ratio may serve as proxy measures of insulin secretion and clearance, respectively (55,56). Basal and postprandial insulin clearance are estimated as the ratio of fasting C–peptide to insulin, and the incremental area under the curve (iAUC) of C-peptide to iAUC glucose, obtained using the trapezoidal method, respectively (57).

1.4 Factors influencing insulin sensitivity and response

Many factors influence insulin sensitivity and response, and are modifiable and non-modifiable.

1.4.1 Ethnicity

Studies have been carried out to understand insulin sensitivity and response in populations of African descent (30,58–63). These studies have consistently shown that populations of African descent have lower insulin sensitivity than their white European counterparts. Lower insulin sensitivity is associated with a greater insulin response, often above that expected for the level of insulin sensitivity. Accordingly, black Africans typically present with a phenotype of low insulin sensitivity and hyperinsulinaemia (Figure 1.2) (37,64).

Hyperinsulinemia is caused by dysregulated insulin secretion and/or clearance from elevated levels of circulating insulin in relationship to the normal levels of blood glucose (65). The hyperinsulinemia in Black African women has been explained as due to alterations in both insulin secretion and clearance depending on age and or level of glycemia (66,67), with studies in African American women shown to have decreased hepatic clearance as the main contributor of hyperinsulinemia (37). In SA, VAT has been shown to be associated with lower insulin secretion and higher hepatic insulin extraction in Black women (61).

Black SA men have been shown to have higher insulin sensitivity than their women counterparts (59). Few studies have examined sex differences in insulin sensitivity and response. In South Africa, Goedecke et al. reported that Black South African men have higher insulin sensitivity but lower compensatory beta-cell response compared to women, but with

increasing age beta-cell function decreased similarly in both men and women but insulin sensitivity did not change (59).

1.4.2 Sex differences

Globally the prevalence of diabetes in 2021 was slightly lower in men (10.2%) than in women (10.8%) aged between 20–79 years (3). Generally, men have lower overweight and obesity prevalence rates than women but men have greater central fat mass particularly more trunk fat mass and VAT and less peripheral fat than women of similar age and BMI associated with higher T2DM risk (59,68–70). In sub-Saharan Africa (SSA) and South Africa (SA), the prevalence of diabetes was similar in men and women (8% vs. 13%) adjusted HbA1c level of 6.5% and above (71). Studies have explored sex differences in insulin sensitivity and response in European and American cohorts (72–75) identifying differences in obesity, body fat distribution, physical activity and fitness, and in glucose metabolism to explain these differences in men and women. The underlying pathophysiology of T2DM in African populations has been explored of recent (14,30,59,66,76) and it has been reported that Black African women have low insulin sensitivity and hyperinsulinemia due to higher insulin secretion and lower hepatic insulin clearance when compared to White SA women (76) and Black SA men (59). It is unclear why the association between overweight/obesity and diabetes risk differs between the sexes in SSA and SA. Only one South African study has compared the insulin dynamics of men and women and reported that women have lower insulin sensitivity, and hyperinsulinemia due to higher insulin secretion and lower hepatic insulin clearance, compared to men (59). These findings maybe confounded by sex differences in overweight and obesity as they are a major contributor to T2DM with a higher prevalence of overweight and obesity in women compared to men (67.4% vs. 27.4%) (71).

Similar results of higher insulin sensitivity and beta-cell function was in women than men have been obtained from a study in central Europe that included participants from the Czech Republic, Austria and Italy using OGTT using quantitative insulin sensitivity check index (QUICKI) and beta-cell function measured as insulinogenic index in 611 females and 361 males with normal glycaemia (77). Another study in Finland using positron emission tomography under hyperinsulinemic normogglycemic conditions, whole body insulin sensitivity was 41% greater in seven women (age 29±2 years and BMI 22±1 kg/m^2) than nine men (age 31±2 years and BMI 23±1 kg/m^2) (78,79) as well as BMI and weight were greater in women than in men aged 20–39 years, in a UK general practice study (78,79).

In SA, men have greater body fat and less lower body peripheral fat compare to women. Also, South African men have been shown to have greater trunk fat mass, higher VAT/SAT ratio and less leg and similar arm fat mass than women (59,80). Adiposity has been reported to be associated with greater diabetes risk (59,67,80,81). Sexual dimorphism in body fat distribution may be mediated be differences in hormones. Indeed, the accumulation of visceral fat and re-distribution of body fat to the abdomen as well as increased glucose intolerance and insulin resistance appear in women undergoing menopausal transition and producing less oestrogens (82), and the decline in testosterone with aging in men is associated with changes in muscle mass and body composition in men (83) and women (84), and the development of insulin resistance and T2DM (85,86).

There is a dearth of studies comparing insulin sensitivity, insulin secretion, insulin clearance and beta-cell function, exploring sex-specific associations with regional and total adiposity in African men and women and in women during menopausal transition at risk of diabetes.

1.4.3 Age

Irrespective of region or income, T2DM prevalence increases with age and as such age is recognised as a risk factor of T2DM (87), with the prevalence expected to increase due to population growth and aging (88). In South Africa, the prevalence of T2DM increases with increasing age and peaks between 65 and 74 years of age (8).

Aging is accompanied by changes in dietary patterns, an increase in sedentary behaviour and decrease in PA, all of which contribute to adiposity and insulin resistance (89). While aging is accompanied by increased weight, adiposity and re-distribution of adipose tissue to the abdominal compartment (90) indeed central obesity, particularly increase in visceral adiposity accompanies aging and contributes to the development of insulin resistance in older adults. With aging there is a decline in insulin sensitivity and, insulin secretion, with insufficient compensation of beta-cell function due to decrease in beta-cell proliferation capacity and enhancement of apoptosis (91–93). Age-associated decline in mitochondrial function, alteration in lipid metabolism, an increase in systemic inflammation, oxidative stress, DNA damage, cell senescence and tissue dysfunction, which exacerbates insulin resistance in the elderly (94–99). Some studies have reported that aging impairs insulin secretion from beta-cells and in addition mitochondrial dysfunction with aging leads to beta-cell death. These abnormalities in insulin sensitivity and insulin secretion in older individuals lead gradually to impaired glucose tolerance and diabetes (100).

Longer life expectancies throughout the world have led to the increase in the prevalence of T2DM in older age and may be related directly to the aging process itself or through other risk factors indirectly related to age such as adiposity.

1.4.4 Adiposity

The prevalence of obesity has increased worldwide in developed as well as low and middle income countries (101). In African, BMI increased from 21kg/m^2 to 23 kg/m^2 in men and from 21.9kg/m^2 to 24.9kg/m^2 in women between 1980 to 2014 (102). Adipose tissue regulates metabolism. In obese individuals, adipose tissue secrete pro-inflammatory cytokines, glycerol, free fatty acids and hormones including leptin and adiponectin which exacerbate the development of insulin resistance and consequently T2DM (103–106). Many epidemiological studies have reported that not only is the increase in adiposity responsible insulin resistance but also the distribution of body fat that is associated with insulin resistance and T2DM, SAT associated with increased risk of T2DM (107–109). In a study of Black SA women it has been shown that baseline trunk fat mass, VAT and the change in VAT were associated with greater odds of developing impaired glucose metabolism/T2DM while baseline leg fat mass was associated with reduced impaired glucose metabolism/T2DM risk after a 13 year follow up (67). Centralisation of fat in pre-menopausal Black SA women have also been reported to be associated with reduced insulin sensitivity and after in 5 year follow up (63). In other populations, studies have reported that VAT is greater risk for T2DM (73,80,110,111). Contrary to these studies, Gradidge et al., showed that abdominal subcutaneous fat as measured by ultrasound was associated with lower odds of having glucose greater than 5.6 mmol/l (112). In the Black SA women, obesity reduces adipogenesis and storage of SAT leading to insulin resistance and T2DM risk (113,114). More studies are warranted to understand the differences in associations between body fat distribution and diabetes risk in Black men and women.

1.4.5 Physical activity

Studies have reported an inverse association between physical activity (PA) and T2DM risk (115–118) with PA of any intensity positively associated with improved glucose metabolism

and insulin sensitivity (119–121). Through its effects on multiple organs and systems physical activity acts as a stimulus to persistent improvement in insulin sensitivity and glucose uptake (106,118,121,122). Accordingly, higher levels of physical activity have been shown to be associated with a significant reduction in the risk of developing T2DM in men and women (123). In fact, less sedentary behaviour and exercise of any type and intensity is associated with reduced T2DM (124–126) even in men and women with high body mass index and elevated glucose levels (127).

On the other hand, sedentary behaviour has been shown to be positively associated with T2DM, cardiovascular disease and mortality (128,129). Some studies have reported that the association between sedentary time and T2DM risk is independent of PA (129), while another study showed that 30–40 minutes per day of PA may attenuates the association between sedentary time and risk of mortality in sedentary individuals (130). A systematic review and meta-analysis showed that participants who reported the greatest sedentary time had a 112% higher relative risk (RR) of T2DM compared to those with the lowest sedentary time (128). Another meta-analysis has showed an increased risk for T2DM to be associated with higher levels of total sitting independent of PA (131).

In SA, most urban Black SA women are physically active despite high prevalence of obesity and metabolic risk factors. A population-based study reported that 57.4% of the population were not physical active (men: 46.9%, women: 67.1%), 14.8% of the population engaged in moderate physical activity (men: 14.4%, women: 15.1%), while 27.8% was engaged in vigorous physical activity (men: 38.7%, women: 17.8%) (132). Further, they showed that men were more likely to be physically active than women and women were less likely to engage in moderate and vigorous PA than men (132). A study comparing physically active women, as

defined by meeting the guideline of 150 min/week of MVPA, had lower body weight, body fat and insulin resistance, but physical activity did not remove risk of T2DM associated with obesity (133,134). Most studies in SA have started exploring the role of PA and T2DM risk but are using self-reported PA (133–138) with limitations in accuracy (139) linked to social desirability bias, response and recall bias and reliability and validity (140). Wearable devices to monitor and measure physical behaviours and are more accurate in providing objective assessment of PA intensity, PA volume and sedentary behaviour than self-reported measures are increasingly being used (141,142). While one device has been used in research to capture physical behaviours combining signals from two accelerometers on the thigh and waist have been shown to have high accuracy and precision in postural classification and intensity (143).

Isotemporal substitution modelling is a new approach to statistical analysis and is considered the gold standard in modelling physical activity and effects of time by substitution of the activity by another for the same amount of time. It has been shown to be accurate and had superior interpretation of the physical activity results presented in absolute rather than relative values (144,145). Isotemporal substitution modelling has been used in European and American cohorts to analyse physical behaviours from wearable devices on the waist, hip and wrist but less accurate in posture classification to distinguish sedentary behaviour from standing which have opposing effects on heath (146–155).

There is a paucity of studies using objectively measured physical activity with one or two accelerometers and adopting the isotemporal substitution analysis in Africa. Such studies are warranted as they will provide comparable results on replacing one physical behaviour with another in African populations.

1.4.6 Other lifestyle factors

Lifestyle factors such as dietary intake, smoking and alcohol consumption are important risk factors in the aetiology of T2DM (156–162). Smoking is an independent risk factor for T2DM (163–169). Tobacco products contain many chemical compounds and free radicals which increase inflammation/oxidative stress (170) and decrease beta-cell function leading to a reduction in insulin secretion (170,171), as well as insulin resistance (172–175) and diabetes complications (158). Accordingly, insulin resistance in smokers may be related to nicotine as long-term use of nicotine gum is associated with insulin resistance (176). Smoking cessation leads to improved insulin sensitivity as shown by an 8 weeks intervention despite a concomitant increase in body weight (174,177).

Long-term, high alcohol intake is an independent risk factor for T2DM (178,179), as it interferes with glucose homeostasis and is associated with the development of insulin resistance leading to T2DM (180,181). As well as contributing to excess caloric intake, which is associated with overweight and obesity, excessive alcohol intake is associated with increased insulin resistance as well as reduced insulin secretion caused by impaired beta-cell function (182). The threshold of alcohol intake related to increased T2DM risk is not clear. Some studies have reported that moderate alcohol intake decreases the risk of T2DM (183), others have shown that heavy alcohol consumption disrupts glycaemic regulation (156,184), while other studies have suggested no effect at all (185).

Dietary modification combined with other lifestyle changes has been shown to improve insulin sensitivity and reduce the risk for T2DM (186). Although there is much debate regarding the dietary recommendations for the prevention and management of T2DM, diets low in glycaemic index and glycaemic load, including a wide variety of fruits and vegetables high in fibre are

generally recommended (186,187). In contrast, hypercaloric diets that are high in process foods and added sugar are associated with increased T2DM risk (188–190).

According to the 2016 SA Demographic and Health survey, the prevalence of tobacco smoking and excessive alcohol intake is high and more common in South African men than women (37% vs. 8%). Similarly, alcohol consumption is higher in SA men than women (61% vs. 26%) and more men than women engaged in risky drinking (28% vs. 5%) and showed signs of problem drinking (16% vs. 3%) (71). The same national survey also reported that 2% of South Africans eat fast food on daily basis and 18% consume fast food at least once a week. While 59% of adults reported that they consumed vegetables, 49% consumed fruits and 36% of the adults indicated they consumed sugar sweetened beverages on the day before the survey (71). These lifestyle factors place South Africans, particularly men, at high risk for T2DM.

1.4.7 HIV/AIDS

In people living with HIV (PLWH), diabetes is often underdiagnosed and undertreated (191). Individuals on antiretroviral therapy (ART) are four times more susceptible to diabetes than HIV negative individuals (192) as metabolic alterations due to HIV infection, antiretroviral therapy and co-morbidities (193). Accordingly, it is important to understand the association between HIV infection, ART treatment and cardio-metabolic disease risk in patients on ART (194,195). The use of ARV therapy in Black Africans has also been associated with an increase in total and central body fat accumulation (196) and dysglycaemia (197), both of which are implicated in T2DM risk. With the new guidelines for ART, it is expected that PLWH will live longer and have near–normal life expectancy and therefore it is important that chronic diseases such as diabetes in PLWH are well managed in order to maintain quality of life and avoid complications from diabetes and / or HIV and treatment (198).

1.4.8 Socio-economic status (SES)

Socio-economic factors such as income, education, housing and access to healthy food and health care are associated with the development and progression of T2DM (199–202). Studies carried out in high income countries have shown that T2DM is more prevalent among individuals of lower socioeconomic status and the socially deprived individuals (203,204). Notably, individuals with low levels of income, occupation and low education attainment are more likely to develop T2DM than those with higher income, better occupation and education (205–209). Further, individuals of lower SES are in a constant struggle to eat a healthy diet, and meet health care costs and as such bear a greater burden of diabetes in terms of morbidity and mortality than those of the higher SES (204,207). Poverty, unmet material needs and social exclusion (199,210) lead to stress triggering psychological and biologic reactions (211,212) that exacerbate depression and anxiety, low self-esteem and motivation. Accordingly, disadvantaged individuals are more susceptible to self-destructive behaviours and choices such as tobacco consumption, excessive alcohol intake and consumption of unhealthy foods (213,214) with negative consequences for T2DM (211,214,215).

In South Africa, poverty is a risk factor for adiposity (216), and according to Bradshaw et al, the poor are at greater risk of being exposed to smoking and exposure to smoky fuels. Excessive alcohol intake was also associated with poverty, and access to and quality of health care was worse for the poor in whom the disease burden is higher compared to the wealthy. Individuals that have less education have less knowledge of the disease and associated risk factors and are therefore less likely to engage in healthy lifestyle behaviours (217). The high financial cost on individuals, families and communities to manage diabetes further exacerbate the effects of poverty (218).

1.4.9 Menopausal transition

Menopausal transition involves many stages of reproductive changes which have been classified using self-reported date of final menstrual period (FMP) (219,220). Women currently having regular periods are classified as pre-menopausal and women who had had no bleeding for more than 12 months were classified as post-menopausal. The post-menopausal class was further classified into early post-menopausal group is they had not had a drop of blood for greater than 12 month but less than six years, and another class of late post-menopausal group if they had not had a drop of blood for more than six years (219).

Menopausal transition is associated with changes in sex hormones, characterised by a decrease in oestrogen and an increase in FSH and LH. These changes are associated with an increase in total body fat and re-distribution of body fat from the peripheral regions to the central region, specifically VAT accumulation (221–224). Cross-sectional studies as well as longitudinal studies have reported that the increase in abdominal adiposity when transitioning from pre- to post-menopause is independent of age and total adiposity (225,226).

The transition from the pre-menopausal to post-menopausal period has been reported to be associated with a decrease in C–peptide and insulin secretion levels, and an increase in fasting glucose concentrations (227–229). Some studies have shown similarities in insulin sensitivity between pre- and post-menopausal women (230,231), while others have reported a decline in insulin sensitivity in post-menopausal women compared to pre-menopausal women (232). However, all these studies were performed in European women and women in United States of America, with no studies that have explored differences in glycaemia or insulin dynamics in women from SSA.

While some studies have explored the differences in cardio-metabolic disease risk between menopausal groups in African women (233,234), others have examined the weight and adiposity differences and metabolic syndrome traits between pre- and post-menopausal women (112,235). One study from SA measuring body composition using DXA showed post-menopausal women had lower whole body lean mass and bone mineral density compared to pre-menopausal subjects but there were no differences in fat mass compared to pre-menopausal women (235).

To the best of my knowledge no studies have been done that have objectively characterised differences in regional fat distribution and glycaemic and insulin dynamics between Black men and women and pre- and post-menopausal Black women with increased risk for T2DM. Most research in women has focussed predominantly on pre-menopausal women. There is a paucity of data assessing sex-specific associations between regional and total fat distribution and insulin sensitivity and response Black men and women. Most studies have used self-reported physical activity measurements. Accordingly, data on objective measures of physical behaviours is scarce sub-Saharan. Africa (SSA). The use of two devices to quantify physical behaviours has not been done in SSA. Further, the association of physical behaviours quantified by combining signals from two accelerometers and T2DM risk markers and isotemporal substitution approach has not been explored. Addressing these gaps will have the following potential clinical implications; identifying people at high risk of T2DM and non-communicable diseases and recommendations with regard to physical activity.

1.5 AIMS AND OBJECTIVES OF THE STUDY

1.5.1 AIMS OF THE STUDY

The aims of this thesis is to determine whether T2DM risk markers are different between middle-aged Black South Africa men and women in Soweto, Johannesburg , and between women at different stages of the menopause transition, and to determine whether there are sex-specific associations between these markers, and body composition and physical behaviours.

1.5.2 OBJECTIVES OF THE STUDY

- To explore sex differences in insulin sensitivity, insulin secretion, insulin clearance and beta-cell function, and sex-specific associations with total and regional fat distribution.
- To describe differences in total and regional adiposity, and glycaemic and insulin measures, between pre-menopausal and early and late post-menopausal Black South African women.
- To determine the associations between physical behaviours and measures of T2DM risk in middle-aged Black South African men and women using isotemporal substitution.

CHAPTER 2

Increased Risk for Type 2 Diabetes in Relation to Adiposity in Middle-Aged Black South African Men compared to Women

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Authors' contributions: CNK reviewed the questionnaire, data collection tools and standard operating procedures, supervised data collection and entry, participated in data collection and entry, data management (including data cleaning and coding) and quality control of the chapter. CNK, JHG and LKM conceptualised the study and CNK analysed the data, drafted and revised the manuscript under the supervision of JHG and LKM. APK, SAN, NJC and OT critically reviewed and commented the manuscript. APK, JHG and LKM advised the statistical approach. All authors reviewed/edited, read and approved all the drafts and the final version of the manuscript.

Abstract

Aims: Despite a higher prevalence of overweight/obesity in black South African women compared to men, the prevalence of type 2 diabetes does not differ. We explored if this could be due to sex differences in insulin sensitivity, clearance and/or beta-cell function, and also sex-specific associations with total and regional adiposity.

Methods: This cross-sectional study included 804 black South African men (n=388) and women (n=416). Dual-energy x-ray absorptiometry was used to measure total and regional adiposity. Insulin sensitivity (Matsuda index), secretion (C-peptide index) and clearance (C-peptide/insulin ratio) were estimated from an oral glucose tolerance test.

Results: After adjusting for sex differences in fat mass index, men were less insulin sensitive and had lower beta cell function than women ($p<0.001$), with the strength of the associations with measures of total and central adiposity being greater in men than women ($p<0.001$ for interactions). Further, the association between total adiposity and type 2 diabetes risk was also greater in men than women (relative risk ratio (95% confidence interval): 2.05 (1.42–2.96), $p<0.001$ vs. 1.38 (1.03–1.85), $p=0.031$).

Conclusion: With increasing adiposity, particularly increased centralisation of body fat linked to decreased insulin sensitivity and beta cell function, black African men are at greater risk for type 2 diabetes than their female counterparts.

Key words: Body fat distribution, insulin sensitivity, insulin secretion, beta-cell function, basal and postprandial insulin clearance, ethnicity.

Introduction

Type 2 diabetes (T2D) is a global health problem, with low-middle income countries particularly affected. It is projected that sub-Saharan Africa (SSA) will have the highest increase in T2D compared to the rest of the world, and in 2019 South Africa (SA) had the highest estimated number of people with diabetes (4.6 million) in the SSA region, and the highest age–adjusted comparative prevalence of diabetes (12.7%) in adults (1), which is higher than the global average (2). Within SSA and SA, the prevalence of T2D does not differ by sex, despite large sexual dimorphism in obesity rates (3). For example, in SA the prevalence of T2D in black SA men and women is similar (10.2% vs. 13.8%) (4) , but the prevalence of overweight and obesity differs markedly (27.4% vs. 67.4%) (5).

The reason for this discrepancy in the association between overweight/obesity and diabetes risk in men and women is not clear. Our group have started to explore the underlying pathophysiology of T2D in Africans (6–10), and shown that black African women present with a phenotype of low insulin sensitivity and hyperinsulinemia due to higher insulin secretion and lower hepatic insulin clearance compared to white SA women (7) and black SA men (8). However, the majority of these studies have been undertaken in premenopausal women (6,7,10), with limited data in middle-aged men and women (8).

Notably, men typically have greater central fat mass (particularly visceral adipose tissue (VAT)) and less peripheral subcutaneous adipose tissue (SAT) than women, which is associated with a higher risk for T2D (8,11,12). However, the sex differences in the association between whole body and regional adiposity, and T2D risk, including insulin sensitivity, secretion and clearance, to our knowledge, has not been studied in African men and women.

Accordingly, the aims of this study were to compare insulin sensitivity, clearance and beta-cell function between middle-aged black South African men and women who differ in obesity prevalence, and to explore sex-specific associations with total and regional adiposity.

Methods

This cross-sectional study includes the analysis of the follow-up data that was part of a longitudinal study designed to investigate the determinants of T2D risk in middle-aged black SA men and women. Data collection for the baseline study, as part of the AWI-Gen (Africa Wits-INDEPTH partnerships for Genomic Research) study (13), took place between 2011 and 2015 in black SA men (n=1027) and women (n=1008) residing in Soweto, South Africa (14). Follow-up data, analysed for this study, was collected between January 2017 and August 2018 on a sample of 502 men and 527 women randomly selected from the original sample. Participants living with HIV were excluded from this data analysis to avoid the confounding effects of the virus and antiretroviral therapy on the outcomes. Complete data was available for 804 participants (388 men and 416 women) and complete oral glucose tolerance (OGTT) data was available on 734 of these participants (Figure 2.1: Sample selection flow chart 1).

The study was conducted in accordance with the tenets of the Helsinki declaration and was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (M160604 and M160975). Prior to inclusion in the study all procedures and possible risks were explained and all participants signed a consent form. Data collection took place at the South African Medical Research Council/University of the Witwatersrand Developmental Pathways for Health Research Unit at the Chris Hani Baragwanath Hospital in Soweto, Johannesburg, South Africa.

Socio–demographic and medical questionnaire

Interviewer administered questionnaires were completed and captured onto REDCap (15). Data collected included age, marital status (married/unmarried), current employment (employed/not employed), highest educational level completed (no formal schooling/elementary school, secondary school level, tertiary education), alcohol intake and tobacco consumption (Yes/No), and self–reported diabetes and/or diabetes medication taken. Menopausal stage was classified according to last menstrual period (16).

Anthropometry

Weight was measured to the nearest 0.1 kg using a TANITA digital scale (model: TBF-410, TANITA Corporation, US). Height was measured to the nearest 0.1cm using a wall–mounted stadiometer (Holtain, UK). Waist circumference (WC) and hip circumference (HC) were measured to the nearest 0.1cm with a non–stretchable tape. For the WC, the tape was placed horizontally between the iliac crest in the mid–axillary plane and the lowest rib margin. For the HC, the tape was placed around the level of the greatest protrusion of the buttocks. Waist-to-hip ratio (WHR) and BMI were calculated, and participants categorised according to the World Health Organisation (WHO) criteria (17).

Body composition and body fat distribution measurements

Dual–energy X-ray absorptiometry (DXA) was used to measure whole body composition, including sub-total (total body minus head to account for any artefacts that may influence the DXA reading) fat mass (FM, kg and % body mass) and fat–free soft tissue mass (FFSTM), and regional FM including trunk, arm and leg FM (QDR 4500A, Hologic Inc., Bedford, USA, APEX software version 4.0.2). Fat mass index (FMI, sub-total fat mass kg/height^2) and FFSTM

index (FFSTM/height^2) were calculated. Regional fat distribution was expressed relative to sub-total FM (%FM), with trunk fat (%FM) representing central fat distribution and arm and leg fat (%FM) representing upper- and lower-body peripheral fat distribution, respectively. Abdominal VAT and SAT areas were estimated from DXA (18).

Blood sampling and analysis

Participants were instructed to not eat, smoke, drink alcohol or exercise for at least 8 hours prior to testing. A single baseline blood sample (10 ml) was drawn for the determination of glycated haemoglobin (HbA1c), plasma glucose, serum insulin, C–peptide and follicle stimulating hormone (FSH) concentrations. Participants then completed a standard 75g oral glucose tolerance test (OGTT) over 2-hours during which blood samples (5 ml) were drawn at 30 min intervals for the determination of glucose, insulin and C-peptide concentrations. Participants with known diabetes and/or those with fasting blood glucose ≥11.1 mmol/l (n=76) (ACCU-CHEK®, MedNet GmbH, Munster, Germany) did not complete the OGTT.

Plasma glucose concentrations were measured on the Randox RX Daytona Chemistry Analyser (Randox Laboratories Ltd., London, UK). HbA1c concentrations were measured using the D–10™ Haemoglobin Analyser (Bio–Rad Laboratories, Inc. USA). Serum insulin and C–peptide concentrations were measured on the Immulite® 1000 Immunoassay System (Siemens Chemiluminescent Healthcare GmbH, Henkestr, Germany). FSH was measured on serum using the ARCHITECT Chemiluminescent Microparticle Immunoassay assay (Abbott Laboratories, Abbott Ireland).

Based on the fasting plasma glucose (FPG) and 2–h OGTT glucose results, participants were classified according to the WHO criteria (19). Participants with impaired fasting glucose and

impaired glucose tolerance were combined and described as having impaired glucose metabolism (IGM).

Calculations from the OGTT

The homeostasis model assessment (HOMA-IR) was used to estimate fasting insulin resistance (20). The Matsuda Index (21), was used to estimate insulin sensitivity for participants with complete OGTT data (n=628), alongside the composite score (22) for participants who only had data for 0 and 120 minutes (n=106). These composite measures have been shown to compare well (22), and were significantly correlated in this study ($r=0.874$; $p<0.001$) to the Matsuda Index. Early phase insulin response to the OGTT was estimated using the insulinogenic index (IGI) (23). Participants without data at 30 minutes or whose insulin response was <0 were excluded from the analysis. Insulin secretion was calculated using the C-peptide index, the ratio of the increment in C-peptide relative to glucose in the first 30 minutes of the OGTT (23). C-peptide is produced in equimolar quantities to endogenous insulin, and unlike insulin, there is negligible hepatic extraction of C-peptide, and hence the C-peptide index and the C-peptide to insulin ratio may serve as proxy measures of insulin secretion and clearance, respectively (24,25). Basal and postprandial insulin clearance were calculated as the ratio of fasting C–peptide to insulin, and the incremental area under the curve (iAUC) of C-peptide to iAUC insulin, calculated using the trapezoidal method, respectively. The oral disposition index (oDI), which reflects insulin secretion adjusted for the level of insulin sensitivity (26–28), was calculated as the product of the C-peptide index and Matsuda index (23) which demonstrated a hyperbolic relationship and was used as the measure of beta-cell function. These calculations were only performed in participants without known T2D and/or not taking medications for T2D, and who underwent an OGTT.

Statistical analysis

Data were analysed using Stata 15.1/IC (StataCorp, College Station, TX, USA). Variables are summarised as percentages for categorical data, mean ± standard deviation (SD) for normally distributed continuous data, and median (25th-75th percentile) if not normally distributed. Normality was assessed using the Shapiro–Wilk test and Q-Q probability plots. Sex differences were determined using Students t-test for normally distributed continuous data, Mann-Whitney U and Kruskal–Wallis tests for skewed continuous data, and Chi-squared test for categorical data. Sex differences in glucose and insulin measures are presented before and after adjusting for FMI using one–way analysis of covariance (ANCOVA). Z-scores were derived for the total and regional adiposity measures for the combined sample, as well as sex-stratified using Fisher's Yates transformation (29). By using Z-scores we were able to compare the risk magnitude per 1 SD change in total and regional adiposity measurements. Multinomial logistic regression was used to explore the relationship between total and regional adiposity measures, and IGM and T2D, using NGT as the reference, and including age, sex, smoking, alcohol intake, education, and FMI (for regional measures), as covariates. All participants with known (n=65) and newly diagnosed (n=42) diabetes were included in the multinomial analyses. We explored sex*adiposity z-score interactions and only found a significant interaction for FMI. Accordingly, the data (excluding FMI) were analysed in the combined sample and the relative risk ratio (RRR) and 95% confidence intervals for IGM and T2D are presented. For the continuous measures of insulin sensitivity (Matsuda index), clearance (fasting C–peptide/insulin ratio) and beta-cell function (oDI), robust regressions were used to explore associations with adiposity z-scores, including age, smoking, alcohol intake, education and FMI (for regional adiposity measures) as covariates. As we were exploring risk factors for T2DM, participants with known diabetes and/or taking medication for diabetes and those without OGTT data were excluded from the robust regression analyses. Due to significant sex

interactions in most models, the analyses were completed separately for men and women using sex-specific total and regional adiposity z-scores. A p–value of <0.05 was considered significant.

Results

Socio-demographic and body composition characteristics

A total of 804 participants (48.3% men) with a mean age of 54.6±6.0 years were included (Table 1). Men were younger than women and significantly more men were married than women. Current employment status was not different between the sexes, however more men than women (18.1 vs. 12.5%) had completed tertiary education. More men currently smoked (46.1% vs. 7.2%) and frequently consumed alcohol (30.4% vs. 4.6%) than women.

Mean BMI was higher in women than men (p<0.001), and accordingly a larger proportion of the women presented with obesity (70.2% vs. 26.6%) (Table 1). While waist circumference was similar, men had higher WHR due to the higher hip circumference of the women. While FFSTM was higher in men, FM (kg and %) and FMI were higher in women. When expressed relative to FM, women had significantly greater leg FM, while men had more central FM (trunk), but arm FM did not differ. Within the central depot, men had less VAT and SAT (both p<0.001), but a higher VAT/SAT ratio.

Differences in glucose and insulin measures between men and women

Although fasting glucose and iAUC for glucose were not different between the sexes, HbA1C and 2 h glucose were higher in women than men (Table 2.1). Fasting insulin and C-peptide, and iAUC for insulin, were also higher in women than men. Accordingly, HOMA-IR was higher and insulin sensitivity (Matsuda index) was lower in women compared to men,

accompanied by a higher insulin response (IGI) characterised by higher insulin secretion (C-peptide index) and lower insulin clearance (basal and postprandial). However, the oDI, a measure of beta-cell function, did not differ by sex.

When adjusting for differences in FMI (Table 2.2), there were no longer sex differences in HbA1C, 2-hour glucose, insulin response, or basal and postprandial insulin clearance, while insulin secretion remained higher in women. In contrast, fasting insulin and C-peptide, as well as HOMA-IR were higher, and insulin sensitivity and beta-cell function were lower in men compared to women.

The prevalence of NGT, IGM and T2DM were not significantly different between men and women.

Associations between total and regional adiposity and risk for IGM and type 2 diabetes

There was a significant sex*FMI z-score interaction ($p<0.001$), such that the RRR for IGM and T2D were greater for men than women (Figure 2.2). Associations between regional adiposity z-scores and risk for IGM and T2D did not differ by sex, and the RRR for the combined sample are presented in Table 2.3. Trunk fat and VAT z-scores were associated with a higher risk for both IGM and T2D, with every 1 SD increase in trunk fat and VAT being associated with a 4.8 fold and 2.6 fold increased risk for T2D, respectively. In contrast, higher leg fat z-score was associated with a 58% and 79% lower risk for IGM and T2D, respectively, while a 1 SD higher arm fat z-score was associated with a 2.2-fold greater risk for T2D only. SAT z-score was not associated with IGM or T2D.

Sex-specific associations between total and regional adiposity z-scores and insulin

measures

There were significant sex*FMI z-score interactions for insulin sensitivity, clearance and beta-cell function, with associations consistently being stronger in men than women (Figure 2.1B–D). There were also significant sex*regional adiposity interactions for most measures of insulin sensitivity and response and therefore the results are presented separately for men and women (Table 2.4). Lower insulin sensitivity was associated with higher central fat mass (trunk fat and VAT), and lower leg fat in both men and women, but the associations with central fat mass were stronger in men than women ($p<0.001$ for all interactions). In contrast, arm fat mass was associated with lower insulin sensitivity in women only ($p<0.001$ for interaction). Beta-cell function (oDI) was negatively associated with VAT in both men and women. In contrast beta-cell function was positively associated with peripheral fat mass in women only ($p=0.040$ for interaction). Basal insulin clearance was negatively associated with trunk fat mass in both men and women, with a stronger association in men ($p=0.017$ for interaction). In contrast, basal insulin clearance was negatively associated with VAT and arm fat, and positively associated with leg fat in women only, but the strength of the association did not differ significantly between sexes. The associations for postprandial insulin clearance were similar to those for basal insulin clearance (data not shown). As the women were at different phases of the menopausal transition with 17.6% being premenopausal, 14.7% perimenopausal and 67.7% being postmenopausal, we wanted to ascertain whether the associations presented above differed by menopausal phase. The associations between total and regional adiposity and insulin sensitivity, secretion and beta-cell function did not differ between menopausal groups. In contrast, the associations between FMI, trunk, leg and arm z-scores and basal insulin clearance differed by menopausal phase, being stronger in the pre- than peri- and postmenopausal women (data not shown).

Discussion

The main and novel findings of this study were that in a sample of Black men and women with a mean age of 54.6 years, after adjustments for differences in body fat, insulin sensitivity, secretion and beta-cell function were lower in Black SA men compared to women, while insulin clearance did not differ by sex. In line with this, the strength of the association between total adiposity and T2D risk was greater in men compared to women. Although black SA women have a higher prevalence of obesity (70.2 vs. 26.6%) and greater whole-body fatness (43.6 vs. 26.3%) than men, they present with a more 'favourable' body fat distribution, characterised by less central fat mass and greater peripheral fat mass. This phenotype has been associated with lower diabetes risk. This together with the greater impact of body fatness on diabetes risk could explain the similar prevalence of diabetes in men and women in this study (11.1 vs. 15.4%) despite the lower adiposity in men.

These findings also suggest that with increasing adiposity, black SA men will be at greater risk for T2D than their female counterparts. We found that the association between total adiposity and risk for T2D was higher in men than women (Figure 2.1A). Further we showed that with increasing FMI the decline in insulin sensitivity was greater in men compared to women, similar to earlier studies from SA (8,30), which was also associated with a more pronounced decrease in beta-cell function in men compared to women. The higher risk in men compared to women was independent of smoking and alcohol intake, lifestyle risk factors were also higher in men compared to women.

The finding of similar T2D prevalence (1) despite marked differences in the prevalence of obesity (3) between sexes are consistent and representative of South Africa and the SSA region. In order to understand the sexual dimorphism in this relationship, it is obviously essential to

account for sex differences in body fatness as well as disentangle the sex-specific associations between regional adiposity and T2D risk. After adjusting for differences in body fatness, men had lower insulin sensitivity, insulin secretion and beta-cell function compared to women, placing the men at higher risk for T2D. Indeed, a lower beta-cell function, estimated using the oDI, has been shown to predict the development of T2D over a 10 year period in a Japanese American cohort (26).

Black African women have been shown to present with hyperinsulinaemia compared to their European counterparts, often beyond that required to maintain normoglycaemia (8,31). Hyperinsulinemia in black African women has previously been attributed to alterations in both insulin secretion and clearance, depending on age, and/or level of glycemia (10,32). Studies in African American women have shown that decreased hepatic insulin clearance is the main contributor to hyperinsulinemia (33). In contrast, we show that the higher IGI in women compared to men, was associated with higher insulin secretion without differences in insulin clearance. Due to limited longitudinal studies, it is not known whether the higher IGI in women is protective or may actually cause insulin resistance (9).

It is well recognised globally and in South Africa that men have greater central body fat and less lower body peripheral fat compared to women (8,30). Similarly, we showed that men had greater trunk fat mass, a higher VAT/SAT ratio, and less leg and similar arm fat mass than women. This adiposity phenotype is associated with greater diabetes risk as previously reported by our group (8,30,32,34). Indeed, we showed that a 1SD increase in trunk z-score was associated with a more than two-fold greater risk for IGM and nearly five times greater risk for T2D, and was also associated with lower insulin sensitivity and lower basal insulin clearance. In contrast, peripheral fat is typically associated with reduced risk for diabetes (30,34) as it acts

as a metabolic sink to sequester excess free fatty acids that may otherwise be directed at ectopic sites such as the liver and pancreas (35). We showed that a 1 SD increase in leg z-score was associated with a 58% lower risk for IGM and a 79% lower risk for T2D, as well as higher insulin sensitivity in both sexes. Notably, the strength of the inverse association between central fat distribution and insulin sensitivity was greater in men compared to women. Several studies in different populations have shown VAT to be more strongly associated with insulin resistance, and therefore a greater risk for T2D, in men than women (30,36–38). A further novel finding of the study was that the positive relationship between beta-cell function and leg FM was weaker in men compared to women, suggesting lower 'protective' effect of leg FM on beta-cell function in men compared to women. Accordingly, despite a lower prevalence of overweight and obesity in men compared to women in our study, this 'unfavourable' regional fat distribution and the sex-specific relationships with insulin sensitivity and beta-cell function places them at greater risk for future T2D.

This is the first study, to our knowledge, in black SA men and women with detailed measures of insulin sensitivity, secretion and clearance, and beta-cell function, based on estimates from an OGTT. We were also able to use DXA, which provides an accurate assessment of body composition and regional adiposity. A limitation is the cross–sectional nature of the study which does not allow us to infer causality. Although the sex differences in obesity and total adiposity may be seen as a limitation, it reflects the status of obesity within South Africa and the sub-Saharan African region (3), and adjustments for total body fatness and the calculation of z-scores were used in the analyses to determine whether these sex differences in adiposity were influencing the insulin- and glucose-related variables. There were no effects of the menopausal transition *per se* on the association between adiposity and insulin sensitivity, secretion and beta-cell function and therefore the sex differences reported cannot be explained

by menopausal status. However, the premenopausal women were not tested at a specific time during their menstrual cycle, which is noted as a limitation of the study. Furthermore, the conclusions for this study are valid only for HIV negative individuals.

In summary, for the same level of body fatness, black South African men are less insulin sensitive and had lower insulin secretion and beta-cell function than women, with the strength of the association between adiposity and T2D risk being greater in men compared to women. This suggests that with increasing adiposity, particularly an increase in central adiposity, black SA men face an increased risk for T2D in comparison with their female counterparts. Longitudinal studies are required to confirm the results of this study.

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Table 2.1: Socio-demographic, body composition, glucose and insulin measures in Black South African men and women (n=804)

Variable	Men	Women	p-value
n (%)	388 (48.3%)	416 (51.7%)	-
Age (years)	54.2±6.2	55.0±5.8	0.047
Socio-demographic characteristics, row: n (%)			
Married, n (%)	219 (56.7)	186 (44.9)	0.001
Currently employed (n, %)	232 (47.3)	258 (52.6)	0.519
Currently smokes, n (%)	179 (46.1)	30 (7.2)	<0.001
Alcohol intake, n (%)			
Never	107 (27.6)	304 (73.1)	
Sometimes (monthly or less and 2-4 times a month)	163 (42.0)	93 (22.4)	
Often (2-3 times and 4 or more times a week)	118 (30.4)	19 (4.6)	<0.001
Educational attainment, n (%)			
No formal schooling/elementary school level	43 (11.1)	38 (9.2)	0.042
Secondary school level	274 (70.8)	325 (78.3)	
Tertiary education	70 (18.1)	52 (12.5)	
Body composition			
Height (cm)	171±6	158±6	<0.001
Weight (kg)	77.4±18.4	85.4±18.0	<0.001
BMI (kg/m^2)	26.4±6.0	34.0±7.0	<0.001
Waist circumference (cm)	96.1±15.4	97.4±13.1	0.191
Hip circumference (cm)	100.6±11.1	116.6±13.6	<0.001
WHR	0.95±0.06	0.84±0.10	<0.001
BMI categories, n (%)			
Underweight (<18.5 kg/m^2)	26 (6.7)	2 (0.5)	<0.001
Normal weight (18.50-24.99 kg/m^2)	145 (37.4)	31 (7.5)	
Overweight (25-29.99 kg/m^2)	115 (29.6)	90 (21.6)	
Obese (≥30.0 kg/m^2)	102 (26.3)	293 (70.4)	
DXA (n=763)			
Fat–free soft tissue mass (kg)	49.4±9.1	41.7±7.0	<0.001
Fat–free soft tissue mass index (kg/m^2)	16.8±2.8	16.6±2.6	0.270
Body fat mass (kg)	20.9±8.9	37.7±10.3	<0.001
Body fat (%)	26.3±6.2	44.0±4.8	<0.001
Fat mass index (kg/m^2)	7.1±3.0	15.1±4.1	<0.001
Trunk (% FM)	46.9±5.3	43.5±5.7	<0.001
Leg (% FM)	40.8±5.0	43.9±6.2	<0.001
Arm (% FM)	12.4±1.3	12.6±1.8	0.055
VAT (cm^2)	91.9±47.4	109.6±44.7	<0.001
SAT (cm^2)	215.8±129.5	474.6±144.3	<0.001
VAT/SAT	0.50±0.19	0.24±0.09	<0.001
Glucose and insulin measures (n=804)			
HbA1c (%)	5.8±1.1	6.3±1.4	<0.001
Fasting glucose (mmol/L)	5.3±1.5	5.5±2.0	0.057
2 h glucose (mmol/L) (n) (n=735)	6.1±2.6	6.6±2.7	0.009
iAUC for glucose (mmol/L) (n=735)	177 (72 – 297)	157 (79 – 262)	0.368
Fasting insulin (mIU/ml)	5.9 (2.3 – 11.9)	9.4 (5.2 – 15.2)	<0.001
iAUC for insulin (mIU/ml) (n=735)	4 132 (2526–7304)	4 692 (3080–7216)	0.021

Fasting C-peptide (ng/ml)	1.84±1.07	2.09±1.17	0.002
iAUC for C-peptide (ng/ml) (n=735)	641 (447 – 925)	638 (465 – 875)	0.915
HOMA–IR	1.37 (0.51 – 2.81)	2.11 (1.13 – 3.69)	<0.001
Matsuda index (mgl^2/mU min) (n=734)	7.1 (3.6 – 13.2)	5.0 (3.1 – 8.4)	<0.001
Insulinogenic Index (mIU/mmol) (n=624)	16.9 (8.3 – 33.0)	23.4 (12.7 – 43.4)	0.001
C-peptide Index (ng/mmol) (n=612)	2.25 (1.27 – 3.79)	2.73 (1.56 – 4.57)	0.002
oDI (mIU/mmol) (n=644)	13.74 (7.22 – 26.09)	13.40 (5.98 – 28.08)	0.917
Basal insulin clearance (ng/mIU)	0.28 (0.20 – 0.39)	0.20 (0.15 – 0.27)	<0.001
Postprandial insulin clearance (ng/mIU) (n=698)	0.18 (0.14 – 0.25)	0.14 (0.12 – 0.17)	<0.001
Glucose tolerance status[1]			
NGT: (FPG <6.1 / 2-h PG <7.8mmol/L)	263 (67.8)	261 (62.7)	0.163
IGM: (FPG: 6.1–6.9 / IGT 2hPG: 7.8–11.0)	82 (21.1)	91 (21.9)	
T2DM: (FPG ≥7/2-h PG≥11.1/diabetes medication)	43 (11.1)	64 (15.4)	

Values expressed as mean±SD, median (25th – 75th percentile) or n (percentage). BMI: body mass index, WHR: waist-hip ratio, DXA: dual x–ray absorptiometry, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue; HbA1c: glycated haemoglobin, iAUC: integrated area under the curve, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance, NGT: normal glucose tolerance, IGM: impaired glucose metabolism, T2DM: type 2 diabetes mellitus.

Matsuda Index: measure of insulin sensitivity (21,22); Insulinogenic Index: measure of insulin response: $\Delta I_{30}/\Delta G_{30}$ (23); C-peptide Index: measure of insulin secretion: $\Delta CP_{30}/\Delta G_{30}$ (23); oDI: oral disposition index, measure of beta-cell function, calculated as product of C-peptide index and Matsuda index (26–28); Glycaemic tolerance status[1]: definition and diagnosis of diabetes and intermediate hyperglycemia: report of a WHO/IDF consultation

Table 2.2: Glucose and insulin measures in Black South African men and women adjusted for fat mass index (FMI)

Variable, n	**Adjusted for fat mass index (FMI)**		
Glucose and insulin measures	**Men**	**Women**	**p-value**
HbA1c (%) (n=761)	6.0 (5.8, 6.1)	6.1 (6.0 – 6.3)	0.256
Fasting glucose (mmol/L) (n=763)	5.5 (5.3 – 5.7)	5.2 (5.0 – 5.5)	0.160
2-h glucose (mmol/L) (n=697)	6.5 (6.2 – 6.9)	6.0 (5.7 – 6.4)	0.116
iAUC for glucose (mmol/L) (n=697)	221 (198 – 244)	163 (140 – 185)	0.003
Fasting insulin (mIU/ml) (n=761)	12.3 (11.1 – 13.5)	7.8 (6.6 – 8.9)	<0.001
iAUC for insulin (mIU/ml) (n=697)	6387 (5842 – 6932)	4704 (4165 – 5244)	0.001
Fasting C-peptide (ng/ml) (n=761)	2.29 (2.16 – 2.41)	1.59 (1.47 – 1.72)	<0.001
iAUC for C-peptide (ng/ml) (n=698)	746 (631 – 861)	895 (781 – 1009)	0.123
HOMA–IR (n=761)	3.03 (2.62 – 3.44)	2.06 (1.66 – 2.45)	0.005
Matsuda Index (mgl^2/mU min) (n=696)	6.3 (5.5 – 7.2)	9.8 (8.9 – 10.6)	<0.001
Insulinogenic index (mIU/mmol) (n=659)	27.4 (16.6 – 38.3)	43.3 (32.3 – 54.2)	0.089
C-peptide index (ng/mmol) (n=613)	2.75 (1.43 – 4.07)	6.91 (5.60 – 8.22)	<0.001
oDI (mIU/mmol) (n=613)	14.0 (1.7 – 26.3)	62.3 (50.0 – 74.5)	<0.001
Basal insulin clearance (ng/mIU) (n=761)	0.27 (0.25 – 0.28)	0.28 (0.26 – 0.29)	0.448
Postprandial insulin clearance (ng/mIU) (n=698)	0.19 (0.18 – 0.21)	0.18 (0.17 – 0.19)	0.403

Data adjusted for fat mass index (FMI) presented as median (95% Confidence Interval);
HbA1c: glycated haemoglobin,
iAUC: integrated area under the curve,
HOMA-IR: Homeostatic Model Assessment of Insulin Resistance,
Matsuda Index: measure of insulin sensitivity (24, 25),
Insulinogenic Index: measure of insulin response: $\Delta I_{30}/\Delta G_{30}$ (26),
C-peptide Index: measure of insulin secretion: $\Delta CP_{30}/\Delta G_{30}$ (26),
oDI: oral disposition index, measure of beta-cell function, calculated as the product of the C-peptide index and Matsuda index (29-31)

Table 2.3: Associations between regional adiposity z-scores and risk for impaired glucose metabolism (IGM) and type 2 diabetes in men and women combined

Men and women	RRR	95% CI	p-value	Model R^2
Trunk z-score (n=761)				
IGM	2.35	1.43 – 3.87	0.001	<0.001
Type 2 diabetes	4.76	2.68 – 8.45	<0.001	
Leg z-score (n=759)				
IGM	0.42	0.24 – 0.71	0.001	<0.001
Type 2 diabetes	0.21	0.11 – 0.39	<0.001	
Arm z-score (n=759)				
IGM	1.35	0.83 – 2.21	0.224	<0.001
Type 2 diabetes	2.19	1.25 – 3.81	0.006	
VAT z-score (n=753)				
IGM	1.76	1.35 – 2.28	<0.001	<0.001
Type 2 diabetes	2.58	1.92 – 3.48	<0.001	
SAT z-score (n=753)				
IGM	1.35	0.74 – 2.48	0.331	<0.001
Type 2 diabetes	1.37	0.68 – 2.75	0.376	

Results of multinomial logistic regression presented as relative risk ratios (RRR) and 95% confidence interval and represent risk of outcome with 1 SD increase in regional adiposity. Model used normal glucose tolerance (NGT) as the reference group compared to impaired glucose metabolism (IGM) and type 2 diabetes, adjusted for: age, smoking, alcohol intake, education attainment, FMI and sex

Table 2.4: Associations between regional adiposity z-scores and insulin sensitivity, basal insulin clearance and Beta-cell function

	ß	95% CI	p-value	Model R^2	Model p-value	ß	95% CI	p-value	Model R^2	Model p-value
	Men					**Women**				
	Insulin Sensitivity (n=344)					**Insulin Sensitivity (n=349)**				
FMI z-score#	-3.442	-4.011 to -2.873	<0.001	0.284	<0.001	-1.225	-1.631 to -0.819	<0.001	0.069	<0.001
Trunk z-score #	-2.680	-4.829 to -0.532	0.015	0.295	<0.001	-1.748	-2.545 to -0.950	<0.001	0.097	<0.001
Leg z-score	1.695	0.233 to 3.157	0.023	0.293	<0.001	1.218	0.454 to 1.982	0.002	0.086	<0.001
Arm z-score#	0.027	-1.667 to 1.722	0.975	0.284	<0.001	-0.893	-1.716 to -0.070	0.033	0.080	<0.001
VAT z-score#	-1.679	-2.540 to -0.818	<0.001	0.309	<0.001	-1.174	-1.657 to -0.691	<0.001	0.106	<0.001
SAT z-score#	-1.043	-2.961 to 0.876	0.286	0.286	<0.001	-0.693	-1.582 to 0.195	0.126	0.075	<0.001
	Beta-cell function (n=304)					Beta-cell function (n=306)				
FMI z-score#	-3.9594	-5.3600 - -2.5589	<0.001	0.065	<0.001	-2.6420	-4.0684 to -1.2156	<0.001	0.027	0.001
Trunk z-score	-4.0597	-9.2112 to 1.0917	0.122	0.068	<0.001	-4.3905	-7.2286 to -1.5524	0.003	0.040	<0.001
Leg z-score#	3.182	-0.3294 to 6.6940	0.076	0.071	<0.001	4.3120	1.7781 to 6.8460	0.001	0.044	<0.001
Arm z-score#	0.1387	-3.8845 to 4.1618	0.946	0.065	<0.001	-0.5693	-3.3668 to 2.2282	0.689	0.028	0.001
VAT z-score	-2.6180	-4.7016 to 0.5344	0.014	0.074	<0.001	-3.8567	-5.5820 to -2.1314	<0.001	0.051	<0.001
SAT z-score	1.6166	-2.9625 to 6.1957	0.488	0.066	<0.001	-1.8141	-4.7217 to 1.0936	0.220	0.029	0.001
	Basal Insulin Clearance (n=346)					Basal Insulin Clearance (n=351)				
FMI z-score#	-0.047	-0.062 to -0.033	<0.001	0.121	<0.001	-0.027	-0.035 to -0.018	<0.001	0.092	<0.001
Trunk z-score#	-0.055	-0.109 to -0.001	0.047	0.129	<0.001	-0.026	-0.044 to -0.009	0.002	0.108	<0.001
Leg z-score	0.006	-0.031 to 0.044	0.738	0.121	<0.001	0.017	0.001 to 0.033	0.036	0.099	<0.001
Arm z-score#	-0.015	-0.058 to 0.027	0.477	0.122	<0.001	-0.022	-0.038 to -0.005	0.011	0.104	<0.001
VAT z-score	0.001	-0.022 to 0.023	0.945	0.121	<0.001	-0.018	-0.028 to -0.008	0.001	0.111	<0.001
SAT z-score#	-0.033	-0.082 to 0.015	0.175	0.125	<0.001	-0.005	-0.024 to 0.013	0.555	0.093	<0.001

Beta coefficients for robust regression models for men and women, adjusted for age, smoking, alcohol intake, education attainment and FMI (except for FMI z-score), VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue,
Insulin sensitivity estimated from the Matsuda Index (24, 25) and beta-cell function was estimated using the oral Disposition index calculated as the product of C-peptide and Matsuda Index (26), and basal insulin clearance calculated as the ratio of fasting C–peptide to fasting insulin
#p<0.05 for sex*z-score body fat interaction term

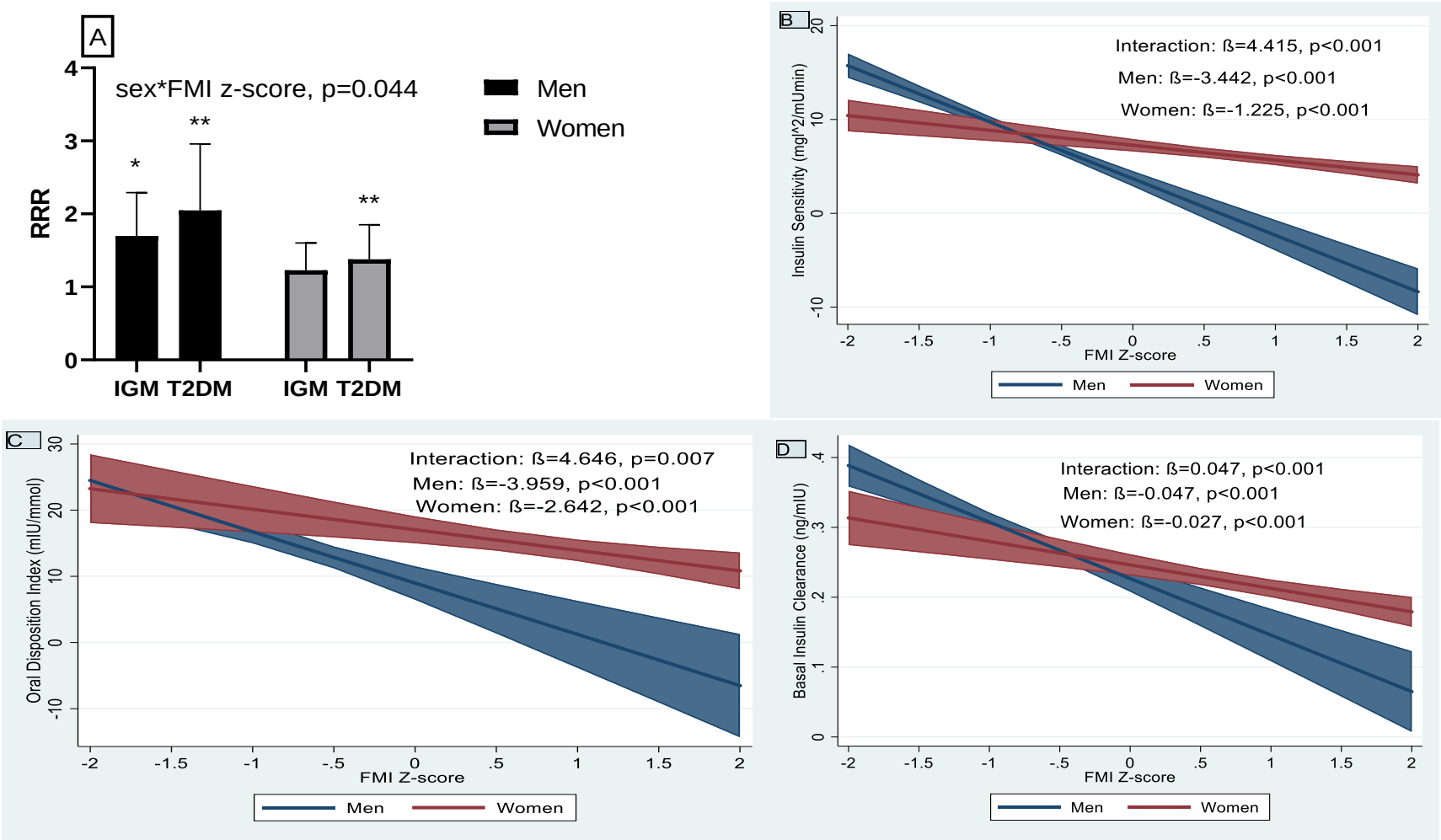
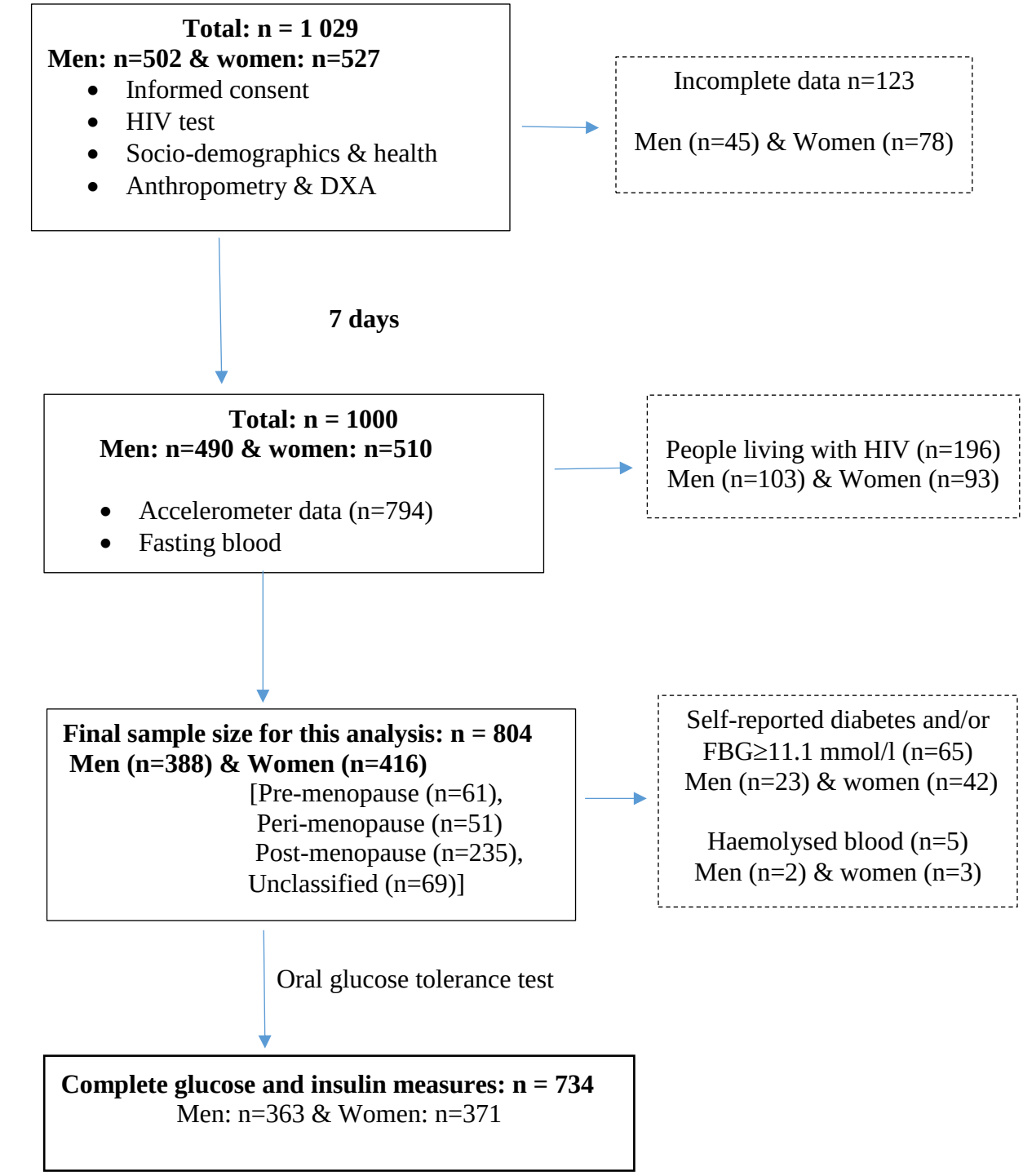


Figure 2.2: Bar graph of the relative risk ratio (RRR) of impaired glucose metabolism (IGM) and type 2 diabetes mellitus compared to the normal glucose tolerant (NGT) in men and women, IGM: (RRR (95%CI): 1.70 (1.27–2.29), p<0.001 vs. 1.23 (0.95–1.60), p=0.115) and T2D: (2.05 (1.42–2.96), p<0.001 vs. 1.38 (1.03–1.85), p=0.031) for men and women, respectively (A); Sex-specific associations between FMI z-scores and insulin sensitivity (Matsuda index) (B), beta-cell function (oral disposition index) (C) and basal insulin clearance (D), modelled as predictive margins of sex with 95% CI



PLWHIV: People living with HIV
FBG: Fasting blood glucose

Figure 2.3: Sample selection flow chart 1

CHAPTER 3

Differences in body fat distribution and glycaemic and insulin measures between pre- and post-menopausal Black South African women

Author's contributions: CNK reviewed the questionnaire, data collection tools and standard operating procedures, participated in data collection and entry, supervised data collection and entry, data management (including data cleaning and coding), quality control and classification by menopausal groups and data analysis of the chapter. CNK, JHG and LKM conceived the study, and CNK drafted and revised the chapter under the supervision of JHG and LKM. NJC and OT critically reviewed and commented the menopausal classification. All authors read and approved the final version of this chapter.

Abstract

Introduction: To describe the differences in total and regional adiposity, and glycaemic and insulin measures, between pre- and early and late post-menopausal Black South African women.

Methods: This cross-sectional study included 298 HIV negative Black South African women who were a subsample of the Middle-aged Soweto Cohort (MASC). Glycated haemoglobin (HbA1c), plasma glucose, serum insulin and C–peptide concentrations were determined after an overnight fast. Insulin sensitivity (Matsuda index), insulin secretion (C–peptide index), insulin clearance (C–peptide/insulin ratio) and beta cell function (oral disposition index), and integrated area under the curve (iAUC) for glucose, insulin and C-peptide were determined from an oral glucose tolerance test. Dual-energy x-ray absorptiometry was used to measure total and regional adiposity. Physical behaviours were measured using accelerometry. Menopausal status was determined by self-reported final menstrual period (FMP) and women were categorised as pre-menopausal, early post-menopausal and late postmenopausal.

Results: Body mass index and all the DXA-derived measures of total and regional adiposity were similar between the menopausal groups. HbA1c and iAUC for glucose were significantly higher in the late post-menopausal than the pre-menopausal group (6.4±1.4% vs. 5.8±0.9%, p=0.019 and 169.4 mmol/L vs 137.9 mmol/L, p=0.026) but this was no longer significant after adjusting for age. Fasting insulin and C–peptide, iAUC for insulin, and insulin sensitivity and clearance were not different between the groups. However, iAUC for C-peptide was higher in the early post-menopausal women compared to the pre-menopausal and the late post-menopausal women before and after adjusting for age (762.9 ng/ml vs. 552.6 ng/ml and 637.5 ng/ml, all p<0.05), whereas

insulin secretion was higher in early than late post-menopause (4.2 ng/mmol vs. 2.3 ng/mmol, p=0.001), but the latter was not significant after adjusting for age. Beta-cell function (20.5 mIU/mmol vs. 11.8 mIU/mmol, p=0.008) was higher in the early compared to the late post-menopause group before and after adjusting for age.

Conclusion: Despite similar adiposity, postprandial glycaemia was higher in late postmenopausal compared to pre-menopausal women, however this was not independent of age. Late post-menopause was associated with lower insulin secretion which has implications for disease risk and needs further exploration in longitudinal studies.

Key words: diabetes risk, body fat distribution, physical behaviours, pre-menopause, early post-menopause, late post-menopause, women.

Introduction

Chapter 2 showed sex differences in total and regional fat distribution, and insulin sensitivity and response. Importantly, central body fat was inversely associated with insulin sensitivity and beta cell function, with the association between central adiposity and insulin sensitivity being stronger in men than women (1). However, the analyses in women did not consider the impact of the menopausal transition on these measures. The menopause transition, which is accompanied by significant changes in sex hormones, is typically associated with an increase in total body fat and redistribution of fat from the gluteo-femoral (peripheral) region to the abdominal (central) region, specifically with an increase in VAT accumulation (2–5). The increase in abdominal adiposity during the menopausal transition has been reported to be independent of age and total adiposity in cross-sectional (6) and longitudinal studies (7). Further, the increase in central fat and reduction in peripheral fat with the onset of menopause has been shown to differ by race/ethnicity in studies from the US and Japan (8–10). Most studies on Black women from Africa have been completed in premenopausal women who have been shown to have a unique phenotype of lower VAT and higher gluteofemoral fat, as well as lower insulin sensitivity and hyperinsulinemia, compared to their white counterparts (11–15).

To our knowledge few studies from Africa have compared body fat distribution and risk factors for type 2 diabetes between pre and postmenopausal women. One study from South Africa, using DXA to characterise differences in body composition showed no differences in whole body or regional fat mass between the menopausal groups (11). Studies from the Congo and Ghana have that postmenopausal women are heavier and have greater number of features of metabolic syndrome compared to pre-menopausal women (16,17). However, to our knowledge no studies

from Africa have specifically explored differences in T2DM risk markers between the menopausal groups.

The pathophysiology of T2DM is dependent on the hyperbolic relationship between insulin sensitivity and insulin response, with the latter incorporating both insulin secretion and insulin clearance (18). Studies in populations of European descent have shown that the transition from pre- to post-menopause has been shown to be associated with substantial decreases in C–peptide and insulin secretion, and an increase in fasting glucose concentrations, that accounts for the higher risk for T2DM in women with the onset of menopause (19,20). While some studies have reported no difference in insulin sensitivity between pre- and post-menopausal women (21,22), others have reported reduced insulin sensitivity in post-menopausal women when compared to pre-menopausal women with similar body mass index (23).

This chapter adds to the knowledge gap and describe the difference in total and regional adiposity, and glycaemic and insulin dynamics, between pre and post-menopausal Black South African women.

Materials and methods

Study design, setting and participants

Women included in this study are from the Middle-aged Soweto Cohort (MASC) which is a longitudinal study of 2 031 participants residing in Soweto, Johannesburg, and was designed to identify the determinants of non-communicable disease and T2DM risk in middle-aged South-African men and women. The details of this cohort are described in the previous chapters. After

exclusion of all men, and women who could not be classified as either pre- or post-menopausal due to missing data (n=10), a hysterectomy (n=45), on contraception (n=24) or menopause replacement therapy (n=1), peri-menopausal (n=53) or HIV positive (n=96), this cross–sectional study analysed data from 298 Black South African (SA) women (Figure 3.1: Consort diagram for sample selection flow 2). Data was collected between January 2017 and August 2018 as described previously (1).

The study was carried out according to the tenets of the Helsinki declaration, and ethical approval was granted by the Human Research Ethics Committee (HREC) Medical (M160604 and M160975) of the University of the Witwatersrand, Johannesburg, South Africa. All study procedures and possible risks were explained to participants who consented and signed the informed consent form prior to inclusion in the study. Data collection took place at the South African Medical Research Council/University of the Witwatersrand Developmental Pathways for Health Research Unit, Chris Hani Baragwanath Hospital in Soweto, Johannesburg, South Africa.

Procedures

Questionnaires were used to collect socio-demographic data and time spent in different physical behaviours was determined by accelerometry. Body composition and body fat distribution were measured using dual energy x-ray absorptiometry. All of these methods are described in detail in previous chapters (1,24). The coefficient of variance for the DXA machine and the coefficient of variation was less than 0.5%. The inter-and intra-assay coefficient of variation (CV) were as follows in men and women respectively, height: 4% and 3.9%, waist circumference: 19.2% and 14.1%, hip circumference: 13% and 12.6%, weight 28.4% and 24%.

Measures of glucose and insulin dynamics

Fasting blood were taken for the measurement of fasting plasma glucose, serum insulin, and C–peptide concentrations and glycated haemoglobin (HbA1c). A 2-hour oral glucose tolerance test (OGTT) was performed and participants and used to calculate insulin resistance (homeostasis model of insulin resistance; HOMA-IR), insulin sensitivity (Matsuda index), insulin secretion (C–peptide index), beta cell function (oral disposition index), basal and postprandial insulin clearance (ratio of C–peptide to insulin), and the incremental area under the curve (iAUC) for glucose, insulin and C–peptide), as described in more detail in Chapter 1. Participants were classified according to the World Health Organisation (WHO) criteria for glucose tolerance using the FPG and the 2 – h OGTT glucose results (25). These are described in more detail in chapter 1 (1). The inter-and intra-assay coefficient of variation (CV) were 1.3% for glucose, 4.7% for insulin, 2.0% for C–peptide, 2.6% for cholesterol, 5.6% for high density lipoprotein (LDL), 3.4% for triglycerides and 1.8% for HbA1c.

Menopausal classification

Menopausal status was classified using self-reported date of final menstrual period (FMP) (26,27). Women currently having regular periods were classified as pre–menopausal and women who reported no bleeding for more than 12 months were classified as post–menopausal. Postmenopausal women were further classified into early postmenopausal group if they had not had a drop of blood for greater than 12 months but less than six years, and late postmenopausal group if they had not had a drop of blood for more than six years (26).

Blood collection and analysis of sex hormones

Serum FSH and LH concentrations were measured on fasting samples using Chemiluminescent Microparticle Immunoassay assay (Architect assays, Abbott Laboratories, IL, USA) at Lancet Laboratories. Serum Oestrogen (E_2) was quantified and oestradiol analysed using mass spectrometry (Acquity® Xevo TQ-XS mass spectrometer (Waters, Manchester, UK)) (28,29).

Statistical analysis

Stata 15.1/IC (StataCorp, College Station, TX, USA) was used for data analysis. To ascertain the distribution, skewness, and kurtosis of data the Shapiro-Wilk test and Q-Q probability plots were completed. The results are summarised as percentages for categorical variables, mean (standard deviation) for normally distributed variables and medians and lower and upper quartiles if skewed. Differences between menopausal groups were explored for normally distributed continuous data using analysis of variance (ANOVA), and one-way analysis of covariance (ANCOVA) when adjusting for age, for skewed continuous data Kruskal-Wallis tests were used, and chi-squared test was used to compare categorical data. Skewed data included 2-hr glucose, iAUC for glucose, insulin, C–peptide, HOMA-IR, fasting C – peptide, fasting insulin, insulin sensitivity, basal insulin clearance, beta-cell function, luteinising hormones, follicle stimulating hormone, and oestrogen. A p–value of <0.05 was considered significant.

Results

Socio-demographic characteristics

The mean age (±SD) of the women was 56±6 years and was significantly different between the menopausal groups ($p<0.001$). While marital status and education did not differ by group, current

employment was higher in the pre- and early post-menopausal groups than the late post-menopausal group (p=0.003). Current smoking status did not differ by group, but more pre-menopausal women consumed alcohol compared to post-menopausal women (p=0.001). Physical behaviours did not differ by menopausal status even after adjusting for differences in age (Table 3.1).

As expected, sex hormones were different between the pre-menopausal and post-menopausal groups with LH and FSH being significantly higher, and oestrogen significantly lower, in the postmenopausal groups compared to the premenopausal group, before and after adjusting for age (Table 3.1).

Body composition and regional adiposity

The prevalence of overweight and obesity were similar between menopausal groups, being 29.5% and 65.6% in pre-menopausal women, 23% and 67.6% in early post-menopausal women and 20.8% and 70% in late post-menopausal women, respectively. Similarly, there were no differences in BMI or any of the anthropometrical and DXA-derived measures of total and regional adiposity between the menopausal groups (Table 3.2).

Glycaemic and insulin dynamics

Table 3.3 presents the glycaemic and insulin measures in the pre-, early and late postmenopausal groups. Although fasting and 2-hour glucose were not different, HbA1c and iAUC for glucose were significantly higher in the late post-menopausal than the pre-menopausal group, but this was no longer significant after adjusting for age. Although 6.5% of the pre-menopausal women, 17.6%

for the early postmenopausal women and 14.7% of the late postmenopausal women had T2DM according to WHO (25), these differences were not statistically significant.

Neither insulin resistance (HOMA-IR) nor insulin sensitivity (Matsuda index) were different between the groups, however insulin secretion and beta-cell function, as reflected by the iAUC, insulin secretion index and the oDI, were higher in the early post-menopausal than the late post-menopausal women. In contrast, fasting measures of insulin and C–peptide did not differ between the groups.

Discussion

This is one of the first studies to compare measures of body fat distribution and OGTT-derived risk markers for T2DM between pre- and post-menopausal Black African women. In this sample of women in whom the overall prevalence of obesity and T2DM was 68.5% and 13.8%, respectively, there were no differences in total or regional adiposity between women categorised at different stages of the menopausal transition. Further, after adjusting for age, the postprandial glycaemia was no longer higher in the late post-menopausal period but the late postmenopausal group had lower postprandial insulin secretion compared to the younger menopausal groups and the higher beta-cell function of the late postmenopausal group compared to the younger menopausal groups was no more observed. Accordingly, with increasing age there is increase in glycaemia which corresponds to the lower insulin secretion.

Several studies have shown that the menopausal transition is accompanied by an increase in abdominal fat (30,31) and redistribution of gluteo-femoral fat from the (peripheral) region to the

abdominal (central) region (2–5). Despite differences between pre- and post-menopausal groups in LH, FSH and oestrogen, this study found no differences in any measures of total or regional adiposity between the menopausal groups, which confirms the results of Jaff et al. (11) in a similar sample of Black SA women from Soweto. These findings may differ to those previously reported in European populations (6,7,9,10,32) due to the high prevalence of overweight and obesity in our sample (67.4%) that is representative of Black South African women (33), thus limiting variation in adiposity over the menopausal transition.

Despite no differences in total or regional adiposity, postprandial glycaemia, characterised by iAUC glucose and HbA1c, was higher in the late post-menopausal compared to the pre-menopausal group. However, this effect was not independent of age, suggesting that the higher glycaemia was because of age rather than menopause *per se*. There were no statistical differences in insulin sensitivity between the groups, but insulin secretion and beta cell function were higher in the early post-menopausal period, presumably as a compensatory response to subtle changes in insulin sensitivity and glycaemia, but then declined in the late post-menopausal period and this corresponded with the increase in post-prandial glycaemia. These findings confirm those of other studies using intravenous glucose tolerance test (IVGTT) (21,23) Notably, the differences in insulin secretion and beta cell function between menopausal groups were independent of age, suggesting that these effects were mediated by the long term effects of the changes in sex hormones over the menopausal period. Indeed, ovariectomy of rodents has consistently been shown to result in a decline in beta-cell function, and decreased oestradiol action through ERα and ERβ appears to affect the survival of beta-cells and insulin secretion (34–36).

Although there is paucity of human studies (37), increasing evidence from experimental studies in rodents and mice have reported that the decreased oestradiol levels and decreased oestradiol action via ERα may cause insulin resistance in the skeletal muscle, liver and adipose tissue (38–41). However, we did not show differences in insulin sensitivity, measured using HOMA-IR or Matsuda index, between the menopausal groups. Notably, postprandial insulin secretion (iAUC C-peptide) was higher in the early post-menopausal women compared to both the premenopausal and late post-menopausal women reflecting hyperinsulinaemia. The higher iAUC C-peptide in the early postmenopausal women reflects higher insulin secretion as a compensatory response for the level of glycaemia while in the late postmenopausal women this is no longer the case, hence the higher glycaemia in the late postmenopausal women. Although not significant when adjusting for age, the oDI was higher in early post-menopausal women compared to pre-menopausal women, suggesting exaggerated insulin response for the level of insulin sensitivity in the early post-menopausal women, which is consistent with other studies in African women (42–44). Hyperinsulinaemia has been consistently reported in Black African women (45–48), but it is still unclear whether this is a cause or consequence of insulin resistance and the subsequent development of T2DM (15). We have previously shown that middle-aged Black SA women pre sent with hyperinsulinaemia, but with increasing age, insulin secretion (and not insulin sensitivity) declines and corresponds with increasing glycaemia (48). Prospective studies are however required to explore the pathogenic sequence leading to T2DM in African women.

Other studies have reported that the increase in T2DM risk with menopausal transition may be linked to factors associated with aging like increase sedentary behaviours, increasing adiposity, and comorbidities such as depression and sleep disorders (49,50). I did not show any differences

in movement behaviours, with time spent in sedentary and movement behaviours and sleep being remarkably similar between groups. However, I did not measure quality of sleep nor did I measure differences in dietary habits, which may alter diabetes risk. Further, I showed no differences in total and regional adiposity between menopausal groups, however, other factors such as inflammatory markers including tumor necrosis factor-α (TNF-α) produced by human fat, have been reported to increase in post-menopausal women and may contribute to decreased insulin-stimulated glucose disposal (51) and promote insulin resistance (52). Future research is required to gain insight into other lifestyle and physiological factors involved in the pathogenesis of T2DM in African women.

No differences were shown across menopausal transition for body composition, body fat distribution and physical activity but a 4–year longitudinal study of 1 393 women aged 47–55 years in central Finland has shown menopause in middle-aged women may accelerate changes in metabolic health, however, physical activity is associated with healthier body lipid profile and body composition but may not modulate the changes of metabolic health indicators during menopausal transition (53). A review reported that central obesity was associated with excess risk of mortality and changes in body composition and reproductive hormones across menopausal transition was associated with increased overall metabolic and cardiovascular disease risk. Similarly, in post-menopausal women a significant dose response for greater body weight, fat and intra-abdominal fat lose with increased exercise duration and longitudinal data has also shown an inverse association between percent body fat increased and moderate or vigorous physical activity among White women (54).

Although no differences in body fat distribution was shown between the menopausal stages in this cohort the decline in oestrogens across menopausal stages in women has been shown to have deleterious effects on body composition and glucose metabolism leading to higher prevalence and incidence of metabolic disorders in post–menopausal women compared to pre-menopausal women (55,56).

Limitations and strengths of the study

This a cross-sectional study and causal conclusions cannot be made from the results. While we had objective measures of sedentary and physical activity behaviours drawn from two accelerometers, we did not have any dietary data. We excluded HIV positive women whose body fat distribution, insulin sensitivity and response may differ and hence these results may not be generalisable to those with HIV. The potential interaction between the sex hormones, beta-cell function and fat metabolism has not been explored. A strength of the study is the completion of OGTTs that are rarely done in population studies from Africa to determine diabetes risk between menopausal groups from which we estimated insulin sensitivity, secretion, basal insulin clearance and beta-cell function. Body fat distribution was measured using dual energy x-ray absorptiometry that provides an objective and more precise estimate of total and regional fat deposits than anthropometric measures.

Conclusion

The menopausal transition in Black women with a high prevalence of overweight and obesity is not accompanied by differences in total or regional adiposity, however lower insulin secretion in late post-menopause may explain higher postprandial glycaemia. Longitudinal studies are warranted to establish the relationship between menopausal transition and glucose and insulin dynamics in the development of T2DM Black African women.

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Table 3.1: Sociodemographic, physical behaviours and sex hormone measures by menopausal status in Black South African women (n=298)

Variable	Pre-menopausal women	Early post-menopause	Late post-menopause	p-value	Adjusted p-value
n (%)	61 (20.5)	74 (24.8)	163 (54.7)		
Age (years)	48.8±3.7[ab]	54.2±3.7[ac]	59.1±4.4[bc]	**<0.001**	
Sociodemographic and lifestyle factors					
Married, n (%)	28 (46.7)	37 (50.0)	67 (41.1)	0.410	
Currently Employed, n (%)	27 (44.3)	32 (43.2)	41 (25.2)	**0.003**	
Education attainment, n (%)					
No formal schooling/elementary school level	2 (3.3)	4 (5.4)	22 (13.5)		
Secondary school level	50 (82.0)	60 (81.1)	126 (77.3)		
Tertiary education	9 (14.7)	10 (13.5)	15 (9.2)	0.080	
Current smokers, n (%)	5 (8.2)	6 (8.1)	7 (4.3)	0.380	
Alcohol intake, n (%)					
Never	35 (57.4)	53 (71.6)	126 (77.3)		
Sometimes (monthly or less and 2-4 times/month)	25 (41.0)	14 (18.9)	31 (19.0)		
Often (2-3 times and 4 or more times/week)	1 (1.6)	7 (9.5)	6 (3.7)	**0.001**	
Consolidated accelerometry (minutes/day)				**p-value**	**p-value[1]**
Light intensity physical activity (LPA) (n=211)	120.1±46.1	131.1±64.4	112.2±46.1	0.084	0.104
Moderate-to-vigorous intensity physical activity (MVPA) (n=211)	36.1±24.9	37.1±21.5	33.0±24.0	0.532	0.693
Sitting/lying (n=193)	618.5±135.2	627.0±143.4	619.8±133.6	0.942	0.732
Standing (n=193)	250.1±102.5	239.8±97.4	269.8±115.0	0.242	0.464
Sleep (n=211)	417.8±62.1	406.1±69.0	401.9±74.3	0.444	0.365
Total movement volume (mg) (n=211)	12.7±3.8	13.0±3.8	12.0±3.4	0.219	0.348
Sex hormones, n (%)	57 (20.1)	73 (25.7)	154 (54.2)	**p-value**	**p-value[1]**
Luteinising hormone (LH) (IU/L) (n=284)	5.5 (3.1 – 16.2)[ab]	27.7 (18.1 – 31.3)[a]	20.9 (16.1 – 25.7)[b]	**<0.001**	**<0.001**
Follicle Stimulating Hormone (FSH) (IU/L) (n=284)	10.7 (5.3 – 23.8)[ab]	59.1 (41.9 – 71.6)[a]	53.2 (43.5 – 64.2)[b]	**<0.001**	**<0.001**
Oestrogen (pmol/L) (n=294)	205.0 (79.0–629.5)[ab]	22.0 (11.0 – 36.0)[a]	25.0 (16.0 – 38.0)[b]	**<0.001**	**<0.001**

Values as mean±SD for normally distributed data or median (25th – 75th percentile) for non-normally distributed data

[1]p-values for accelerometry and sex hormones adjusted for age only

[a]: Difference between pre-menopausal and early post-menopausal women after adjusting for age

[b]: Difference between pre-menopausal and late post-menopausal women after adjusting for age

[c] Difference between early post-menopausal and late post-menopausal women after adjusting for age
All pre-menopausal women reported regular menstrual periods, bold represents significant difference

Table 3.2: Body composition in pre- and early and late post-menopausal Black South African women (n=298)

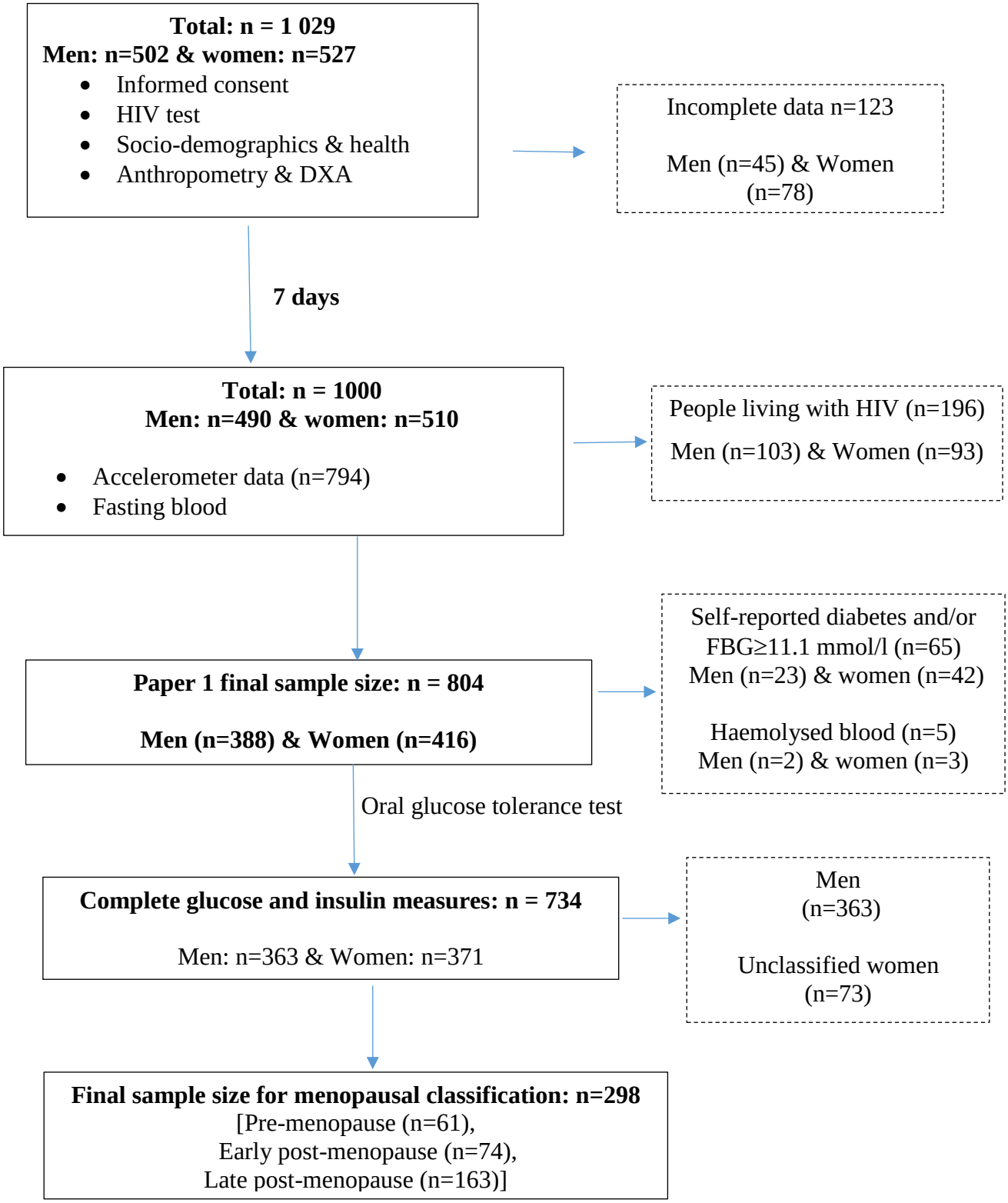
Body composition	**Pre-menopausal women**	**Early post-menopause**	**Late post-menopause**	**p-value**	**p-value[1]**
Height (cm)	158.8±7.2	158.0±5.8	158.0±5.5	0.659	0.866
Weight (kg)	85.2±18.5	82.1±16.5	84.9±18.6	0.487	0.288
BMI (kg/m^2)	33.8±7.4	32.8±6.4	33.9±7.2	0.510	0.377
Waist circumference (cm)	97.3±14.3	96.8±13.4	97.8±13.3	0.336	0.536
Hip circumference (cm)	116.5±13.2	113.1±15.5	117.0±12.9	0.119	0.065
WHR	0.82±0.07	0.85±0.14	0.83±0.07	0.133	0.125
BMI categories in kg/m^2, n (%)					
Underweight (<18.5 kg/m^2)	0 (0.0)	1 (1.4)	1 (0.6)	0.771	0.673
Normal weight (18.5-24.9 kg/m^2)	3 (4.9)	6 (8.1)	14 (8.6)		
Overweight (25-29.9 kg/m^2)	18 (29.5)	17 (23.0)	34 (20.8)		
Obese (≥30.0 kg/m^2)	40 (65.6)	50 (67.6)	114 (70.0)		
DXA (n=283)	57 (20.1)	73 (25.8)	153 (54.1)		
Fat Mass (FM, kg) (n=283)	36.0±8.4	36.5±9.8	37.9±11.0	0.425	0.175
% Body Fat, (n=283)	42.7±4.1	43.8±4.6	44.4±5.1	0.080	0.229
Fat mass index (FMI, kg/m^2) (n=283)	14.3±3.4	14.6±3.9	15.2±4.4	0.337	0.164
Fat–Free Soft Tissue Mass (FFSTM, kg) (n=282)	42.1±6.7	40.5±7.3	40.8±6.7	0.418	0.614
FFSTM index (FFSTMI, kg/m^2) (n=282)	16.7±2.4	16.2±2.6	16.3±2.6	0.563	0.611
Trunk % FM (n=283)	39.9±5.3	41.3±5.3	42.0±6.1	0.073	0.194
Leg %FM (n=283)	44.2±6.3	43.8±5.5	43.6±6.3	0.850	0.986
Arm %FM (n=283)	12.6±1.5	12.4±1.4	12.5±1.8	0.727	0.513
VAT area (cm^2) (n=283)	99.3±48.9	103.6±37.2	112.8±47.0	0.105	0.793
SAT area (cm^2) (n=283)	442.3±125.2	466.4±144.3	480.6±150.3	0.229	0.226
VAT/SAT	0.22±0.11	0.22±0.08	0.23±0.08	0.610	0.669

Values as mean±SD for normally distributed data or median (25th – 75th percentile) for non-normally distributed data
[1]p-values for body fat distribution adjusted for age only

Table 3.3: Glycaemic and insulin measures in pre- and early and late post-menopausal Black South African women (n=298)

Glucose and insulin measures	Pre-menopause	Early post-menopause	Late post-menopause	p-value	p-value[1]
HbA1c (%) (n=295)	**5.8±0.9**	6.3±1.4	**6.4±1.4**	**0.019**	0.545
Fasting glucose (mmol/L) (n=298)	5.2±1.2	5.5±2.2	5.4±1.7	0.618	0.736
2 h glucose (mmol/L) (n=268)	5.7 (4.9 – 7.0)	5.7 (4.4 – 7.1)	6.0 (5.2 – 8.2)	0.128	0.439
iAUC for glucose (mmol/L) (n=250)	**137.9 (74.2–228.6)**	147.9 (70.8–243.8)	**169.4 (106.3–302.2)**	**0.026**	0.764
Fasting insulin (mIU/ml) (n=297)	8.5 (5.7 – 13.2)	8.1 (5.0 – 15.2)	8.5 (4.6 – 13.5)	0.963	0.730
iAUC for insulin (mIU/ml) (n=267)	4 939 (3544–7277)	4 545 (2911–7154)	4 540 (3009–6712)	0.632	0.895
Fasting C-peptide (ng/ml) (n=297)	1.68 (1.30 – 2.29)	1.78 (1.33 – 2.64)	1.71 (1.33 – 2.29)	0.772	0.670
iAUC for C-peptide (ng/ml) (n=268)	**552.6 (474.0–798.9)[a]**	**762.9 (497.4–1107.3)[ac]**	**663.5 (459.0–823.2)[c]**	**0.011**	**0.010**
Basal Insulin Clearance (ng/mIU) (n=297)	0.195 (0.152–0.266)	0.223 (0.174– 0.289)	0.199 (0.158– 0.276)	0.555	0.440
HOMA–IR (n=297)	1.75 (1.19 – 3.51)	1.65 (1.02 – 3.30)	1.81 (0.97 – 3.35)	0.970	0.731
Insulin sensitivity (mgI^2/mUmin) (n=268)	5.1 (3.0 – 7.8)	5.0 (3.1 – 9.6)	5.2 (3.2 – 8.3)	0.873	0.608
Insulinogenic index (mIU/mmol) (n=217)	32.6 (15.4 – 54.6)	24.1 (12.9 – 59.2)	21.6 (11.3 – 34.9)	0.130	0.961
Insulin secretion (ng/mmol) (n=230)	3.0 (1.7 – 5.0)	**4.2 (1.8 – 8.4)[c]**	**2.3 (1.2 – 3.5)[c]**	**0.001**	**0.008**
oDI-C-peptide (mIU/mmol) (n=230)	15.0 (6.8 – 29.0)	**20.5 (8.8 – 60.2)[c]**	**11.8 (5.1 – 22.5)[c]**	**0.008**	***0.052***
Glycaemic categories, n (%) (n=298)					
NGT: (FPG <6.1/2-h PG <7.8mmol/L)	45 (73.8)	47 (63.5)	96 (58.9)		
IFG: (FPG:6.1–6.9)/IGT (2hPG:7.8–11.0)	12 (19.7)	14 (18.9)	43 (26.4)		
T2DM: (FPG ≥7/2-hPG≥11.1)	4 (6.5)	13 (17.6)	24 (14.7)	0.173	0.053

Values as mean±SD for normally distributed data or median (25th – 75th percentile) for non-normally distributed data
[1]p-values for glycaemic and insulin measures after adjusting for age.
HbA1c: glycated haemoglobin
iAUC: integrated area under the curve
HOMA–IR: Homeostatic Model Assessment of Insulin Resistance
oDI: oral disposition index, measure of beta-cell function, calculated as product of c-peptide index and Matsuda index
NGT: Normal glucose tolerance
IFG: Impaired fasting glucose
T2DM: Type 2 diabetes mellitus
FPG: Fasting plasma glucose
[a]: difference between pre-menopausal and early post-menopausal women
[c]: difference between early post-menopausal and late post-menopausal women
bold represents significant difference



PLWHIV: People living with HIV
FBG: Fasting blood glucose

Figure 3.1: Consort diagram for sample selection flow 2

CHAPTER 4

Physical behaviours and their association with type 2 diabetes mellitus risk markers in urban South African middle-aged adults: An isotemporal substitution approach

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Author contributions: CNK reviewed the questionnaire, data collection tools and standard operating procedures, participated in data collection and entry, supervised data collection and entry, data management (including data cleaning and coding), quality control of physical activity data from the Actigraphs and activPALs and data analysis. AS and KW processed the physical activity data. CNK, JHG, SB and LKM conceived the study and CNK drafted and revised the manuscript under the supervision of JHG, SB and LKM. SB and KW advised on the statistical methods. KW and SB critically reviewed and commented on the manuscript. All authors read and approved the final version of the manuscript.

Abstract

Introduction: To examine the associations between physical behaviours and type 2 diabetes mellitus (T2DM) risk in middle-aged South African men and women.

Research design and methods: This cross-sectional study included middle-aged men (n=403; age: median [IQR], 53.0 [47.8–58.8] years) and women (n=324; 53.4 [49.1–58.1] years) from Soweto, South Africa. Total movement volume (average movement in milli-g) and time (minutes/day) spent in different physical behaviours, including awake sitting/lying, standing, light intensity physical activity (LPA) and moderate-to-vigorous intensity physical activity (MVPA), were determined by combining the signals from two triaxial accelerometers worn simultaneously on the hip and thigh. All participants completed an oral glucose tolerance test, from which indicators of diabetes risk were derived. Associations between physical behaviours and T2DM risk were adjusted for sociodemographic factors and body composition.

Results: Total movement volume was inversely associated with measures of fasting and 2-h glucose and directly associated with insulin sensitivity, basal insulin clearance, beta-cell function, but these associations were not independent of fat mass, except for basal insulin clearance in women. In men, replacing 30 minutes of sitting/lying, standing or LPA with the same amount of MVPA time was associated with 1.2–1.4 mmol/L lower fasting glucose and 12.3–13.4 mgl^2/mUmin higher insulin sensitivity. In women, substituting sitting/lying with the same amount of standing time or LPA was associated with 0.5–0.8 mmol/L lower fasting glucose. Substituting 30 minutes sitting/lying with the same amount of standing time was also associated with 3.2 mgl^2/mUmin higher insulin sensitivity, and substituting 30 minutes of

sitting/lying, standing or LPA with the same amount of MVPA time was associated with 0.25-0.29 ng/mIU higher basal insulin clearance in women.

Conclusion: MVPA is important in reducing T2DM risk in men and women but LPA appears to be important in women only. Longitudinal and intervention studies warranted to provide more specific PA recommendations.

Keywords: sitting/lying, standing, light physical activity, moderate-to-vigorous physical activity, isotemporal substitution, diabetes risk.

What is already known about this topic

Extensive evidence has shown an inverse association between physical activity and type 2 diabetes risk, and report that time spent in sedentary behaviours is a recognised risk factor for type 2 diabetes. Most of these studies have only considered the association between the physical behaviours themselves and type 2 diabetes risk, without also considering the behaviour that is replaced for that time. There is also controversy in the literature as to whether the association between physical activity and type 2 diabetes risk is independent of adiposity. It is well recognised that wearable devices provide a more accurate and objective measure of physical behaviours compared to self-report, with limited data combining the signals from two accelerometers to more accurately measure intensity and posture and limited data from Africa.

What this study adds

- Total movement volume was inversely associated with fasting and 2-h glucose, and positively associated with insulin sensitivity, basal insulin clearance, and beta-cell function, but these associations were not independent of fat mass, except for basal insulin clearance in women.

- In men, substituting 30 minutes of awake sitting/lying, standing or light intensity physical activity with the same amount of time in moderate-to-vigorous intensity physical activity was associated with lower fasting glucose and higher insulin sensitivity.

- In women, substituting 30 minutes of awake sitting/lying with the same amount of standing or light intensity physical activity was associated with lower fasting glucose, and

substituting 30 minutes of awake sitting/lying with the same amount of standing was associated with higher insulin sensitivity.

- In women, substituting 30 minutes of awake sitting/lying, standing or light intensity physical activity with the same amount of time in moderate-to-vigorous intensity physical activity was associated with higher insulin clearance.

How this study might affect research, practice or policy

Intervention studies are needed to determine whether sex-specific physical activity recommendations are needed as although moderate-to-vigorous intensity physical activity is associated with lower risk type 2 diabetes risk markers in men and women, light intensity physical activity seems to be beneficial in women only.

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) is increasing globally and sub-Saharan Africa (SSA) is projected to have the greatest estimated increase compared to all other regions by 2045 (1). Within SSA, South Africa (SA) has the highest prevalence of T2DM, with the latest national prevalence for adult men and women at 8% and 13%, respectively (2). Extensive evidence reports an inverse association between physical activity (PA) and T2DM risk (3–5). Studies have shown that physical activity of any intensity positively influences glucose regulation and insulin sensitivity in a dose–response manner (6,7).

Sedentary time is also recognised as a risk factor for T2DM with a systematic review and meta-analysis showing that participants who reported the greatest sedentary time were at a 112% higher relative risk (RR) of T2DM compared to those with the lowest sedentary time (8). While some studies have shown this association to be independent of physical activity (9), a meta-analysis by Patterson et al., showed an increased risk for T2DM with higher levels of total sitting independent of PA (10).

Time spent in sedentary behaviour, PA and sleep are mutually exclusive and the total minutes available in a day are fixed and finite. Therefore, understanding the beneficial effects of physical activity depend not only on the considered aspect of PA but also on the activity type displaced (11). A recent meta-analysis reported that replacing 30 minutes of sedentary time with the same amount of time in light intensity physical activity (LPA) was associated with reductions in fasting insulin, waist circumference and all-cause mortality, and replacing sedentary time with moderate-to-vigorous intensity physical activity (MVPA) was associated

with reductions in body mass index (BMI), waist circumference, fasting glucose and insulin concentrations, and all-cause mortality (12). Further, results from the UK Biobank study (n= 475,502) reported that replacing sedentary behaviour with 30 minutes/day of physical activities or structured exercise was associated with a 6–31% lower incidence of T2DM 11 years later (13). Isotemporal substitution analysis simultaneously models a specific activity and the effects of time in substitution of the activity by another for the same amount of time (14). However, the use of isotemporal substitution has been limited to studies including European populations, with a dearth of studies in Africa.

A South African population-based survey reported that only 14.8% were moderately physically active and 27.8% were vigorously physically active, and that men were more likely to be physically active whereby women were less likely to engage in moderate as well as vigorous PA (15). Few South African studies have explored the association between physical behaviours and T2DM risk (16–21). Globally, the majority of evidence reporting the association between PA and health outcomes is based on self-reported PA which has several limitations (22). Wearable devices are increasingly being used and provide a more accurate, objective assessment of sedentary behaviour and PA intensity and volume than subjective self-reported measures (23,24). Edwardson et al., have shown that high accuracy can be obtained using two wearable devices with postural categorization from an accelerometer on the thigh, and intensity also using information from an accelerometer on the waist (25). This has however, never been undertaken in Africa.

There is controversy as to whether the association between PA and T2DM risk is mediated or independent of adiposity (4). This is relevant to the South African context where there is a high prevalence of obesity and where adiposity differs significantly between men and women (2).

The aim of this study is therefore to examine the association between physical behaviours quantified by combining signals from two accelerometers, and risk markers for T2DM in middle-aged men and women from urban South Africa using an isotemporal substitution approach. We hypothesised that replacing 30 minutes of sedentary time or LPA with the same amount of time in higher intensity behaviours is associated with a reduction in the risk markers for T2DM.

Materials and methods

Research design, setting and participants

This cross–sectional study used data from the Middle-aged Soweto Cohort (MASC) collected between January 2017 and August 2018 (502 men and 527 women), as described previously (26). For this analysis, complete accelerometry (27) and oral glucose tolerance test (OGTT) data were available on 727 participants (n=403 men and n=324 women) (Figure 4.1: Sample selection flow chart 3).

Ethical approval was granted by the Human Research Ethics Committee (HREC) Medical (M160604 and M160975) of the University of the Witwatersrand, Johannesburg, South Africa. All study procedures and possible risks were explained to participants who consented and signed the informed consent form prior to inclusion in the study. Data collection took place in

accordance with the guidelines of Helsinki at the South African Medical Research Council/University of the Witwatersrand Developmental Pathways for Health Research Unit, Chris Hani Baragwanath Hospital in Soweto, Johannesburg, South Africa.

Procedures

Physical behaviour assessment and data processing

Physical behaviours were objectively measured using two accelerometers, an ActiGraph GT3X+ (AG) (ActiGraph, Pensacola, USA) on the right hip, and an activPAL (AP) (PAL technologies Ltd., Glasgow, UK) on the right thigh. The participants were advised to wear the accelerometers continuously for seven days and nights including sleep times and the weekend, and only to remove the ActiGraph GT3X+ during bathing or water-based activities. They were also requested to continue with their normal daily activities. Participants received a sleep diary and were asked to record their daily sleep and wake times for the same period.

At the end of the seven days, participants returned the accelerometers and raw data was downloaded using the Actilife software (ActiGraph, Pensacola, USA) and actiPAL software (PAL Technologies Ltd., Glasgow, UK). Complete data from ActiGraph GT3X+ and activPAL for four to seven days was obtained by a combination of processing scripts (PAMPRO) and post-processing scripts (28). The raw tri-axial signals from the two accelerometers were calibrated to local gravity (29) and acceleration and pitch angles converted to minute-by-minute time series. The signals were combined with the self-reported sleep times and reported at participant level to estimate total volume and time spent in postures and intensities of behaviours. The behaviour outcomes were summarised as total movement volume (Euclidian

norm minus one, ENMO expressed as milli-g (mg)), time spent in sleep, and awake time in sitting/lying, standing, LPA, and MVPA, all in minutes per day (min/day). Details of the objectively measured PA data acquisition, processing, development of the algorithm and classification are described elsewhere (26).

Measures of type 2 diabetes mellitus risk markers

From each participant, blood samples were drawn after an overnight fast for determination of plasma glucose, serum insulin, C–peptide, and glycated haemoglobin (HbA1c). Participants then underwent a standard oral glucose tolerance test (OGTT). Participants ingested 75g anhydrous glucose in 250ml water and then 5ml blood samples were drawn every 30 minutes for 2 hours. Randox RX Daytona Chemistry Analyser (Randox Laboratories Ltd., London, UK) was used to measure plasma glucose concentrations and D–10^{TM} Haemoglobin Analyser (Bio–Rad Laboratories, Inc. USA) was used to measure HbA1c concentrations. Immulite® 1000 Immunoassay System (Siemens Healthcare Diagnostics, Tarrytown, NY) was used to measure serum insulin and C-peptide concentrations.

The homeostasis model assessment (HOMA-IR) was used to estimate fasting insulin resistance (30). Insulin sensitivity was estimated by the Matsuda Index for participants with complete OGTT results, and the composite insulin sensitivity index for those with data at 0 and 120 minutes (31,32). Insulin secretion was calculated as the C-peptide index, which was the ratio of the increment in C-peptide to glucose during the first 30 minutes of the OGTT. The oral disposition index (oDI), an estimate of beta-cell function, was calculated as a product of C-peptide index and Matsuda index (33). Basal insulin clearance were estimated as fasting C-

peptide/insulin concentrations, and postprandial insulin clearance was estimated from the incremental area under the curve (iAUC) of C-peptide to iAUC insulin using the trapezoidal method.

Sociodemographic questionnaires

Questionnaires were completed on Research Electronic Data Capture (REDCap) (34) and included data on age, marital status (married/unmarried), highest level of education attained (no formal schooling/elementary school, secondary school, and tertiary education), 12 item household assets (electricity, television, radio, motor vehicle, fridge, washing machine, telephone/cell phone, microwave, bicycle, tablet/laptop/personal desktop, DSTV/satellite TV, Mnet) classified into 3 categories (0-4 for category 1, 5-8 for category 2 and 9-12 for category 3) and employment status (employed/not employed).

Anthropometry and body composition

Weight was measured with a TANITA digital scale (model: TBF-410, TANITA Corporation, US) to the nearest 0.1 kg. Height was measured with a wall-mounted stadiometer (Holtain, UK) to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg)/(height in m)^2. Sub-total fat mass (FM, kg) (total fat mass minus the head) was measured with a Hologic QDR 4500A dual–energy x-ray absorptiometry (DXA) machine (Hologic Inc., Bedford, USA) and analysed with APEX software version 13.4.2.3 according to standard procedures. Fat mass index (FMI) was calculated as sub-total fat mass (kg)/height^2 (m^2). Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) areas were estimated from DXA, as described elsewhere (35) .

HIV/AIDS tests

Pre- and post-HIV counselling was provided and an HIV antibody test, Wondfo® One Step HIV–1/2 Whole Blood/Serum/Plasma: Test 2 lines (Guanghu Wondfo Biotech Co., Ltd) was completed for all consented participants, except those previously known as HIV positive. Newly diagnosed HIV positive participants were referred to an HIV clinic for follow up and retained in the study. Participants were subsequently categorised into HIV negative (HIV-) or HIV positive (HIV+).

Statistical analysis

Data was analysed using Stata 15.1/IC (StataCorp, College Station, TX, USA). Shapiro-Wilk test and Q-Q probability plots were used to assess distribution and ascertain skewness and kurtosis of the data. Variables were summarised as count (percentages) for categorical data, mean (standard deviation) if normally distributed continuous data, and median (25th–75th percentiles) if not normally distributed continuous data. Student's t-tests were used to explore the sex differences for normally distributed continuous data, and Mann-Whitney U and Kruskal-Wallis tests were used to compare skewed continuous data. Multivariable robust regression analyses were used to explore the relationship between total movement volume and outcome variables (fasting and 2-hr glucose, insulin sensitivity, basal insulin clearance, and beta-cell function) and covariates which have been shown in the literature to be associated with the outcomes were included. In model 1, age was included as a covariate, in model 2; age, HIV status, education, asset category and employment were included as covariates; in model 3, FMI

was added to covariates in model 2; while in model 4, VAT was included with the covariates from model 3.

Isotemporal substitution modelling was used to estimate the effect of replacing time spent in one physical behaviour (sleep, sitting/lying, standing, LPA and MVPA) with another (14); specifically, we ascertained the theoretical effect of reallocating 30 minutes of one physical behaviour to 30 minutes of another physical behaviour on the glucose and insulin measures listed previously. Regression coefficients (and 95%CI) represent the replacement of one physical behaviour with another, while other behaviours remain constant for the same time. Isotemporal substitution models were adjusted for age, HIV status, education, asset category and employment (model 2 in total movement volume robust regression analyses), and then subsequently for FMI. Due to marked differences in levels of PA and adiposity between men and women (table 4.1), all analyses were stratified by sex. A p–value of <0.05 was considered significant and 95% CI stated.

Results

Sample characteristics

Physical behaviours, body fat, and measures of glycaemia and insulin dynamics for the whole sample, and men and women separately, are presented in Table 4.1. Men and women were of similar age (~53 years) and a similar proportion were living with HIV (21.1% vs. 19.8%, p=0.657). Women had higher BMI and DXA-derived measures of fat mass, FMI, VAT and SAT than men (all p<0.001).

There were no sex differences in LPA or sleep time, but men had higher total movement volume (mg) and spent more time in MVPA than women (both $p<0.001$). Men spent more time sitting/lying and less time standing than women (both $p<0.001$). There were no sex differences in fasting or 2-hour glucose, while all the measures of insulin dynamics were different between the sexes, with women having higher HOMA-IR, insulin secretion and beta-cell function, and lower insulin sensitivity and basal insulin clearance than men.

Associations between total movement volume and measures of glycaemia and insulin dynamics

In men and women, total movement volume was inversely associated with fasting glucose and positively associated with insulin sensitivity in models adjusted for age, HIV status and socio-economic status (SES) (Table 4.2). After adjusting for FMI and VAT these associations were no longer significant. In men only, total movement volume was also inversely associated with 2-h glucose, but after adjusting for FMI and VAT this association was no longer significant. In women only, total movement volume was also significantly associated with basal insulin clearance, and this remained significant when adjusting for FMI and VAT. There was no association between total movement volume and beta-cell function in either men or women.

Isotemporal substitution of physical behaviours in men

Replacing 30 minutes of sitting/lying, standing or LPA with 30 minutes of MVPA was associated with 1.2–1.4 mmol/L lower fasting glucose and 12.3–13.4 mgl^2/mUmin higher insulin sensitivity in men (Table 4.3 and 4.4). Replacing 30 minutes of sitting/lying with the same amount of time standing or in MVPA was associated with 1.4 mmol/L and 3.3 mmol/L

lower 2-hour glucose, respectively. Although replacing 30 minutes of LPA with the same amount of time in MVPA was associated with 3.5 mmol/L lower 2-hour glucose, it was also associated with 1.6 mmol/L lower 2-hour glucose when replaced by standing. Replacing 30 minutes of sitting/lying and LPA with the same amount of time in MVPA was associated with 17 mIU/mmol and 19.1 mIU/mmol higher beta-cell function, respectively. Replacing physical behaviours was not associated with basal insulin clearance in men.

After further adjusting for FMI, the associations between physical behaviours and fasting glucose, insulin sensitivity, and beta-cell function were no longer significant (data not shown). However, replacing 30 minutes of sitting/lying with 30 minutes of standing time (1.3 mmol/L, CI: 0.4–2.2, p=0.003) and replacing 30 minutes of standing with the same amount of LPA time (1.7 mmol/L, CI: 0.4–3.0, p=0.007) both remained significantly associated with 2-hour glucose after adjusting for FMI.

Isotemporal substitution of physical behaviours in women

Replacing 30 minutes of sitting/lying with standing or LPA were associated with 0.5 mmol/L and 0.8 mmol/L lower fasting glucose, respectively, but were not associated with 2-hour glucose. Replacing 30 minutes of sitting/lying with the same amount of time standing was associated with 3.2 mgl^2/mUmin higher insulin sensitivity, 0.05 ng/mIU higher basal insulin clearance, and 11.3 mIU/mmol higher beta-cell function. Replacing 30 minutes of sitting/lying or standing or LPA with the same amount of time in MVPA was associated with 0.25–0.30 ng/mIU higher basal insulin clearance (Table 4.3 and 4.4).

After further adjusting for FMI, the associations with fasting glucose, 2-hour glucose, and insulin sensitivity were no longer significant (data not shown). In contrast, the significant associations with basal insulin clearance were maintained when 30 minutes of sitting/lying (0.21 ng/mIU, CI: 0.41–0.02, p=0.029) or standing (0.19 ng/mIU, CI: 0.39–0.002, p=0.048) were replaced by MVPA. Replacing 30 minutes of sitting/lying with the same amount of standing was also still associated with higher beta-cell function (8.68 mIU/mmol, CI: 0.93–16.44, p=0.028) after adjusting for FMI.

Discussion

This study in a middle-aged African population of men and women showed that physical activity was associated with lower risk for T2DM. In support of our hypothesis, we showed that replacing 30 minutes of sedentary time or LPA with the same amount of time in higher intensity behaviours was associated with a reduction in the risk markers for T2DM. Specifically, substituting 30 minutes of awake sitting/lying, standing or LPA with the same amount of time in MVPA in men was associated with lower fasting glucose and higher insulin sensitivity, both well-accepted indicators of T2DM risk. In women, just replacing 30 minutes of sitting/lying with the same amount of time standing was associated with lower fasting glucose and higher insulin sensitivity. We have also shown that the associations between physical behaviours and these measures of T2DM risk are mediated by adiposity in both men and women.

In both men and women our study showed that total movement volume was associated with lower fasting glucose and higher insulin sensitivity, and in men only was also associated with lower 2-hr glucose, although none of these associations remained significant after adjusting for adiposity. Physical activity through its effects on multiple organs and systems is associated with

improved insulin sensitivity and glycaemia (5,36). Data from the EPIC-InterAct case-control study of incident T2DM reported a significant reduction in the risk of developing T2DM in men and women who had higher levels of physical activity (37). Indeed, epidemiological studies have shown that PA reduces the risk of insulin resistance and T2DM in healthy individuals and appropriate exercise training is an effective intervention for individuals at risk of T2DM (38,39). Physical activity can reduce the risk of T2DM in men and women with high body mass index and elevated glucose levels. Even relatively modest exercise can stimulate immediate and persistent insulin sensitivity the next day in adults at risk of T2DM (40). The men in our study completed on average 410 minutes of MVPA/week while average MVPA/week in the women was 240 minutes, which may account for the additional effect of PA on postprandial glucose uptake, with skeletal muscle being the major site of uptake. Extensive literature has explored the association between objectively measured physical activity and measures of T2DM risk (4,8,9). Not all of these studies have accounted for adiposity, while others have found the association to be mediated by adiposity (36), and still other studies have found the significant association between PA and T2DM remains after adjusting for adiposity (37,41). The differences in the results may be due to marked heterogeneity in research designs and assessment of physical behaviours and T2DM risk. In our study, the associations between PA and T2DM risk in both men and women were mediated by total adiposity. Balkau et al. reported that objectively measured daily total activity and the association with insulin sensitivity in healthy adult men and women between 30–60 years remains even after adjusting for overall body mass index or abdominal adiposity (42).

Using the combination of signals from two accelerometers for objective measurement of physical behaviours, we used isotemporal substitution to account for the displacement of time in particular behaviours within a 24-hr day (11,14). We showed that replacing sitting/lying with standing time was only associated with lower 2-hour glucose in the men, while in women, replacing sitting with the same amount of time standing was associated with improvements in fasting glucose, insulin sensitivity, insulin clearance and beta-cell function. This difference when modelling the hypothetical effect of posture change resulting in all aspects of insulin dynamics being affected in women compared to only post-prandial glucose in men may be due to the higher adiposity in women. An Australian study in men and women (36-80 years) using the isotemporal substitution approach, showed that replacing sitting with standing for 2 h/day was associated with 2% lowering of fasting plasma glucose in both men and women (43). These differences may be accounted for by the vast disparities in adiposity between men and women in our study compared to the study in Australia.

Replacing sedentary time i.e. sitting or standing with movement, irrespective of intensity, has been associated with a wide array of health benefits. Yates et al., reported that reallocating 30 minutes per day of sedentary time to LPA and MVPA was associated with a 5% and 18% difference in insulin sensitivity (Matsuda-ISI) in adult men and women of average age 65 years after adjusting for ethnicity, sex, age, medication, social deprivation and BMI (44). In a South African study, LPA has been shown to be associated with reduced cardiovascular disease risk (45) and has also been included in the recent WHO recommendations for physical activity (46). Interestingly in this study, the only significant associations when replacing sedentary behaviour with LPA was a decrease in fasting glucose in the women only when replacing sitting with

LPA, and an increase in 2-hour glucose in the men when replacing standing with LPA. The men and women in this study spent an average of 2 hours a day in LPA, and previous studies in similar populations has shown that this is largely time spent in incidental activity or walking for transport rather than leisure time activity which is typically low in populations from low and middle income countries (18,47). As physical behaviours have been accurately measured in this study by combining the signals from two accelerometers, it can be concluded time spent in LPA in this population is not sufficient to influence diabetes risk significantly.

Time spent in higher intensity physical activity, in many studies described as MVPA, has been repeatedly shown in the literature to be associated with lower T2DM risk (3,6,48). In this study replacing sitting/lying, standing and LPA with MVPA was associated with improvements in the measures of T2DM risk in men including a decrease in fasting glucose and 2-h glucose, and an increase in insulin sensitivity and beta cell function. In contrast in the women, replacing sitting/lying, standing and LPA with MVPA was only associated with an increase in basal insulin clearance. Although we did not specifically examine time spent in vigorous intensity physical activity, previous research has shown that SA men spend more time in higher intensity activity than women (49), which may have explained the greater associations between MVPA and diabetes risk in men than women. Another explanation may be the 'legacy effect' of earlier patterns of activity which have been shown to be higher in men compared to women throughout adolescence in a longitudinal South African cohort (47), and which may then result in alterations in muscle physiology and improved cardiorespiratory fitness that may be maintained into adulthood. This is consistent with the higher levels of cardiorespiratory fitness in young adult men compared to women from Soweto (49), and another study from Cape Town that

reported very low levels of cardiorespiratory fitness in young women (45). Indeed, cardiorespiratory fitness, and not objectively measured MVPA, has previously been associated with higher insulin sensitivity in black African women (45). Results from an exercise intervention study in black African women with obesity and low baseline levels of cardiorespiratory fitness demonstrated that 12 weeks of intensive training only resulted in a small improvement in insulin sensitivity with no changes in glycaemia (50). This may be explained by low levels of fitness and high levels of obesity and insulin resistance in these women (45).

Another novel finding of the study was the improvement in insulin clearance when replacing sitting/lying, standing, and LPA, with MVPA, in women only. Previous research has shown that black South African women have low insulin clearance compared to their European counterparts, which contributes to their characteristic hyperinsulinemia (51). Further, we showed that women had lower insulin clearance compared to men. It is still unclear if low insulin clearance and/or the resultant hyperinsulinemia is a compensatory response to low insulin sensitivity or is the driver of T2D in the Black African women (51). Nonetheless, this is the first study to show that MVPA is associated with higher insulin clearance, independent of the effects of adiposity, and that this association is specific to women and not observed in men. Prospective studies are required to investigate whether an increase in insulin clearance, and consequently a reduction in hyperinsulinemia, does confer reduced risk from T2D in black Africans.

The strengths of this study included the combination of signals from two accelerometers for objective measurement of physical behaviours. Most studies have used regression analysis to determine the associations between PA and diabetes risk. Our study used isotemporal substitution and 30 minutes as this links closely with public health recommendations. These results highlight the importance of measuring different physical activity behaviours, which are differentially associated with glycaemia and insulin dynamics. Notably, we included both men and women and showed differences in the relationship between physical activity behaviours and diabetes risk. The cross-sectional design is a limitation of the study. Self-reported sleep diaries are prone to recall bias, and social desirability coupled with reporting of time in bed, rather than sleep duration, may introduce misclassification. However, the accelerometry adjunct to sleep diaries assisted in verifying the sleep classification.

In conclusion, this study provides novel evidence on the potential benefits of engaging in more active behaviours on risk factors for T2DM in men and women. This is critical in public health given the high amount of time spent sitting/lying and standing in our study population. Further, longitudinal and intervention research is required to determine whether the effects of different intensity physical behaviours are sex-specific and need to be taken into account when designing public health interventions to reduce non-communicable disease risk.

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Author contributions: CNK analysed the data. AS and KW processed the physical activity. CNK, JHG, SB and LKM conceived the study and CNK drafted and revised the manuscript under the supervision of JHG, SB and LKM. All authors read and approved the final version of the manuscript.

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Table 4.1: Body fat distribution and physical behaviours in men and women

Characteristic	n	Men (n=403)	Women (n=324)	p-value
Age (years)	727	53.3±6.2	53.7±5.9	0.450
Height (cm)	727	171.1±6.5	158.3±6.1	<0.001
Weight (kg)	727	73.7±17.7	83.5±18.8	<0.001
BMI (kg/m^2)	727	25.2±5.8	33.3±7.0	<0.001
Accelerometry (minutes/day)				
LPA	727	116.5±77.4	121.3±49.6	0.333
MVPA	727	58.6±42.8	34.3±23.2	<0.001
Sitting/lying	675	645.9±124.0	613.9±128.7	0.001
Standing	675	197.5±86.7	258.1±99.3	<0.001
Sleep	727	421.0±84.3	409.5±75.9	0.057
Total movement volume (mg)	727	15.1±6.0	12.4±3.5	<0.001
DXA				
Fat Mass (FM, kg)	692	19.2±8.4	36.5±10.7	<0.001
Fat Mass Index (FMI, kg/m^2)	692	6.6±2.9	14.6±4.2	<0.001
VAT (cm^2)	692	83.4±42.8	102.7±43.7	<0.001
SAT (cm^2)	692	194.4±122.0	456.9±149.1	<0.001
Glycaemia and insulin dynamics				
Fasting glucose (mmol/L)	725	5.0±1.0	5.1±0.9	0.692
2-h glucose (mmol/L)	720	5.9±2.5	6.3±2.3	0.064
Fasting insulin (mIU/ml)	723	5.1 (2.1–10.3)	8.9 (5.2–14.5)	<0.001
Fasting C–peptide (ng/ml)	723	1.5 (1.1–2.3)	1.8 (1.3–2.5)	<0.001
HOMA–IR	723	1.1 (0.5–2.3)	1.9 (1.1–3.4)	<0.001
Insulin sensitivity (mgl^2/mUmin)	719	7.5 (4.0–14.0)	5.0 (3.1–8.2)	<0.001
Insulin secretion (ng/mmol)	628	2.3 (1.3–3.8)	3.1 (1.8–5.1)	<0.001
Basal insulin clearance (ng/mIU)	723	0.29 (0.20–0.40)	0.20 (0.15–0.26)	<0.001
Beta-cell function (mIU/mmol)	610	107.0 (56.6–195.2)	138.2 (61.1–251.3)	0.046

Values for DXA, glycaemia and insulin dynamics are mean±standard deviation (SD) or median (25th – 75th)
HOMA–IR: Homeostasis model assessment of insulin resistance

Table 4.2: Multiple robust regression analyses of total movement volume and measures of glycaemic and insulin dynamics

		Men		Women	
Model 1	n	**β**	**95%CI**	**β**	**95%CI**
Fasting glucose (mmol/L)	725	**-0.014**	**-0.028 to -0.003**	**-0.031**	**-0.054 to -0.009**
2-h glucose (mmol/L)	720	**-0.033**	**-0.063 to -0.002**	-0.052	-0.109 to 0.004
Insulin sensitivity (mgl^2/mUmin)	719	**0.240**	**0.138 to 0.343**	**0.162**	**0.049 to 0.275**
Basal insulin clearance (ng/mIU)	723	0.001	-0.001 to 0.003	**0.004**	**0.002 to 0.006**
Beta-cell function (mIU/mmol)	628	0.129	-0.079 to 0.338	0.336	-0.095 to 0.768
Model 2					
Fasting glucose (mmol/L)	726	**-0.014**	**-0.027 to -0.0004**	**-0.026**	**-0.050 to -0.002**
2-h glucose (mmol/L)	720	**-0.033**	**-0.065 to 0.002**	-0.042	-0.103 to 0.018
Insulin sensitivity (mgl^2/mUmin)	719	**0.199**	**0.096 to 0.301**	**0.138**	**0.022 to 0.255**
Basal insulin clearance (ng/mIU)	723	0.001	-0.002 to 0.003	**0.005**	**0.002 to 0.007**
Beta-cell function (mIU/mmol)	628	0.168	-0.049 to 0.385	0.393	-0.070 to 0.855
Model 3					
Fasting glucose (mmol/L)	690	-0.006	-0.019 to 0.008	-0.019	-0.041 to 0.008
2-h glucose (mmol/L)	685	-0.007	-0.038 to 0.025	-0.011	-0.073 to 0.050
Insulin sensitivity (mgl^2/mUmin)	684	0.073	-0.015 to 0.161	0.071	-0.059 to 0.201
Basal insulin clearance (ng/mIU)	688	-0.001	-0.003 to 0.002	**0.003**	**0.0001 to 0.005**
Beta-cell function (mIU/mmol)	599	-0.006	-0.227 to 0.215	0.306	-0.194 to 0.807
Model 4					
Fasting glucose (mmol/L)	690	-0.004	-0.018 to 0.009	-0.013	-0.037 to 0.011
2-h glucose (mmol/L)	685	-0.0003	-0.0311 to 0.0317	-0.003	-0.062 to 0.057
Insulin sensitivity (mgl^2/mUmin)	684	0.053	-0.034 to 0.141	0.072	-0.052 to 0.195
Basal insulin clearance (ng/mIU)	688	-0.001	-0.003 to 0.002	**0.003**	**0.0001 to 0.005**
Beta-cell function (mIU/mmol)	599	-0.061	-0.286 to 0.164	0.236	-0.241 to 0.713

Beta coefficients represent the difference in the outcomes listed per 1 mg difference in movement volume; bold represents significant associations.
Model 1: included age
Model 2: included age, HIV status, education, asset category and employment,
Model 3: included age, HIV status, education, asset category and employment and FMI,
Model 4: included age, HIV status, education, asset category and employment, FMI and VAT

Table 4.3: Associations of reallocating 30 minutes of physical behaviours on glycaemia for men and women

		β	95%CI	β	95%CI
	Fasting glucose (mmol/L)		**Men (n=372)**		**Women(n=301)**
From SITTING/LYING to:	SLEEP	-0.106	-0.561 to 0.348	-0.435	-0.956 to 0.085
	STANDING	-0.317	-0.270 to 0.534	**-0.547**	**-0.908 to -0.187**
	LPA	-0.062	-0.528 to 0.040	**-0.790**	**-1.553 to -0.028**
	MVPA	**-1.254**	**-2.148 to -0.359**	0.389	-1.287 to 2.064
From STANDING to:	SLEEP	-0.238	-0.810 to 0.333	0.112	-0.458 to 0.681
	SITTING/LYING	-0.132	-0.534 to 0.270	**0.547**	**0.187 to 0.908**
	LPA	-0.194	-0.781 to 0.392	-0.242	-1.114 to 0.629
	MVPA	**-1.386**	**-2.338 to -0.433**	0.937	-0.795 to 2.667
From LPA to:	SLEEP	-0.044	-0.583 to 0.494	0.355	-0.510 to 1.220
	SITTING/LYING	0.062	-0.404 to 0.529	**0.790**	**0.028 to 1.553**
	STANDING	0.194	-0.392 to 0.781	0.242	-0.629 to 1.114
	MVPA	**-1.191**	**-2.243 to -0.139**	1.179	-0.868 to 3.227
From MPVA to:	SLEEP	1.147	0.177 to 2.117	-0.824	-2.620 to 0.971
	SITTING/LYING	**1.254**	**0.359 to 2.148**	-0.389	-2.065 to 1.286
	STANDING	**1.385**	**0.433 to 2.338**	-0.936	-2.669 to 0.795
	LPA	**1.191**	**0.139 to 2.243**	-1.179	-3.227 to 0.868
	2-h glucose (mmol/L)		**Men (n=371)**		**Women(n=298)**
From SITTING/LYING to:	SLEEP	-1.297	-2.325 to -0.269	-1.198	-2.253 to 0.126
	STANDING	**-1.385**	**-2.293 to -0.476**	-0.877	-1.791 to 0.035
	LPA	0.199	-0.854 to 1.253	-1.203	-3.134 to 0.726
	MVPA	**-3.261**	**-5.289 to -1.232**	1.102	-3.147 to 5.349
From STANDING to:	SLEEP	0.087	-1.204 to 1.380	-0.320	-1.768 to 1.127
	SITTING/LYING	**1.385**	**0.476 to 2.293**	0.877	-0.035 to 1.791
	LPA	**1.584**	**0.259 to 2.909**	-0.325	-2.534 to 1.882
	MVPA	-1.875	-4.033 to 0.282	1.980	-2.415 to 6.376
From LPA to:	SLEEP	-1.496	-2.838 to -0.279	0.005	-2.190 to 2.201
	SITTING/LYING	-0.199	-1.253 to 0.854	1.203	-0.726 to 3.134
	STANDING	**-1.584**	**-2.909 to -0.259**	0.325	-1.882 to 2.534
	MVPA	**-3.460**	**-5.845 to -1.075**	2.306	-2.882 to 7.494
From MVPA to:	SLEEP	1.963	-0.237 to 4.165	-2.300	-6.864 to 2.262
	SITTING/LYING	**3.261**	**1.232 to 5.289**	-1.102	-5.351 to 3.147
	STANDING	1.875	-0.282 to 4.033	-1.980	-6.376 to 2.415
	LPA	**3.460**	**1.075 to 5.845**	-2.306	-7.494 to 2.882

Models are adjusted for age, HIV status, education, asset category and employment; bold represents significant associations.

Table 4.4: Associations of reallocating 30 minutes of physical behaviours on insulin dynamics for men and women

		β	95%CI	β	95%CI
Insulin Sensitivity (mgl^2/mUmin)			**Men (n=371)**		**Women (n=297)**
From SITTING/LYING to:	SLEEP	2.507	-0.988 to 6.003	-0.986	-3.631 to 1.658
	STANDING	-0.685	-3.775 to 2.403	**3.229**	**1.404 to 5.054**
	LPA	0.366	-3.217 to 3.951	-0.054	-3.907 to 3.798
	MVPA	**12.714**	**5.819 to 19.610**	6.866	-1.612 to 15.345
From STANDING to:	SLEEP	3.193	-1.201 to 7.587	-4.216	-7.105 to -1.326
	SITTING/LYING	0.685	-2.403 to 3.775	**-3.229**	**-5.054 to -1.404**
	LPA	1.052	-3.452 to 5.557	-3.284	-7.692 to 1.124
	MVPA	**13.400**	**6.063 to 20.738**	3.636	-5.136 to 12.409
From LPA to:	SLEEP	2.140	-1.997 to 6.279	-0.932	-5.316 to 3.452
	SITTING/LYING	-0.366	-3.951 to 3.217	0.054	-3.798 to 3.907
	STAND	-1.052	-5.557 to 3.452	3.284	-1.124 to 7.692
	MVPA	**12.348**	**4.239 to 20.457**	6.920	3.431 to 17.272
From MVPA to:	SLEEP	-10.207	-17.691 to -2.722	-7.852	-16.960 to 1.254
	SITTING/LYING	**-12.714**	**-19.610 to -5.818**	-6.866	-15.345 to 1.612
	STANDING	**-13.400**	**-20.738 to -6.063**	-3.636	-12.409 to 5.136
	LPA	**-12.348**	**-20.457 to -4.239**	-6.920	-17.272 to 3.431
Basal Insulin Clearance (ng/mIU)			**Men (n=371)**		**Women (n=300)**
From SITTING to:	SLEEP	0.030	-0.052 to 0.113	-0.009	-0.070 to 0.051
	STANDING	-0.003	-0.077 to 0.069	**0.048**	**0.006 to 0.090**
	LPA	0.008	-0.076 to 0.093	0.014	-0.074 to 0.104
	MVPA	0.026	-0.137 to 0.190	**0.298**	**0.101 to 0.495**
From STANDING to:	SLEEP	0.034	-0.069 to 0.138	-0.058	-0.125 to 0.008
	SITTING/LYING	0.003	-0.069 to 0.077	**-0.048**	**-0.090 to -0.006**
	LPA	0.012	-0.094 to 0.119	-0.033	-0.136 to 0.068
	MVPA	0.030	-0.143 to 0.204	**0.250**	**0.046 to 0.453**
From LPA to:	SLEEP	0.022	-0.076 to 0.120	-0.024	-0.126 to 0.077
	SITTING/LYING	-0.008	-0.093 to 0.076	-0.014	-0.104 to 0.074
	STANDING	-0.123	-0.119 to 0.094	0.033	-0.068 to 0.136
	MVPA	0.018	-0.174 to 0.210	**0.284**	**0.043 to 0.524**
From MVPA to:	SLEEP	0.004	-0.173 to 0.181	-0.308	-0.519 to -0.097
	SITTING/LYING	-0.026	-0.190 to 0.137	**-0.298**	**-0.495 to -0.101**
	STANDING	-0.030	-0.204 to 0.143	**-0.250**	**-0.453 to -0.046**
	LPA	-0.018	-0.210 to 0.174	**-0.284**	**-0.524 to -0.043**
Beta-cell function (mIU/mmol)			**Men (n=329)**		**Women (n=256)**
From SITTING/LYING to:	SLEEP	3.505	-3.687 to 10.698	-1.843	-11.917 to 8.231

	STANDING	3.827	-2.535 to 10.191	**11.304**	**4.210 to 18.397**
	LPA	-2.088	-9.269 to 5.091	2.960	-12.031 to 17.951
	MVPA	**16.996**	**2.886 to 31.104**	8.607	-23.341 to 40.556
From STANDING to:	SLEEP	0.322	-9.519 to 8.874	-13.147	-24.246 to -2.048
	SITTING/LYING	-3.827	-10.191 to 2.535	**-11.304**	**-18.397 to -4.210**
	LPA	-5.915	-15.069 to 3.237	-8.343	-25.643 to 8.955
	MVPA	13.168	-1.790 to 28.127	-2.696	-35.778 to 30.384
From LPA to:	SLEEP	5.593	-2.715 to 13.903	-4.803	-21.936 to 12.329
	SITTING/LYING	2.088	-5.093 to 9.269	-2.960	-17.951 to 12.031
	STANDING	5.915	-3.237 to 15.069	8.343	-8.955 to 25.643
	MVPA	**19.084**	**2.562 to 35.606**	-5.647	-33.324 to 44.618
From MVPA to:	SLEEP	-13.491	-28.962 to 1.980	-10.450	-44.714 to 23.813
	SITTING/LYING	**-16.996**	**-31.106 to -2.886**	-8.607	-40.556 to 23.341
	STANDING	-13.168	-28.127 to 1.790	2.696	-30.384 to 35.778
	LPA	**-19.084**	**-35.606 to -2.562**	-5.647	-44.618 to 33.324

Models adjusted for age, HIV status, education, asset category and employment

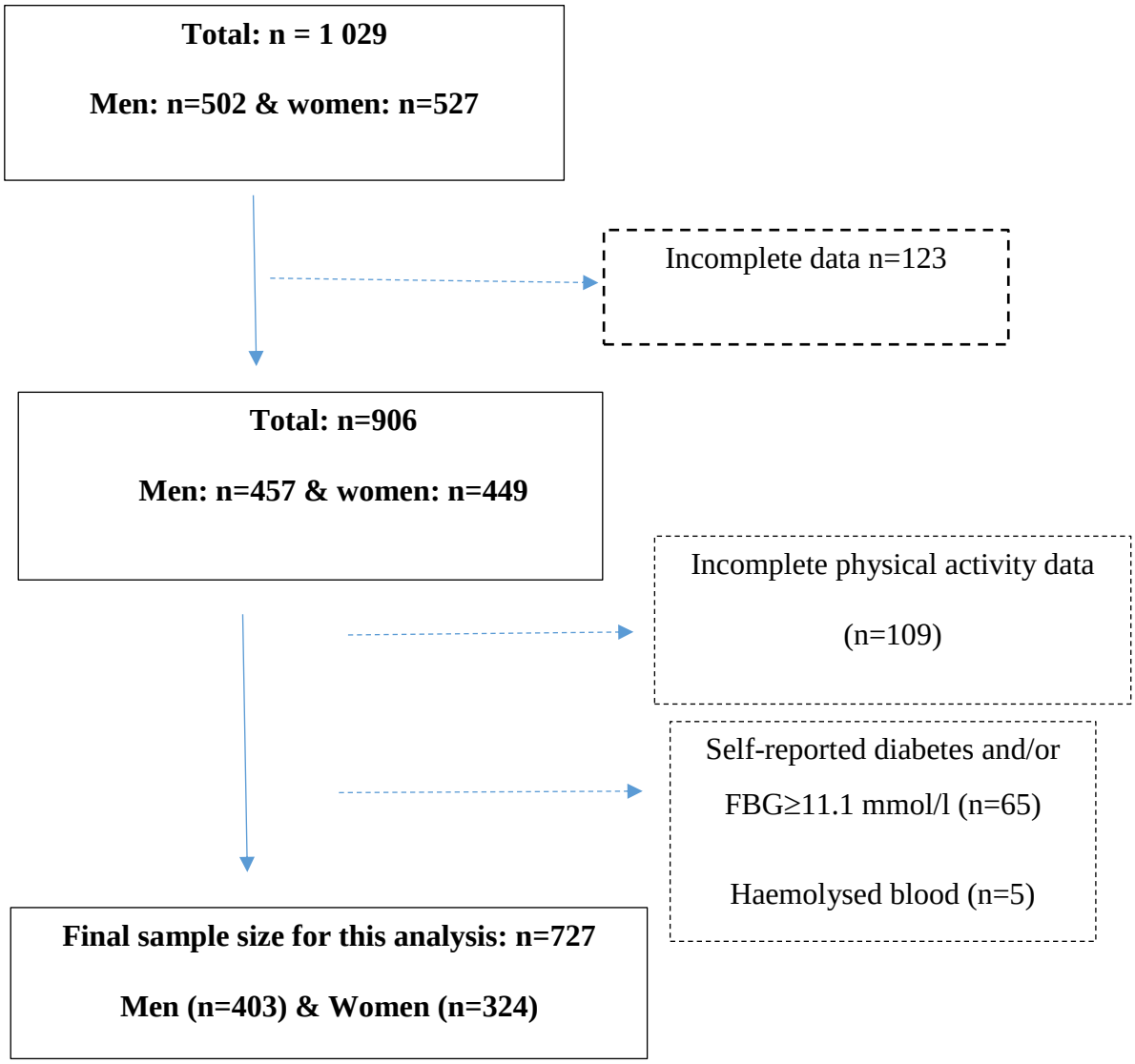

Figure 4.1: Sample selection flow chart 3

CHAPTER 5

SUMMARY CONCLUSIONS AND RECOMMENDATIONS

This thesis aimed to determine whether markers of type 2 diabetes mellitus risk are different between middle-aged Black South Africa men and women, and between women at different stages of the menopause transition, and whether there are sex-specific associations between these T2DM markers and body fat distribution and physical behaviours.

The key findings relating to the specific objectives are summarised below in table 5.1.

5.1 Summary of the objectives and key findings

Table 5.1: Summary of objectives and key findings

Chapter	Objectives	Key findings
2	To explore sex differences in insulin sensitivity, secretion, clearance and beta-cell function, and sex-specific associations with total and regional fat distribution	• Mean body mass index and fat mass were higher in women than men and a larger proportion of women than men presented with obesity (70.2% vs. 26.6%). • After adjusting for differences in body fat, insulin sensitivity, secretion and beta cell function were lower in men compared to women, while insulin clearance was not different. • The strength of the association between total adiposity and measures of T2DM risk were greater in men than women.
3	To describe differences in total and regional adiposity, and glycaemic and insulin measures, between pre-menopausal and early and late post-menopausal Black South African women	• Body mass index and total and regional adiposity measures were similar between the menopausal groups. • HbA1c and iAUC for glucose were significantly higher in the late post-menopausal than the pre-menopausal group but this was no longer significant after adjusting for age. • Despite similarities in insulin sensitivity and clearance between the menopausal groups, insulin secretion and beta cell function were

		higher in the early compared to the late post-menopause group before and after adjusting for age.
4	To determine the associations between physical behaviours and measures of T2DM risk in middle-aged Black South African men and women using isotemporal substitution.	• Total movement volume was associated with lower fasting and 2-h glucose concentrations and with higher insulin sensitivity, basal insulin clearance and beta-cell function, however these associations were not independent of fat mass. • In men, replacing 30 minutes of sitting/lying, standing or LPA with the same amount of MVPA time was associated with lower fasting glucose and higher insulin sensitivity. • In women, substituting sitting/lying with the same amount of standing time or LPA was associated with lower fasting glucose. Substituting 30 minutes sitting/lying with the same amount of standing time was also associated with higher insulin sensitivity, and substituting 30 minutes of sitting/lying, standing or LPA with the same amount of MVPA time was associated with higher basal insulin clearance in women.

5.2 Summary of results

The findings of this thesis have provided insights on insulin sensitivity, secretion, clearance and beta-cell function in middle-aged Black SA men and women. The first study (chapter 2) of this thesis showed that although men have a lower mean BMI than women they have a more "unfavourable" fat distribution ie. more central fat mass and less peripheral fat mass. Related to their higher BMI, women have lower insulin sensitivity and a higher compensatory insulin response due to higher insulin secretion than men. However, for the same level of body fatness, Black South African men are less insulin sensitive and have lower insulin secretion and beta-cell function than women. Further the strength of the association between adiposity and T2DM risk is greater in men compared to women which suggests that with increasing adiposity, particularly an increase in central adiposity, Black SA men may have an increased risk for T2DM when compared to their female counterparts.

The second study (chapter 3) explored the menopausal transition in the women to determine whether there were differences in body fat distribution and markers of T2DM risk between women at different stages of the menopausal transition. The mean BMI of the women in this study was over 30 kg/m^2 which could explain why there were no differences in either total or regional adiposity between the menopausal groups. Interestingly however both insulin secretion and beta cell function were lower in the late compared to the early post-menopausal women, even after adjusting for age, which may explain the higher postprandial glycaemia in this group, although this difference was not independent of age. Commensurate with previous studies in SA including mainly pre-menopausal women, we show that middle-aged Black SA women have low insulin sensitivity and present with hyperinsulinaemia, due to a reduced insulin clearance and high insulin secretion. However, with increasing age, and the transition

to the late post-menopausal period, insulin secretion, and not clearance declines. The decrease in beta cell function corresponds to an increase in glycaemia and risk for T2DM. Accordingly, interventions to reduce the burden on the beta-cell are required for the prevention of T2DM in Black African women.

Finally, the third study (chapter 4) explored the associations between physical behaviours and measures of T2DM risk markers. Total movement volume was inversely associated with measures of fasting and 2-h glucose and directly associated with insulin sensitivity, basal insulin clearance and beta-cell function independent of body fat mass. This study provides novel evidence on the potential benefits of engaging in more active behaviours on risk factors for T2DM in our sample of Black, South African men and women which is critical in public health given the high amount of sedentary behaviour in our study population. However, the intensity of the physical behaviours necessary in order to improve the T2DM risk profiles of men and women seems to differ. While women only need to replace sitting/lying with a more active physical behaviour, men are required to replace less active behaviours with MVPA in order to reduce risk. But at the same time, men sit more which could be very problematic. This may have implications for public health practitioners, clinicians and policy makers in designing interventions, prevention and education programmes to reduce the health burden from diabetes and its complications in a population, although intervention studies are required. These programmes should target individuals at risk early enough to reduce the economic and adverse health risk outcomes from diabetes later on in life.

The results have clinical implications. With the general upward trend in adiposity especially central adiposity Black South African men are at increased risk of T2DM than women. Menopausal transition in Black women with high prevalence of overweight and obesity is

accompanied by lower insulin secretion in late post-menopausal women which may explain higher postprandial glycaemia and lower insulin secretion with increasing age. Engaging in more active behaviours has potential benefits on the risk factors for T2DM in men and women.

5.3 Strengths and limitations of the thesis

5.3.1 Strengths

One of the major strengths of this thesis was the inclusion of very accurate and robust measures of the various exposures and the markers of T2DM risk. This included measuring body composition and regional adiposity using dual energy x-ray absorptiometry (DXA), and the oral glucose tolerance test to measure the various markers of T2DM risk. Equal numbers of men and women were included in this study and sex comparisons in risk factors and markers, for which there is a dearth of information were used. Further, the use of objective measure of physical behaviours by combining the signals from two accelerometers which was shown to be accurate in describing and distinguishing postural changes particularly lying/sitting to standing. Validated questionnaires were used to collect data on potential confounders such as sociodemographic, asset category and employment, and lifestyle factors.

The statistical analyses explored sex-specific associations and differences were also a strength of this thesis. The Z–scores were derived for total as well as regional adiposity measures for the combined sample and stratified by sex using Fisher's and Yate's transformation which permitted the determination of risk magnitude per 1 SD change to directly compare relationships between different regional adiposity measures and glycaemic and insulin dynamics. Further, for the physical behaviours analyses, isotemporal substitution was used, and 30 minutes was selected to link better to public health messaging and recommendations.

By using the isotemporal substitution approach the theoretical effect of reallocating 30 minutes of one physical behaviour to 30 minutes of another physical behaviour was determined. Accordingly, regression coefficients and 95% confidence interval represented the replacement of one physical behaviour with another when the other behaviours remain constant over the same time, adjusted for potential confounders. Robust regression analysis which is less sensitive to not normally distributed data and outliers than standard linear regression was used. These analyses were stratified by sex due to differences in levels of physical activity and adiposity in men and women.

Our sample reflects Black South Africans and our findings are generalizable to middle-aged Black South African populations.

5.3.2 Limitations

This a cross-sectional study and causal conclusions cannot be made from the results. Another limitation of this study was the exclusion of the HIV positive participants as it is well accepted that body fat distribution as well as insulin sensitivity and response may be influenced by not only the infection itself but also the effects of and duration of ART. Although the sex differences in obesity and total adiposity may be seen as a limitation due to marked differences in men and women, it reflects the status of obesity within South Africa and the sub-Saharan African region(236) (233), and adjustments for total body fatness and the calculation of z-scores were used in the analyses. Also, the premenopausal women were not tested at a specific time during their menstrual cycle, which is noted as a limitation of the study. Furthermore, the findings relate to middle-aged Black SA men and women may not be generalizable to the general Black African populations. Although detailed measures of physical behaviours were

used, the influence of dietary intake was not explored, which impacts on body composition and T2DM risk. Medication use, comorbidity, socio-economic status, tobacco consumption, alcohol intake and family history were not adjusted for in the analysis. The large confidence intervals of some variables reflecting large variability in the results is also another limitation of this thesis. However, the findings add to our understanding of the pathophysiology of T2DM in Black men and women. Direct measures of insulin sensitivity and response by the Gold standard or FSIVGTT was not done however, these have been shown to correlate closely with the OGTT used in this study (46). Moreover, they provide a more physiological assessment and may be more appropriate in Black African populations who present with a hyperinsulinaemic response to glucose (15).

5.4 Future research and recommendations

This study has highlighted sex-specific differences in the associations between body fat and its distribution, and objectively measured physical behaviours, and markers of T2DM in Black men and women. I have also shown differences in some of these markers between men and women, as well as between menopausal groups. After this study I propose the following to fill in the research gaps:

Prospective studies to measures the change in body composition and physical behaviours in both men and women and how these are associated, not only with markers of T2DM risk but also the disease itself, which is expected to increase in prevalence with increasing age.

The prevalence of HIV is about 20% in SA. HIV positive individuals should be included in future studies to elucidate the influence of HIV infection, ART and duration on ART on body fat distribution and its association with insulin sensitivity and response.

Given the disparate socio-economic conditions among South Africans, future studies should include measures of dietary intake that impact body fatness and risk for T2DM, especially as African populations transition to "western'' lifestyles. Other ethnic groups whose body composition, fat distribution, insulin sensitivity and response may not be same as Black SA men and women should be included in future studies for comparison and generalisability.

Further research should also include genetic traits, epigenetics and other environmental factors such as higher levels of walkability, green space and increased levels of noise and air pollution which remain largely unexplored and may influence glycaemic and insulin dynamics.

5.5 Conclusions

My thesis is based on middle-aged Black South African men and women living in Soweto, Johannesburg, South Africa. This thesis has demonstrated the importance of body composition and body fat distribution in insulin sensitivity and response, and at the same time how physical behaviours and the menopausal transition can influence insulin sensitivity and response. It demonstrates the sex differences in T2DM risk and highlights future risks associated with increasing adiposity especially in men. With increasing urbanisation, the changing lifestyle of many citizens with a tendency to consume a westernised diet and increase sedentary behaviour is likely that the increase in the prevalence of overweight and obesity and further exacerbate the burden of T2DM.

This thesis contributes to our understanding of insulin sensitivity, insulin secretion, insulin clearance and beta-cell function in Black South African men and women, the results of which may provide information to be used in the formulation of population-specific prospective

studies to better understand insulin sensitivity and response and at the same time provide evidence-informed interventions to reduce the bourgeoning epidemic of T2DM in a population at risk.

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APPENDICES

APPENDIX A: PLAGIARISM DECLARATION

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY:

I, **NYUYKI Clement Kufe** (Student number: **395736**) am a student registered for the degree of

Doctor of Philosophy (PhD) in the academic year 2022.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: 

Date: 31st August 2022_____

APPENDIX B: PLAGIARISM REPORT

PhD THESIS_Clement Kufe_05APRIL2022_TURNITIN2.docx

ORIGINALITY REPORT

18%	17%	19%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.medrxiv.org Internet Source	11%
2	hdl.handle.net Internet Source	2%
3	Clement Kufe, Lisa Micklesfield, maphoko Masemola, Tinashe Chikowore et al. "Increased Risk for Type 2 Diabetes in Relation to Adiposity in Middle-Aged Black South African Men compared to Women", Cold Spring Harbor Laboratory, 2022 Publication	1%
4	Julia H. Goedecke, Tommy Olsson. "Pathogenesis of type 2 diabetes risk in Black Africans: A South African perspective", Journal of Internal Medicine, 2020 Publication	1%
5	Clement N. Kufe, Julia H. Goedecke, Maphoko Masemola, Tinashe Chikowore et al. "Physical behaviours and their association with type 2 diabetes risk in urban South African middle-aged adults: An isotemporal substitution	1%

approach", Cold Spring Harbor Laboratory, 2022
Publication

6	care.diabetesjournals.org Internet Source	1 %
7	eje.bioscientifica.com Internet Source	1 %
8	"Handbook of Growth and Growth Monitoring in Health and Disease", Springer Science and Business Media LLC, 2012 Publication	1 %
9	ajpendo.physiology.org Internet Source	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		

APPENDIX C: HREC (MEDICAL) CLEARANCE CERTIFICATE for main study

R14/49 Prof Lisa Micklesfield

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160604

NAME: (Principal Investigator)	Prof Lisa Micklesfield
DEPARTMENT:	Paediatrics MRC/Wits Developmental Pathways for Health Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Determinants of Type 2 Diabeles Mellitus (T2D) Risk in Middle-Aged Black South African (SA) Men and Woman: Dissecting the Role of Sex Hormones, Inflammation and Glucocorticoids
DATE CONSIDERED:	24/06/2016
DECISION:	Approved with conditions
CONDITIONS:	Providing the name and status of the Doctor once appointed. The MTA must be submitted and approved by the HREC (Medical) before shipment of the specimen.
SUPERVISOR:	
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	26/09/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in June and will therefore be due in the month of June each year.

Principal Investigator Signature Date 26-09-2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX D: PhD HREC (MEDICAL) CLEARANCE CERTIFICATE-Clement Kufe

R14/49 Mr Nyuyki Clement Kufe

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160975

NAME: (Principal Investigator)	Mr Nyuyki Clement Kufe
DEPARTMENT:	Paediatrics and Child Health MRC/Wits Developmental Pathways for Health Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Determinants of Insulin Sensitivity in Middle-Aged Black South African Men and Women: The Role of Sex Hormones, Inflammation, Glucocorticoids and Lifestyle Factors
DATE CONSIDERED:	30/09/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	A/Prof L. Micklesfield and A/Prof J. Goedecke
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	18/01/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in September and will therefore be due in the month of September each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

19/01/2017
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX E: RADIATION AUTHORISATION LETTER

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Radiation and Health Physics Unit,
East Campus

Date: **7th June 2016**

To; Prof. Cleaton – Jones,
Chairman, HREC,
University of the Witwatersrand,
Johannesburg

Research Protocol Title: *Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors.*

Research Protocol Number:

Dear Sir,

I have reviewed the above mentioned research protocol, and I am satisfied that it may proceed subject to the following conditions;

A. No additional requirements ☑

B. The following requirements are needed; ☐

Yours sincerely,
James Larkin,
Director, Radiation and Health Physics Unit

(Digital signature)

James F. S. Larkin
2016.06.07
12:39:06 +02'00'

Radiation and Health Physics Unit, Professional Development Hub, Gate 6, 92 Empire Road, Braamfontein, Johannesburg, South Africa.
Tel: +27 11 717-6931 | Email: james.larkin@wits.ac.za | www.wits.ac.za

APPENDIX F: PERMISSION TO CONDUCT RESEARCH AT THE CHRIS HANI BARAGWANATH HOSPITAL

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 20 July 2016

TITLE OF PROJECT: Determinants of type 2 diabetes mellitus (T2D) risk in middle-aged black South African (SA) men and women: dissecting the role of sex hormones, inflammation and glucocorticoids

UNIVERSITY: Witwatersrand

Principal Investigator: L Micklesfield

Department: DPHRU, Paediatrics

Supervisor (If relevant):

Permission Head Department (where research conducted): Yes

Date of start of proposed study: July 2016
Date of completion of data collection: Dec 2021

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Human Research Ethics Committee of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 20 July 2016

.....
Approved/Not Approved
Hospital Management
Date: 27/07/16

APPENDIX G: PERMISSION LETTER TO USE RESEARCH SITE

MRC/Wits Developmental Pathways for Health Research Unit,
Department of Paediatrics,
School of Clinical Medicine,
Faculty of Health Sciences,
University of the Witwatersrand, Johannesburg
Tel: 011-9331122

05 September 2016

Human Research Ethics Committee (Medical)
University of Witwatersrand
Johannesburg

To whom it may concern

RE: Permission for NYUYKI Clement KUFE to use the research site

I, Prof Shane Norris, Director of the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU), Department of Paediatrics, Faculty of Health Sciences, University of the Witwatersrand hereby give permission to NYUYKI Clement KUFE under the supervision of Associate Professor Lisa Micklesfield and Assoiciate Professor Julia Goedecke to use our research site for his PhD research study titled: **Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors.** NYUYKI Clement KUFE's research is extremely important and will add significantly to the knowledge on type 2 diabetes mellitus in middle-aged black South African women and men. There is little known about black South African men and women insulin resistance, whose phenotype differs to Caucasian men and women, and no data is available on middle-aged black South African men.

If there is any further information that you require, please do not hesitate to contact me.

Sincerely,

Professor Shane Norris
Director
MRC/WITS Developmental Pathways for Health Research Unit
011-9331122
Shane.Norris@wits.ac.za

APPENDIX H: CHANGE OF TITLE

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

20 January 2022
Person No: 395736
TAA

Mr CK Nyuyki
Houghton Village
6 Boundary Rd
Houghton Estate
Berea
2198
South Africa

Dear Mr Clement Kufe Nyuyki

Doctor of Philosophy: Change of title of research

I am pleased to inform you that the following change in the title of your Thesis for the degree of **Doctor of Philosophy** has been approved:

From:	**Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors**
To:	**Insulin sensitivity and response in middle-aged Black South African men and women: associations with body fat distribution, menopause and objectively measured physical behaviours.**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX I: INFORMATION SHEET AND INFORMED CONSENT

Participant's Code:

PARTICIPANT INFORMATION SHEET 1

Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors

Invitation

Hello, my name is Clement NYUYKI and I am a PhD Student at the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU) from the University of the Witwatersrand.

I would like to invite you to participate in the research study entitled; Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors. Before, agreeing to participate, it is important that you understand the purpose of the study, the study procedures, benefits and risks as well as your right to withdraw from the study at any time. If you have any questions, do not hesitate to ask me. You should not agree to take part unless you are satisfied with all the procedures involved. If you decide to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will also be given a copy to keep.

Why is the study being done?

Studies have shown that body composition (the amount of muscle and fat in your body) and body fat distribution (where the fat is located in your body, for example, fat around your stomach) changes as one gets older, and that this is associated with an increased risk of certain diseases such as type 2 diabetes (sugar disease). However, most of these studies were undertaken in white populations from developed countries. It is important to study this in the black African population as many factors such as socio-economic status, physical activity and nutrition, are specific to this population, and may have an important impact on how your body works and how this changes with aging. Further, it is not known how body fat distribution and the risk of diabetes is affected by HIV infection, which is known to change body fat and its distribution. This study will examine different changes that happen in the body with aging and how these influence body fat distribution and type 2 diabetes risk in middle-aged black men and women. The factors that we are interested in examining include male and female sex hormones (for example, testosterone and oestrogen), inflammatory markers (involved in the immunity) and circulating cortisol (stress hormone).

Who can participate?

We are going to be testing women who participated in the Birth to Twenty Study of Women Entering and in Endocrine Transition (SWEET study) between 2011 and 2014, and men who participated in the H3Africa/AWIGEN study in 2014.

You will only be eligible for this follow-up study if you participated in these studies.

What will happen if you decide to take part in the study?

If you meet the criteria and decide to take part in the study, you will be required to complete two testing sessions described below, at DPHRU at Chris Hani Baragwanath Academic Hospital in Soweto.
You are under no obligation to take part in the study and are not required to give a reason if you do not wish to participate. If you decide to take part in the study, you are free to withdraw

at any time and without giving a reason and without prejudice. If you decide to withdraw from the study, we will discuss with you what will happen to any information or samples that you have provided. If the incomplete samples and information can usefully contribute to the study, we will ask your permission to store them and use them in our analysis. Alternatively, on your request all your information and samples will be destroyed.

Testing Session 1: Testing at any time of the day and will take 2 hours of your time.

You will be requested to complete a series of questionnaires in two visits (40 pages) by interview, which will include questions on measures of socioeconomic status (i.e. housing, employment and income), personal and family history of disease (e.g. diabetes, obesity, high blood pressure, and cardiovascular disease), reproductive history, access to food, and stress. In addition, questions on lifestyle factors, including smoking and alcohol intake, medication and supplement use will be included. Furthermore, your dietary food intake over the past 7 days will be measured using a questionnaire that lists all possible foods. You will also be asked to wear two small devices, one on your hip and one on your thigh, for 7 days to measure your physical activity and sedentary time. Your weight and height will be measured, as well as your waist and hip circumferences. In addition, you be required to undergo a scan to measure your body fat, muscle mass, and bone density, using a special X-ray scan. If there is any risk that you may be pregnant, you will be requested to have a pregnancy test prior to the scan, as the scan will expose you to a small amount of radiation. Your blood pressure will also be measured and you will also be requested to have an HIV test. You will also be required to bring your ID document, clinic card as well as any medication that you are currently taking.

Further details of these procedures are provided below:

Demographic, socioeconomic status and lifestyle questionnaire

You will be asked questions about various measures of social and economic status (e.g. if you are employed or not, what do you do at work, your source (s) of income) and questions about whether there are people in your family with diseases such as high blood pressure or heart problems. You will also be asked questions about your personal health, stress and reproductive history. In addition questions on lifestyle factors including smoking and alcohol intake, medication and supplement use will be included. We will fill in the answers for you. You can skip any questions that make you uncomfortable.

Food frequency questionnaire (FFQ)

You will also be asked to fill in a food frequency questionnaire to measure your usual dietary intake (what you normally eat). This questionnaire will give us a sense of what and how much you have eaten over the last week. In addition, we will ask you a few questions about your food security, in other words, your access to food and if you or your family ever experience periods of hunger.

Body composition and DXA (dual-energy X-ray absorptiometry) measurements

Your weight, height, waist and hip circumference will be measured as part of your body composition assessment. In addition, you will undergo a special X-ray scan (DXA) that will tell us about your muscle mass, body fat and bone density. The scan will take approximately 20 minutes to perform during which you will lie quietly on the scanning table in a medical gown provided. You will be asked whether or not you are pregnant. If there is any possibility that you may be pregnant please tell the technician and we will perform a pregnancy test. If you are pregnant, you will <u>not</u> have the scan. The only risk associated with the DXA scan is exposure to radiation. However, the radiation exposure with a DXA scan is less than half that of a chest x-ray.

Blood pressure:
After a 5-minute relaxation period, blood pressure will be measured 3 times in a row, separated by 5 minutes of relaxation between readings. A standard blood pressure monitor will be used.

Physical activity

You will be asked to wear two motion sensors (accelerometer and activPAL) for 7 days, to measure your activity and sedentary patterns. They are the size of a small matchbox, and one is worn on the waist with a lightweight belt and the other is attached to the thigh using a waterproof plaster. You will be instructed on how to use the monitors. There are no side-effects associated with wearing the monitors.

HIV test

We would like to test your HIV status. This is very important as it may have effects on your body composition, risk for diabetes and the factors that we will measure in your blood. The HIV test is voluntary, however If you have previously been tested as HIV positive this is not necessary. If you refuse the test, you will not be able to participate in the study. It is also important to note that if you decide to test, you will be given pre-test counseling. This test is always strictly confidential and can only happen if you agree. There is no way in which anyone can link your HIV status to your name as all results in this study are coded with a number. No one including your doctor, family, or work colleagues will be told about this test without your permission. The advantage of a rapid test is that you do not have to return to get your test result. Results will be available when you check out, after all the other tests, measurements and questionnaires are completed.

You will have your finger pricked with a sterile needle and the drop of blood (less than 0.5ml) will be tested on specific HIV testing kits to check for HIV antibodies. Test results will be given to you in private by a registered trained counsellor. If the report states negative it means that there are no antibodies to HIV. The window period will be explained. If the report states positive, it means that you are HIV positive and that there are antibodies to HIV. You will be given a letter to refer you to a clinic specialising in HIV treatment and you will be given a second test at the clinic to confirm this result. Sometimes we cannot clearly tell if the results are negative or positive. We will then refer you to a clinic specialising in HIV treatment and you will be given a second test at the clinic to confirm this result.

There is a chance that some of the questions in the questionnaire might trigger some emotional distress. If this happens, we will refer you to our counselling nures on site and she will make the necessary appointment for you to see a psychologist at the Psychology Unit at Chris Hani Baragwanath Hospital

Testing session 2: Testing in the early morning (between 7:30 and 8:00 am) and will take 2½ hours of your time

You will be requested to visit the DPHRU offices at the Chris Hani Barangwanath Academic Hospital between 7:30 and 8:00 am in the morning approximately 7 days after testing session 1, but after an overnight fast (nothing to eat or drink, except water, from 10pm the night before). You will be asked to donate a sample of blood and then undergo an oral glucose tolerance test (OGTT) to determine whether you are at risk for developing diabetes. You will also be required to return the two physical activity monitors that were given to you in the first testing session.

Blood sampling and oral glucose tolerance test (OGTT)

Blood sampling and the OGTT can only be performed in the morning after an overnight fast. Therefore it is important that you do not eat or drink anything (except water) from 10 PM the night before, or for 10-12 hours before your test begins. You are not allowed to take any medication, food or drink, chew gum or sweets, before your blood test.

Blood sampling will be performed by inserting a small plastic tube into a vein in your arm and a small plastic tap or valve will be attached onto it so that blood samples can be drawn before the 2 hour OGTT test. The first blood sample (50 ml) will be drawn for the measurement of blood lipids (fats), glucose (sugar) and various hormones (e.g. insulin and cortisol) and inflammatory markers in the fasted state (i.e. before you eat anything). You will then be asked to drink a cup of water containing 75 g of glucose (sugar). Thereafter, blood samples (5 ml or 1 teaspoon each) will be drawn from your arm at 30, 60, 90 and 120 minutes after the glucose ingestion (total 20ml, 4 teaspoons) for the later measurement of changes in glucose and insulin levels. After your test we will give you something to eat and drink.

What are the risks and discomforts of this study?

There are no risks or discomforts associated with the administration of the questionnaires. Strict confidentiality of results will be maintained. Your name will be removed from all data, and you will be assigned a number, which will be used to identify data relating to you. All records will be kept in a locked room and in a secure computer database in the research unit. Your name will not be used in any publication of the results.

There are no risks associated with the use of the physical activity monitors. The only risk associated with the DXA scan is exposure to radiation. However, the radiation exposure with a DXA scan is less than half that of a chest x-ray (11.3 microSieverts).

There are no appreciable risks associated with the fasting blood sampling and OGTT, other than those associated with routine blood sampling. Sometimes when blood is taken you may feel a prick at the place where the needle enters your body. Afterwards there may be a little bruise, and pain (which are associated with normal blood sampling), and in very unusual circumstances, local infection. Very occasionally participants may 'faint'. This is a stress response to a trigger (e.g. the sight of blood) and has no long-term effects. This test is used routinely for both research and medical purposes. The total amount of blood drawn will be 70 ml, which is substantially less ($1/7^{th}$) than that of a standard blood donation (500 ml). All procedures will be supervised and carried out by a nursing sister and appropriately trained medical personnel using sterile techniques to minimise any risks of infection.

If we discover that you have any health problems based on our tests, you will be contacted and referred to the appropriate clinic for treatment and/or management.

Are there any benefits to you for being in the study?

You will receive your own results from the study, including your body composition (e.g. muscle and fat mass), your blood pressure, lipids (fats in your blood) and glucose tolerance (your risk for diabetes). In addition, you will contribute to our understanding of the how body fat distribution and the risk for type 2 diabetes differs between black South African men and women (pre-menopausal and post-menopausal), with and without HIV. This information can be used to provide evidence for future strategies for the prevention, treatment and management of diabetes risk in middle-aged black South Africans.

What will happen when the study is over?

Detailed analysis of the samples will take some time, but once these analyses have been completed, the final results of the study will be shared with you. In addition, the results of the study will be published in scientific journals and to complete a PhD thesis report, as well as in the local media. Your name will not be used in any publication of the results. You may be contacted for a follow-up study.

Will you receive reimbursement for transport?

You will receive R150 to cover transport costs to DPHRU for each of the two testing sessions. The transport money will be paid to you at the end of each session.

Who will see the information that is collected about you during the study?

Strict confidentiality of results will be maintained. Your name will be removed from all data, and you will be assigned a number, which will be used to identify data relating to you. All records will be kept in a locked room and in a secure computer database in the research unit. Your name will not be used in any publication of the results.

Storage of BLOOD samples

Blood samples for biochemical analysis will be temporarily stored in the DPHRU laboratory at Chris Hani Baragwanath Academic hospital

Who do I contact if I have any questions about the study?

If you have any questions or you experience any problems during or after the tests, please contact NYUYKI Clement Kufe (PhD student in Wits, working here) or a representative of the Human Research Ethics Committee.

NYUYKI Clement KUFE (PhD student)
Principal Investigator
MRC/Wits Developmental Pathways for Health Research Unit
Chris Hani Baragwanath Hospital, Soweto Johannesburg
Tel: 063 092 6943 (cell)
Email : kufekle@yahoo.co.uk / nyuyki.kufe@students.wits.ac.za

Human Research Ethics Committee contact details:
Prof P Cleaton-Jones, Tel 011 717 2301, email: peter.cleaton-jones1@wits.ac.za or Ms Zanele Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng Administrative Officers 011 717 2700/2656/1234/1252 zanele.ndlovu@wits.ac.za; Rhulani.mkansi@wits.ac.za; and Lebo.moeng@wits.ac.za

Participant's Code:

INFORMED CONSENT 1

Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors

Consent to participate in the study:

"I, _____, hereby give consent to participate in this research trial to be conducted by DPHRU, within the Department of Paediatrics at the University of Witwatersrand.

I understand that the study will involve completion of questionnaires by interview, an HIV test, routine body measurements (i.e. height, weight, hip and waist circumference), an X-ray scan to measure my body fat and muscle mass, and bone density, blood pressure, collection of blood samples after an overnight fast (10-12 hours) and during a 2-hour oral glucose tolerance test, as well as wearing an accelerometer and ActivPAL for 7 days to measure physical activity and sedentary time, respectively. The purpose and all the details of this study have been explained to me.

I have read and have had explained to me the procedures described. I have had an opportunity to ask questions and my questions have been answered in a satisfactory way.

I understand that all the information collected during the study will be treated with the strictest confidentiality and will only be used for scientific research purposes. All samples will be kept in a freezer in a secure facility with access limited to research personnel. All records will be kept in a locked room and in a secure computer database in the research unit. My name will not be used in any publication of the results. I understand that for data verification and quality control purposes regulatory authorities and/or members of the Wits Human Research Ethics Committee (Medical) may be allowed access to my personal data under conditions of strict confidentiality.

I understand that I may be contacted for a follow-up study.

I agree to participation in the study on the condition that:

1. I can withdraw voluntarily from the study at any time and that no adverse consequences will follow on withdrawal from the study.

2. I have the right not to answer any or all questions posed in the questionnaire.

3. The University of the Witwatersrand's Human Research Ethics committee has approved the study protocol and procedures.

4. All results will be treated with the strictest confidentiality.

5. Only group results, and not my individual results, will be published in scientific journals and in the media.

6. The study scientific team are committed to treating participants with respect and privacy through interviews conducted in private and follow-up counselling available on request.

7. I will receive a referral note to psychological services if I experience any psychological distress during the study.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily and understand that I have the right to withdraw my consent without this affecting the current research study or my medical care.

Print Name of Participant _____

Signature of Participant _____

Date _____

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

RESEARCH ASSISTANT:

_____

Printed Name Signature/Mark Date and Time

Copy provided to participant _____ (initialed by researcher)

WITNESS: (If applicable)

_____

Printed Name Signature/Mark Date and Time

Participant's Code:

PARTICIPANT INFORMATION SHEET 2

INFORMED CONSENT 2

Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors

Certificate of Consent:

1) If any of the **BLOOD** that I have provided for this research project is unused or leftover when the project is completed

   ☐ I give permission for my **blood** sample to be stored indefinitely

   AND if my **blood** sample is to be stored:
   ☐ I give my permission for my **blood** sample to be stored and used in future research of any type, which has been properly approved

   AND if any research on my **blood** sample cannot easily be done in South Africa:
   ☐ I give my permission for a portion of my **blood** sample to be sent out of the country for analysis if appropriately approved

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily and understand that I have the right to withdraw my consent without this affecting the current research study or my medical care.

Print Name of Participant _____

Signature of Participant _____

Date _____

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

RESEARCH ASSISTANT:

_____

Printed Name Signature/Mark Date and Time

Copy provided to participant _____ **(initialed by researcher)**

WITNESS: (If applicable)

_____

Printed Name Signature/Mark Date and Time

Participant's code:

INFORMED CONSENT 3

Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors

The information around the blood sample taken from me is clear and the purpose of consent is for me to inform the study what they can or cannot do with these samples.

I understand that all procedure/tests on the stored blood will be approved by the Human Research Ethics Committee of the University of the Witwatersrand.

YES ☐ NO ☐

I am in agreement that my blood sample may be stored and used for the purposes described above.

YES ☐ NO ☐

I am in agreement that the data generated from my blood sample may be made available as stated above.

YES ☐ NO ☐

I am in agreement that the information I have supplied in the list of questions and the information from the tests and measurements taken from me may be used as stated above.

YES ☐ NO ☐

I agree that a small volume of my blood sample may be sent out of the country if the research cannot easily be done in South Africa.

YES ☐ NO ☐

I agree that an portion of my blood sample may be stored in a biobank (laboratory) and that some data may be stored in a database as stipulated and that these may be shared according to the processes and procedures by using my study code or another code that de-identifies my sample and data.

YES ☐ NO ☐

I understand that every time a new study is done on my blood sample, permission will be obtained from the ethics committee for the study to make sure that it is used only for the purposes stated above.

YES ☐ NO ☐

I understand that I will not benefit directly from the research done on my blood sample.

YES ☐ NO ☐

I understand that I may withdraw from the study at any time.

YES ☐ NO ☐

PARTICIPANT

_____

Printed name Signature/Mark Date and Time

RESEARCH ASSISTANT:

_____

Printed Name Signature/Mark Date and Time

Copy provided to participant _____ (initialed by researcher)

WITNESS: (If applicable)

_____

Printed Name Signature/Mark Date and Time

Participant's code:

HIV COUNSELLING FORM AND INFORMED CONSENT

Hello! My name is _____. First, we would like to discuss some matters with you. Information collected will be not be used in any identification form outside this facility. Therefore confidentiality will be maintained. We will provide you with information you need to know about HIV and AIDS. This will then be followed by information to help you understand your risk exposure to HIV and then you could be able to take an HIV test.

CLIENTS HIV HISTORY

Have you been tested for HIV before?	Yes	No	If Yes, when did you test? What was the HIV results		Negative	Positive
If positive, do you have a copy of the results	Yes	No	If no, would you like to do another test?		No	Yes
What was your reason for testing	Illness	Insurance	Partner died	Pregnancy (Females Only)	Employment	General Check up
If other please state reason						

CLIENT SUPPORT SYSTEM

Have you ever had a loss in your life?	Yes	No			
If yes,	Who				
	When				
If the test is HIV positive, will you tell someone?	Yes	No	If Yes Who?		
Who else will you tell if you are HIV positive?	Family	Partner	Friend	Other (State)	
How will you tell this person you trust?					
Do you think you will get support from that person?	Yes	No	Would you like us to offer support?	Yes	No

PRE COUNSELLING SESSION

UNDERSTANDING HIV AND AIDS

COUNSELLOR TO USE CUE CARDS FOR COUNSELLING

Understanding of HIV/AIDS, client should be explaining mode of transmission and exchange of fluids.		Meaning of Window Period (What is it?)	
Benefits for HIV Testing		Importance of knowing ones HIV status	

		(What does it mean?)	
Meaning of HIV testing		Meaning of HIV Negative Result	
Meaning of Confidentiality. (Counsellor to clarify confidentiality)		Meaning of HIV Positive Result	
		Perception of risk to HIV exposure. (Does the client think they are at risk to HIV infection?)	

HIV TESTING

Counsellor: Explain rapid testing processes. A rapid test for HIV will be done by the DPHRU lab. About a teaspoon full of blood will be collected (5ml to 10ml) and tested on specific HIV testing kits to check for HIV antibodies. Test results will be given to you in private when you check out today by a registered trained counsellor. If the report states negative it means that there are no antibodies to HIV. The window period will be explained. If the report states positive, it means that you are HIV positive and that there are antibodies to HIV. You will be given a letter to refer you to a clinic specialising in HIV treatment and you will be given a second test at the clinic to confirm this result. Sometimes we cannot clearly tell if the results are negative or positive. We will then repeat the test and if it is still indeterminate we will refer you to a clininc for a second testing that will help confirm the results

PATIENT CONSENT

I agree to have the HIV Rapid test. The procedure to be carried out has been explained to me. The possible discomforts, risks and benefits involved in taking part in the test have also been described to me. I understand that I can leave the study at any point. I also understand that if I have any questions concerning the test then the investigator will explain these to me.

Date: _____ Patient: _____

Contact details of researchers:

NYUYKI Clement KUFE (PhD student)
Principal Investigator
MRC/Wits Developmental Pathways for Health Research Unit
Chris Hani Baragwanath Hospital
Soweto Johannesburg
Tel: 063 092 6943 (cell)
Email : kufekle@yahoo.co.uk / nyuyki.kufe@students.wits.ac.za

Human Research Ethics Committee contact details:
Prof P Cleaton-Jones, Tel 011 717 2301, email peter.cleaton-jones1@wits.ac.za or Ms Zanele Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng Administrative Officers 011 717 2700/2656/1234/1252 zanele.ndlovu@wits.ac.za; Rhulani.mkansi@wits.ac.za; and Lebo.moeng@wits.ac.za

Participant's code:

POST TEST COUNSELING SESSION

NB. COUNSELOR: Identify Client with Name and ID number against HIV Test Results

HIV NEGATIVE TEST RESULT

We spoke earlier about what HIV positive and HIV negative results mean. Explain again. Your results are back and you are HIV negative; you do not have the HIV virus in your body

COUNSELORS KEY TASKS	CLIENTS NOTES	COMMENTS
Explain the implications of the negative test result		
Identify and prioritize the behaviours that correspond to the client's risk		
Motivate the client to develop a risk reduction plan		
Encourage clients to discuss their HIV status with current and future partners		

POST TEST COUNSELLING

HIV POSITIVE TEST RESULTS

We spoke earlier about what HIV positive and HIV negative results mean. Explain again. Your results are back and you are HIV positive; you do have the HIV virus in your body

COUNSELORS KEY TASKS	CLIENTS NOTES	COMMENTS
Inform client that the test results are available		
Provide results clearly and simply		
Review the meaning of the result		
Allow the client time to absorb the meaning of the result		
Explore the client's understanding of the result		
Assess how client is coping with the result		
Acknowledge the challenges of dealing with an initial positive result		

IDENTIFY SOURCES OF SUPPORT

COUNSELORS KEY TASKS	CLIENTS NOTES	COMMENTS
Identify current health care resources		
Address the need for health care providers to know client's test result		
Explore client's access to medical services		
Identify needed medical referrals		
Discuss situations in which the client may want to consider protecting his or her own confidentiality		
Discuss options of support groups (i.e. post test club)		
Provide appropriate referrals		

REFERRAL TO OTHER PROGRAMS

Refer the client with letter to Thandekile Essien and the clinic (ZAZI VCT service at Baragwanath Hospital); she will then ensure that the client has the appropriate support.

COUNSELLORS NOTES:

_____

_____

_____

_____

_____

_____

Counsellor's Signature:……

APPENDIX J: QUESTIONNAIRE

University of the Witwatersrand, Johannesburg
Developmental Pathways for Health Research Unit
Department of Paediatrics and Child Health

Determinants of insulin sensitivity in middle-aged black South African men and women: the role of sex hormones, inflammation, glucocorticoids and lifestyle factors

PARTICIPANT QUESTIONNAIRE

DATE: Day ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

BTT ID NUMBER: ☐☐☐☐☐☐☐

BONE ID NUMBER: ☐☐

Components	Tick
Study information sheet	
Consent form	
ID COPY	
Participant Questionnaire	
Food Frequency Questionnaire	
Anthropometric Measurements	
DXA scan	
Fasting bloods samples	
OGTT	
Accelerometer (ActiGraph)	
ActivPAL	
Dietary intake	
Payment	
Feedback (comment)	
Date : _____	
Signature: _____	

IDENTIFICATION AND CONTACT DETAILS	
Name _____ ID number: _____ Date of Birth : _____ Age: _____ Physical Address: _____ _____ Postal Address: _____ _____ E-mail: _____ Tel No's: _____ (h) _____(w) _____ (Cell) _____ **Contact details of a relative or a friend who will <u>always</u> know where you live:** Alternative contact Person: _____ Relationship:_____ Tel No's: _____ (h) _____(w) _____ (Cell) _____ **TO BE KEPT SEPARATE FROM QUESTIONNAIRE DATA**	**SUBJECT CODE:**

SECTION A: DEMOGRAPHIC AND SOCIEO-ECONOMIC DETAILS

1. CURRENT MARITUS STATUS (tick option that applies)

Single	
Married/cohabiting	
Widowed	
Separated/Divorced	

2. HIGHEST LEVEL OF EDUCATION ATTENDED (tick options that applies)

No education	
Grade 1-2	
Std (1-3) Grade (3-5)	
Std (4-5) Grade (6-7)	
Std (6-7) Grade (8-9)	
Std (8) Grade (10)	
Std (9) Grade (11)	
Matric Grade (12)	
Post Matric qualification	
Diploma	
Tertiary education (university / technikon)	

3. CAREGIVER'S CONFIRMATION DETAILS

Question	Answer
Where were you born? (City/Town & Province SA) (Country & Rural/Urban)	
Where did you spend most of your school years, which includes primary and high school?	
How many years have you been living in Gauteng?	

4. GENERAL HOUSEHOLD INFORMATION

Questions	Answers
4.1 How many people live in your house including you?	
4.2 How many rooms are in your house (including kitchen, dining room, bedrooms, excluding bathrooms)	
4.3 How many bathrooms are in your house?	
4.4 How many rooms are there for sleeping?	

5. WHICH OF THE FOLLOWING DO YOU HAVE IN YOUR HOUSEHOLD (Tick for YES and X for NO)?

Electricity		Television		Radio	
Motor vehicle		Fridge		Washing machine	
Telephone/Cell phone		Microwave		Bicycle	
Tablet/Laptop/Personal Desktop		DSTV/Satellite		MNet	

6. HOW WOULD YOU DESCRIBE YOUR HOME (tick the one that best describes it)?

House		Flat/Cottage/Townhouse		Residence/hostel	
Shack/Zozo		Government housing (e.g. municipal/RDP housing)		Room in backyard of house (or shared house)	

7. WHAT ARE THE WALLS OF YOUR HOUSE MADE OF? (tick appropriate box)

Brick/concrete		Mud/ cement		Plastic/cardboard	
Clay/Mud		Corrugated iron/zinc		Other	
Prefab		Plaster/finished			

8. WHAT IS THE **ROOF** OF YOUR HOUSE MADE OF? (tick appropriate box)

Straw/Thatch		Galvanised iron		Other (specify)	
Earth/sand/Mud		Wood/planks			
Concrete		Tiles/slates			

9. WHAT IS THE **FLOOR** OF YOUR HOUSE MADE OF (tick one box only)

Earth/sand/mud		Stone/Brick		Cement	
Wood		Vinyl/linoleum		Other	
Carpet		Ceramic tiles			

10. WHAT IS THE MAIN SOURCE OF DRINKING WATER IN THE HOUSE? (Tick one box only)

Bottled water		Protected dug out well		Public tap/standpipe	
Running water (tap water)		Unprotected dug well		Tanker truck/cart with small tank	
Piped water into yard/plot		Protected spring		Piped water into dwelling	

Surface water		Rain water		Other	

11. WHAT IS THE TYPE OF TOILET FACILITY IN THE HOUSE (tick one box only)

Flush to piped sewer system		Protected dug out well		Bucket system	
Flush to septic tank		Ventilated improved pit (VIP) latrine		Other	
Traditional pit toilet		No facility or bush or field			

12. HOUSEHOLD FOOD INSECURITY ACCESS SCALE

1. In the past four weeks, did you worry that your household would not have enough food?			
1. No	2. Rarely	3. Sometimes	4. Often
2. In the past four weeks, were you or any household member not able to eat <u>the kinds of foods you preferred</u> (i.e. VEGETABLES, FRUIT, MEAT/CHICKEN <u>NOT</u> "luxury" food such as pizza, burgers or fried chicken) because of a lack of resources?			
1. No	2. Rarely	3. Sometimes	4. Often
3. In the past four weeks, did you or any household member have to eat a <u>limited variety of foods</u> (e.g. Pap with <u>NO meat</u> OR pap with sweetened water) due to a lack of resources?			
1. No	2. Rarely	3. Sometimes	4. Often
4. In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food?			
1. No	2. Rarely	3. Sometimes	4. Often
5. In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?			

1. No	2. Rarely	3. Sometimes	4. Often
6. In the past four weeks, did you or any other household member have to eat fewer meals in a day because there was not enough food?			
1. No	2. Rarely	3. Sometimes	4. Often
7. In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food?			
1. No	2. Rarely	3. Sometimes	4. Often
8. In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food?			
1. No	2. Rarely	3. Sometimes	4. Often
9. In the past four weeks, did you or any household member go a whole day and night without eating anything because there was not enough food?			
1. No	2. Rarely	3. Sometimes	4. Often

13. EMPLOYMENT AND INCOME

13. 1 Are you currently employed? (tick)	YES NO
13.2 If YES TO 13.1, what type of employment?	FORMAL INFORMAL
13.3 If YES to 13.1, which best describes the work that you are employed to do? (tick relevant option)	

Skilled manual work (i.e. sewing, beadwork, arts and craft, administrative assistants)	
Unskilled manual work (i.e. hotel maids, cleaner, sweepers or farm worker, domestic work)	
Clerical support, service or sales (i.e hairdresser, taxi service)	
Managerial/professional	
Own business	
Other (specify)	

15.4 Other household incomes (tick appropriate box):

Grants(PLUS number of people receiving grant)			
Child support grant		Support from a partner	
Disability grant		Support from family	
Care dependency Grant		Other (specify)	
Grants for older persons			
Foster care grant			

Income is a sensitive question to many people. However, it is very important for the study to have an idea of your monthly income.

13. 5 Monthly Household Income (including all sources of income e.g. grant, spousal support or family support) (Tick appropriate range):

R 1 to 2499		R 10 000 to 14 999		No income	
R 2500 to 4999		R 15 000 to 19 999		Not willing to disclose	
R 5000 to 7499		R 20 000 to 29 999			
R 7500 to 9999		Give other range			

13.6 How many people do you support with this income? _____Adults _____Children

SECTION B: GENERAL HEALTH

1. Are you pregnant?

Y	N

2. Have you had a hysterectomy (i.e. ovaries removed)?

Y	N

2.1 If YES, what date was it?_____

3. Do you have regular periods?

Y	N

3.1 If YES to 3, when was the start date of your last menstrual period?

3.2 If NO to 3, when was your LAST period?

3 months ago	6 months ago	1 year ago	More than 1 year

Y	N

3.3 How would you classify your menopausal stage?

Pre-menopausal	
Peri-menopausal “menopausal transition” e.g. menstrual irregularity, hot flashes, mood changes	

Post-menopausal (e.g. 12 month period without menstruation)	

4. How many children do you have?_____

5. How many pregnancies have you had? _____

6. When was your last pregnancy?_____

7. Contraception and fertility control

Contraceptive method	**Tick the appropriate box (give name where appropriate)**	**Duration**
None		
Oral contraceptive pill		
Injection (state frequency of injection)		
Female sterilisation		
IUD “Intra-Uterine Device” loop		
Patch		
Other and specify		

8. MEDICATION AND SUPPLEMENT USE

8.1	Do you use any medicine regularly or daily that a doctor or nurse has prescribed?	YES..... NO.....

8.2	Please provide the following information of your medication (s): **Name of medication(s)?** **What are they used for?** **What is the dosage and frequency of use of use? (e.g. 3 tablets per day)** **What long have you been using the medication?** **Examples of medical conditions that you could be using the medication (s) for:** high blood pressure, heart attack or angina (chest pains), stroke, high cholesterol (fats in the blood), diabetes, emphysema, bronchitis, asthma, osteoporosis, TB, epilepsy, cancer OR state don't know if you are unsure what the medication is used for)	
8.3	Do you use nutritional or other supplements?	YES ..... NO .....
8.4	If YES to 8.3, Name the supplement, what is it used for, dosage, frequency and duration of use.	
8.5	Do you use any herbal medicine?	YES ..... NO .....
8.6	Name of herbal medicine, what you are using for, state the dosage, frequency and duration of use.	

8.7	Have you been sick in the past week?	YES ..... NO .....
8.8	If YES, what sickness? 8.8.1 Did you take medicine? (yes/no) What medication(s) did the nurse or doctor prescribe you? State the dosage, frequency and duration of use.	YES ..... NO .....

9. CLINICAL CONDITIONS

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
9.1	Would you say your health is poor, average, good, or very good/excellent?	POOR..... AVERAGE ..... GOOD..... VERY GOOD/EXCELLENT .....
9.2	Do you personally think that you are underweight, normal weight or overweight?	UNDERWEIGHT..... NORMAL WEIGHT ..... OVERWEIGHT. ..... OBESE..... DON'T KNOW.....
	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:	
9.3	High Blood Pressure?	YES..... NO..... DON'T KNOW.....

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
9.4	Heart attack or angina (chest pains)?	YES..... NO..... DON'T KNOW.....
9.5	Stroke?	YES..... NO..... DON'T KNOW.....
9.6	High blood cholesterol or fats in the blood?	YES..... NO..... DON'T KNOW.....
9.7	Diabetes or Blood Sugar?	YES..... NO..... DON'T KNOW.....
9.8	Emphysema/Bronchitis?	YES..... NO..... DON'T KNOW.....
9.9	Asthma?	YES..... NO..... DON'T KNOW.....
9.10	Sore joints, e.g. Arthritis, gout?	YES..... NO..... DON'T KNOW.....
9.11	Osteoporosis?	YES..... NO..... DON'T KNOW.....

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
9.12	Epilepsy / fits?	YES..... NO..... DON'T KNOW.....
9.13	TB?	YES..... NO..... DON'T KNOW.....
9.14	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: …….....
9.15	Cancer?	YES..... NO..... DON'T KNOW..... If yes, what? ……...

10. FAMILY AND MEDICAL HISTORY

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
	Now I would like to ask you about your family. Do you have a **close blood relative** (father, mother, brother, sister or child) who has ever had any of the following conditions:	

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
10. 1	High Blood Pressure?	YES ..... NO ..... DON'T KNOW ..... If yes, who? …… …… …… ……
10.2	Heart attack or angina or chest pain when exerting himself/herself?	YES ..... NO ..... DON'T KNOW ..... If yes, who…… ……...…… …… …… ……...
10.3	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 ..... …… …….....

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

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
		…… …… …… ……

SECTION C: LIFESTYE

	1. TOBACCO USE (WHO STEPwise Questionnaire)	
1.1	Do you **currently smoke** any tobacco products, such as cigarettes, cigars, or pipes?	YES ..... NO .....
1.2	Do you currently smoke tobacco products **daily**?	YES ..... NO .....
1.3	**How old** were you when you first **started** smoking daily?	YES OLD _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ IF "YOU DON'T REMEMBER", **.....**
1.4	If you do not remember how old you were, do you remember how long ago it was?	WEEKS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _
		MONTHS AGO _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
		YEARS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
1.5	On average, how many of the following items do you smoke each day?	MANUFACTURED CIGARETTES _ _ _

	(CHECK EACH ITEM, IF NOT SMOKING AN ITEM, CODE 00)	HAND-ROLLED CIGARETTES _ _ _ _
		PIPES FULL OF TOBACCO _ _ _ _ _ _
		CIGARS/CHEROOTS/CIGARILLOS _ _
		OTHER_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
1.6	**In the past,** did you ever smoke daily?	YES ..... NO .....
1.7	**How long ago did you stop** smoking daily?	WEEKS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ _

2. Do you use snuff?

2.1 IF YES, how often do you use snuff?

Once a day	
Twice a day	
Three times a day	
More than three times a day	
Other: Specify _____	

3. Do you use e-cigarettes (electronic cigarettes?)

3.1 IF YES, how often do you use e-cigarettes?

Once a day	
Twice a day	
Three times a day	
More than three times a day	
Other: Specify _____	

4. 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

4.1 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			

4.2 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	

4.3 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	

SECTION D: Clinical measurements (OGTT, Anthropometry, DXA and BP)

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: _____

Date of last menstrual cycle: _____

OGTT:

Fasting blood sample: Tubes 5 x SST – 27 epp (500µL each); 3 x EDTA – 9 epp (500µL each); 1 x LH – 1 epp (500 µL); 1 x FO – 1 epp (500µL)

Drink: ☐ Time: _____ Comments: _____

30 min: ☐ Time: _____ Comments: _____

60 min ☐ Time: _____ Comments: _____

90 min: ☐ Time: _____ Comments: _____

120 min: ☐ Time: _____ Comments: _____

DXA: ☐ Full Body ☐ Half Body

Comments: _____

Anthropometry	**(1)**	**(2)**	**(3)**
• Height (cm)			
• Weight (kg)			
• Waist (cm)			
• Hip (cm)			

BLOOD PRESSURE	**(1)**	**(2)**	**(3)**
• SYSTOLIC BP			
• DIASTOLIC BP			
• PULSE			
• TIME OF BP	**h**		

HIV RAPID TEST

Name _____

ID number: _____

Date of Birth _____ Age: _____

Physical Address: _____

_____

You can only take the HIV test if you have signed the HIV consent form.

Have you signed the HIV consent?

YES	NO

HIV test results (click appropriate box)

Negative	
Positive	

TO BE KEPT SEPARATE FROM QUESTIONNAIRE DATA

SUBJECT CODE:

PHYSICAL ACTIVITY SHEET

1. Accelerometer

1.1 Serial number of the monitor:_____

1.2 Date collected:_____

1.3 Date returned:_____

RECORDING DAY	**Intensity**		
	Total moderate intensity	Total vigorous intensity	Moderate-to-vigorous intensity
DAY 1			
DAY 2			
DAY 3			
DAY 4			
DAY 5			
DAY 6			
DAY 7			

2. ActivPAL

1.1 Serial number of the monitor:_____

1.2 Date collected:_____

1.3 Date returned:_____

RECORDING DAY	Sedentary time
DAY 1	
DAY 2	
DAY 3	
DAY 4	
DAY 5	
DAY 6	
DAY 7	

PARTICIPANT:		**DATE:**	
RESEARCH ASSISTANT:		**DATE:**	

APPENDIX K: SLEEP DIARY

Adults Study_Sleep Diary						
SUBJECT CODE:						
Name:						
Please write in this diary on all the days that you wear the accelerometer						
Date	**Wake-up time**	**Bed time**	**Did you nap?**	**Nap start time**	**Nap end time**	**Did you work?**
			Yes / No			Yes / No
			Yes / No			Yes / No
			Yes / No			Yes / No
			Yes / No			Yes / No
			Yes / No			Yes / No
			Yes / No			Yes / No
			Yes / No			Yes / No
Example	*6am*	*10:30pm*	*Yes*	*3pm*	*4:30pm*	*Yes*
Notes:						
Wake-up time:	The time on the clock when you open your eyes in the morning, and are ready to get out of bed					
Bed time:	The time on the clock at night when you close your eyes to start trying to sleep					
Napping:	Any time during the day when you lie down, close your eyes, and try to sleep					

APPENDIX L: Student's contribution to articles and agreement of co-authors

I, **Clement Kufe, NYUYKI**, student number; **395736**, declare that this thesis is my own work and that I contributed adequately towards research findings published in the articles stated below which are included in my thesis.

Signature of Student…… ..... **Date:** 23rd March 2022..…

Name of Primary Supervisor: Professor Lisa Micklesfield

Signature of Primary Supervisor **Date:** 31 March 2022

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of his Thesis. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article 1:

Kufe CN, Micklesfield LK, Masemola M, Chikowore T, Kengne AP, Karpe F, Norris SA, Crowther NJ, Olsson T, Goedecke JH. Increased Risk for Type 2 Diabetes in Relation to Adiposity in Middle-Aged Black South African Men compared to Women. *Eur J Endocrinol*.2022; 186(5): 523-533. doi: https://doi.org/10.1530/EJE-21-0527. PMID: 35225824

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Comments by primary supervisor: CNK reviewed the questionnaire, data collection tools and standard operating procedures, supervised data collection and entry, participated in data collection and entry, data management (including data cleaning and coding) and quality control of the chapter. CNK, JHG and LKM conceptualised the study and CNK analysed the data, drafted and revised the manuscript under the supervision of JHG and LKM. All authors reviewed/edited, read and approved all the drafts and the final version of the manuscript.

Article 2:

Kufe CN, Masemola M, Soboyisi M, Smith A, Westgate K, Goedecke JH, Brage S, Micklesfield LK. **"Physical behaviours and their association with type 2 diabetes mellitus risk markers in urban South African middle-aged adults: An isotemporal substitution approach".** *BMJ Open Diabetes Research & Care.* July 2022; **10:**e002815. DOI: 10.1136/bmjdrc-2022-002815

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Comments by primary supervisor: CNK reviewed the questionnaire, data collection tools and standard operating procedures, participated in data collection and entry, supervised data collection and entry, data management (including data cleaning and coding), quality control of physical activity data from the Actigraphs and activPALs and data analysis. AS and KW processed the physical activity data. CNK, JHG, SB and LKM conceived the study and CNK drafted and revised the manuscript under the supervision of JHG, SB and LKM. SB and WK advised on the statistical methods. WK and SB critically reviewed and commented the manuscript. All authors read and approved the final version of the manuscript.