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Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans

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Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans

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Student number: 1864260

Supervised by:
Dr Tinashe Chikowore
Prof Julia Goedecke



UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

A research report (in the format of a "submissible" paper) submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Medicine (Genomic Medicine)

Johannesburg, 10 May 2023

NATIONAL HEALTH LABORATORY SERVICE

School of Pathology, University of the Witwatersrand

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10 May 2023

Attention: Examiner

Dear Examiner,

Re: Research report submitted to the Faculty of Health Sciences by Ms Bontle Masango in partial fulfilment of the requirements for the degree of Master of Science in Medicine (Genomic Medicine)

Thank you for agreeing to examine a research report entitled "Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans".

Bontle Masango (ID 1864260) is submitting her research report for the degree MSc (Med) Genomic Medicine in the format of a "submissible" paper. Bontle Masango and her supervisors, Dr Tinashe Chikowore and Prof Julia Goedecke, have chosen to submit the article to BMJ Open Diabetes Research & Care. The submissible paper attached is written following the submission guidelines of the stipulated journal, which are attached in the appendix for your reference. The only deviations from these guidelines are that the manuscript has been presented as one complete document (including short abstract, tables, and figures) for ease of marking, and the Vancouver style is not numbered within the text of the main document.

The approved research protocol and ethics clearance certificate are attached in the appendix. Please refer to the Table of Contents for a complete list of the contents provided in this research report.

Yours sincerely,

Student: Bontle Masango Supervisor: Dr Tinashe Chikowore Co-supervisor: Prof Julia Goedecke

This report is intended solely to record the observations and/or opinion of the writer. It does not constitute a medico-legal report

Declaration

I, Bontle Masango, declare this research report (in the format of a “submissible” paper) is my own, unaided work. It is being submitted for the Master of Science in Medicine (Genomic Medicine) degree at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

10th day in May 2023, in Johannesburg

Dedication

This report is dedicated to my mother, Mavis Seagele Masango and my late father, Emmelin Masango. I hope this achievement has fulfilled the dream they have envisioned for me.

Contribution of the candidate to the paper

Declaration: Student's contribution to the article(s) and agreement of co-authors

I, Bontle Masango, student number 1864260, declare that this Research Report is my own work and that I contributed significantly towards research findings presented in the paper intended for publication.



Signature of Student

Date: 12/12/2022

Name of Primary Supervisor: Dr Tinashe Chikowore **Name of Co-Supervisor:** Prof Julia Goedecke



Signature of Primary Supervisor Date:

12/12/2022



Signature of Co-Supervisor

Date: 12/12/2022

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the student's use of the article(s) as part of her Research Project.

Article title: Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans.

Co-authors	Signature	Date
Tinashe Chikowore		12/12/2022
Julia Goedecke		12/12/2022

Abstract

This study aimed to determine the metabolic and genetic factors that account for the variation in postprandial glucose (PPG) in Black South Africans (SA). The study included 794 participants from the middle-aged Sowetan Cohort (MASC). PPG was calculated using the integrated area under the curve (iAUC) in response to an oral glucose tolerance test. Principal component analysis was applied to 31 metabolic factors to generate clusters represented by principal component variables. Polygenic risk scores (PRS) were computed for each participant using variants and weights from a validated African type 2 diabetes PRS. Linear regression models were used to evaluate the variance. The PRS did not contribute to the PPG variability in men or women. Central fat, serum lipids, and liver enzymes explained 10.8% of PPG variability in women. In men, peripheral fat, serum lipids, liver enzymes, and steroid hormones explained 10.6% of PPG variability. Our work has identified metabolic factors that predict PPG variability in Black SA.

Acknowledgements

I would like to acknowledge and give my warmest thanks to my supervisor, Dr Tinashe Chikowore, and co-supervisor, Prof Julia Goedecke, for their constant support, guidance, and encouragement. Thank you for taking the time to go through my drafts and presentations.

Thank you to my family for their endless love and kindness and my friends for making this journey truly enjoyable and memorable.

Lastly, thank you to the University of the Witwatersrand (WITS) and National Health Laboratory Service (NHLS) for allowing me to pursue the Master's degree and the National Research Foundation (NRF) for funding my studies. We are grateful to all MASC participants as well as DPHRU field staff.

The study was funded by the South African Medical Research Council (SAMRC) with funds received from the South African National Department of Health, the UK MRC (via the Newton Fund), and GSK Africa Non-Communicable Disease Open Lab (via a supporting Grant project no: ES/N013891/1). Supplemental funds were also received from the South African National Research Foundation (Grant no: UID:98561).

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List of Abbreviations

AADM	Africa America Diabetes Mellitus
ALT	Alanine transaminase
AST	Aspartate transaminase
AUC	Area under the curve
BMI	Body mass index
GGT	Gamma-glutamyl transferase
HbA1c	Glycated haemoglobin
HC	Hip circumference
DBP	diastolic blood pressure
DHEA	Dehydroepiandrosterone
DXA	Dual-energy X-ray absorptiometry
FFSTM	Fat-free soft tissue mass
GWAS	Genome-wide association studies
IDF	International Diabetes Federation
IGM	Impaired glucose metabolism
OGTT	Oral glucose tolerance test
MASC	Middle-aged Soweto Cohort
MVP	Million Veteran Program
NGT	Normal glucose tolerance
PC	Principal component
PCA	Principal component analysis
PPAR	Peroxisome proliferator-activated receptor
PPG	Postprandial glucose
T2D	Type 2 diabetes
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SES	Socio-economic status
SD	Standard deviation
SHBG	Sex hormone-binding globulin.
SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
VAT	Visceral adipose tissue
WC	Waist circumference
WHR	Waist-to-hip ratio

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Research report in the format of a “submissible” paper

This work has been written up as a paper for submission to BMJ Open Diabetes Research & Care. The author’s guidelines, such as abstract, referencing, and word count, have been adhered to and can be found in Appendix.

Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans

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SUMMARY

What is already known on this topic There is high inter-individual variability in postprandial glucose (PPG) in response to identical meals. High PPG levels can be a risk factor for cardiovascular diseases and type 2 diabetes (T2D), even in individuals with normal glucose tolerance.

What this study adds The precise metabolic and genetic factors that contribute to PPG variability in Black South Africans are not known. Our study identified metabolic factors (body fat distribution, serum lipids, liver enzymes, and steroid hormones) that predict PPG variability in Black middle-aged South Africans using metabolic clustering on an extensive range of phenotypic data.

How this study might affect research, practice or policy This is the first study in Africa to explore factors associated with PPG variability and this information is key to our understanding of the pathophysiology of T2D in the Sub-Saharan Africa (SSA) region which has the highest projected rates of increase in diabetes. Also, characterising the factors that contribute to inter-individual variability in PPG is the first step towards risk stratification and implementing future precision medicine approaches for people in Africa.

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ABSTRACT

Introduction The study aimed to determine the metabolic and genetic factors that account for the variation of postprandial glucose (PPG) in Black South Africans (SA) men and women.

Research design and methods This study included 794 participants from the Middle-aged Sowetan Cohort (MASC). The integrated area under the curve (iAUC) for glucose during the oral glucose tolerance test (OGTT) was calculated using the trapezoidal rule. T2D polygenic risk scores (PRS) were computed using PRSice for each participant, utilising variants and weights from a validated African T2D PRS. Principal component analysis was used separately to cluster 31

metabolic factors in men and women. Multivariable linear regression was used to assess the proportion of variance in PPG accounted for by metabolic factors and PRS in men and women while adjusting for selected covariates.

Results Three different principal components (PCs) were identified in women and men, accounting for 39.1% and 44.1% of the variance in metabolic factors, respectively. In men, the metabolic factors explained 10.6% of the variability in PPG, with PC1 (peripheral fat; β -0.339; 95% CI -0.511–-0.167, *P*<0.001), PC2 (liver enzymes and steroid hormones; β 0.476; 95% CI 0.210–0.742; *P*<0.001), and PC3 (lipids and peripheral fat; β -0.328; 95% CI -0.631–-0.026; *P*=0.033) contributing significantly to PPG. In women, metabolic factors explained 10.8% of the variability in PPG, with PC1 (central fat; β 0.377; 95% CI 0.171–0.581; *P*<0.001) and PC2 (lipids and liver enzymes; β 0.448; 95% CI 0.145–0.750; *P*=0.003) contributing significantly to PPG. The T2D PRS did not contribute to the PPG variability in men or women.

Conclusion We identified metabolic factors that predict PPG variability in Black SA. Further multiomic studies are required to identify other factors that can help predict PPG variability.

INTRODUCTION

Type 2 diabetes (T2D) is a global healthcare burden affecting approximately 463 million individuals worldwide, with 19.4 million from Africa (Saeedi *et al*., 2019). The Sub-Saharan Africa (SSA) region has the highest projected rates of increase in diabetes compared to the other International Diabetes Federation (IDF) regions (Saeedi *et al*., 2019). SSA has limited resources to manage the burden, and as a result, it has a high proportion (59.7%) of undiagnosed people living with diabetes (Saeedi *et al*., 2019). There is a need for cost-effective risk stratification tools that allow for early detection to delay and prevent the progression of T2D.

Postprandial glucose (PPG) refers to the blood glucose levels after a meal and is a measure of diabetes risk (Berry *et al*., 2020). Even in individuals with normal glucose tolerance, postprandial hyperglycaemia can be a risk factor for cardiovascular diseases and T2D (Berry *et al*., 2020). The measure of PPG provides insight into the inter-individual variability in response to identical meals and is evaluated to assess the ability to regulate glucose metabolism and insulin sensitivity (de Andrade Mesquita *et al*., 2018). Understanding the determinants of PPG variability is essential to determine factors that can be targeted in interventions for preventing and delaying the progression diseases such as T2D. These findings are applicable in determining variability in dietary responses, which can assist with risk stratification, and the implementation of future precision medicine approaches for people in Africa, which have not been previously explored.

PPG can be measured in various ways. Studies have examined PPG variability from the oral glucose tolerance test (OGTT) using the shape index (Tschritter *et al*., 2003) and the integrated area under the curve (iAUC) calculated from the trapezoidal method (Allison *et al*., 1995). The shape index gives relevant information about different metabolic risk profiles (Tschritter *et al*., 2003); however, it only uses the 90- and 120-minutes glucose readings and does not account for the total variability in the OGTT (Tschritter *et al*., 2003). The iAUC method indicates whole glucose variability and glycaemic control (DeVries, 2013). It considers the changes in the entire glucose curve after glucose load and provides more information about glucose tolerance status (Truswell, 1992).

Several risk factors are thought to affect PPG variability. These include genetics, meal composition and context, lifestyle factors such as physical activity, sedentary behaviour, sleep, body composition and fat distribution, health status, and medication (Palmnäs *et al*., 2020). However, these factors do not act in isolation. Hence cluster analysis of demographic, anthropometric, lifestyle, and biochemical measures have been suggested to reveal important determinants of postprandial variability (Palmnäs *et al*., 2020). Studies have used cluster and principal component analysis (PCA) to group metabolic variables such as anthropometrics, blood pressure, blood glucose, lipid measures, liver enzymes, and hormones. Their findings illustrated the ability of cluster analysis to stratify participants based on their PPG variability and determine how postprandial metabolism reflects an individual's metabolic health status (Riedl *et al*., 2018) (Krishnan *et al*., 2012). Although these are fundamental approaches, these studies have only been performed in the European context and are yet to be evaluated amongst Africans.

Evidence exists that genetic factors influence PPG variability (Langenberg & Lotta, 2018). In a heritability study of twins, genetic factors accounted for approximately 48% of PPG variation (Berry *et al*., 2020). While the study showed the proportion of the variation accounted for by genetic factors, the specific genetic factors contributing to the PPG variation are still unknown. Genome-wide association studies (GWAS) have helped identify specific genetic factors in Europeans and those in Africa are yet to be explored (Langenberg & Lotta, 2018). For example, a European study found that only 9% of specific genetic factors accounted for the variation in PPG, and the remaining genetic factors are still unknown (Berry *et al*., 2020). To our knowledge, no similar studies have been done in Africa, and GWAS studies are still limited. This highlights the need for more studies to determine genetic variants associated with PPG variability in Africa. Ongoing research evaluates polygenic risk scores (PRS) for T2D in Africa (Chikowore *et al.*, 2022), but no study has developed PRS for PPG. The usefulness of stratifying individuals based on their genetic susceptibility, using PRS to evaluate PPG variability, is yet to be explored.

To our knowledge, no studies in African populations have evaluated the impact of lifestyle, metabolic and genetic factors on PPG variability. Accordingly, our study aimed to assess the genetic factors indicated by PRS and metabolic factors associated with PPG variability in Black South African men and women.

RESEARCH DESIGN AND METHODS

This is a cross-sectional study of the African Wits-IN-DEPTH partnerships for Genomic Research (AWI-Gen) (Ali *et al*., 2018) and Middle-aged Soweto Cohort (MASC) (Goedecke *et al*., 2022), which were used to provide the genotype and phenotype data, respectively. In this study, PPG was calculated using the glucose iAUC, the metabolic factors were clustered using PCA, and PRS for each participant was computed using PRSice. The independent contribution of metabolic factors and PRS to PPG variability was assessed using multivariable linear regression.

Study participants

This study included follow-up data of the MASC, a longitudinal study of 2031 participants residing in Soweto, Johannesburg on whom baseline data were collected between 2011 and 2014 as part of the AWI-Gen Collaborative Centre, which is a Human Heredity and Health in Africa (H3A) study (Ramsay *et al*., 2016). The follow-up was performed between January 2017 and August 2018 (figure 1) and formed part of this study. The MASC study is designed to identify the determinants of noncommunicable disease and T2D risk in middle-aged South African (SA) men and women (Goedecke *et al*., 2022). Data collection for the primary study occurred at the South African Medical Research Council (SAMRC)/University of the Witwatersrand, Developmental Pathways for Health Research Unit at the Chris Hani Baragwanath Hospital in Soweto, Johannesburg, South Africa. The study was conducted following the Declaration of Helsinki and was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (clearance certificate no. M160604 and M160975). Before participation in the research trial, all participants provided a broad informed consent available in the appendix. The data collected from the participants in this study was compiled with the already defined standard operating procedures of data management and curation in the Developmental Pathways for Health Research Unit (DPHRU) and was integrated into the main database. These data are kept in accordance with the Data Protection Act, 1998. The DPHRU endorses a policy that promotes data preservation and sharing. All data are strictly confidential and locked up securely.

The inclusion criteria for this PPG variability study were men and women in MASC with complete OGTT data. The following exclusion criteria were applied: participants with known diabetes or those taking diabetes medication, missing fasting glucose and incomplete OGTT (figure 1). The

final sample from the MASC included 794 participants (406 men and 388 women). Of these participants, 617 had genotype data as part of the AWI-Gen study. AWI-Gen study ethical clearance was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Wits) (protocol numbers M121029 and M170880). This PPG variability study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (clearance certificate no. M220296). The study ethics is available in the appendix.

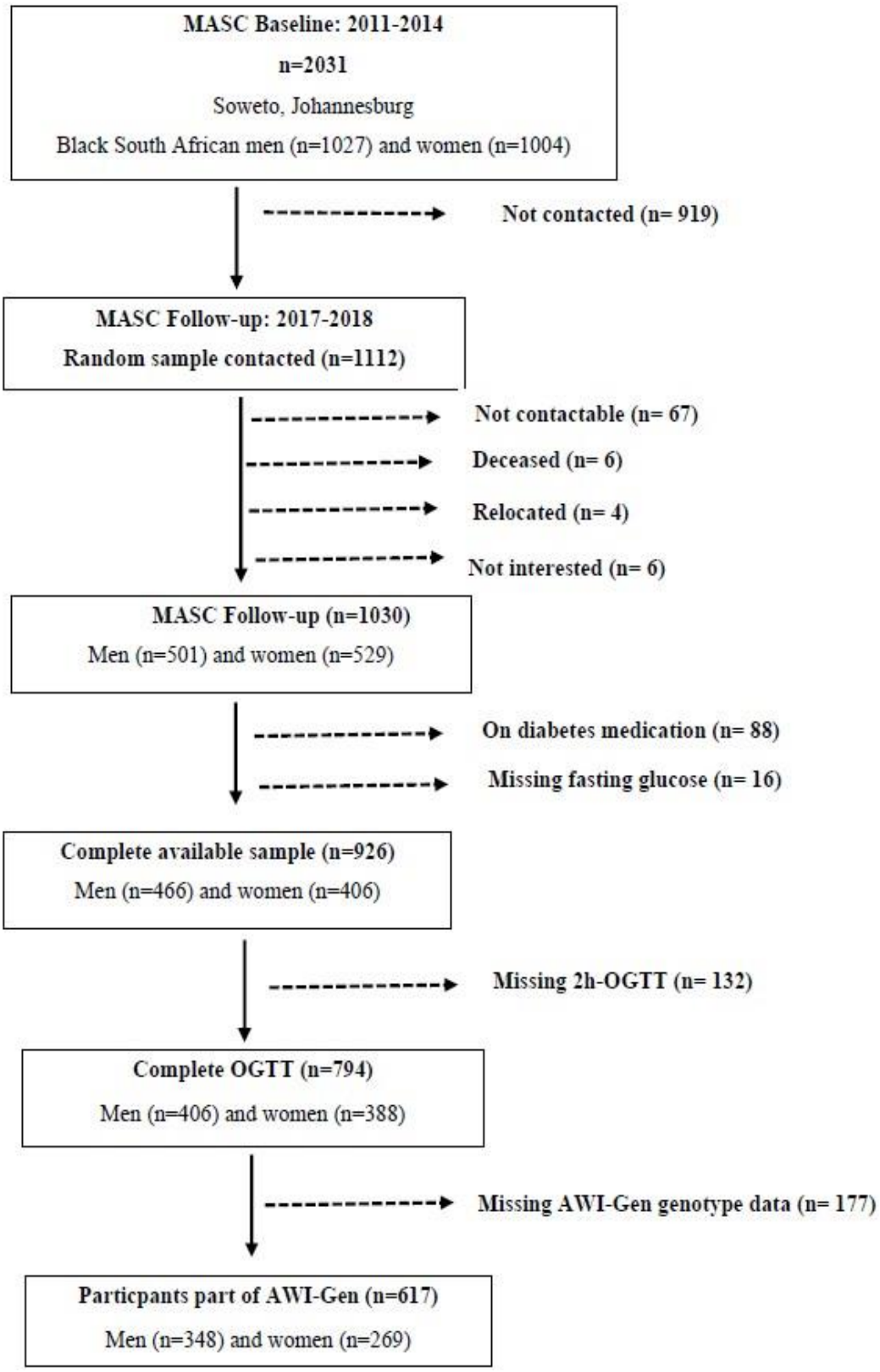


Figure 1: Consort diagram for the Middle-aged Soweto Cohort (MASC) postprandial glucose (PPG) variability study.

The break lines show participants excluded from the MASC follow-up due to incomplete OGTT data and without AWI-Gen genotype data.

Socio-demographic factors and health questionnaires

Demographic factors, socioeconomic status (SES), and medical history were collected through interviewer-administrated questionnaires and captured on Research electronic data capture (REDCap) (Harris *et al*., 2009). This included age, sex, marital status (married vs unmarried), the highest education level (primary, secondary or tertiary), alcohol consumption (Yes or No), smoking tobacco (Yes or No), and asset index. Asset index was calculated by summing assets reported to be owned in the household (Munthali *et al*., 2018). HIV status was assessed using an antibody HIV test in HIV-negative participants (Wondfo One Step HIV–1/2 Whole Blood/Serum/Plasma: Test 2 lines Guanghu Wondfo Biotech Co., Ltd., Hong Kong, China). Those that were found to be living with HIV were referred to a local clinic but retained in the study. In addition, participants were asked to bring in their chronic medications, which were documented, e.g. diabetic medication, hypertension medication, lipid medication, and hormone replacement therapy (Yes or No). The menstrual stage was assessed in women using the final menstrual cycle, which was classified as pre-menopausal, perimenopausal or post-menopausal (Jaff *et al*., 2014).

Blood pressure, body composition, and body fat distribution measures

The participant's weight and height were measured to the nearest 0.1 kg and 0.1 cm, using a digital scale and wall-mounted stadiometer, respectively (Goedecke *et al*., 2022). The waist circumference (WC) and hip circumference (HC) were measured to the nearest 0.1 cm with a non-stretchable tape. The tape was placed horizontally between the iliac crest in the mid-axillary plane and the lowest rib margin for WC and HC; it was placed around the level of the most significant protrusion of the buttocks. The waist-to-hip ratio (WHR) was calculated. Sub-total (total body minus head) fat mass, fat-free soft tissue mass (FFSTM), and regional adiposity (trunk, arm, gynoid, android, and leg fat mass, expressed as a percentage of total fat mass (%FM)) were measured using a dual-energy X-ray absorptiometry (DXA) (QDR 4500A, Hologic Inc., Bedford, NY, USA, APEX software version 4.0.2). Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were estimated as described before (Micklesfield *et al*., 2012). Systolic and diastolic blood pressures were measured to the nearest 0.1 mmHg on the left arm with a digital blood pressure monitor (Omron M6, Kyoto, Japan) and appropriate cuffs. After the participant had been seated for at least 5 minutes, three blood pressure readings were taken at 2-minute intervals. For each participant, the average of the second and third readings was used in the analyses.

Physical activity and sleep

Participants were asked to wear two accelerometers, ActivPAL (PAL Technologies Ltd) and ActiGraph (GTX3+, ActiGraph LLC), on the mid-anterior right thigh and right hip, respectively, to

assess their physical activity and sedentary behaviour. Participants were asked to wear the accelerometers for seven days and nights and record their daily sleep (including naps) and waking times in a dairy. Monitors were used to record the raw triaxial signals from the accelerometers. These were calibrated to local gravity, periods of non-wear were removed, and activity-related acceleration (Euclidean norm minus one (ENMO)) and thigh pitch angles were derived at a minute level. The signals from the monitors were combined, and self-reported sleep times were overlaid onto these time series (Mendham *et al*., 2022). Data were summarized at the participant level to derive total movement volume, and the magnitude and angle of the signals were used to derive time spent in postures and intensities of activity (Micklesfield *et al*., 2022). The variables reported were total movement volume (ENMO expressed as milli- gravitational units) and time (in minutes per day) spent sleeping.

Blood sampling and biochemical measures

At ~8 am and after an overnight (10-12 hours) fast, blood samples were taken for the measurement of fasting insulin, glucose, liver enzymes (alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT)), sex hormones (luteinising hormone (LH), folliclestimulating hormone (FSH), testosterone, and estrogen, androstenedione and dehydroepiandrosterone (DHEA)), lipids (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG)), and glucocorticoids (cortisol and cortisone). After that, a standard 2-hour OGTT was conducted. After ingesting 75 g of glucose (dissolved in 250 ml water). Blood samples were drawn at 30 minutes intervals for 2 hours to measure plasma glucose concentrations.

FSH and LH were measured with the ARCHITECT Chemiluminescent Microparticle Immunoassay assay (Abbott Laboratories), testosterone, androstenedione, DHEA and glucocorticoids with a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, USA). Estrogen was measured using a Waters Acquity® Xevo TQ-XS mass spectrometer. Serum Insulin and lipid concentrations were measured with Immulite® 1000 Immunoassay System (Siemens Chemiluminescent Healthcare GmbH, Henkestr, Germany) and glucose concentrations with a Randox RX Daytona Chemistry Analyser (Randox Laboratories Ltd., London, UK), respectively (Kufe *et al*., 2022). TC, TG, and HDL-C were measured directly from the Immulite® 1000 Immunoassay System (Siemens Chemiluminescent Healthcare GmbH, Henkestr, Germany). LDL-C was calculated with the Friedewald equation (Friedewald *et al*., 1972).

Classification of OGTT

Participants were classified as normal glucose tolerance (NGT, fasting glucose <6.1 mmol/L and 2hour OGTT <7.8 mmol/L), impaired glucose metabolism (IGM fasting glucose: 6.1- 6.9 mmol/L and 2-hour post glucose load: 7.8 - 11.0 mmol/L), and T2D (fasting glucose ≥7 mmol/L and 2-hour post glucose load ≥11.1 mmol/L) according to the World Health Organisation criteria (World Health Organization, 2006).

Genotyping and imputation

The participants, who formed part of the AWI-Gen cohort, were genotyped using the 2.3 M SNP H3Africa array at Illumina® FastTrack™ Microarray services (Illumina, San Diego, USA) and the Illumina pipeline was used for genotype calling (Choudhury *et al*., 2022). The pre-imputation and imputation quality control (QC) were carried out using the H3ABioNet/H3Agwas pipeline and the African Genome Resources reference panel (EAGLE2 +PBWT pipeline) at the Sanger Imputation Server. Detailed post-imputation QC resulted in the final AWI-Gen dataset containing 10,603 participants and 13.98 M SNPs, as described before (Choudhury *et al*., 2022). For this study, we used the AWI-Gen genotype data for the subset of the Soweto site participants (617) who are also participants of the MASC cohort that had complete OGTT data.

Statistical analysis

Descriptive statistics. Data were analyzed using R Studio version 2022.02.0, which uses R version 4.1.3. Categorical variables are represented as frequencies (n) and percentages (%), continuous variables that were normally distributed are represented as mean ± standard deviation (SD), and continuous variables that were not normally distributed are represented as median (25th - 75th percentiles). A Shapiro-Wilks test was used to check if continuous variables followed a normal distribution. Between men and women, Mann-Whitney U and t-test were used for non-parametric and parametric continuous variables, respectively. The chi-squared test was used to compare categorical variables between men and women.

AUC calculation. The glucose iAUC was calculated in R (version 2022.02.0) using the trapezoidal rule (Sakaguchi *et al*., 2016).

Multivariable analysis. Due to the significant differences in lifestyle and metabolic factors, as well as the differential association between body fatness and T2D risk (Kufe *et al*., 2022), separate principal component analysis (PCA) for men and women were performed. PCA was used for multivariable dimension reduction to derive latent variables termed principal components (PCs) from 31 metabolic factors for men and women separately. PCA was chosen because it is a data-

driven approach that can calculate the covariances of data, find patterns in the data, and reduce the dimension of large datasets (Fiamoncini *et al*., 2018). The following variables were included in the PCA: central fats (WC, SAT, android fat, and trunk fat), peripheral fats (HC, gynoid fat, and leg fat), liver enzymes (AST, GGT, and ALT), serum lipids (TG, TC, and LDL), sex hormones (FSH and LH), and steroid hormones (androstenedione, oestradiol, cortisol, cortisone, and DHEA). These variables were standardized to ensure they were on the same scale before the PCA analysis. The scree plot was used to determine the number of PCs to retain. (Supplemental figure 1). The PCs were named based on the variables that had higher factor loadings.

Polygenic risk scores. We utilised a PRS developed and validated for T2D in African populations (Chikowore *et al*., 2022). This PRS was developed using PRSice 2 software and computed using the following parameters for pruning (clumping distance of 500 kb and R^2 of 0.5) and using the African Americans GWAS from the Million Veterans Program (MVP) as discovery (figure 2) while adjusting for age, sex and population structures as described elsewhere (Chikowore *et al*., 2022) For our study, we used the summary statistics comprising of the SNPs and weights of this PRS to compute genetic risk scores for the participants using PRSice for the 617 MASC participants .This genetic risk score was then utilised in the linear models described below.

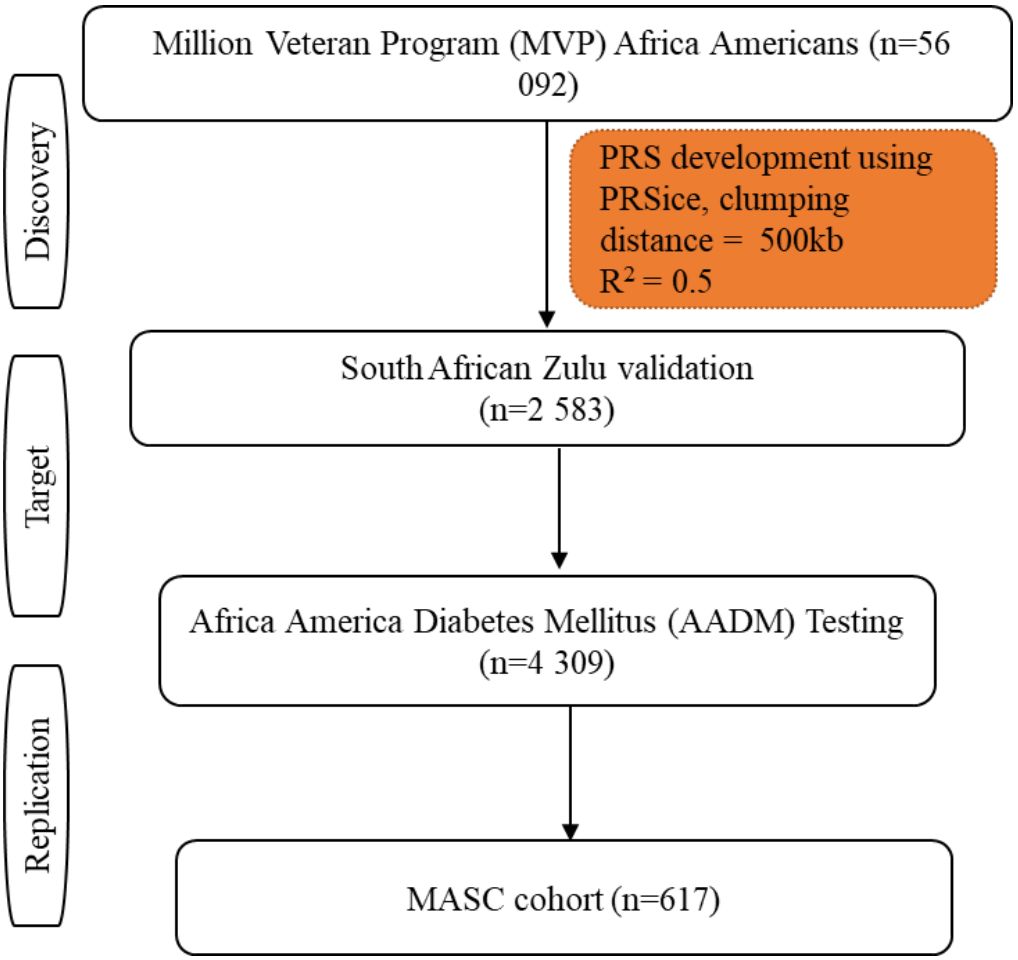


Figure 2: Schematic diagram for the development of the T2D PRSs and its application in the MASC cohort.

Linear regression models. Multivariable linear regression was used to evaluate the independent contribution of PRS and metabolic factors to PPG variability, estimated as the glucose iAUC in men and women separately. Four separate regression models were performed to predict PPG variability. Null model: included only covariates that are known to alter the glycaemic response to glucose: age, menopausal stage, smoking status, alcohol consumption, blood pressure medications, lipid medication, HIV status, total physical activity, the total amount of sleep, asset index. Although lifestyle, environmental, and socio-economic variables describe the study population, these were removed from the models because they were not significant and did not add to the variability explained. Genetic principal components were added to the null model for the PRS to correct for population structure; Model 1: null model and metabolic factors (PCs); Model 2: null model and PRS; Model 3: null model, PRS and metabolic factors (PCs). To determine the relative contribution to the PPG variability, the variance from the null model (R^2) was subtracted from the variance (R^2) of models 1, 2 and 3. This enabled us to determine variance accounted by metabolic factors, PRS and these two combined in models 1, 2, and 3, respectively.

RESULTS

Participants socio-demographic and body fat distribution

A total of 794 participants with a mean age of 53 years were included. More men were married than women (52.1% vs 41.3%), more men smoked tobacco (49.6% vs 6.5%) and consumed alcohol (33.6% vs 5.2%) compared to women.

The mean BMI was higher in women than men (P<0.001), and more women were classified as living with obesity than men (92.5% vs 20.9%) (table 1). Accordingly, women had significantly higher waist and hip circumferences, but the waist-to-hip ratio (WHR) was lower in women than in men. The fat-free soft tissue mass (FFSTM) was higher in men (P<0.001), but body fat (%), body fat mass (kg), and fat mass index (kg/m^2) were higher in women than in men. When expressed relative to FM, men had more central fat mass (trunk FM and android FM), while women had higher fat in the lower extremities (leg FM and gynoid FM). The arm FM in both men and women did not differ. The central measures, VAT and SAT (both P<0.001), were higher in women, but the VAT/SAT ratio was higher in men than in women (table 1).

Biochemical measures, and AUC

Fasting glucose and glucose tolerance categories did not differ between men and women. Although the glucose iAUC was slightly higher in men compared to women (P=0.042) (table 1), there were still considerable inter-individual differences in PPG responses to the OGTT in both men and women (figure 3).

While HDL concentrations were similar, TC and LDL concentrations were higher and TG lower in women compared to men. The liver enzymes AST, ALT, and GGT were higher in men than in women (P<0.001). As expected, the sex hormones LH, FSH, and dehydroepiandrosterone (DHEA) were more elevated in women, whereas androstenedione, estrogen, progesterone, and testosterone were higher in men. Cortisol and cortisone were higher in men than in women.

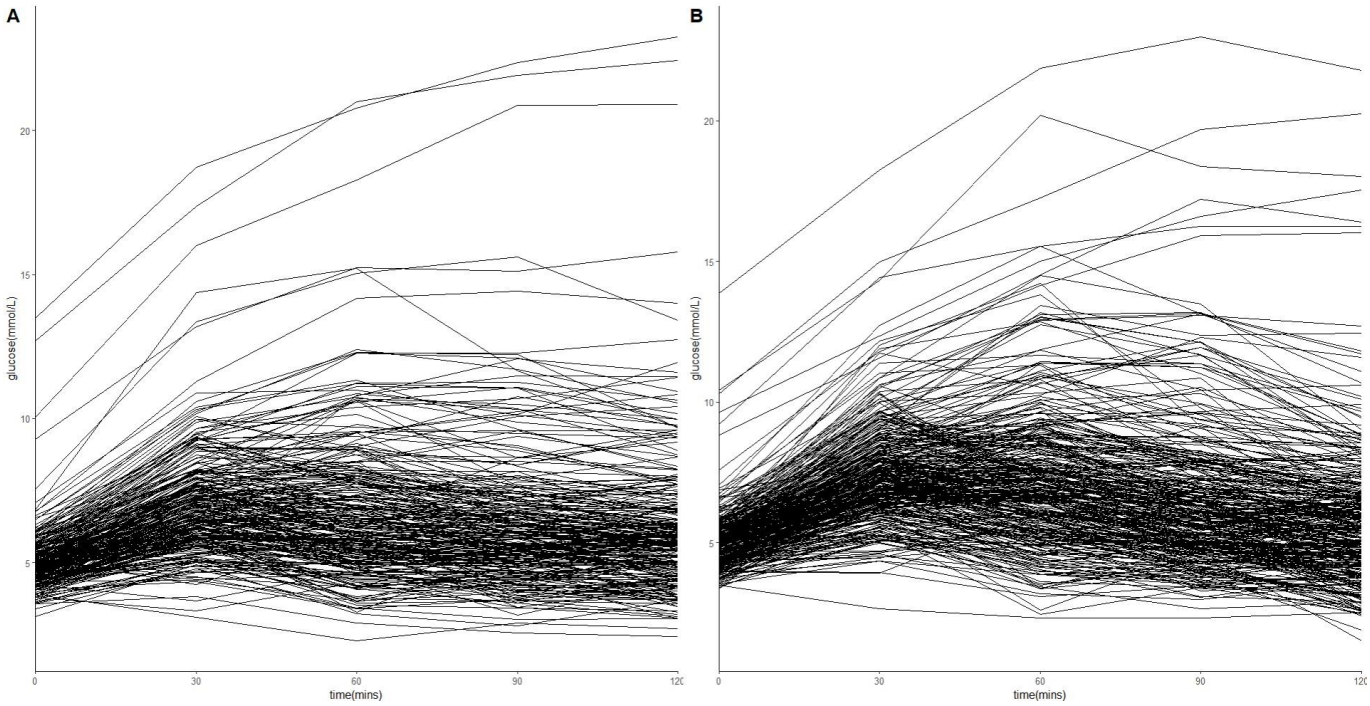


Figure 3: Glucose curves showing PPG inter-individual variability in women (A) and men (B) during a 2h OGTT.

Table 1: Socio-demographic status, body composition, and biochemical measures in Black South African men and women.

Variable	Men	Women	*P-value*
n (%)	406 (51.1)	388 (48.9)	-
Socio-demographic characteristics			
Age (years)	52.0 (47.0 – 58.0)	53.4 (49.0 – 58.0)	0.112
Married/cohabiting (n (%))	214 (52.8)	162 (41.9)	<0.001
Employed (n (%))	163 (40.2)	153 (39.5)	0.895
Lifestyle characteristics			
Sleep (min/d) (n = 599)	424.0 (371.7 – 471.7)	411.8 (362.2 – 455.65)	0.059
ENMO (milli-g) (n = 600)	14.3 (11.4 – 17.5)	12.3 (10.0 – 14.5)	<0.001
Smoking tobacco (n (%))	206 (50.9)	24 (6.2)	<0.001
alcohol intake (n (%))			<0.001
No Alcohol intake	103 (25.4)	277 (71.6)	
Alcohol intake (monthly or less and 2-4 times a month)	166 (41.0)	90 (23.3)	
Alcohol intake (2-3 times per week or more)	136 (33.6)	20 (5.2)	
Education (n (%))			0.204
No formal schooling/elementary school level (n (%))	47 (33.3)	31 (8.7)	
Secondary school level (n (%))	287 (17.6)	311 (79.8)	
Tertiary education (n (%))	135 (48.9)	45 (11.3)	
Body composition measures			
Height (cm)	171.4 (167.0 – 175.2)	158.2 (154.0 – 162.3)	<0.001
Weight (kg)	72.2 (60.8 – 84.2)	80.9 (71.7 – 94.1)	<0.001
BMI (kg/m^2)	24.5 (20.8 – 29.1)	32.8 (28.5 – 37.3)	<0.001
Waist circumference (cm)	92.4 (82.0 – 102.7)	95.5 (87.4 – 103.6)	0.003
Hip circumference (cm)	98.1 (90.8 – 105.4)	114.6(106.4 – 123.4)	<0.001
WHR	0.94 (0.90 – 0.98)	0.85 (0.77 – 0.88)	<0.001
BMI groups, n (%)			<0.001
Underweight (<18.5 kg/m^2)	38 (9.4)	18 (4.6)	
Normal weight (18.50-24.99 kg/m^2)	169 (41.6)	6 (1.5)	
Overweight (25-29.99 kg/m^2)	114 (28.1)	5 (1.3)	
Obese (≥30.0 kg/m^2)	85 (20.9)	359 (92.5)	
DXA-derived body composition (n = 756)			
Fat–free soft tissue mass (kg)	47.5 (41.6 – 53.1)	40.4 (36.4 – 44.9)	<0.001
Body fat mass (kg)	17.4 (11.6 – 22.8)	35.0 (28.5 – 41.7)	<0.001
Body fat (%)	26.0 (20.7 – 30.4)	45.2 (41.6 – 48.4)	<0.001
Trunk (% FM)	46.6 ± 5.1	43.3 ± 5.9	<0.001
Leg (% FM)	41.0 ± 4.8	44.2 ± 6.3	<0.001
Arm (%FM)	12.4 ± 1.3	12.5 ± 1.8	0.738
Gynoid (%FM)	17.1 ± 1.8	17.8 ± 2.3	<0.001
Android (%FM)	8.4 ± 1.5	7.4 ± 1.4	<0.001
VAT (cm^2)	85.5 ± 43.3	104.1 ± 43.3	<0.001
SAT (cm^2)	197.0 ± 119.7	461.4 ± 145.8	<0.001
VAT/SAT	0.51 (0.36 – 0.66)	0.23 (0.17 – 0.27)	<0.001
Glucose and insulin measures (n = 794)			
Fasting glucose (mmol/L)	5.0 (4.4 – 5.5)	5.0 (4.5 – 5.4)	0.911

2 h glucose (mmol/L)	5. 7 (4.4 – 6.8)	5.8 (4.9 – 7.1)	0.017
Glucose $_{iAUC}$ (mmol/L)	13.0 (11.0 – 14.8)	12.4 (10.7 – 14.8)	0.042
Glucose tolerance status n (%)			0.237
NGT: (FPG <6.1 / 2-h PG <7.8 mmol/L)	331 (81.5)	308 (79.6)	
IGM: (FPG 6.1–6.9 / IGT 2hPG: 7.8–11.0)	56 (13.8)	61 (15.8)	
T2DM: (FPG ≥7 /2-h PG ≥11.1 mmol/L)	19 (4.7)	18 (4.7)	
Serum lipids			
TG (mmol/L) (n = 793)	0.9 (0.7 – 1.4)	0.8 (0.6 – 1.0)	<0.001
TC (mmol/L) (n = 793)	4.3 (3.6 – 4.9)	4.5 (3.9 – 5.2)	0.003
LDL (mmol/L) (n = 793)	2.4 (1.8 – 3.0)	2.8 (2.2 – 3.4)	<0.001
HDL (mmol/L) (n = 793)	1.3 (1.0 – 1.5)	1.3 (1.1 – 1.5)	0.208
Liver enzymes			
ALT (µKat/L) (n = 793)	0.4 (0.3 – 0.5)	0.3 (0.3 – 0.4)	<0.001
AST (µKat/L) (n = 793)	0.7 (0.5 – 0.9)	0.7 (0.6 – 0.8)	<0.001
GGT (µKat/L) (n 793)	0.7 (0.5 – 1.3)	0.5 (0.3 – 0.7)	<0.001
Sex hormones			
Estrogen (pmol/L) (n = 767)	125.5 (96.0 -154.5)	88.0 (57.0 – 135.9)	<0.001
Testosterone (nM) (n = 774)	15.3 (11. 9 – 19.5)	0.3 (0.2 – 0.5)	<0.001
LH (pmol/L) (n = 768)	3.6 (2.5 – 5.0)	18.6 (10.4 – 19.4)	<0.001
FSH (pmol/L) (n = 768)	4.9 (3.5 – 7.4)	46.5 (23.5 – 64.0)	<0.001
Steroid hormones			
Androstenedione (nmol/L) (n = 774)	2.6 (1.8 – 3.5)	1.3 (0.9 – 1.8)	<0.001
DHEA (nmol/L) (n = 774)	1.8 (0.0 – 8.9)	5.2 (1.8 – 7.9)	<0.001
Cortisol (pmol/L) (n = 774)	322.8 (246.0 – 412.3)	250.9 (194.5 – 321.7)	<0.001
Cortisone (pmol/L) (n = 774)	60.7 (51.1 – 69.7)	46.1 9 (39.3 – 55.5)	<0.001

Values are expressed as mean±sd, median (25th – 75th percentile), or n (%). Abbreviations: BMI, body mass index; DXA, dual-energy X-ray absorptiometry; ENMO, Euclidean norm minus one; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist-hip ratio; IGM, impaired glucose metabolism; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; LH, luteinising hormone; FSH, follicle-stimulating hormone; DHEA, dehydroepiandrosterone; glucose $_{iAUC}$, integrated area under the curve

Principal component analysis

Based on the scree plots, three PCs were selected as they accounted for most of the variance and adding extra PCs did not account for extra variance (supplemental figure 1). In women, PC1 captured the greatest variance (21.3%) (supplemental figure 1A) and was driven by central fat (WC, SAT, android fat, and trunk fat) as shown by the factor loadings in figure 4A and supplemental table 1. This was followed by PC2 (9.8%) (supplemental figure 1A), driven by serum lipids (TG, TC, and LDL) and liver enzymes (AST, GGT, and ALT). The major contributors of PC3 (8.0%) (supplemental figure 1A) in women were steroid hormones (androstenedione, oestradiol, cortisol, cortisone, and DHEA).

In men, PC1 (27.0%) captured the most variance (supplemental figure 1B) and the major contributors were peripheral fat (gynoid fat, leg fat) as shown by the factor loadings in figure 4B

and supplemental table 1. This was followed by PC2 (10.6%) (supplemental figure 1B), driven by liver enzymes (AST, GGT, and ALT) and steroid hormones (androstenedione, cortisol, cortisone, and DHEA). Serum lipids (TG, TC, and HDL) and peripheral fat (gynoid fat, leg fat, and hip circumference) were the major contributing factors for PC3 (6.5%) as shown in figure 4B and supplemental table 1.

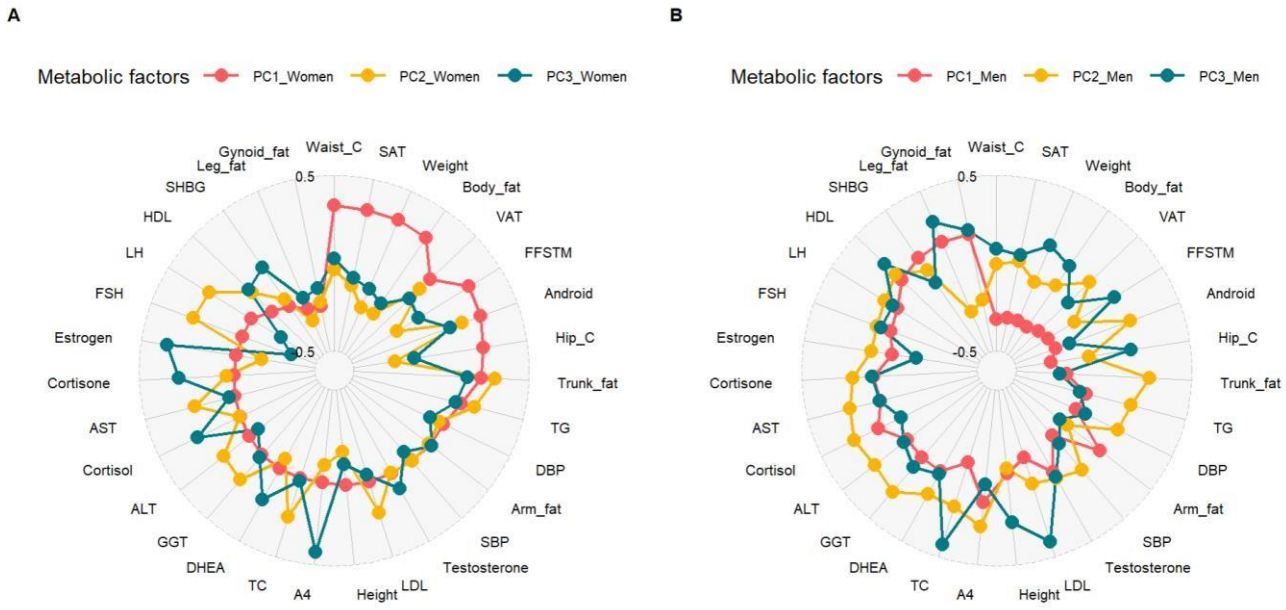


Figure 4: Radar plot showing factor loadings contributions of the metabolic factors to the principal components in women (A) and men (B).

Abbreviations: PC, principal component; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; LH, luteinising hormone; FSH, follicle-stimulating hormone; DHEA, dehydroepiandrosterone; HC, hip circumference; WC, waist circumference; DBP, diastolic blood pressure; SBP, systolic blood pressure; FFSTM, Fat–free soft tissue mass; SHBG, sex hormone binding globulin.

Association of PRS and Metabolic factors with PPG

In women, the null model including all covariates accounted for 14.1% (P=0.035) of the variability in PPG. The metabolic factors (model 1) contributed an additional 10.8% to the variability and only PC1 (central fat) and PC2 (lipids and liver enzymes) were significantly (P<0.001 and P=0.003) associated with PPG (table 3). The addition of PRS (model 2) to the null model, did not contribute significantly to the variance (P=0.050) and PRS was not associated with PPG (P=0.564). Accordingly, when adding both metabolic factors and PRS (model 3) to the null model, only PC1 (central fat) and PC2 (lipids and liver enzymes) were significantly associated with PPG (P<0.001 and P=0.005) and the model did not account for any additional variability above that which was explained by the metabolic factors, as reported in Model 1 (10.9% of the variance).

In men, the null model including the covariates contributed an insignificant 4.5% (P=0.398) to the variability in PPG. The metabolic factors (model 1) contributed an additional 10.6% to the variability, with PC1 (peripheral fat), PC2 (liver enzymes and steroid hormones), and PC3 (serum lipids and peripheral fat) significantly associated with PPG (P<0.001, P<0.001, and P=0.033, respectively) (table 4.) PRS (model 2) did not add significantly to the model (P=0.453). When PRS and metabolic factors were combined in Model 3, these factors explained 11.1% of the variance, and only PC1(peripheral fat), PC2 (liver enzymes and steroid hormones), and PC3 (serum lipids and peripheral fat) remained significantly associated with PPG (P<0.001, P<0.001, and P=0.035, respectively) (table
4).

Table 2: Multivariable regression associations between the iAUC and metabolic factors and polygenic risk scores in women (n = 269)

	Null Model **Model R^2** =0.141 Model ***P*-value** =0.035			**Model 1** **Model R^2** =0.249 **Model *P*-value** <0.001			**Model 2** **Model R^2** =**0.**142 **Model *P*-value** =0.051			**Model 3** **Model R^2** = 0.250 **Model *P*-value** <0.001		
Variables	**β**	**95% CI**	***P* Value**	**β**	**95% CI**	***P* Value**	**β**	**95% CI**	***P* Value**	**β**	**95% CI**	***P* Value**
Age	0.135	0.011 to 0.260	0.033	0.098	-0.021 to 0.219	0.108	0.133	0.008 to 0.258	0.036	0.098	-0.022 to 0.219	0.111
Asset index	0.133	-0.152 to 0.418	0.360	0.166	-0.103 to 0.436	0.226	0.140	-0.146 to 0.428	0.334	0.174	-0.098 to 0.446	0.208
Peri-menopausal stage	0.384	-1.569 to 2.337	0.699	0.835	-1.050 to 2.721	0.383	0.408	-1.550 to 2.368	0.681	0.858	-1.033 to 2.750	0.371
Post-menopausal stage	-0.154	-1.784 to 1.476	0.852	-0.105	-1.656 to 1.445	0.893	-0.102	-1.745 to 1.541	0.902	-0.050	-1.617 to 1.517	0.949
Pre-menopausal stage	0.434	-1.403 to 2.277	0.640	0.222	-1.572 to 2.017	0.806	0.480	-1.369 to 2.330	0.608	0.248	-1.552 to 2.049	0.785
Smoking tobacco	0.437	-2.056 to 3.129	0.684	0.043	-2.443 to 2.531	0.972	0.590	-2.014 to 3.194	0.655	0.088	-2.409 to 2.586	0.944
HBP med	0.890	-0.350 to 2.131	0.159	0.747	-0.438 to 1.933	0.215	0.876	-0.367 to 2.120	0.166	0.736	-0.452 to 1.926	0.223
Lipid med	-0.277	-2.742 to 2.188	0.825	-0.517	-2.849 to 1.814	0.661	-0.262	-2.733 to 2.208	0.834	-0.505	-2.842 to 1.831	0.669
Total sleep	0.000	-0.007 to 0.008	0.928	-0.000	-0.007 to 0.007	0.943	0.000	-0.007 to 0.008	0.919	-0.000	-0.007 to 0.007	0.943
Physical activity	-0.074	-0.237 to 0.090	0.377	-0.036	-0.193 to 0.120	0.643	-0.075	-0.240 to 0.088	0.364	-0.038	-0.196 to 0.118	0.627
HIV + treated	-1.575	-3.214 to 0.064	0.059	**-2.140**	**-3.717 to -0.562**	**0.008**	-1.567	-3.210 to 0.075	0.061	**-2.124**	**-3.706 to -0.542**	**0.008**
HIV + untreated	-0.327	-3.490 to 2.835	0.838	-0.759	-3.747 to 2.229	0.616	-0.281	-3.454 to 2.891	0.861	-0.715	-3.714 to 2.283	0.638
Drink Alcohol	-5.332	-9.260 to -1.404	0.008	**-3.838**	**-7.036 to -0.639**	**0.018**	-5.277	-9.217 to -1.337	0.008	**-3.881**	**-7.091 to -0.672**	**0.018**
Metabolic factors												

PC1	-	-	-	**0.377**	**0.171 to 0.581**	**<0.001**	-	-	-	**0.378**	**0.173 to 0.584**	**<0.001**
PC2	-	-	-	**0.448**	**0.145 to 0.750**	**0.003**	-	-	-	**0.436**	**0.130 to 0.742**	**0.005**
PC3	-	-	-	0.249	-0.099 to 0.599	0.160	-	-	-	0.259	-0.092 to 0.612	0.147
PRS	-	-	-	-	-	-	0.139	-0.336 to 0.615	0.564	0.124	-0.330 to 0.580	0.588

Abbreviations: HBP med, high blood pressure medication; HIV+ human immunodeficiency virus-positive; PC, principal component; PRS, polygenic risk score. *Note*: Null model: All the covariates that were included. Model 1: All the covariates with the metabolic factors (PC1, PC2, PC3). Model 2: the covariates and PRS. Model 3: the covariates and metabolic factors + PRS

Table 3: Multivariable regression associations between the iAUC and metabolic factors and polygenic risk scores in men (n = 348)

	Null Model **Model R^2** =0.453 Model ***P*-value** =0.398			**Model 1** **Model R^2** =0.155 Model ***P*-value** <0.001			**Model 2** **Model R^2** =0.050 Model ***P*-value** =0.589			**Model 3** **Model R^2** =0.161 Model ***P*-value** <0.001		
Variables	**β**	**95% CI**	***P* Value**	**β**	**95% CI**	***P* Value**	**β**	**95% CI**	***P* Value**	**β**	**95% CI**	***P* Value**
Age	0.034	-0.040 to 0.190	0.365	0.033	-0.038 to 0.104	0.360	0.035	-0.039 to 0.110	0.349	0.035	-0.036 to 0.106	0.331
Asset index	-0.116	-0.318 to 0.084	0.254	-0.143	-0.339 to 0.052	0.151	-0.122	-0.324 to 0.079	0.234	-0.153	-0.350 to 0.042	0.124
Smoking tobacco	-0.383	-1.350 to 0.583	0.435	0.046	-0.894 to 0.987	0.922	-0.366	-1.335 to 0.602	0.457	0.085	-0.856 to 1.027	0.858
HBP med	0.617	-0.530 to 1.765	0.290	-0.109	-1.229 to 1.011	0.848	0.644	-0.506 to 1.795	0.271	-0.077	-1.197 to 1.042	0.891
Lipid med	-1.064	-3.506 to 1.377	0.391	-0.629	-2.965 to 1.705	0.596	-1.068	-3.513 to 1.375	0.390	-0.639	-2.972 to 1.693	0.589
Total sleep	-0.005	-0.011 to -0.000	0.042	-0.005	-0.010 to 0.000	0.066	-0.006	-0.011 to -0.000	0.038	-0.005	-0.010 to 0.000	0.055
Physical activity	-0.080	-0.161 to 0.000	0.051	-0.041	-0.119 to 0.036	0.300	-0.081	-0.162 to -0.000	0.048	-0.041	-0.119 to 0.036	0.292
HIV + treated	-0.625	-1.843 to 0.591	0.312	-0.980	-2.215 to 0.254	0.119	-0.626	-1.845 to 0.591	0.311	-0.991	-2.225 to 0.241	0.114
HIV + untreated	-0.195	-2.616 to 2.224	0.873	-0.559	-2.872 to 1.753	0.634	-0.118	-2.549 to 2.312	0.923	-0.430	-2.749 to 1.888	0.717
Drink Alcohol	0.107	-1.205 to 1.419	0.872	0.178	-1.068 to 1.426	0.777	0.112	-1.201 to 1.425	0.866	0.191	-1.055 to 1.437	0.763
Metabolic factors												
PC1	-	-	-	**-0.342**	**-0.516 to -0.168**	**<0.001**	-	-	-	**-0.348**	**-0.522 to -0.173**	**<0.001**
PC2	-	-	-	**0.475**	**0.210 to 0.742**	**<0.001**	-	-	-	**0.488**	**0.220 to 0.755**	**<0.001**
PC3	-	-	-	**-0.346**	**-0.652 to -0.040**	**0.027**	-	-	-	**-0.342**	**-0.647 to -0.003**	**0.028**
PRS	-	-	-	-	-	-	0.143	-0.232 to 0.518	0.453	0.233	-0.123 to 0.590	0.198

Abbreviations: HBP med, high blood pressure medication; HIV+ human immunodeficiency virus-positive; PC, principal component; PRS, polygenic risk score. *Note*: Null model: All the covariates that were included. Model 1: All the covariates with the metabolic factors (PC1, PC2, PC3). Model 2: the covariates and PRS. Model 3: the covariates and metabolic factors + PRS.

DISCUSSION

This is the first study to evaluate PRS and metabolic factors associated with PPG variability in middle-aged Black SA men and women. In women, metabolic factor principal components driven by; central fat distribution, serum lipids, and liver enzymes were associated with higher PPG and these metabolic factors explained 10.8% of the variability in women. Other variables such as drinking alcohol and being treated for HIV were significant and their models as well, but these were covariates, and the aim was to explore if PRS and metabolic factors association were independent of these covariates. In men, only liver enzymes and steroid hormones driven metabolic PCs were associated with elevated PPG, while peripheral fat and serum lipids were associated with lower PPG. These metabolic factors in men explained 10.6% of the variability in PPG. The PRS was not associated with PPG variability in both men and women.

In our study, we did comparisons in a sex specific manner because previous studies reported women to have a higher 2-hour OGTT compared to men, and this may be a consequence of using a fixed glucose load in the OGTT, irrespective of body composition measures such as body size and height which can impact the glucose uptake (Mauvais-Jarvis, 2018) (Sicree *et al*., 2008). Also, the analyses were performed separately in men and women due to the differences observed in body composition measures, liver enzymes, serum lipids, sex hormones, and steroid hormones.

In this study, central fat driven metabolic PC was closely associated with higher PPG in women. This is expected as it has been reported that the accumulation of central fat is linked to glucose intolerance (Cefalu *et al*., 1995). Similarly, studies in SA have shown that central obesity indicated by the increase in WC and VAT as a risk factor for developing T2D among middle-aged urban Black SA women with WC and VAT measures as key determinants (Mtintsilana *et al*., 2019) (Chantler *et al*., 2015). Another study reported that Black SA women were less insulin sensitive and had greater SAT than VAT (Goedecke *et al*., 2009). Moreover, central fat distribution is linked to insulin resistance, fatty liver, and dyslipidaemia in Black SA women (Keswell *et al*., 2016). Indeed, we found that higher PPG was also associated with elevated serum lipids (LDL, TC, and TG) and liver enzymes driven metabolic PC. Elevated liver enzymes are a marker of a fatty liver reflecting on liver pathology and these have recently been shown to be associated with dyslipidaemia in adults (Kathak *et al*., 2022). Massive studies in the USA have shown that fatty liver is lower in Black Americans than in their white counterpart (Guerrero *et al*., 2009). But even so, for a given amount of fatty liver, Black women were more insulin resistant than white women (Goedecke *et al*., 2015). Interestingly, this is the first study in an African population to find liver enzymes having a direct correlation with PPG levels in both women and men. It is possible to hypothesize that it could

reflect fatty liver and further studies are required to measure hepatic steatosis to find the association.

In contrast to women, we showed that elevated peripheral body fat driven metabolic PC was associated with lower PPG in men. Peripheral body fat distribution is a more 'favourable' as it is associated with reduced diabetes and cardiovascular disease risk (Vasan *et al*., 2018). Our findings on peripheral fat are consistent with a novel finding by Kufe *et al*., (2022), where increased leg fat was associated with a 79% lower risk for T2D and higher insulin sensitivity in Black SA men. Consequently, lipid measures (HDL, LDL, and TC) and peripheral fat distribution driven metabolic PCs, were associated with lower PPG levels. A recent African study found elevated HDL in men to exert a protective effect against T2D by associating with improved PPG measures (Soremekun *et al*., 2022). However, elevated LDL and TC are often associated with dyslipidaemia and increased T2D risk (Soremekun *et al*., 2022). These lipids are known to diminish insulin sensitivity by altering the pancreatic β-cell function (Mooradian, 2009). To our knowledge, this is the African study to find the association between serum lipids (HDL, LDL, and TC) and lower PPG levels. The mechanism mediating this observation is not known. It is possible to hypothesize that the glucose metabolism may be linked to the HMG-CoA-reductase activity or the intracellular lipid concentration compartments and further studies are required to find the association.

Elevated steroid hormones (cortisol, cortisone, DHEA, and androstenedione) driven metabolic PC was associated with higher PPG in Black SA men. High cortisol concentrations are linked to a high risk for T2D because cortisol regulates glucose metabolism in the liver by increasing gluconeogenesis and decreasing glycogen synthesis (Kuo *et al*., 2015). DHEA and androstenedione are precursors of sex hormones in men and women (Phillips, 1996). Studies on the role of DHEA and androstenedione in T2D risk remain scarce. A recent European study involving a healthy population of men and women found no association of DHEA and androstenedione levels with T2D incidence (Brahimaj *et al*., 2017). Instead, the study suggested that DHEA may offer a protective role against T2D because DHEA is a peroxisome proliferator-activated receptor (PPAR) α agonist, which when bound by a PPARα ligand, it can reduce the onset of T2D. Overall, the results from previous studies have different findings on the effect of steroid hormones and the risk for T2D. To our knowledge, this is the first African study to find the association between steroid hormones (cortisol, cortisone, DHEA, and androstenedione) and PPG levels in men.

Polygenic risk scores have demonstrated the potential to improve risk stratification in large populations, assist in diagnosis and refine risk management and treatment strategies (Yanes *et al*., 2020). Contrary to expectations, this research did not find T2D PRS significantly associated with

PPG variability. In a previous UK study, the additive genetic factors explained 48% of the variance in PPG (Berry *et al*., 2020). However, the study used GWAS specific to PPG, was performed in twin studies, and comprised of younger healthy adults of European ancestry (Berry *et al*., 2020). Our study used the T2D PRS in middle-age Black Africans because of the unavailability of GWAS specific to PPG. PPG assessed from the OGTT is a stronger predictor of T2D than fasting plasma glucose due to PPG being reflective of peripheral or whole body glucose metabolism than hepatic glucose metabolism in individuals of African ancestry (Cavalot *et al*., 2006) (Briker *et al*., 2020). But even so, the African T2D PRS that were used still did not explain the observed PPG variability in Black middle-aged SA men and women due to the unavailability of GWAS specific to PPG in Black Africans, hence no association was found in this study. Therefore, developing GWAS and PRS for PPG in African populations can be considered.

Limitations and strength

Our study was limited in that we used the glucose iAUC to capture the full variability of PPG, but other methods such as the mean absolute glucose change, coefficient of variation, and Bayesian analysis of glucose dynamics during OGTT may be considered for future studies. Our study was a cross-sectional design from which we cannot infer causality and thus, it is difficult to say anything certain about the direction of the associations. We did not make use of the longitudinal analyses because the OGTT was only measured in the follow-up and not in the baseline collection point. The detailed phenotyping was only performed in the follow-up and not at baseline. However, this is the plan for future analyses. Lastly, the African American population that was used to generate the PRS differs genetically from continental Africans and this might have contributed to the limited predictive power of the T2D PRS.

Nonetheless, our study had the following strengths of having participants in the study that were well-phenotyped with DXA measures of body composition and fat distribution, detailed biochemical measures, and steroid hormones measured using a mass spectrometer which is a gold standard. Our study is among the first to use metabolic clustering on such an extensive range of phenotypic data. However, future studies should include the gut microbiome and other multi-omics approaches, which have been found to be associated with PPG variability (Berry *et al*., 2020).

CONCLUSION

We show for the first time using data from Black middle-aged SA that inter-individual differences in PPG responses to the OGTT can be explained by body fat distribution, serum lipids, liver enzymes, and steroid hormones. Our findings also show that there are still unknown factors contributing to inter-individual variability. Understanding the factors associated with the PPG

variability from identical meals will help in precision medicine approaches for people in Africa. Hence, multi-omic studies are required to identify other contributing factors to the variability in Black South Africans.

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APPENDICES

A. Research protocol

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PART-TIME OR FULL-TIME: **Full-time**		
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DEPARTMENT: **Human Genetics**		
TITLE OF PROPOSED RESEARCH: **Evaluation of the genetic and metabolic determinants of postprandial glucose variability in black South Africans**		
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WITS ETHICS NOT REQUIRED:	Yes	No	IF Y SUPPLY ETHICS CLEARANCE CERTIFICATE AS ATTACHMENT AND INCLUDE ETHICS NUMBER HERE: **M220296**
WITS ETHICS PENDING:	Yes	No	
WITS ETHICS APPROVED:	(Yes)	No	

***Please note the final human ethics clearance certificate or**

animal ethics certificate must be available prior to starting research		
As supervisor/s, I/we confirm that I have read the protocol which has been submitted for assessment.		
SIGNATURE OF SUPERVISOR/S:		
SIGNATURE PG OFFICE STAFF ……	REGISTERED YES….. NO…..	STAMP

SYNOPSIS OF RESEARCH:

Introduction: Postprandial glucose (PPG) is the amount of glucose in the blood after a meal. It is determined by a person's unique biological and lifestyle characteristics. Elevated PPG is a risk factor for type 2 diabetes (T2D), even in individuals with normal fasting glucose. Notably, there is high inter-individual variability in PPG responses to identical meals. The interplay of genetic and environmental factors is thought to result in PPG variability. However, the precise genetic factors and intermediary metabolic predictors (metabotypes) that account for the variation in PPG have not been determined in black South Africans.

Aim: To evaluate the genetic factors in polygenic risk scores and metabotypes associated with postprandial glucose variability in black South Africans.

Justification for study: Understanding the factors associated with the PPG variability from identical amounts of foods/nutrients, will help with precision nutrition dietary approaches for people in Africa

Proposed methodology: This study is a secondary analysis of 1002 Black South African men (n=500) and women (n=502) from the Middle-aged Sowetan Cohort (MASC). Baseline measurements included anthropometry, fasting glucose, liver enzymes, hormones, and body composition measures. The participants underwent an oral glucose tolerance test (OGTT) from which plasma glucose was measured every 30 minutes for 2-hours. Principal component analysis (PCA) will be used to derive metabotypes from body composition, anthropometric and biochemical measures. PRSs for the participants will be computed using variants and weights from validated T2D PRS for continental Africans. Linear regression models will then be used to find the PPG variance accounted for by PRSs, metabotypes separately and combined.

Anticipated outcomes: The predictivity of the PRS and metabotypes for PPG will be determined. This will help in clarifying the usefulness of these factors for stratifying the response of black South Africans to carbohydrate-based foods and future precision nutrition efforts.



Research Proposal

Evaluation of the genetic and metabolic determinants of postprandial glucose variability in black South Africans

by

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Master of Science (Med) in Genomic Medicine

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List of abbreviations

ALT	Alanine transaminase
AST	Aspartate transaminase
GGT	Gamma-glutamyl transferase
BMI	Body mass index
DXA	Dual-energy X-ray absorptiometry
FFSTM	fat-free soft tissue mass
GWAS	Genome-wide association studies
IDF	International Diabetes Federation
IGM	Impaired glucose metabolism
OGTT	Oral glucose tolerance test
MASC	Middle-aged Soweto Cohort
NGT	Normal glucose tolerance
PC	Principal component
PCA	Principal component analysis
PPG	Postprandial glucose
T2D	Type 2 diabetes
SAT	Subcutaneous adipose tissue
SES	Socio-economic status
SD	Standard deviation

SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
VAT	Visceral adipose tissue
WHR	Waist-to-hip ratio

List of figures

Figure 1: Factors affecting inter-individual PPG variability

Figure 2: Plasma glucose concentration during a 2-hour OGTT

Figure 3: Schematic diagram for approaches used in the development of PRSs for PPG in black South Africans using African and multi ancestry groups

1. Introduction

Type 2 diabetes (T2D) mellitus is a multifactorial disease caused by the complex interaction of genetic and environmental factors (Sirdah & Reading, 2020). As of 2019, the prevalence of diabetes in Africa was 4.7 %, and the Sub-Saharan Africa (SSA) region has the highest projected rates of increase in diabetes compared to the other International Diabetes Federation (IDF) regions (Saeedi et al., 2019). African countries have a high proportion of undiagnosed people living with diabetes (59.7%) and have minimal resources to manage the burden (Saeedi et al., 2019). It is important to understand the risk factors for T2D, to enable the development of interventions to curb the burden of T2D in Africa.

Postprandial glucose (PPG) is one of the measures used to assess diabetes risk. PPG refers to blood glucose levels after a meal (Berry et al., 2020). Dysregulation of PPG may lead to postprandial hyperglycaemia (elevated blood glucose) and postprandial hypoglycaemia (low blood glucose) (Zeevi et al., 2015). Elevated PPG is a risk for T2D and cardiovascular diseases even in individuals with normal fasting glucose (Berry et al., 2020).

Notably, there is high inter-individual variability in the PPG responses to identical meals (Berry et al., 2020). This variability is due to the complex interactions of genetic and environmental factors.

1.1. PPG Assessment

PPG is assessed using the oral glucose tolerance test (OGTT), which is widely used in clinical settings to diagnose T2D mellitus (Tschritter et al., 2003). The OGTT is conducted by administrating 75 grams of a glucose load, followed by measuring plasma glucose levels every 30 minutes for 2 hours (Tschritter et al., 2003).

The two most common approaches to measure PPG variability are the shape index and glucose area under the curve (AUC) (Cook, 2007). The glucose AUC is an index for assessing the increase in blood glucose levels after the glucose load and it is commonly used for calculating the glycaemic index (Pigsborg & Magkos, 2022). However, the AUC is a less sensitive metric than the shape index (Cook, 2007).

The shape index is computed by subtracting plasma glucose levels at 90 minutes from those at 120 minutes during the OGTT. A shape index greater than zero represents a biphasic shape (two peaks) and a shape index less than zero represents a monophasic shape (one peak) (Tschritter et al., 2003). More peaks are associated with higher variability and correspond with improved metabolic conditions such as lower glucose concentrations (Tschritter et al., 2003). Patients with T2D mellitus show monophasic shape indices whereas participants with normal glucose tolerance show biphasic indices (Tura et al., 2022). Therefore, the shape index can be used in studies exploring diabetes risk or in studies seeking to understand factors that contribute to PPG in normal healthy individuals.

1.2. Factors affecting inter-individual PPG variability

The factors that affect inter-individual variability in PPG include but are not limited to demographic factors, lifestyle, genetics, and metabolic phenotypes (Figure 1) (Handakumbura et al., 2019). The interaction of these factors results in intermediatory phenotypes, which influence the PPG variability through various pathways. These are further discussed in subsequent sections.

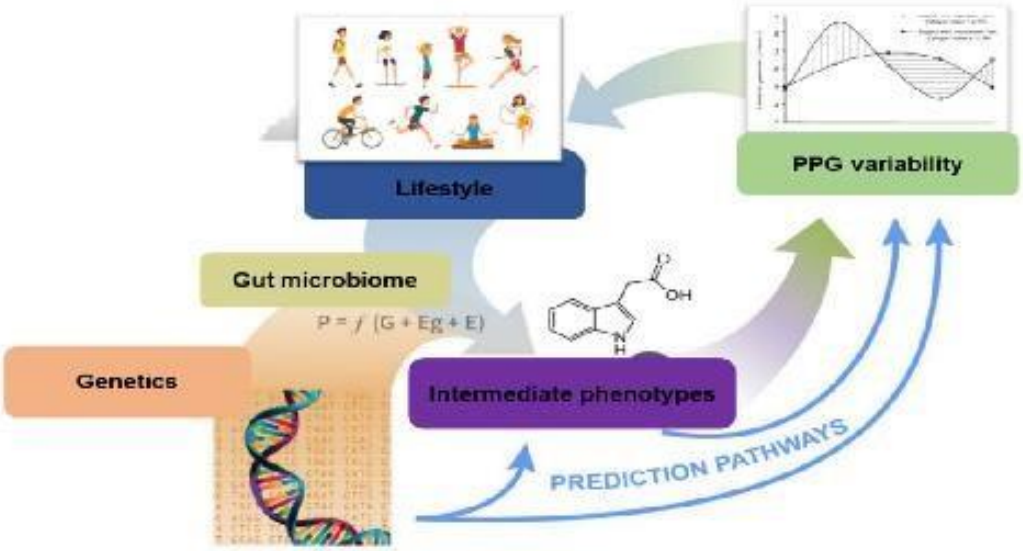


Figure 1: Factors affecting inter-individual PPG variability. Adapted from 'Metabotyping as a Stopover in Genome-to-Phenome Mapping' by Handakumbura et al., (2019).

1.2.1. Demographic factors

Demographic factors such as age and sex affect PPG levels (Tschritter et al., 2003). Elderly people have higher PPG levels than young individuals (Basu et al., 2006). With ageing, muscles and tissues become less sensitive to insulin causing reduced glucose clearance after a meal and poor blood glucose control (Kalyani & Egan, 2013). Men have more skeletal muscle mass, the major site of glucose uptake, than women, which results in men having faster, greater glucose uptake and disposal and, accordingly, lower PPG than women. However, it has been noted that after adjusting for skeletal muscle mass (using height as a proxy), PPG no longer differs by sex (Leone et al., 2022). Therefore, age affects variability in PPG levels, whereas sex results in variability when there are no height adjustments.

Education level, household crowding, and employment status are markers for socio-demographic status (SES) in South Africa. SES is found to impact diet and lifestyle factors. It is also a risk for T2D through complex processes involving access to good health care services, healthy foods, and places to exercise (Agardh et al., 2011). In South Africa there is a difference in SES between ethnicities. The Black population in South Africa has lower SES, affected by less formal education, crowded households, and higher unemployment rates. There is a need to characterize the relationship between SES and PPG variability.

1.2.2. Lifestyle factors

Lifestyle factors, including meal timing and physical exercise, influence PPG variability (Langenberg & Lotta, 2018). The order and timing of meal consumption elicit different glycaemic responses (Berry et al., 2020). The lack of physical exercise affects glucose metabolism by decreasing insulin sensitivity and increasing the risk of insulin resistance resulting in increased PPG. Light to moderate physical exercises influences PPG levels by increasing insulin and non-insulin mediated glucose uptake (Berry et al., 2020). The different physical activities undertaken by individuals result in variability in PPG levels.

1.2.3. Genetics

Evidence exists of the significant contribution of genetic factors to PPG (Langenberg & Lotta, 2018). For example, in a heritability study in twins, genetic factors accounted for approximately 48 % of PPG variation (Berry et al., 2020). While this study shows us the proportion of the variation accounted for by genetic factors, the specific genetic factors contributing to the PPG variation are unknown. Genome wide-association studies (GWAS) have been useful in identifying genetic factors in heritability studies (Langenberg & Lotta, 2018). However, few genetic studies have explored associations with PPG variability and none in Africa. The existing few studies found that only 9% variation of postprandial glucose could explain the 48% depicted in twin-based studies (Berry et al., 2020). This highlights the need for more studies to determine genetic variants associated with PPG variability.

GWAS studies are still limited in Africa. However, ongoing research is evaluating polygenic risk scores (PRSs) for T2D in Africa (Chikowore et al., 2022). The PRSs are computed by summing up weighted risk alleles carried by individuals. These PRSs enable individuals to be stratified based on their genetic susceptibility to diseases such as T2D. This makes them useful for evaluating the contribution of genetic susceptibility to PPG variation. Recent studies evaluating PPG variability have used SNPs with smaller effect sizes and found that the SNPs are significantly associated with PPG (Berry et al., 2020). This indicates that genetic factors might have affected PPG variability at a small significant scale. The usefulness of PRSs in evaluating PPG variability is yet to be explored.

1.2.4. Metabolic phenotype

Genetic and environmental interactions directly influence PPG. However, these are complex and difficult to measure. Nonetheless, intermediary factors of these complex processes can be used as predictors for the external phenotypes such as PPG (figure 1) (Handakumbura et al., 2019). Metabotyping is a process that involves the selection of specific metabolic phenotypes accompanied by cluster analysis approaches to stratify people (Riedl et al., 2018). This process can be used to assess PPG variability.

There is no consensus on the particular metabolic phenotypes that should be considered, and several varied approaches have been used in recent literature. For example, untargeted metabolomic approaches to discover dietary intake biomarker studies have been explored (Tebani & Bekri, 2019). This approach uses biological samples such as urine to assess the postprandial responses to dietary intake. However, it should be noted that even though several food biomarkers have been identified, they are still yet to be validated (Tebani & Bekri, 2019). Therefore, for such reasons, the untargeted approach will not be the focus of this study.

Alternatively, metabotypes comprising cardiometabolic measures, biochemical parameters, and anthropometrics have been proposed as better approaches to evaluating the variability of postprandial levels (Palmnäs et al., 2020). For example, (Riedl et al., 2018) used anthropometric measures (BMI) together with biochemical parameters (glucose, lipids, liver enzymes and hormones) that resulted in three metabotypes that stratified individuals based on the variable responses to nutritional exposures. However, these studies have been done in the European context and they are yet to be evaluated amongst Africans.

In summary, elevated PPG is a risk factor for T2D. Variability in PPG gives insights into how people respond differently when exposed to identical amounts of food or nutrients. Understanding the factors that affect PPG variability is pivotal for applying precision nutrition. Several factors affect PPG variability, including demographics, lifestyle, and genetic factors, which may be characterised as metabotypes reflecting their interaction. Limited studies have been done to evaluate the role of genetic factors in the form of PRSs and metabotypes in the variability of PPG in Africa. This study seeks to explore this phenomenon.

2. Aim and Objectives

2.1. Aim

The study aims to evaluate the genetic factors in polygenic risk scores and metabotypes associated with postprandial glucose variability in black South Africans.

2.2. Objectives

1. To determine PPG using the shape index and the integrated AUC.
2. To determine the metabotypes by constructing a principal component analysis (PCA) using the participants anthropometric, body composition and biochemical measures.
3. To evaluate the association between the metabotypes and PPG variability in middle-aged Black South Africans.
4. To compute the polygenic risk score for each participant using variants and weights from validated T2D polygenic risk scores.
5. To evaluate the variance in PPG accounted by T2D polygenic risk scores using linear regression models.
6. To assess the variance in PPG accounted by both T2D polygenic risk scores and metabotypes using linear regression models.

3. Methods

This is a secondary data analysis of the AWI-Gen Soweto and Middle-aged Soweto Cohort (MASC) which will be used to provide the genotype and phenotype data, respectively. The data collection from the primary study that will be used in this research project has been published before and is described in brief below (Kufe et al., 2022). The methods that will be used in this research project include power calculations for both PCA and linear regression models, calculating PPG using the shape index and integrated AUC (iAUC), determining the metabotypes using PCA, computing PRSs for each participant, constructing linear regression models with metabotypes and PRSs, and using statistical analysis evaluate the variances.

3.1. Study Participants

The secondary data analysis study will include the MASC, which is a longitudinal study of 2031 participants residing in Soweto, Johannesburg, and was designed to identify the determinants of non-communicable disease and T2D risk in middle-aged South-African men and women (Goedecke et al.,

2022). Participants with known diabetes or those taking diabetes medication will be excluded from this study to avoid the confounding effect of the medication. From the available data, only 1002 participants (men=500 and women= 502) data will be used, and the inclusion criteria will be participants with OGTT results.

3.2. Power calculation

A power calculation will be estimated for the PCA analysis and linear models. According to (Mundfrom et al., 2009), the number of variables, the number of factors, the number of variables per factor, and the level of communality are required to estimate sample sizes. For this study, we will use 25 variables to extract three metabotypes. We assume minimal communality, and according to (Mundfrom et al., 2009), a minimum sample size of 160 participants will be required. For the linear models, assuming we have seven predictor variables for multiple regression, with a critical predictor with an anticipated effect size of 0.3, as has been reported for the PRS by (Chikowore et al., 2022), we will require 170 participants to have a power of 80%.

3.3. OGTT

A standard 2-hour OGTT was conducted in the primary study, blood samples (5 ml) were drawn at 30 minutes intervals after ingesting 75 g of glucose (dissolved in 250 ml water).

For this research project, the participants fasting plasma glucose and 2-hour OGTT glucose results will be used to classify participants as impaired glucose metabolism (IGM), normal glucose tolerance (NGT) or T2D. NGT will be participants with fasting glucose <6.1 mmol/L and 2-hour OGTT <7.8 mmol/L, IGM fasting glucose will be between 6.1- 6.9 mmol/L and 2-hour post glucose load will be 7.8 to 11.0 mmol/L, and T2D if fasting glucose is ≥7 mmol/L and 2-hour post glucose load is ≥11.1 mmol/L (World Health Organization, 2006).

3.4. Postprandial glycemia

3.4.1. Shape index

The postprandial glycemia will be assessed using the shape index. The shape index for each participant will be computed by subtracting plasma glucose levels at 90 minutes from those at 120 minutes (Figure 2).

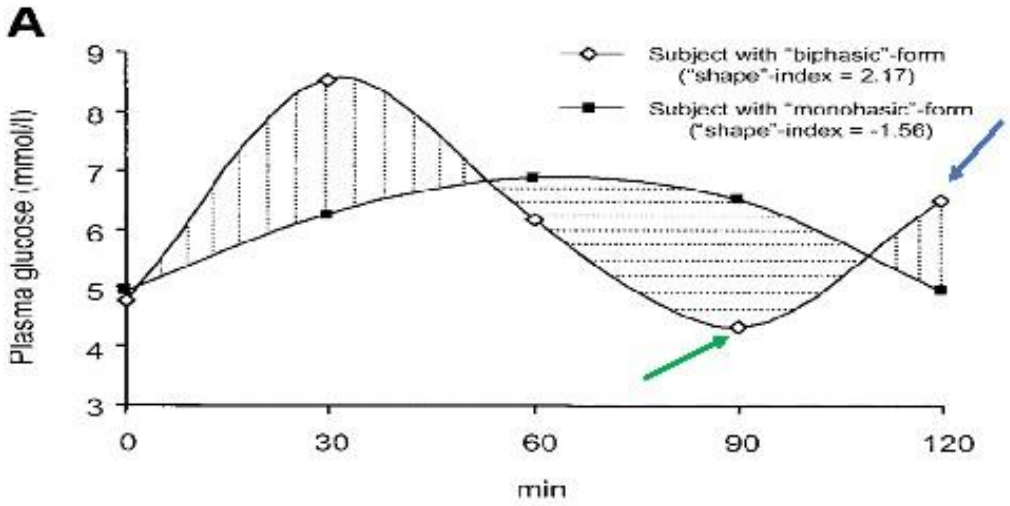


Figure 2: Plasma glucose concentration during a 2-hour OGTT. The green arrow represents the plasma glucose concentration at 90 minutes and the blue arrow at 120 minutes. Adapted from ' Assessing the shape of the glucose curve during an oral glucose tolerance test' by Tschritter et al., (2003).

3.4.2. Integrated area under the curve

The glucose iAUC will be calculated in R studio (version 2022.02.0) using the trapezoidal method. The dataset will be loaded on R studio and two vectors will be defined; the actual (time) variable and predicted (plasma glucose) variable. Subsequently, we will use the auc() function to calculate the iAUC.

3.5. Polygenic risk scores

This study will use published T2D PRSs for African and multi ancestry populations (Polfus et al., 2021). Summary statistics from published African and multi ancestry populations PRSs will be used as discovery datasets. The genotype data of these participants, which are part of the AWI-Gen study, will be the target dataset. PRSice 2 software will then be used to generate polygenic scores for the participants. A clumping distance (kb), r^2, and optimal P-value thresholds will be required to compute the PRSs. The P-values of these PRSs will evaluate the transferability and the R^2 will assess the predictability. The generated PRSs from each discovery dataset will first be tested on a few participants then validated on the rest of the cohort. The discovery dataset with the best PRSs will be used in subsequent steps.

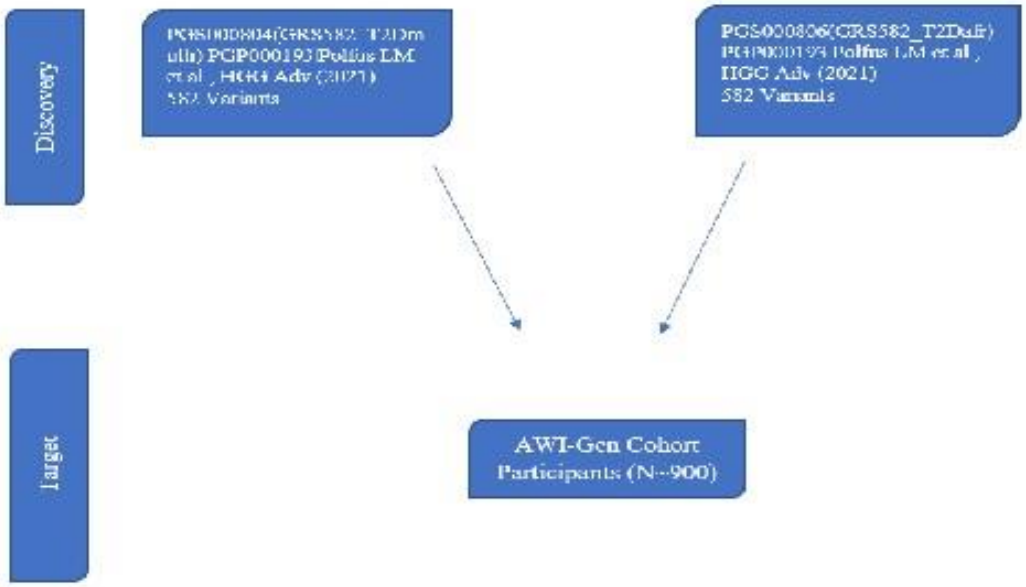


Figure 3: Schematic diagram for approaches used in the development of PRSs for PPG in black South Africans using African and multi ancestry groups.

3.6. Metabotypes

The variables from the MASC cohort will be used to construct a PCA. The variables include: anthropometrics, body composition measures, and biochemical measurements. The PCA will derive the clusters that will form the metabotype groups. The variables will be log-transformed to reduce scale before PCA. The log transformation gives an advantage because it offers the covariates and variances independent of scale.

The PCA will first be performed separately for both men and women. Should men and women have similar biochemical measures, body composition measures and anthropometrics, the two groups will be combined for subsequent analyses.

4. Primary study data collection

Demographic and Socio-economic factors

Demographic factors and SES were collected through the interviewer-administrated questionnaires. This included age, sex, employment status (employed vs unemployed), marital status (married vs unmarried), the highest education level (primary, secondary or tertiary), and housing density.

Anthropometry

The participant's weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. The waist and hip circumference were measured to the nearest 0.1 cm.

Body composition measures

The whole-body composition was measured using Dual-energy X-ray absorptiometry (DXA). DXA measured the sub-total (total body minus head) fat mass, fat-free soft tissue mass (FFSTM), and regional adiposity (trunk, arm, and leg fat mass), abdominal visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT).

Biochemical measures

The following biochemical measures were recorded; fasting insulin, glucose, liver enzymes (alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT)), sex hormones (testosterone and estrogen), lipids (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride), and glucocorticoids (cortisol and corticosterone).

5. Statistical analysis

Descriptive statistics will be used to examine the distribution of the data. Data will be analyzed using R Studio version 2022.02.0 which uses R version 4.1.3. Categorical variables will be represented as frequencies (n) and percentages (%), continuous variables that are normally distributed will be represented as mean ± standard deviation (SD) and continuous variables that are not normally distributed will be represented as median (25^{th} - 75^{th} percentiles). A Shapiro-Wilks test will be used to check if a continuous variable follows a normal distribution. A student t-test will be used to determine sex difference, Mann-Whitney U and Kruskal-Wallis tests for skewed continuous variables, and the chi-squared test for categorical variables.

For multivariable data analysis, PCA will be used to derive principal components (PCs). PCA was chosen because it is a data driven approach that can calculate the covariances of data, find patterns in the data, and reduce the dimension of large datasets. Many of the variables that will be assessed may be associated with PPG and correlated with one another. The PCA analysis enables one to see its overall contribution to PPG, while showing unique patterns that are related to the population studied. The PCs will represent metabotypes, with PC1 capturing the most variance, followed by PC2, etc. The Kaiser–Meyer–Olkin test will measure the sampling adequacy which should be 0.5< for the satisfactory factor analysis to proceed and Bartlett's test of sphericity will be used to determine the significant difference.

Linear regression will be used to derive three models. The linear regression analysis will allow us to assess if there is a linear relationship between the predictor variable and the outcome variable when the covariates

are added in the model. It will also allow us to see if the relationship changes after including additional variables to the regression model. All three full models will have the same outcome variable (shape index) and covariates (sex, age, SES, anthropometrics, body composition measures, biochemical measures) but different predictor variables. The predictor variables will be PRSs, metabotypes, PRSs and metabotypes together. The null model, with only the outcome variable and covariates, will be used to get the variance accounted by each predictor variable of concern (PRS; PRS + Metabotype).

To determine whether the regression models differed by sex, regression analyses will be repeated, and a sex interaction term will be introduced into the models. If there is any significant difference, it will be presented as sex stratified analysis.

6. Ethics

The study was granted ethical clearance (M220296) by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand.

7. Timeline

	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Literature review										
Ethics application										
Protocol preparation										
Protocol assessment										
Data analysis										
Write up										

8. Funding

No funding will be required. This is a secondary data analysis study.

9. Problems

- The shape index may not capture all the variability.
- The variance explained may be different in men and women.
- The metabotypes might be limited because we do not have metabolomics which would capture lifestyle factors.

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B. Ethics clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Ms B Masango
School of Pathology
Division of Human Genetics
Medical School
University

E-mail: 1864260@students.wits.ac.za

cc: Supervisor: Dr T Chikowore and Professor J Goedecke <Tinashechikowore1@wits.ac.za> and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2022/03/17

REF: R14/49

PROTOCOL NO: **M220296** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Ms B Masango

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M220296

NAME: (Principal Investigator)	Ms B Masango
DEPARTMENT:	School of Pathology Division of Human Genetics Medical School University
PROJECT TITLE:	Evaluation of the genetic and metabolic determinants of postprandial glucose variability in Black South Africans
DATE CONSIDERED:	Ad hoc
DECISION:	Approved conditionally
CONDITIONS:	Sub-study under M160604 and Ml 70880 The HREC requires evidence of Faculty protocol approval
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr T Chikowore and Professor J Goedecke

Dr CB Penny, Chairperson, HREC (Medical)

APPROVED BY:

DATE OF APPROVAL: 2022/03/17

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and ONE COPY returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

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 submit details to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the

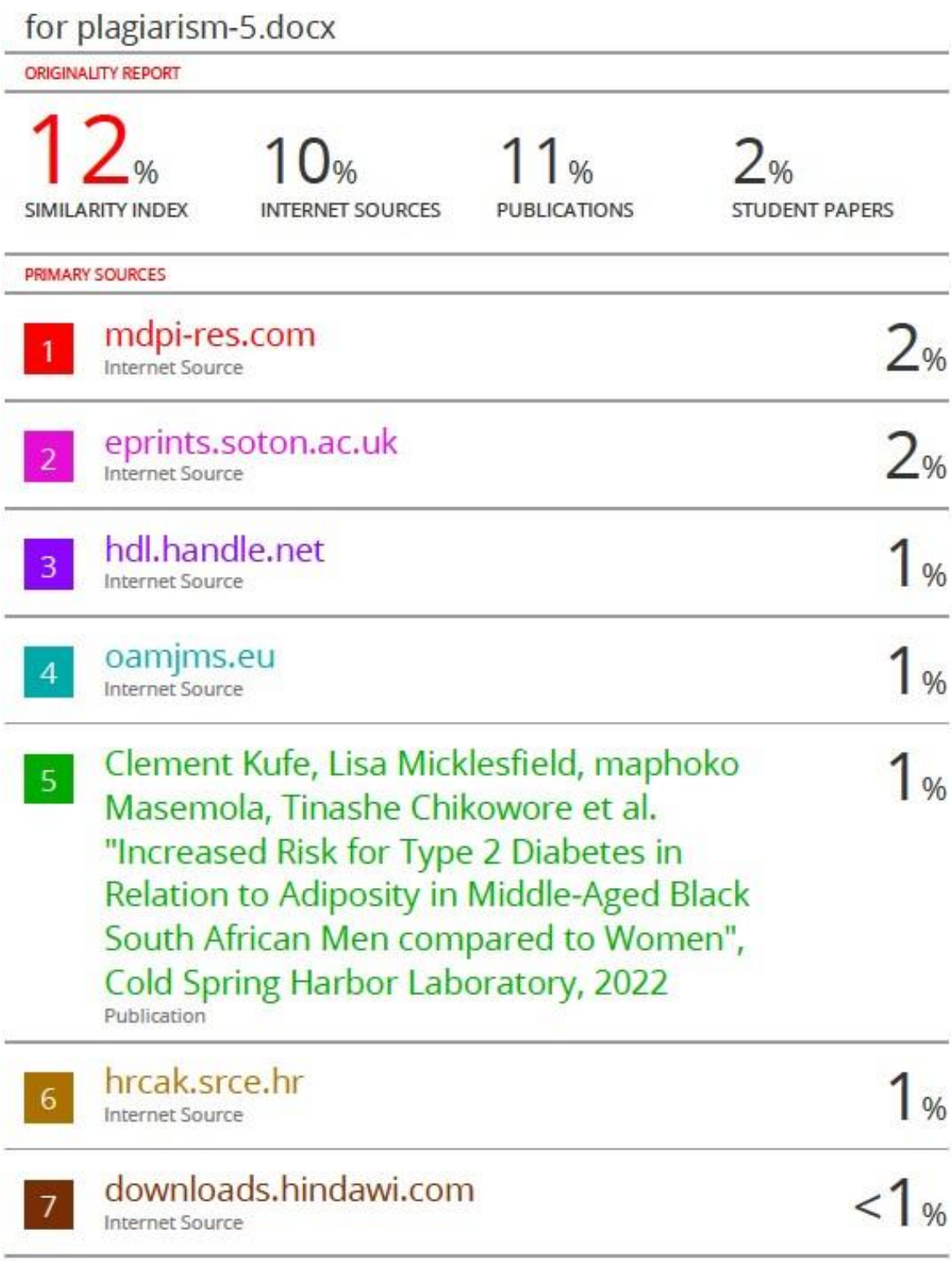
study was initially reviewed in February and therefore reports and re-certification will be due in the month of February each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).



08/04/2022

Signature of Principal Investigator | Date

C. Turn-it-in report



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D. Primary study Informed consent

INFORMED CONSENT 1

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

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, grip strength, 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 or Thumbprint
Date and Time

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

WITNESS: (If applicable)

Printed Name Signature/Mark or Thumbprint
Date and Time

PARTICIPANT INFORMATION SHEET 2

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

Information sheet:

Will any of your blood samples be stored and used for research in the future?

The researchers will ask your permission to store your blood samples for future research indefinitely. Also, we will be extracting DNA from your blood and this will be stored indefinitely. All samples will be kept in a freezer in a secure facility with access limited to research personnel only; 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. For data verification and quality control purposes regulatory authorities and/or members of the University of the Witwatersrand Human Research Ethics Committee may be allowed access to your personal data under conditions of strict confidentiality.

Future research analyses on the stored blood samples and DNA will be based on new research that we are at present not aware of, but may be important in our understanding of the risk for disease. Any research done in the future will only be executed once it has been approved by the Human Research Ethics Committee (Medical) of the University of Witwatersrand that is set up to determine that the research is done according to accepted standards. You will not be penalized in any way for not allowing the use of your blood or tissue for future research. If you decide not to donate blood for future research, it will be destroyed on completion of this study.

When entering into the study, you will receive a unique code that will be used for sample and data analysis, which serves to maintain your confidentiality. When storing samples, you may choose that we keep the unique code on the sample so that we can link any new results to your existing data. If any clinically relevant information relating to this sample is found, we will inform you of the results. Alternatively, you can remove the identifying number, so that your information will not be linked to the sample and you will not be informed of any clinical results relating to the new analyses.

Associate Professor Lisa Micklesfield (PhD)
Principal Investigator
MRC/Wits Developmental Pathways for Health Research Unit
Chris Hani Baragwanath Hospital
Soweto Johannesburg
Tel: 021-650 3153(w) 083 9433172 (cell)
Email : lisa.micklesfield@wits.ac.za or
Associate Professor Julia Goedecke (PhD)
Principal Investigator
Honorary Associate Professor, School of Clinical Medicine, Wits University
Address: Room 230 RIND Building, South African Medical Research Council, Francie van Zijl Drive, Parow, 7725, Cape Town
Tel: 021-9380862(w) 0828255616 (cell)
Email : julia.goedecke@mrc.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 INFORMATION SHEET 2

INFORMED CONSENT 2

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

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 or Thumbprint
Date and Time

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

WITNESS: (If applicable)

Printed Name Signature/Mark or Thumbprint
Date and Time

INFORMED CONSENT 3

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

The information around the blood sample taken from me and the DNA that will be extracted from the blood 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 and DNA samples will be approved by the Human Research Ethics Committee of the University of the Witwatersrand.

YES ☐ NO ☐

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

YES ☐ NO ☐

I am in agreement that the data generated from my DNA 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 bit of my DNA 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 DNA 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 of the H3Africa initiative 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 DNA, 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 DNA.

YES ☐ NO ☐

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

YES ☐ NO ☐

PARTICIPANT

Printed name Signature/Mark or Thumbprint
Date and Time

RESEARCH ASSISTANT:

Printed Name Signature/Mark or Thumbprint
Date and Time

Copy provided to participant _____ (initialed by researcher)

WITNESS: (If applicable)

Printed Name Signature/Mark or Thumbprint
Date and Time

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:

Associate Professor Lisa Micklesfield (PhD)
Principal Investigator
MRC/Wits Developmental Pathways for Health Research Unit
Chris Hani Baragwanath Hospital
Soweto Johannesburg
Tel: 021-650 3153(w) 083 9433172 (cell)
Email : lisa.micklesfield@wits.ac.za or
Associate Professor Julia Goedecke (PhD)
Principal Investigator
Honorary Associate Professor, School of Clinical Medicine, Wits University
Address: Room 230 RIND Building, South African Medical Research Council, Francie van Zijl Drive, Parow, 7725, Cape Town
Tel: 021-9380862(w) 0828255616 (cell)
Email : julia.goedecke@mrc.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

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:.....

Print Name of Researcher_____

Signature of Researcher _____

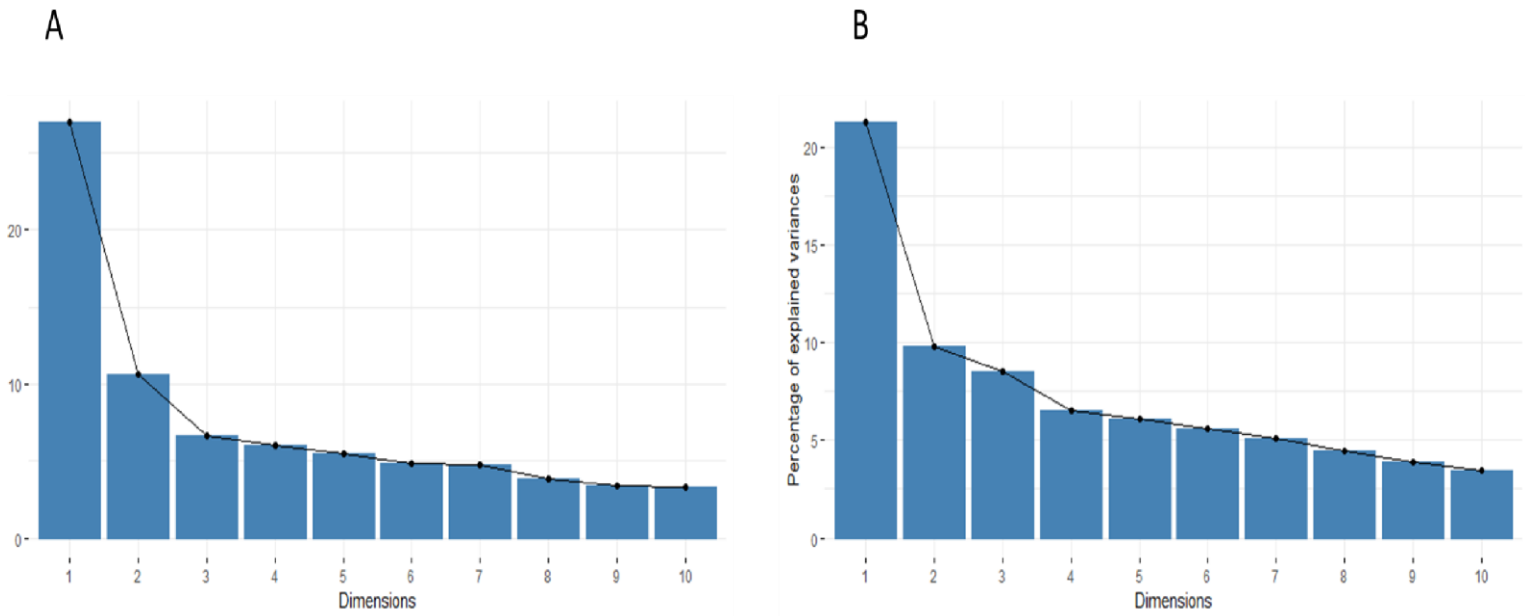
Date _____

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

WITNESS:
Printed name:_____ **Signature/Mark or Thumbprint**

Date and Time: _____

E. Supplemental material - List of Figures



Supplemental figure 1: Scree plots for women (A) and men (B) showing the distribution of principal components by their variance contribution to PPG.

F. Supplemental material - List of Tables

Supplemental table 1: Metabolic factor loadings of the major contributing principal components in women and men.

Metabolic factors	Women			Men		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
WC (cm)	**0.329**	-0.038	0.028	-0.318	-0.004	0.081
SAT (cm^2)	**0.322**	-0.116	-0.073	-0.306	0.024	0.058
Weight (kg)	**0.321**	-0.220	-0.107	-0.304	-0.065	0.164
Body fat (%)	**0.308**	-0.218	-0.145	-0.308	-0.022	0.115
VAT (cm^2)	0.145	0.064	-0.018	-0.285	0.120	-0.049
FFSTM (kg)	**0.295**	-0.19	-0.046	-0.268	-0.090	0.180
Android (%FM)	**0.280**	0.167	0.093	-0.252	0.201	-0.167
HC (cm)	0.226	-0.260	-0.149	-0.298	-0.079	**0.162**
Trunk (%FM)	**0.247**	0.108	0.149	-0.210	0.261	-0.251
TG (mmol/L)	0.138	**0.213**	0.103	-0.086	**0.178**	-0.123
DBP (mmHg)	0.083	0.053	-0.001	-0.108	0.156	-0.046
Arm (%FM)	0.081	0.068	0.089	0.131	-0.101	-0.156
SBP (mmHg)	0.063	0.070	-0.000	-0.126	0.133	-0.063
Free testosterone (pmol/L)	0.059	0.054	**0.158**	0.052	0.091	0.083
LDL (mmol/L)	0.053	**0.237**	0.013	-0.093	0.065	**0.410**

Height (cm)	0.045	-0.146	-0.073	-0.024	-0.049	0.259
Androstenedione (nmol/L)	0.031	-0.070	0.430	0.142	0.283	0.040
TC (mmol/L)	0.030	**0.263**	0.050	-0.062	**0.200**	**0.426**
DHEA (nmol/L)	0.027	-0.034	**0.230**	0.046	**0.192**	0.065
GGT (µKat/L)	0.021	**0.209**	0.041	0.041	0.299	0.116
ALT (µKat/L)	0.001	**0.187**	-0.063	0.032	0.269	0.058
Cortisol (pmol/L)	-0.016	-0.010	**0.258**	0.142	**0.294**	-0.006
AST (µKat/L)	-0.026	**0.210**	0.005	0.075	0.254	0.076
Cortisone (pmol/L)	-0.039	0.005	**0.273**	0.091	**0.208**	0.098
Estrogen (pmol/L)	-0.048	-0.193	**0.352**	-0.009	0.109	-0.148
FSH (pmol/L)	-0.052	0.037	-0.350	0.032	0.117	0.093
LH (pmol/L)	-0.056	**0.227**	-0.255	0.055	0.142	0.088
HDL (mmol/L)	-0.123	**0.246**	0.059	0.135	**0.183**	**0.269**
SHBG (nmol/L)	-0.164	-0.119	0.104	0.170	0.082	-0.001
Leg (% FM)	-0.23	-0.304	-0.163	**0.185**	-0.247	**0.309**
Gynoid (% FM)	-0.239	-0.215	-0.134	**0.182**	-0.203	**0.204**

Abbreviations: PC, principal component; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; LH, luteinising hormone;
FSH, follicle-stimulating hormone; DHEA, dehydroepiandrosterone; HC, hip circumference; WC, waist circumference; DBP, diastolic blood pressure; SBP, systolic blood pressure; FFSTM, Fat–free soft tissue mass; SHBG, sex hormonebinding globulin. *Note*: those in bold are the major contributing metabolic factors

G. Supplemental material - BMJ Open Diabetes Research & Care author guidelines

BMJ Open Diabetes & Research Care is an open access diabetes journal dedicated to publishing high-quality medical research from all disciplines and therapeutic areas of diabetes research, management and treatment. A continuous publication model facilitates rapid publication of basic and clinical research regarding type 1 and type 2 diabetes and associated symptoms, complications, and treatments. The online-only format allows for continuous updates and serves as an invaluable resource to the multidisciplinary community of endocrinology, public health/prevention and internal medicine. All submissions are subject to peer review. Articles should not be under review or under consideration by any other journal when submitted to *BMJ Open Diabetes and Research Care*.

FORMATTING YOUR PAPER

These are general formatting guidelines across BMJ, please always refer to journal-specific instructions for authors for article type specifications. You can browse the titles on our Journals website. If you are looking to submit to *The BMJ*, please visit this section.
If you are unable to find the answer to your question, our editorial team will be on hand to offer assistance throughout the submission process. Contact details for the editorial team are on the journal's Contact Us page.
You can also refer to our formatting checklist to make sure you have covered everything on submission.

Title page

The title page must contain the following information:

- Title of the article
- Full name, postal address and e-mail of the corresponding author
- Full name, department, institution, city and country of all co-authors
- Word count, excluding title page, abstract, references, figures and tables

This is excluded for the journal *BMJ Quality and Safety* which operates triple-anonymised peer review. *Back to top >>*

Keywords

Keywords are specific terms that define what your paper is about. Keywords are important for search engine optimisation and enhance the discoverability of your work and its impact. They also help editors to identify peer reviewers for your manuscript.
We ask authors to use Medical Subject Headings (MeSH) descriptors as keywords to optimise discoverability. MeSH provides two tools to help authors select MeSH descriptors as keywords:

- **MeSH on Demand** – input text from an abstract to automatically identify related terms
- **MeSH Browser** – search for related terms and descriptors using an existing list of keywords

You can start to type in a term and select from a list of suggested matches or search the full list of keywords. If your required MeSH descriptor is not available in the keyword list please contact the editorial office who will arrange for it to be added. You will be able to include this at revision. *Back to top >>*

Authors and Institutions

On submission of your article through our submission system you will be asked to provide a name, email address and institutional affiliation for all contributing authors. In the final published article author names, institutions and addresses will be taken from these completed fields and not from the submitted Word document. Refer to the BMJ policy on authorship for more information. *Back to top >>*

Manuscript format

The manuscript must be submitted as a Word document (*BMJ Case Reports* and *Veterinary Record Case Reports* request that authors submit using a template which should also be in Word format). PDF is not accepted.
The manuscript should be presented in the following order:

- Title page
- Abstract, or a summary for case reports (Note: references should not be included in abstracts or summaries)
- Main text separated under appropriate headings and subheadings using the following hierarchy: BOLD CAPS, bold lower case, Plain text, Italics
- Tables should be in Word format and placed in the main text where the table is first cited. Tables should also be cited in numerical order
- Acknowledgments, Competing Interests, Funding and all other required statements
- References. All references should be cited in the main text in numerical order

We have added a "submission prefill" tool to all of our journals, which typically reduces time taken to submit a paper by 25 percent. Just upload your manuscript and the system will automatically extract and populate the following submission fields: Title, Abstract, Authors, Institutions, Funders.
Figures must be uploaded as separate files (view further details under the Figures/illustrations section). All figures must be cited within the main text in numerical order and legends should be provided at the end of the manuscript.
Online Supplementary materials should be uploaded using the File Designation "Supplementary File" on the submission site and cited in the main text.
Please remove any hidden text headers or footers from your file before submission. *Back to top >>*

Style

Acronyms and abbreviations should be used sparingly and fully explained when first used. Abbreviations and symbols must be standard. SI units should be used throughout, except for blood pressure values which should be reported in mm Hg.
Whenever possible, drugs should be given their approved generic name. Where a proprietary (brand) name is used, it should begin with a capital letter.
To ensure a consistent approach, submitted articles should not include Trademark or Registered trademark symbols in the main text, tables or figures.
Back to top >>

Figures and illustrations

Images must be uploaded as separate files. All images must be cited within the main text in numerical order and legends must be provided (ideally at the end of the manuscript). Video: How to improve your graphs and tables

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Colour images

For certain journals, authors of unsolicited manuscripts that wish to publish colour figures in print will be charged a fee to cover the cost of printing. Refer to the specific journal's instructions for authors for more information.

Alternatively, authors are encouraged to supply colour illustrations for online publication and Black and white versions for print publication. Colour publication online is offered at no charge, but the figure legend must not refer to the use of colours. Detailed guidance on figure preparation *Back to top >>*

File types

Figures should be submitted in TIFF, EPS, JPEG or PDF formats. Please note, figures submitted in TIFF formats should be a single-layered flat file; we can not accept TIFF files which contain multiple pages. In EPS files, text (if present) should be outlined. For non-vector files (eg TIFF, JPEG) a minimum resolution of 300 dpi is required, except for line art which should be 1200 dpi. Histograms should be presented in a simple, two-dimensional format, with no background grid.
For figures consisting of multiple images/parts, please ensure these are submitted as a single composite file for processing. We are unable to accept figures that are submitted as multiple files. During submission, ensure that the figure files are labelled with the correct File Designation of "Mono
Image" for Black and white figures and "Colour Image" for colour figures.
Figures are checked using automated quality control and if they are below the minimum standard you will be alerted and asked to resupply them.
Please ensure that any specific patient/hospital details are removed or Black ed out (e.g. X-rays, MRI scans, etc). Figures that use a Black bar to obscure a patient's identity are not accepted. *Back to top >>*

Tables

Tables should be in Word format and placed in the main text where the table is first cited. Tables must be cited in the main text in numerical order. Please note that tables embedded as Excel files within the manuscript are NOT accepted. Tables in Excel should be copied and pasted into the manuscript Word file.
Tables should be self-explanatory and the data they contain must not be duplicated in the text or figures. Any tables submitted that are longer/larger than 2 pages will be published as online only supplementary material. Video: How to improve your graphs and tables *Back to top >>*

Multimedia files

You may submit multimedia files to enhance your article. Video files are preferred in .WMF or .AVI formats, but can also be supplied as .FLV, .Mov, and .MP4. When submitting, please ensure you upload them using the File Designation "Supplementary File – Video". *Back to top >>*

References

BMJ reference style
BMJ formats references using Vancouver style; references are sequentially numbered within the text of the main document and match the reference list at the end of the article. The first three

authors are listed by last name and initials, with additional authors acknowledged by the use of ‘et al’ if applicable.
Depending on the type of reference, we may also include: the publication name, date of publication, volume and page numbers, chapter, DOI, URL, PubMed ID, access date, and any other necessary information.
Exception: Medical Humanities uses Chicago author-date referencing which is more commonly used in social sciences; references are listed by author and date within the text of the main document with the an alphabetical reference list at the end of the article. Please see the online style manual for details and this published article for examples. Preparing references

- Authors are asked to follow these guidelines when formatting their references:
- References should be cited in numerical order (i.e. 1,2,3) in the text and be listed numerically in the reference list at the end of the article
- The reference list should be included as part of the main text document and not in the footnotes
- References cited in the text should be presented in square brackets [6] or parentheses (6) rather than superscript
- Multiple reference citations should be separated by commas [6, 9, 12] or by hyphens if numbers are sequential [12-15]
- Reference citations within figures and tables (or their legends/footnotes) should be listed in the reference list

MANUSCRIPT CATEGORIES AND REQUIREMENTS
Original research

Original research should contribute to the diabetes literature and patient care. Research reviews that systematically synthesise evidence (e.g. Systematic reviews, Meta-analysis, Scoping reviews, Mixed methods reviews, etc) should be submitted as Systematic reviews.

The abstract should include four sections:

- **Introduction** (the purpose or hypothesis of study)
- **Research Design and Methods** (the basic design, setting, animal model, cell line or number of participants and
  selection criteria, treatment or intervention, and methods of assessment)
- **Results** (significant data found)
- **Conclusions** (the validity, limitations and clinical applicability of the study and its results Please include the key messages of your article after your abstract using the following headings. This section should be no more than 3-5 sentences and should be distinct from the abstract; be succinct, specific and accurate.
- **What is already known on this topic** – *summarise the state of scientific knowledge on this subject before you did your study and why this study needed to be done*
- **What this study adds** – *summarise what we now know as a result of this study that we did not know before*
- **How this study might affect research, practice or policy** – *summarise the implications of this study*

This will be published as a summary box after the abstract in the final published article.
Word Count: up to 5,000 words
Abstract: up to 300 words and structured including the headings Introduction, Research design and methods, Results and Conclusions

Tables/Illustrations: up to five tables and/or figures
References: up to 50

Review

BMJ Open Diabetes & Research Care publishes review articles that are comprehensive, scientifically accurate summaries of topics in clinical or basic science dealing with areas of current clinical relevance, promising experimental therapies, or emerging scientific concepts.

Research reviews that systematically synthesise evidence (e.g. Systematic reviews, Meta-analysis, Scoping reviews, Mixed methods reviews, etc) are classified by the journal as Systematic reviews and must be submitted as such.

Review Articles should describe either basic and/or clinical investigations, discuss the physiological and clinical significance of the work and place it in the context of previously published information. Review articles are usually commissioned but the journal will consider unsolicited submissions.
Word Count: up to 5,000 words
Abstract: up to 250 words, unstructured
Tables/Illustrations: Maximum four tables and/or figures
References: up to 65

Invited systematic review

In addition to reviews, *BMJ Open Diabetes & Research Care* solicits systematic reviews on subjects that fall under the journal's scope. At this time, we are not considering suggestions for submissions.

Please include the key messages of your article after your abstract using the following headings. This section should be no more than 3-5 sentences and should be distinct from the abstract; be succinct, specific and accurate.

- **What is already known on this topic** – *summarise the state of scientific knowledge on this subject before you did your study and why this study needed to be done*
- **What this study adds** – *summarise what we now know as a result of this study that we did not know before*
- **How this study might affect research, practice or policy** – *summarise the implications of this study*

This will be published as a summary box after the abstract in the final published article.
Word Count: up to 5,000 words
Abstract: up to 250 words, unstructured
Tables/Illustrations: Maximum four tables and/or figures
References: up to 65

Editorial

Editorials are mostly commissioned pieces about an article that invites direct commentary because it concerns a particularly timely subject within the field or offers innovative or controversial findings. While the journal will accept unsolicited submissions, it will not consider them for publication unless they address an urgent or immediately relevant topic that directly pertains to the journal's scope. **Word Count:** up to 1,200 words
Abstract: not required
Tables/Illustrations: Maximum one tables and/or figure

References: up to 10

Letter to the editor

Letters to the editor are generally commissioned, but unsolicited submissions are welcome.
Word Count: up to 600 words
Abstract: not required
Tables/Illustrations: Maximum one table and/or figure
References: up to 10

Supplements

The BMJ Publishing Group journals are willing to consider publishing supplements to regular issues. Supplement proposals may be made at the request of:

- The journal editor, an editorial board member or a learned society may wish to organize a meeting. Sponsorship may be sought and the proceedings published as a supplement.
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