

https://dspace.org/

Delineating the pathophysiology of type 2 diabetes in middle-aged black South African women

Mtintsilana, Asanda

2021
https://hdl.handle.net/10539/32041

Downloaded from a DSpace repository. https://dspace.org/

DELINEATING THE PATHOPHYSIOLOGY OF TYPE 2 DIABETES IN MIDDLE-AGED BLACK SOUTH AFRICAN WOMEN

By
Asanda Mtintsilana

A thesis submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of

Doctor of Philosophy



Date of Submission
7 May 2021

Supervisors
Professor Julia H. Goedecke
Associate Professor Lisa K. Micklesfield
Dr Elin Chorell

PLAGIARISM DECLARATION

I **Asanda Mtintsilana** (Student number: **607019**) am a student registered for a **PhD degree** in the year **2021.**

I hereby declare the following:

I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.

I confirm that ALL the work submitted for assessment for the above course is my own unaided work except where I have explicitly indicated otherwise.

I have followed the required conventions in referencing the thoughts and ideas of others.

I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signature:  Date: **7 May 2021**

DEDICATION

I would like to dedicate this thesis to my wonderful family (my grandmother, mother, aunt, brother and cousins) whose unwavering love, support, encouragement has always propelled me during this journey and towards my dreams.

ACKNOWLEDGEMENTS

Funding

I would like to thank the Swedish Research Council (Swedish Development Grant), the South African Medical Research Council (SA MRC), the Wellcome Trust of the United Kingdom (funded the Birth to Twenty plus (Bt20+) cohort) for funding this PhD project. I would also like to thank the National Research Foundation (NRF) of SA for funding the PhD scholarship and the Swedish Research Council (Swedish Development Grant) for providing additional funding of the PhD scholarship.

Participants

I am eternally grateful to all the female caregiver participants of the Bt20+ cohort for taking part in this project. Without your participation, this project would not have been possible. Thank you for your cooperation and allowing me to contribute in the advancement of knowledge to better understand the pathophysiology of type 2 diabetes (T2D), which is currently the leading cause of death in SA women.

Principal investigators and supervisors

To Professors Tommy Olsson, Julia Goedecke and Lisa Micklesfield and Dr Elin Chorell, thank you so much for this great opportunity to conduct my PhD project under you guidance and supervision. Your unwavering support, commitment and dedication towards my PhD journey have been consistent and unmeasurable. I am filled with so much gratitude and forever indebted to you for choosing me to be part of this project. You have given me a platform to grow, not only as a researcher, but as a person. When I started this PhD degree I was filled with so much anxiety and self-doubt as I thought I wasn't the ideal person for this PhD project due to my lack of knowledge and skills, in particular in diabetes research. Thank you for taking a chance on me- your kindness and patience with my learning has given me much confidence as a researcher. I have gained so much knowledge that has given me a sense of pride to continue to learn and not be

afraid to make mistakes and learn from them. This has been my greatest joy of doing my PhD, the feeling of finally understanding something that was terrifying me or giving me so much anxiety. This would not have been possible without your excellent guidance and the style of supervision; which created a safe place for me to challenge and push-myself beyond measure to become the best that I can be. Thank you for your motivation, valuable opinions and critical advice, which at times, felt like the worst but was needed and has shaped me to be a better researcher. For this, I am eternally grateful.

To my main supervisor, Professor Goedecke, my gratitude to you is impossible to quantify. As a main supervisor, you have perhaps had the most difficult job but the grace and patience with which you supervised me, generosity with even your personal time and never-ending patience will be cherished in my heart for many years. You were the first person to go over my first drafts, most of which were in the poorest form. You were the first person I always contacted when I was anxious, frustrated and unsure about something. Often, I fear I may have unfairly given you too much workload. But true to your character, every single time, you listened to my worries and helped me resolve any issue I had. To highlight how excellent of a supervisor you have been to me (and to other PhD students), I remember how we were so happy to have a bit of “extra free-time” when you had your twins, but you left us disappointed and in disbelief when things did not change during your maternity leave! You remained available to us, even on weekends, and your home became our meeting place to discuss our PhD projects. It has truly been my absolute pleasure and privilege to work with you. I hope that I will be able to pay forward the same gestures of kindness, knowledge, supervision, work-ethic, drive, and passion to the next generation of researchers.

DPHRU staff

Thank you to the current and former DPHRU staff, in particular the “Metabo” research team: Mrs Vukosi Nomses Baloyi, Mrs Siphumelele Thango, Ms Tshifhiwa Ratshikombo and Mr Melikhaya Soboyisi. It was an absolute pleasure to work with you all. I will always cherish the respect and love we had for each other and the commitment we had towards this project. Your kindness, support, dedication and overall work ethic made this

project one of the best projects I have ever partaken in. Thank you for going beyond what you were tasked with. My first two and half years of my PhD project at Bara were the best years of my academic life to date. I created so many happy memories working with all of you; from recruiting participants to doing field work which allowed me to explore Soweto and learn more about the participants. I hope our paths will cross again in the near future.

A special thank you to Professor Shane Norris for the warm welcome at DPHRU and for valuable knowledge and assistance, in particular during the first few years of my PhD. I would also like to give thanks to Mr Yusuf Guman, Mr Onke Godongwana, Mr Luigi Malgas, Ms Mosadiapula Lydia Nakedi, Ms Thabile Sibiya, Mrs Nokuthula Jada, Mrs Patience Smangele Sigasa and to everyone who played a part in my research project.

Family and friends

To my family, thank you so much for allowing me to pursue my lifelong dream of obtaining a PhD degree and for believing in me, encouraging and supporting me all these years. Thank you for understanding and not making me feeling guilty of the path I have chosen and the duration I have taken to complete my studies, despite the circumstances and challenges at home. To my close friends, thank you so much for being present, for cheering and supporting me throughout these years. Your presence in my life keeps me focused, driven and gives me the energy to succeed in life. I am truly blessed to have you and be able to complete this milestone with you.

TABLE OF CONTENTS

PLAGIARISM DECLARATION……i
DEDICATION……ii
ACKNOWLEDGEMENTS……iii
TABLE OF CONTENTS……vi
LIST OF FIGURES……xi
LIST OF TABLES……xii
SCIENTIFIC OUTPUTS FROM THIS THESIS……xiii
ABBREVATIONS……xvi
GLOSSARY OF TERMS USED IN THIS THESIS……xvii
ABSTRACT……xviii

CHAPTER ONE……1

1. Literature review……1

1.1. Diabetes……2
1.2. Scope of the problem……2
1.3. Diagnosis of T2D……3
1.4. A brief overview of the pathophysiology of T2D……5
1.5. The assessment of insulin sensitivity/insulin resistance, beta-cell function and insulin clearance……9
1.6. Proposed theories and mechanisms for T2D……12
1.7. Ethnic differences in the pathophysiology of T2D: focus on insulin sensitivity and hyperinsulinaemia……16
1.7.1. Insulin sensitivity/ Insulin resistance (IR)……16
1.7.2. Hyperinsulinemia, described in the context of insulin secretion and clearance....23
1.8. Determinants of insulin sensitivity/IR and hyperinsulinaemia: focus on black SA women……37
1.8.1. Body fat and fat distribution……38
1.8.2. Ectopic fat deposition: liver fat accumulation and NAFLD prevalence……48
1.8.3. Age, gender and genetic factors……53
1.8.4. Socio-cultural, socio-demographic and lifestyle factors……54
1.8.4.1. Socio-cultural and demographic factors……54

1.8.4.2. Lifestyle factors……...57
1.9. A brief overview of metabolomics in T2D……...59
1.9.1. Branched chain amino acids and lipids……60
1.9.2. Bile acids……..63
1.9.3. Metabolites and prediction of T2D……66
1.10. Summary, gaps in literature and motivation for research……68
1.11. Aim and objectives……..72

CHAPTER TWO……74

2. Fat redistribution and accumulation of visceral adipose tissue predicts type 2 diabetes risk in middle-aged black South African women: a 13-year longitudinal study……74

2.1. Introduction……75
2.2. Material and methods……76
2.2.1. Study population……..76
2.2.2. Body composition……79
2.2.3. Menopausal status……..79
2.2.4. Blood sampling and biochemical analysis at follow-up……79
2.3. Statistical analysis……...81
2.4. Results……81
2.4.1. Changes in body composition over the 13-year follow-up period……81
2.4.2. Differences in body composition between NGT and IGM/T2D groups at baseline and follow-up……84
2.4.3. DXA-derived body composition measures as predictors of IGM/T2D risk at follow-up……...86
2.4.4. Associations between DXA-derived body composition measures, and markers of glycaemia at follow-up……87
2.5. Discussion……...90
2.6. Conclusion……92

CHAPTER THREE……94

3. Hyperinsulinaemia in middle-aged black South African women: contribution of insulin

secretion and clearance……..94

3.1. Introduction……95
3.2. Material and methods……96
3.2.1. Study design……96
3.2.2. Body composition……97
3.2.3. Socio-demographic and behavioural factors……..97
3.2.4. Blood sampling and biochemical analysis……...98
3.2.5. Beta-cell function and insulin clearance parameters……99
3.3. Statistical analysis……..99
3.4. Results……100
3.4.1. Socio-demographic and behavioural factors by glycaemic groups……...100
3.4.2. Basic anthropometry, body fat and fat distribution and biomarker characteristics according to glycaemic groups……102
3.4.3. Contribution of insulin secretion and clearance to basal and postprandial hyperinsulinaemia……....107
3.4.4. Associations between insulin sensitivity, body fat and fat distribution, and FLI, and insulin secretion and clearance ……108
3.5. Discussion……110
3.6. Conclusion……114

CHAPTER FOUR……....116

4. Adiposity mediates the association between the dietary inflammatory index and markers of type 2 Diabetes risk in middle-aged black South African women……..116

4.1. Introduction……...117
4.2. Material and methods……..118
4.2.1. Study design……..118
4.2.2. Body composition……119
4.2.3. Socio-demographic assessment……120
4.2.4. The DII®……..120
4.2.5. Biochemical analysis……....123
4.3. Statistical analysis……124
4.4. Results……126

4.4.1. Participants' characteristics according to E-DII score……126
4.4.2. Associations between E-DII and markers of T2D Risk in black SA women……128
4.4.3. Associations between E-DII and markers of T2D Risk in black SA women with body composition as mediators……133
4.4.4. Direct and Indirect effects of E-DII on markers of T2D in black SA women with inflammatory cytokines as mediators……133
4.5. Discussion……134
4.6. Conclusion……138

CHAPTER FIVE……139

5. Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women: a prospective cohort study……139

5.1. Introduction……140
5.2. Material and methods……141
5.2.1. Study design and population……141
5.2.2. Clinical measurements……142
5.2.3. Sample preparation and metabolomics analyses……142
5.3. Statistical analysis……143
5.4. Results……143
5.4.1. Subject characteristics……143
5.4.2. Metabolomics analysis……146
5.4.3. Metabolite profiles that describe the development and risk of T2D in black SA women……146
5.5. Discussion……152
5.6. Conclusion……156

CHAPTER SIX……157

6. Concluding chapter……157

6.1 Overview of research question and aim of thesis ……158
6.2 Summary of the objectives, hypotheses and key findings……162

6.3 Summary and implication of study findings……..165
6.4 Strengths and limitations of the thesis……..168
6.5 Future research and recommendations……173
6.6 Conclusion……175

REFERENCES……177

APPENDICES……..204

Appendix A: Declaration by student and co-authors……205
Appendix B: Supporting information……208
Appendix C: Ethics clearance certificates ……237
Appendix D: Participant questionnaire……241
Appendix E: Turnitin report……274

LIST OF FIGURES

Figure 1.1: A simple diagram for the pathogenesis of T2D, based on existing literature……8
Figure 1.2: Adipose tissue dysfunction in the development of T2D, as illustrated in the context of spillover, portal and twin-cycle theories.....15
Figure 1.3: Alternative model for the development of T2D theories.....37
Figure 1.4: A schematic diagram of the bile acid synthetic pathway……64

Figure 2.1: Sample selection flow chart of the female caregivers (e.g. mother, grandmother, aunt) of the Birth to Twenty (Bt20+) plus cohort…….....78

Figure 3.1: Represents plasma glucose (A), insulin (B) and C-peptide (C) serum concentrations during an oral glucose tolerance tests for the three glycaemic groups; Normal glucose tolerance (NGT) group, Impaired glucose metabolism (IGM) group and type 2 diabetes (T2D) group…..105
Figure 3.2: Represents insulin secretion rate (A) and β-CGS (presented as the slope mean slope of the insulin secretion rates over the observed glucose concentration range during the OGTT) (B), calculated from the mathematical model during an oral glucose tolerance test (OGTT) for the normal glucose tolerance (NGT) group, impaired glucose metabolism (IGM) group and type 2 diabetes (T2D) group……...106

Figure 4.1: Sequence of steps in creating the energy-adjusted- DII (dietary inflammatory index) in middle-aged black South African women……....122
Figure 4.2: Represent a single mediator model used to test the association between the energy-adjusted-dietary inflammatory index (E-DII) (i.e., independent variable**)** and markers of type 2 diabetes (T2D) (i.e., dependent variable), body composition and inflammatory cytokines as mediators……....125

Figure 5.1: Two separate multivariate models (OPLS-DA) that describe circulating type 2 diabetes metabolite profiles in black South African women, before and after disease diagnosis……...148
Figure 5.2: Branched-chain amino acid catabolism before (baseline) and after type 2 diabetes diagnosis (13-year follow-up)……...150
Figure 5.3. Heatmap of Spearman correlation coefficients between baseline metabolites and 13-year follow-up fasting clinical end points……151
Figure 5.4: Metabolite pathways altered before and after type 2 diabetes development in black South African women. ……...155

Figure 6.1: A schematic diagram of the pathophysiology of T2D in black African women…….160

LIST OF TABLES

Table 1.1: Diabetes diagnostic criteria……4
Table 1.2: Ethnic comparison of insulin sensitivity between black African and white European individuals……...18
Table 1.3: Ethnic comparison of hyperinsulinaemia, beta-cell function and insulin clearance between black African and white European participants……24
Table 1.4: Ethnic comparison of visceral adipose tissue between black African and white European individuals……43
Table 1.5: Ethnic comparisons of liver fat accumulation and NAFLD prevalence between black African and white European participants…….....51

Table 2.1: Participant characteristics at baseline and follow-up (n=144)……83
Table 2.2: Body composition characteristics between the NGT and IGM/T2D groups at baseline and follow-up……..85
Table 2.3: Body fat and fat distribution measures as predictors of IGM/T2D risk at follow-up….87
Table 2.4: Regression coefficients from robust multiple linear models for the prediction of HbA1c, fasting insulin resistance (HOMA2-IR) and OGTT-derived insulin sensitivity (Matsuda Index) at follow-up……89

Table 3.1: Socio-demographic and behavioural factors of middle-aged black South African women by by glycaemic groups (dark grey)(n=188)…… 100
Table 3.2: Basic anthropometry, body fat and fat distribution and biomarker characteristics of middle-aged black South African women by glycaemic groups (dark grey) (n=188)……...104
Table 3.3: Contribution of insulin secretion and clearance to basal and postprandial hyperinsulinaemia ($AUCins_{120}/AUCglu_{120}$), explained by insulin secretion and clearance (n=188)……..108
Table 3.4: Associations between insulin sensitivity, body fat and fat distribution, and FLI, and insulin secretion and clearance (n=188)……112

Table 4.1. Body composition and clinical characteristics of the participants according to E-DII scores……126
Table 4.2. Direct and indirect effects of E-DII on markers of T2D risk with body composition as mediators in black South African (SA) women……...128
Table 4.3. Total, direct and indirect effects of E-DII on markers of T2D risk with inflammatory cytokines as mediators in black SA women…….....130
Table 4.4. Regression coefficients (95% CIs) for the association between E-DII and measures of body composition and inflammatory cytokines in black SA women…….....131
Table 4.5. Regression coefficients (95% CIs) for the associations between body composition and inflammatory cytokines and markers of T2D risk in black SA women……132

Table 5.1: Characteristics of participants in the three diagnostic groups, at baseline and at 13-year follow-up. All participants exhibited normal glucose tolerance (NGT) at baseline…….....145

Table 6.1: Summary of the objectives, hypotheses and key findings……162

SCIENTIFIC OUTPUTS FROM THIS THESIS

Contribution of the PhD student

As a PhD student and study coordinator, I was in charge of setting up procedures and overall project coordination, field work and participant recruitment, data collection, biochemical analysis, and data cleaning. For each of the publications, I was responsible for conceptualisation, statistical analysis, interpretation of the data, and drafting and editing of the manuscripts. For a detailed description of all the author contributions, please refer to the relevant chapters. A declaration document signed by myself and all the co-authors is included in Appendix A, Declaration: Student's contribution to article(s) and agreement of co-author(s).

Below is the list of publications and conference presentations (oral and poster) produced for this thesis:

Publications from this study

Mtintsilana A, Micklesfield LK, Chorell E, *et al* Fat redistribution and accumulation of visceral adipose tissue predicts type 2 diabetes risk in middle-aged black South African women: a 13-year longitudinal study. *Nutrition and Diabetes* 2019; **9**:1-12.

Mtintsilana A, Micklesfield LK, Chorell E, *et al* Adiposity mediates the association between the dietary inflammatory index and markers of type 2 diabetes risk in middle-aged Black South African women. *Nutrients* 2019; **11**: 1-19.

Zeng Y, **Mtintsilana A,** Goedecke JH, *et al* Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women. *Metabolism, Clinical and Experimental* 2019; **95**: 57-64.

Conference presentations: oral and poster

Mtintsilana A, Micklesfield LK, Chorell E, *et al* Visceral adipose tissue (VAT) and not subcutaneous adipose tissue (SAT) is associated with type 2 diabetes (T2D) and cardiometabolic risk in middle-aged black South African women: a longitudinal analysis. The Society for Endocrinology, Diabetes and Metabolism of South Africa (SEMDSA) annual congress, 4-7 May 2017, Johannesburg, South Africa. (Oral presentation)

Mtintsilana A, Micklesfield LK, Chorell E, *et al* Baseline body fat and fat distribution predicts T2D risk 11 years later in black SA women. The Society for Endocrinology, International Diabetes Federation Congress, 4-8 December 2017, Abu Dhabi, UAE. (Poster presentation)

Mtintsilana A, Micklesfield LK, Chorell E, *et al* Adiposity mediates the association between the dietary inflammatory index and markers of type 2 diabetes risk in middle-aged Black South African women. 18th International Congress of Endocrinology (ICE)/53rd SEMDSA Congress**,** 1-4 December 2018, Cape Town, South Africa. (Poster presentation)

Mtintsilana A, Micklesfield LK, Chorell E, *et al* The determinants of hyperinsulinaemia in middle-aged black South African women. Berzelius Symposium: Obesity and Type 2 diabetes, understanding the role of ethnicity, 11-12 September 2019, Umeå, Sweden**.** (Oral and poster presentation).

ABBREVIATIONS

^{1}H-MRS	Proton magnetic resonance spectroscopy
β-CGS	Beta-cell glucose sensitivity
BCAA	Branched-chain amino acids
BCKA	Branched-chain keto acids
BMI	Body mass index
Bt20+	Birth-to-twenty cohort plus
CDCA	Chenodeoxycholic acid
CEACAM 1	Carcinoembryonic antigen–related cell adhesion molecule 1
CRP	C-reactive protein
CT	Computed tomography
CVD	Cardiovascular diseases
DCA	Deoxycholic acid
DI	Disposition index
DII	Dietary inflammatory index
DXA	Dual-energy x-ray absorptiometry
E-DII	Energy-adjusted dietary inflammatory index
EGP	Endogenous glucose production
FFA	Free fatty acids
FFQ	Food frequency questionnaire
FLI	Fatty liver index
FM	Fat mass
IVGTT	Intravenous glucose tolerance test
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GC-TOF/MS	Gas chromatography coupled with time-of-flight mass spectrometry
HbA1c	Glycated haemoglobin
HC	Hyperglyceamic clamp
HDL-C	High-density lipoprotein cholesterol
HEC	Hyperinsulinaemic-euglycaemi clamp
HOMA2-IR	Homeostatic model of insulin resistance- HOMA2 is an updated computer based version of the HOMA-IR with additional parameters such as the beta-cell function (HOMA-%B) and insulin sensitivity (HOMA-%S).
HOMA-IR	Homeostatic model of insulin resistance
IDE	Insulin degrading enzyme
IFG	Impaired fasting glucose
IGI	Insulinogenic index
IGM	Impaired glucose metabolism
IGT	Impaired glucose tolerance
IHL	Intra-hepatic lipid content
IL	Interleukins (e.g., IL-6)
IR	Insulin resistance
IVGTT	Intravenous glucose tolerance test
LC-MS	Liquid chromatography-mass spectrometry

LC-TOF/MS	Liquid chromatography coupled with time-of-flight mass spectrometry
LDL-C	Low-density lipoprotein cholesterol
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolamine
MCP-1	Monocyte chemotactic protein
MMTT	Mixed meal tolerance test
MRI	Magnetic resonance image
MS	Mass Spectrometry
MVPA	Moderate- to vigorous-intensity physical activity
NAFLD	Non-alcoholic fatty liver disease
NCDs	Non-communicable diseases
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
OPLS	Orthogonal partial least squares
OPLS-DA	Orthogonal partial least squares-discriminant analysis
OR	Odds ratios
PC	Phosphatidylcholine
PCA	Principal component analysis
PE	Phosphatidylethanolamine
PLS	Partial least square
SA	South Africa
SAT	Subcutaneous adipose tissue
SES	Socio-economic status
SSA	Sub-Saharan Africa
T2D	Type 2 diabetes
TCA	Tricarboxylic acid
TG	Triacylglycerol or Triglyceride
TNF-α	Tumour necrosis factor- alpha
UDCA	Ursodeoxycholic acid
UK	United Kingdom
USA	United States of America
VAT	Visceral adipose tissue
VLDL	Very-low dense lipoprotein
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist-to-hip ratio

GLOSSARY OF TERMS USED IN THIS THESIS

Black African and white European

A cohort of middle-aged and older black South African (SA) women from Soweto Township, Johannesburg metropolitan area, Gauteng province, was recruited for this study. Notably, Soweto Township is one of the most densely populated urban areas in SA, with the population including Bantu-Speakers of various ethnolinguistic groups (i.e., Xhosa, Zulu, Sotho, Pedi, Tswana, Venda, Tsonga, and Swati). It is also important to highlight that literature from SA, the United States of America (USA) and the United Kingdom (UK) was reviewed in order to delineate the pathophysiology of type 2 diabetes (T2D) in urban black SA women. Therefore, throughout this thesis the term black African is used to refer to individuals of African ancestral origin as an ethnic group. This includes black African individuals from SA (e.g., black SA), USA (e.g., African-Americans) and UK (e.g., UK Afro-Caribbean). Furthermore, the term white European is used to describe individuals of European ancestral origin from SA (e.g. white SA), USA (e.g., European-American) and UK (e.g., white European), as an ethnic comparator group.

Middle-aged women

The term “middle-aged women” refers to both pre- and post-menopausal women, from late 30s to 55 years of age. However, for the purpose of this thesis, it is roughly used to refer to women who are mostly in menopausal transition and/or already in post-menopausal stage. This is the group that is scarcely studied in SA.

ABSTRACT

Introduction

The global prevalence of type 2 diabetes (T2D) is increasing at an alarming rate, particularly in developing countries such as South Africa (SA). Black African women have a higher risk and T2D prevalence than white European women, but the pathophysiology of T2D appears to differ. Compared to white European women, black African women have lower visceral adipose tissue (VAT) and liver fat accumulation, and greater peripheral adiposity, but paradoxically present with lower peripheral insulin sensitivity and hyperinsulinaemia, often exceeding that required to maintain normoglycaemia. The exact causes and biological mechanisms underlying this phenotype are poorly understood.

Aim

To identify risk factors for T2D and elucidate putative biological mechanisms implicated in the pathophysiology of T2D in middle-aged urban black SA women. This aim was addressed in four parts:

- Using a longitudinal design, the first study **(chapter 2)** aimed to: (i) explore the association between body fat and fat distribution, and the risk for developing T2D 13 years later; and (ii) investigate the independent associations between baseline and/or change in body fat and fat distribution, and measures of glycaemia at follow-up.
- A cross-sectional design was used to (i) examine the relative contributions of insulin secretion and clearance to basal and postprandial hyperinsulinaemia; (ii) and to explore the associations between insulin sensitivity, adiposity and fatty liver index (FLI, as a proxy of liver fat)**,** and insulin secretion and clearance **(chapter 3).**
- The third study **(chapter 4)** used a cross-sectional design to determine whether dietary-induced inflammation, measured by the dietary inflammatory index (DII),

is associated with markers of T2D risk, and if this association is mediated by adiposity and/or inflammatory markers.

- Using metabolomics analysis, the final study of the thesis **(chapter 5)** used a longitudinal design to identify circulating metabolite patterns that predict the development of T2D**.**

Methods

A sample of 221 black SA women were recruited between 2015-2016 as they fulfilled the criteria of being a caregiver from the Birth-to-Twenty plus cohort (BT20+), on whom we have phenotype data and stored biochemical samples from 2002-2003. These criteria included being younger than 65 years of age, HIV negative and willing to be tested for HIV, and had normal fasting glucose (<6.1 mmol/l) at baseline. At both time points the following measurements were collected; basic anthropometry and dual-energy X-ray absorptiometry (DXA)-derived body composition including estimates of VAT and abdominal subcutaneous adipose tissue (SAT). An oral glucose tolerance test (OGTT) was completed at follow-up only and included analysis of glucose, insulin and C-peptide concentrations at 10, 20, 30, 60, 90 and 120 min. At follow-up, fasting bloods were used for additional analyses including the Homeostatic Model Assessment of Insulin Resistance (HOMA2-IR), HbA1c and inflammatory cytokines, as well as liver enzymes from which the FLI was estimated. A validated food frequency questionnaire was collected at follow-up and was used to calculate the energy-adjusted-DII (E-DII). At follow-up, fasting and OGTT-derived samples were used for the analysis of insulin sensitivity (Matsuda Index) and calculation of insulin secretion and clearance using the Mari mathematical model of beta-cell function. The World Health Organisation (WHO) diabetes diagnostic criteria were used for the categorisation of glycaemic groups: normal glucose tolerance (NGT, fasting glucose < 6.1 mmol/l and/or 2-h post glucose load < 7.8 mmol/l), impaired fasting glucose (IFG, fasting glucose 6.1-6.9 mmol/L), impaired glucose tolerance (IGT, 2-h post glucose load 7.8-11.0 mmol/l) and T2D (fasting glucose ≥ 7.0 mmol/l and/or 2-h post glucose load ≥ 11.1 mmol/l). For statistical analyses the IFG and IGT groups were combined and referred to as impaired glucose metabolism, IGM, as appropriate. Multi-platform mass spectrometry-based metabolomics techniques,

followed by multivariate analyses were used to elucidate metabolite signatures that were associated with T2D development at the baseline and follow-up sampling.

Results

Over the 13-year follow-up period, 64% of the participants remained NGT, while 25% developed IGM and 11% developed T2D. Body weight and fat mass (FM) increased by 7.0 ± 9.0kg and 5.8 ± 6.3kg, respectively, with a relative decrease in peripheral adiposity (leg %FM) and increase in central adiposity (trunk %FM and VAT) (all $p < 0.05$). The IGM/T2D group at follow-up had higher trunk %FM and VAT, as well as lower leg %FM at baseline, compared to the NGT group ($p < 0.0001$). Independent of age and body fat, measures of central obesity, baseline trunk FM (odds ratio (OR) (95% confidence interval (CI)) per 1 kg increase, 1.95 (1.43–2.67)), and baseline VAT (OR (95% CI) per 10 cm^2 increase, 1.25 (1.10–1.42)), and the change in VAT (1.12 (1.03–1.23)), were associated with greater odds of developing IGM/T2D at follow-up. In contrast, baseline leg FM (OR (95% CI) per 1 kg increase, 0.55 (0.41–0.73)) was associated with reduced IGM/T2D risk at the 13-year follow-up period. Furthermore, both baseline and change in trunk FM and VAT were inversely associated with insulin sensitivity, while baseline leg FM was positively associated with insulin sensitivity at follow-up.

In the second study of the thesis, it was reported that both insulin secretion and clearance were associated with basal and postprandial hyperinsulinaemia, however, insulin secretion explained more of the variance (47%) than insulin clearance (37%) in the basal state. In contrast, insulin secretion and clearance accounted for a similar proportion of the variance (12 vs 15%) for the total hyperinsulinaemic response to an OGTT. Insulin sensitivity and leg %FM were inversely associated with basal and total insulin secretion ($p<0.0001$). In contrast, FLI and VAT were positively associated with both measures of insulin secretion ($p<0.05$). Insulin sensitivity was positively, and total adiposity, FLI and VAT were inversely, associated with measures of insulin clearance ($p<0.05$). Furthermore, insulin sensitivity accounted for a greater amount of the variance in both insulin secretion (18 and 38%) and clearance (21 and 26%) in comparison to

measures of adiposity (insulin secretion, 8 and 23%; insulin clearance, 7 and 10%) and FLI (insulin secretion, 7 and 23%; insulin clearance, 7 and 9%).

The third study of the thesis showed that E-DII was associated with all markers of T2D risk, namely, fasting glucose and insulin, two-hour glucose, and insulin sensitivity, independent of age (all $p< 0.05$). These associations were mediated by measures of adiposity, but not inflammatory cytokines. Notably, measures of central obesity had proportionally higher (24–100%) mediation effects than total adiposity measures (10–60%).

The results of the final study of this thesis showed that the metabolite profile characterised by phospholipids, branched-chain amino acids (BCAA) and bile acid metabolites, was significantly different between the participants that developed T2D (i.e., NGT-T2D) and those that remained NGT (i.e., NGT-NGT) over the 13-year follow-up period. At baseline, the NGT-T2D group had lower proliferation-related bile acids (ursodeoxycholic acid, UDCA)- and chenodeoxycholic acid, CDCA) and higher lysophosphatidylcholine:lysophosphatidylethanolamine ratio containing linoleic acid (LPC(C18:2):LPE(C18:2)), and higher levels of leucine and its catabolic intermediates (ketoleucine and C5-carnitine), compared to the NGT-NGT group. At follow-up, these metabolites and/or metabolic pathways remained significantly different between the NGT-T2D and NGT-NGT groups. Accordingly, the NGT-T2D group had higher apoptosis-related bile acids (deoxycholic- and glycodeoxycholic acid) and lower LPC(C18:2) levels than the NGT-NGT group. In contrast, BCAAs and their catabolic intermediates remained significantly higher in the NGT-T2D group.

Conclusion

In summary, the findings of this thesis suggest that obesity, in particular central obesity, is a key risk factor for developing IR, and consequently hyperinsulinemia, and T2D in middle-aged black SA women. In addition to BCAA metabolism, which is extensively linked to the development of T2D in white Europeans, the current findings suggest that changes in phospholipid and bile acid metabolism might be important biomarkers for the

early detection, prevention and management of the T2D in middle-aged black SA women. It is postulated that the promotion of healthy eating, in particular diets containing foods with low inflammatory properties, may be crucial in addressing the rapid increase in obesity and T2D in black SA women. Intervention and longitudinal studies are needed to determine causality and identify biological mechanisms that predict the development of VAT and liver fat accumulation, insulin resistance and hyperinsulinemia, as well as alterations in BCAA, phospholipid and bile acid metabolic pathways in the African population. This knowledge will assist in formulating intervention programmes to reduce the risk of developing obesity and T2D in black SA women.

CHAPTER ONE

Literature review

1.1. Diabetes

Diabetes or diabetes mellitus is defined as a chronic condition that is characterised by excessive blood glucose concentrations (i.e., hyperglycaemia) due to a collection of complex disorders that include cellular insulin resistance (IR), loss of compensatory insulin secretion by the pancreatic beta-cells and reduced insulin clearance, as well as hormonal abnormalities (1). Notably, there are several types of diabetes, but type 1 diabetes, type 2 diabetes (T2D) and gestational diabetes are the most common (1). Compared to the other two, T2D is the most prevalent type of diabetes, affecting approximately 90-95% of adults with diabetes (1), and is the main focus of this thesis. While most cases of diabetes in adults are likely to be caused by T2D (1), it is important to highlight that some reports do not differentiate between type 1 diabetes and T2D. In such cases, the term diabetes will be used throughout the thesis.

1.2. Scope of the problem

The global prevalence of diabetes is increasing at an alarming rate, particularly in low- and middle-income countries within the Sub-Saharan African (SSA) region (1). In 2019, approximately 19 million adults between the ages of 20-79 years were living with diabetes in the SSA region, and SA had the largest affected population (~4.6 million). Of major concern is that this number in the SSA region is estimated to reach 47 million by 2045, and expected to have the highest projected increase in diabetes of all the IDF regions (1). The SSA region also had the highest percentage of diabetes-related deaths under the age of 60 years (75%), with greater diabetes-related mortality in women than men (1). Accordingly, diabetes was the second leading cause of mortality in SA, after tuberculosis, accounting for 5.5% of all deaths reported by Statistics South Africa in 2016 (2). When stratified by sex, diabetes-attributable mortality in SA was greater in women than men (7.2 vs. 4.0%), and ranked first and fifth as the cause of death in women and men, respectively (2). It is important to note that while black SA had the highest proportion of deaths (70%) from all causes, diabetes-related deaths were not reported by ethnicity (2). Nonetheless, the risk of developing T2D and the prevalence of T2D are higher in black Africans than in individuals of white European ethnicity,

irrespective of geographical location (3–8). In SA, urban black SA women have higher T2D risk and prevalence compared to their white European, and male, counterparts (3,4,9). Of great concern is that amongst black SA women, middle-aged and older urban women have a higher T2D prevalence (24.3%) than their pre-menopausal counterparts (10.1%), and yet remain the least studied group (10).

Although the high risk and prevalence of T2D in urban black SA women is likely attributed to urbanisation, socio-economic status (SES), and socio-cultural and environmental factors through their effect on lifestyle (e.g., diet and physical activity) (11–16), weak or absent associations between these factors and markers of T2D such as insulin sensitivity have been reported in black SA women (17). In addition to these factors, it is also important to consider that physiological or biological factors might have a prominent role in driving the high risk and prevalence of T2D in black African women. Indeed, physiological and biological differences between black African and white European individuals are well documented, and include differences in insulin sensitivity (3–5,7,18), insulin response to glucose (3,5–7,18,19), and body fat and fat distribution (5,17,20–22). Therefore, this literature review will examine the role of ethnicity, in particular in women of black African and white European descent, in the pathophysiology of T2D, with greater focus on black SA women who are the research group of interest for this thesis. A better understanding of the pathophysiology of T2D in black SA women will assist in formulating policies to curb the rapid increase in the prevalence of T2D and reduce the long-term health consequences and economic costs associated with T2D, especially in developing countries such as SA (23).

1.3. Diagnosis of T2D

The oral glucose tolerance test (OGTT) is considered the gold standard test for the diagnosis of T2D (24). According to the World Health Organisation (WHO) and the American Diabetes Association (ADA) diagnostic criteria (Table 1.1), T2D is diagnosed as a fasting glucose level of greater or equal to 7.0 mmol/L and/or a 2-hour plasma glucose level that is greater or equal to 11.1 mmol/L (24,25). Moreover, T2D can also be diagnosed using a random blood glucose cut off of ≥ 11.1 mmol/L, and also the glycated

haemoglobin (HbA1c) ≥ 6.5% (Table 1.1) (24–26). It is important to note that the classification and diagnosis of T2D by the European Association for the Study of Diabetes (EASD) and the Society for Endocrinology, Metabolism and Diabetes of South Africa are also based on the WHO and ADA criteria (27,28). In contrast, normal glucose tolerance (NGT) reflects a functional system in which the regulatory hormones (e.g., insulin and glucagon) and metabolic tissue cells are working together in an efficient manner to maintain glucose levels at a steady state (24) (Table 1.1). On the other hand, impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) levels are referred to as prediabetes. Prediabetes is characterised by raised blood glucose levels that are not sufficient for a diagnosis of T2D (Table 1.1).

Table 1.1: Diabetes diagnostic criteria

	NGT	IFG/IGT	T2D
World Health Organisation (WHO)			
Fasting plasma glucose	< 6.1 mmol/L	≥ 6.1 and < 7.0 mmol/L	≥ 7.0 mmol/L
Two-hour plasma glucose following a 75g oral glucose load	< 7.8 mmol/L	≥ 7.8 and < 11.1 mmol/L	≥ 11.1 mmol/L
Random plasma glucose			≥ 11.1 mmol/L
HbA1c			≥ 6.5%
American Diabetes Association (ADA)			
Fasting plasma glucose	< 5.6 mmol/L	≥ 6.1 and < 6.9 mmol/L	≥ 7.0 mmol/L
Two-hour plasma glucose following a 75g oral glucose load	< 7.8 mmol/L	≥ 7.8 and < 11.1 mmol/L	≥ 11.1 mmol/L
Random plasma glucose			≥ 11.1 mmol/L
HbA1c	< 5.7%	5.7-6.4%	≥ 6.5%

Abbreviations: IFG, impaired fasting glycaemia; IGT, impaired glucose tolerance; HbA1c Glycated haemoglobin; NGT, normal glucose tolerance; T2D, type 2 diabetes.

1.4. A brief overview of the pathophysiology of T2D

Although the pathophysiology of T2D is an ongoing debate (29–32), T2D is often characterised by impairments in the relationship between insulin sensitivity and beta-cell insulin secretion, including disruptions in other biological processes such as insulin clearance and adipose tissue dysfunction. These are presented diagrammatically in Figure 1.1 (1,33,34). Although there is evidence for some genetic contribution to these metabolic defects (35–37), obesity or excess fat accumulation is a prominent feature underlying their development (17,20,21,38–43). Accordingly, in the majority of individuals, IR, beta-cell failure, insulin clearance, as well as adipose tissue dysfunction, are closely related to obesity (17,20,21,38–43), which in turn is often attributed to lifestyle and environmental factors (10,11,13,15,44,45). Notably, these biological defects and their subsequent role in the development of T2D are described in detail under sections 1.6, 1.7, and 1.8.

In brief, insulin sensitivity refers to the responsiveness of insulin-sensitive tissues (i.e., muscles, adipose tissue and liver) to insulin, which facilitates glucose uptake and utilisation, as well as suppressing hepatic endogenous glucose production (EGP) (33,34). These insulin-mediated actions are crucial for maintaining normoglycaemia in the basal and postprandial states (1,24,33,34). Notably, the relationship between insulin sensitivity and insulin secretion is hyperbolic in nature (46–48), which means that at low insulin sensitivity, the beta-cells respond to the small change in insulin sensitivity by hypersecreting insulin, resulting in high peripheral insulin concentrations in order to maintain normoglycaemia (46–48). In contrast, at high insulin sensitivity, the beta-cell responsivity to a small change in insulin sensitivity causes a small increase in insulin secretion, which results in low peripheral insulin levels and/or insulin response to glucose (46–48). It is important to note that the stimulation of pancreatic beta-cells results in the rapid release of both insulin and C-peptide, which are secreted in equimolar amounts (49–51). Notably, the amount of insulin in the peripheral circulation varies markedly from the total amount of insulin secreted by the beta-cells. This is due to the fact that peripheral insulin levels include the amount of insulin secreted by the beta-cells and that cleared by the hepatocytes (49–51). Unlike insulin, C-peptide is

presumably not cleared by the liver and has a longer half-life (i.e., 20-30 vs 3-5 minutes), hence it is a preferred marker of endogenous pre-hepatic insulin secretion (49–51). In contrast, approximately 50-80% of insulin is cleared by the liver (49–51), which is referred to as hepatic insulin clearance. Insulin not cleared during this phase exits the liver via the hepatic vein, where it is transported to the heart and gets distributed to the rest of the body through the arterial circulation and further undergoes clearance by the metabolic tissues such as skeletal muscle, adipose tissue and kidneys (49–51). Insulin clearance that occurs in these tissues is known as extra-hepatic insulin clearance (49–51). Insulin not cleared by the metabolic tissues is delivered to, and finally degraded by, the kidney (49–51). Notably, hepatic and extra-hepatic insulin clearance are distinct processes that are independently regulated and differ significantly between individuals (52–54).

The product of insulin secretion and insulin sensitivity, known as the disposition index (DI), serves as an important marker of beta-cell function (46–48). The DI remains unchanged if the individual's beta-cell response to a change in insulin sensitivity results in an adequate increase or decrease in insulin secretion (46–48). This appropriate response by the beta-cells to changes in insulin sensitivity maintains normoglycaemia. However, in the presence of IR and/or obesity, insulin secretion increases and/or insulin clearance decreases, leading to compensatory hyperinsulinaemia (3,5,9,55–58) (Figure 1.1).Therefore, hyperinsulinaemia is a biological condition characterised by high peripheral insulin levels, often exceeding that which is required to maintain normoglycaemia (3,5,9,55–58). Several factors such as genetics (59–61), excessive fat accumulation and ectopic fat deposition (41–43,56,57), incretin hormones (62,63), and lifestyle factors (64) have been implicated in the regulation of hyperinsulinaemia, via insulin secretion and/or clearance.

When the beta cells can no longer secrete sufficient insulin to compensate for IR and/or obesity, blood glucose levels increase above the normal range, resulting in glucose intolerance or hyperglycaemia (46–48). This stage is also characterised by enhanced EGP in the basal state and impaired post-prandial suppression of glucose production (Figure 1.1). Moreover, the ability of insulin to metabolise glucose and facilitate glucose

uptake in insulin-sensitive tissues is impaired (Figure 1.1). Notably, when the beta-cells can no longer compensate for the degree of IR and/or obesity and the loss of compensatory response by the beta-cell worsens, beta-cell failure develops. This marks the onset of T2D (Figure 1.1).

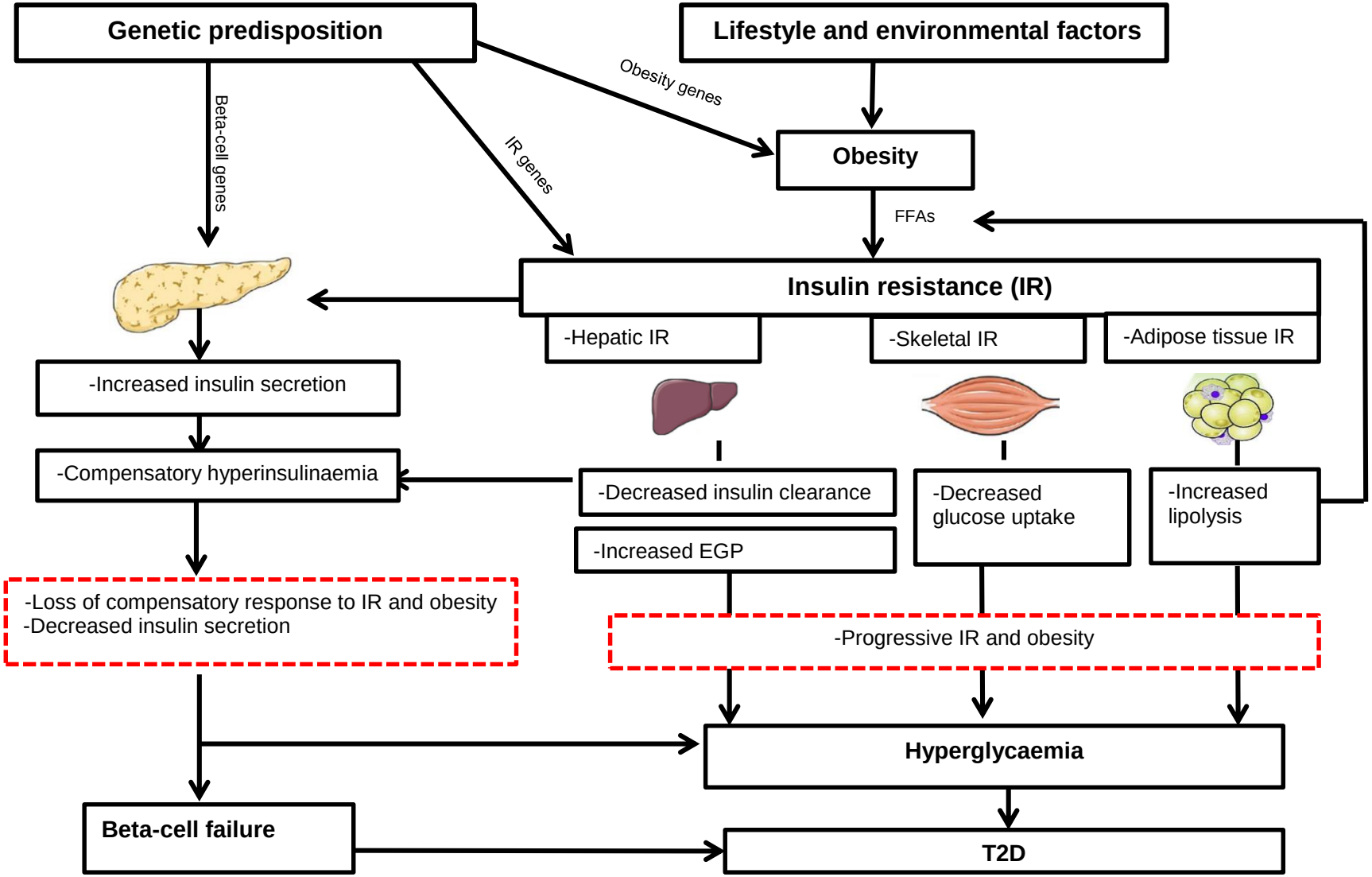


Figure 1.1: A simple diagram describing the pathogenesis of T2D, based on existing literature. Abbreviations: EGP, Endogenous glucose production; FFA, Free fatty acids; IR, Insulin resistance; T2D, Type 2 diabetes.

1.5. The assessment of insulin sensitivity/insulin resistance, beta-cell function and insulin clearance

There are several common methods and/or tests used to estimate insulin sensitivity, beta-cell function and insulin clearance, which are described in detail in previous reviews (65–67). These methods and/or tests include the fasting homeostasis model assessment of IR (i.e., HOMA-IR), oral (e.g., OGTT and mixed meal tolerance test (MMTT)) and intravenous tests (e.g., intravenous glucose tolerance test, IVGTT). Another set of tests include the metabolic clamp procedures such as the hyperglycaemic clamp (HC) and the hyperinsulinaemic-euglycaemic clamp (HEC) (65–67). For the purpose of this thesis, parameters such as the DI, insulin secretion and beta-cell glucose sensitivity (βCGS) are referred to as estimates of beta-cell function.

Although, there are various methods and/or tests for the assessment of insulin sensitivity, beta-cell function and insulin clearance, it is important to acknowledge that all the tests have strengths and limitations. There is no gold standard test for measuring beta-cell function, however, the IVGTT is the most commonly used test as it allows for the most accurate quantification of the first phase insulin secretion response (from 2-10 minutes post-glucose load) (65–67), which is an important marker in the progression from NGT to IGT. On the other hand, the HEC is considered a gold standard test for the measurement of peripheral insulin sensitivity (65–67). When combined with isotope tracers, the HEC can be used to directly assess tissue-specific insulin sensitivity, for example at the liver, and without the use of isotope tracers the HEC can provide a direct measure of whole-body insulin sensitivity (65–67). Despite these strengths, the intravenous tests or metabolic clamp procedures are labour intensive, require trained personnel, and are time consuming and expensive. Thus, these are not feasible for large epidemiological studies and clinical settings (65–67). Furthermore, these tests are less physiological compared to the oral tests, as they do not release incretins, which are important hormones that regulate glucose homeostasis (65–67). Hence, surrogate indices such as the HOMA-IR and the OGTT, which are simpler to administer in a routine clinical setting are preferred, in particular for large studies and in under-resourced settings.

The estimation of insulin sensitivity, beta-cell function and insulin clearance is often calculated using empirical equations or mathematical-derived model indices (65–67). For example, insulin sensitivity can be calculated from fasting glucose and insulin concentrations or from OGTT-derived glucose and insulin data using equations or algorithms such as the HOMA-IR and Matsuda index, respectively (68,69). Notably, HOMA-IR and Matsuda index have been shown to be strong correlates of HEC (69,70). There are few studies that have examined the correlations between HOMA-IR and Matsuda index, and HEC, in black Africans, and findings have been inconsistent (71–73). In a study by Pisprasert et al. (72), Matsuda Index was significantly correlated with HEC in African-American men (72), but this and other studies in Afro-Caribbean people (72) and African-American children (73), have not shown a significant correlation between HOMA-IR and HEC (71)

Beta-cell function can also be estimated using empirical equations such as the commonly used DI or insulinogenic index (IGI) (3,9,46–48). The IGI, which is calculated as the ratio of change in insulin to the change in glucose from 0-30 minutes during the OGTT, is used as a measure of the early phase insulin response. Despite its frequent use, it is important to note that the IGI uses insulin instead of C-peptide concentrations, which reflect both insulin secretion and clearance (49–51). For these reasons, the use of IGI or the DI when calculated from peripheral insulin levels, may not accurately estimate the relationship between insulin secretion and prevailing glucose concentrations. However, if C-peptide levels are measured, indices such as the DI can be used to estimate beta-cell function. Furthermore, C-peptide levels can be used to accurately estimate endogenous pre-hepatic insulin secretion using models of C-peptide deconvolution such as the Mari mathematical model of beta-cell function (65–67,74–76).

The Mari mathematical model is a widely used mathematical model of beta-cell function that can be applied with data from multiple tests (e.g., MMTT, OGTT and IVGTT) (74,75,77). Like all mathematical models of beta-cell function, the Mari model attempts to describe the relationship between insulin secretion and glucose concentration (74,75). The Mari model also provides an estimate of endogenous insulin clearance (41,61,78)., which has been validated against hepatic insulin clearance calculated by modelling of

data collected in individuals who underwent an HEC (78). Further, modelling by Polidori et al. (52) provides estimates of hepatic and extrahepatic insulin clearance as distinct processes that regulate insulin clearance. This is of relevance for some ethnic groups such as black Africans, where it has been shown that hepatic rather than extra-hepatic insulin clearance is a major contributor to hyperinsulinaemia (52–54).

One of the advantages of the Mari model is that it enables researchers to explore the dynamic relationship that exists between glucose, insulin, and beta-cell response over time. Furthermore, the model takes into account physiological factors (e.g., potentiation factor) that are involved in the ability of the beta-cells to respond to changes in plasma glucose concentrations. Despite these advantages, the Mari model has some limitations. The selection of data entered into the model is subjective, thus can lead to under- or over-estimation of the beta-cell function. Furthermore, the model involves complex mathematics, thus requires an expert or trained personnel to analyse and interpret the data. Moreover, there is no validated and widely available computer program yet, which means the calculation of the beta-cell function requires assistance from the developers of the model. Nonetheless, there is a need for studies to utilise the mathematical models of beta-cell function, which provide better description of the beta-cell function and glucose concentration relationship than indices such as IGI. This is of great importance since the mathematical models of beta-cell function also quantify insulin secretion and clearance parameters as distinct processes that regulate peripheral insulin concentrations, thus allowing us to gain an in depth understanding of hyperinsulinaemia. This is of relevance for black Africans, in particular black African women, who present with hyperinsulinaemia, and who have a higher T2D risk and prevalence than their white European counterparts (3,5,18,55,79), yet the pathophysiology of hyperinsulinaemia and its contribution to the development of T2D remains poorly understood in Africans. The mathematic model of beta-cell function and insulin clearance has been explored in white Europeans and in African-Americans (41,61,74,75,77,78). Therefore, quantification of beta-cell function and clearance using a mathematical model of beta-cell function is required to provide more insights into the role of hyperinsulinaemia in the development of T2D in black Africans from Africa.

1.6. Proposed theories and mechanisms for the development of T2D

The exact underlying causes of T2D are unknown. Currently, it is believed that IR is the primary cause of T2D; however, this view is debated (29–32). Some authors have proposed that factors such as hyperinsulinaemia or hepatic insulin clearance may be initial drivers of T2D, in particular in some ethnic groups such as Africans (31,32,80). Further evidence to refute IR as a proposed primary driver in T2D pathophysiology is supported by the findings that hyperinsulinaemia is an independent predictor of T2D (81,82). Although there is no consensus about the initial cause of T2D, the prominent features of T2D pathophysiology (e.g., IR and beta-cell dysfunction) have close associations with obesity, in particular central obesity and ectopic fat deposition (i.e., accumulation of fat in non-adipose tissues) (5,17,40–42,83–87). Furthermore, T2D patients are either obese or were formerly obese (10,86,88,89).

The role of obesity or excessive fat accumulation in the development of metabolic defects such as IR and hyperinsulinaemia is described in the context of adipose tissue dysfunction (38,39,42,90,91). It is also important to note that in addition to adipose tissue dysfunction, there are also other biological mechanisms such as inflammation, mitochondrial dysfunction and endoplasmic reticulum stress, which have also been linked to the development of IR, hyperinsulinaemia and T2D, which are reviewed elsewhere (92–94). Notably, these biological processes have close associations with obesity, which makes obesity a key underlying mechanism for the development of T2D. For the purpose of this review, three complementary theories, namely, the spillover or adipose tissue expandability theory, portal theory and the twin-cycle theory, will be briefly discussed below and presented in Figure 1.2, to provide better insights on how adipose tissue dysfunction is linked to the development of T2D (39,90,91,95).

In brief, subcutaneous adipose tissue (SAT), which is the adipose tissue located under the skin, plays an essential role in regulating energy balance by acting as a safe depot or metabolic sink for storing excess fat during excess energy intake and/or reduced energy expenditure (39,90,96). Notably, SAT includes abdominal and gluteo-femoral depots, with the latter having larger adipocytes, higher lipoprotein lipase activity,

“favourable” adipokines and lower rate of lipolysis and pro-inflammatory cytokines compared to abdominal SAT (97,98). Furthermore, SAT has less lipolytic activity, adipokines and pro-inflammatory profile than visceral adipose tissue (VAT, i.e., adipose tissue surrounding the internal organs) (84,90,96,99). Compared to VAT, SAT also has greater storage capacity or expandability, however, with increasing fat accumulation this capacity becomes limited (39,90,96). To elaborate, in the event of excess energy balance or over nutrition, adipocytes expand to accumulate excess free fatty acids (FFAs), which are then stored as triglycerides (39,90,96,100). This expansion occurs by mature adipocytes increasing in size known as hypertrophy, and/or by undergoing hyperplasia, which is when new small adipocytes are generated, thereby increasing the number of adipose tissue cells (39,90,96,100). Notably, adipose tissue hypertrophy is more strongly associated with T2D risk than adipose tissue hyperplasia (92,93,101). This might be partly due to the fact that cell hypertrophy, often termed ‘unhealthy adipose tissue expansion’ is associated with hypoxia and fibrosis which in turn cause low-grade inflammation (92,93,98,101,102) (Figure 1.2). Low-grade inflammation is a biological condition characterised by elevated expression or secretion of chemokines, adipose tissue macrophages and pro-inflammatory cytokines, which infiltrate insulin-sensitive organs such as the liver and the muscle, and impairs their ability to respond adequately to insulin, and subsequently cause IR (92,93,98,101) (Figure 1.2).

The adipose tissue expandability or spillover hypothesis suggests that SATs loss of capacity to store excess fat and suppress lipolysis, results in FFAs and other lipid intermediates (e.g., triglycerides and cholesterol) to spill over into the VAT depot and in non-adipose tissue such as muscles, liver and pancreas, thereby interfering with their metabolic functions including the sensitivity of the tissues to insulin (39,90,91,100) (Figure 1.2). The accumulation of FFAs in the VAT depot is involved in the pathophysiology of T2D as VAT releases excess FFAs and pro-inflammatory cytokines directly into the portal circulation, which become deposited in the liver, leading to increased liver fat accumulation and hepatic IR (40,84,90,96,99) (Figure 1.2). This in turn leads to the production and deposition of toxic lipid intermediates (e.g., triglyceride-rich very low dense-lipoprotein, TG-VLDL) in metabolic tissues including the pancreatic beta-cells (42,43,83,86), creating a vicious cycle of lipotoxicity, as described by the twin-

cycle hypothesis (95). It is believed that lipotoxicity or excessive fat accumulation in ectopic tissues, which is also accompanied by IR, beta-cell dysfunction, as well as other metabolic defects, such as decreased glucose uptake in the skeletal muscle and enhanced hepatic EGP, is the key underlying mechanism for the development of T2D (42,43,79,83,86) (Figure 1.2). Notably, gradual increases in FFA levels in the pancreas may inhibit insulin secretion and induce beta-cell apoptosis, leading to beta-cell failure and overt T2D (42,43,83,86) (Figure 1.2).

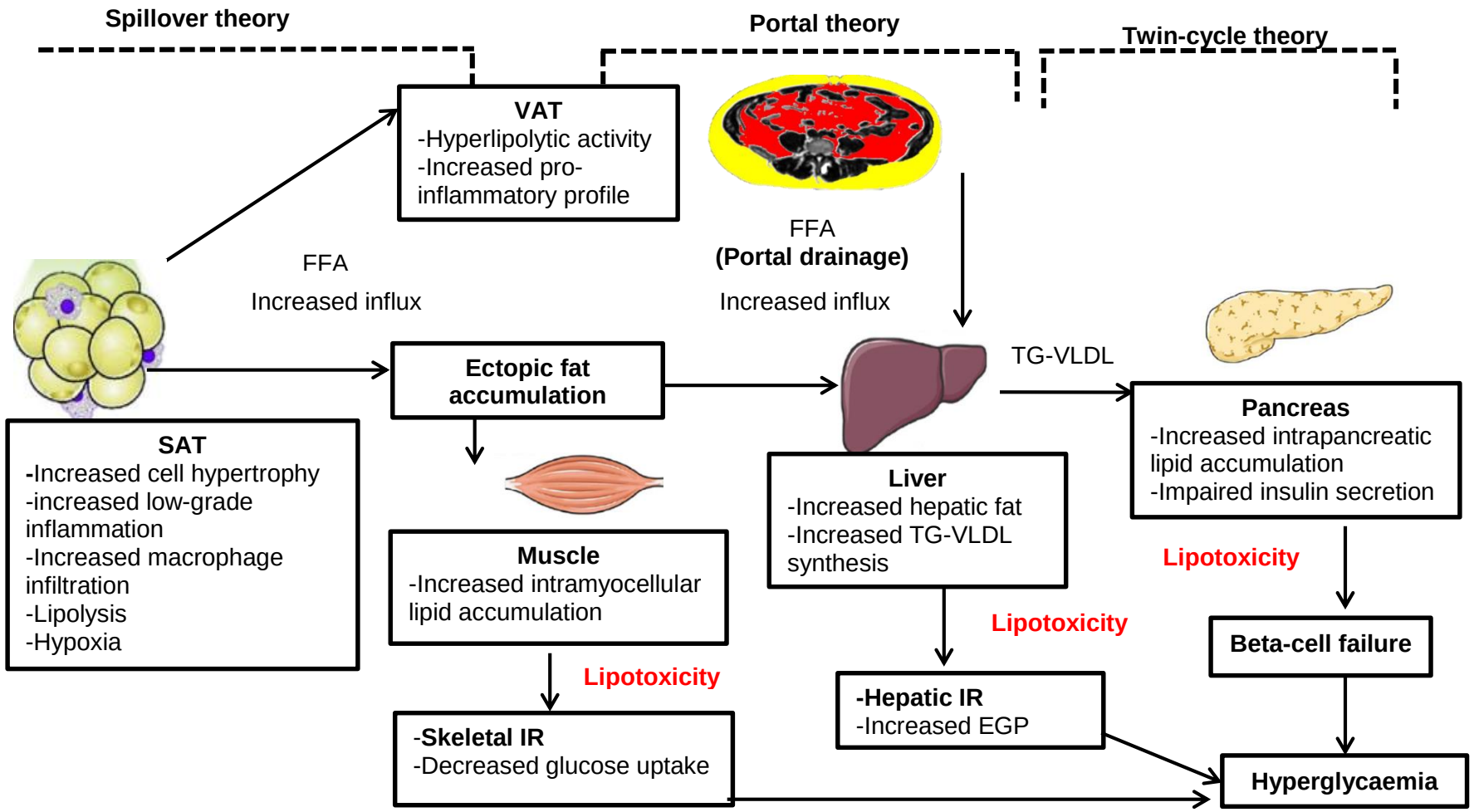


Figure 1.2: Adipose tissue dysfunction in the development of T2D, as illustrated in the context of spillover, portal and twin-cycle theories (Adapted from Goff et al. (6)). Abbreviations: EGP, Endogenous glucose production; FFA, Free fatty acids; IR, Insulin resistance; SAT, Subcutaneous adipose tissue; T2D, Type 2 diabetes; TG, Triglycerides; VAT, Visceral adipose tissue; VLDL, Very-low density lipoprotein.

1.7. Ethnic differences in the pathophysiology of T2D: focus on insulin sensitivity and hyperinsulinaemia

1.7.1. Insulin sensitivity/ Insulin resistance (IR)

Ethnic differences in insulin sensitivity between black African and white European individuals have been well described (Table 1.2). The majority of studies examining insulin sensitivity in black Africans from the USA, SA and the UK show that black Africans have lower whole-body and/or peripheral insulin sensitivity compared to their white European counterparts (3,5–7,18,19,55). In contrast, assessment of hepatic insulin sensitivity using different techniques, has shown that black Africans, irrespective of geographical location, have similar hepatic insulin sensitivity to their white European counterparts (20,103–105) (Table 1.2).

It is also important to note that a significant number of studies have reported contradictory findings, where whole-body and/or peripheral insulin sensitivity is similar (61,79,87,106,107) and hepatic insulin sensitivity is lower (6) or higher (87) in black African and white European individuals. In a SA study of pre-menopausal women, it was reported that while whole-body insulin sensitivity was similar, hepatic insulin sensitivity characterised by enhanced suppression of EGP, was higher in black compared to white women (87). Similarly, a later study by Chung et al. (103) revealed that although both parameters of insulin sensitivity were not significantly different between black African and white European pre-menopausal women; at any given level of whole-body insulin sensitivity black African pre-menopausal women had greater hepatic insulin sensitivity than their white European counterparts (103). Notably in both of these studies, greater hepatic insulin sensitivity in black African pre-menopausal women was found in the context of lower VAT and liver fat accumulation (87,103), and was linked to lower rates of gluconeogenesis (103) compared to white European counterparts. Indeed, one of the major determinants of IR is the centralisation of body fat, in particular VAT and liver fat accumulation (20,40,41,84,96,99). Of relevance is that black Africans typically present with lower levels of VAT and liver fat accumulation compared to their white European counterparts (18,20,22,87), which partly explains why black African women had greater hepatic insulin sensitivity than white European women (87,103). Notably, the role of VAT

and liver fat accumulation in T2D pathophysiology between black African and white European women is discussed in detail in sections 1.8.1 and 1.8.2. Despite the ethnic differences in VAT and liver fat, it is important to highlight that these factors are associated with unfavourable lipid profile, insulin levels, IR and T2D risk (17,21,99,105). Furthermore, in the limited prospective studies investigating VAT accumulation in relation to T2D risk, it was reported that VAT accumulation was independently associated with incident prediabetes and/or T2D in a multi-ethnic cohort study from the USA (108) and in white Europeans in the Rotterdam cohort (109). Notably, VAT, liver fat and IR are all inter-related and have been implicated in hyperinsulinaemia via their association with insulin secretion and clearance in various studies conducted in USA and Europe (41,57,58,61,83,110), as described in detail in the next section, 1.7.2. Therefore, it is proposed that the discrepancies in insulin sensitivity between the two ethnic groups might also be attributed to hyperinsulinaemia.

Of relevance to this study is that it is not known if these associations will be replicated in an African population. Hence, more studies, especially longitudinal study designs, are needed to examine whether VAT is a predictor of IR and T2D in black Africans. Further, the lack of studies investigating the associations of insulin sensitivity with insulin secretion and clearance in Africans warrants the need for more studies. Of major concern is that there are very limited studies (both cross-sectional and longitudinal) investigating insulin sensitivity in relation to T2D risk in black African post-menopausal women, despite having greater IR, T2D risk and T2D prevalence than their pre-menopausal counterparts (10,18,55). No study has investigated the associations between VAT and insulin sensitivity in black SA post-menopausal women, despite this group having the highest levels of overweight and obesity (111), which have been linked to the increased T2D prevalence in SA (10,89). Furthermore, the association between insulin sensitivity and insulin secretion and clearance, is yet to be explored in black SA post-menopausal women.

Table 1.2: Ethnic comparison of insulin sensitivity between black African and white European individuals

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Whole-body and/or peripheral insulin sensitivity								
Chandler-Laney et al. (5)	Cross-sectional	African-American (n=76) 7–12 years (n=33) 18–32 years (n=25) 40–70 years (n=18)	European-American (n=92) 7–12 years (n=29) 18–32 years (n=32) 40–70 years (n=31)	F	African-American Pre-pubertal: 10.3 ± 1.5 years Pre-menopausal: 25.9 ± 3.4 years Post-menopausal: 55.7 ± 4.2 years European-American Pre-pubertal: 10.3 ± 1.5 years Pre-menopausal: 25.9 ± 3.4 years Post-menopausal: 55.7 ± 4.2 years	IVGTT	NGT	Across all age groups, African-Americans had lower insulin sensitivity than European-Americans, even after adjusting for body fat.
Chung et al. (18)	Cross-sectional	Black African women (n=71) Pre-menopausal (n=52) Post-menopausal (n=19)	White European women (=48) Pre-menopausal (n=35) Post-menopausal (n=13)	F	Black African women: 42.3 ± 9 years Pre-menopausal: 39 ± 7 years Post-menopausal: 53 ± 4 years White European women: 43.3 ± 2 years Pre-menopausal: 38 ± 7 years Post-menopausal: 57 ± 4 years	IVGTT	NGT and IGT	Insulin sensitivity was similar between black African women (both pre- and post-menopausal) women compared to white European women. Insulin sensitivity was not significantly different between black African and white European pre-menopausal women Black African post-menopausal women had lower insulin sensitivity than their white European counterparts.

Abbreviations: F, Female; IGT, Impaired glucose tolerance; IVGTT, Intravenous glucose tolerance test; M, Male; NGT, Normal glucose tolerance; NW, Normal weight; OB, Obese; OGTT, Oral glucose tolerance tests; SA, South Africa; T2D, Type 2 diabetes.

Continuation of Table 1.2

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Goree et al. (55)	Cross-sectional	African-American women (n=76) Pre-pubertal (n=34) Pre- (n=27) and Post-menopausal (n=15)	European-American women (n=78) Pre-pubertal (n=14) Pre- (n=33) and Post-menopausal (n=21)	F	African-American Pre-pubertal girls: 9.3 ±1.5 years Pre-menopausal: 24.8 ± 3.3 years Post-menopausal: 56.6± 5.1 years European-American Pre-pubertal: 9.6 ±1.6 years Pre-menopausal: 25.7 ± 3.5 years Post-menopausal: 55.7 ± 4.2 years	IVGTT	Non-diabetic	Insulin sensitivity was lower in African-American women compared to their white European counterparts, independent of age.
Smith et al. (79)	Cross-sectional	African-American women (n=34)	European-American (n=83)	F	African-American: 39 ±1.9 years European-American: 44 ±0.9 years	OGTT IVGTT	Non-diabetic	Similar insulin sensitivity between the ethnic groups.
Chung et al. (103)	Cross-sectional	Black African pre-menopausal womn (n=24)	White European pre-menopausal women (n=22)	F	Black African pre-menopausal women (37 ± 1 years) White European pre-menopausal women (37 ± 1 years)	Two-step isotope labelled, HEC.	Non-diabetic	Whole insulin sensitivity was similar between the two ethnic groups. Adipose tissue insulin sensitivity similar between black African and white European women.
Goedecke et al. (3)	Cross-sectional	Black SA women (n=29) 13 NW and 16 OB	White SA (n=28) 14 NW and 14 OB)	F	Black SA women NW: 24 ± 2 years OB: 28 ± 1 years White SA women NW: 26 ± 2 years OB: 31 ±2 years	OGTT IVGTT	Non-diabetic	Matched by BMI, black SA women (both NW and OB groups) had lower whole-body insulin sensitivity.

Abbreviations: F, Female; HEC, Hyperinsulinaemic-euglycaemic clamp; IGT, impaired glucose tolerance; IVGTT, Intravenous glucose tolerance test; NGT, Normal glucose tolerance; NW, Normal weight; OB, Obese; OGTT, Oral glucose tolerance tests; SA, South Africa; UK, United Kingdom; WAB, Without abdominal obesity.

Continuation of Table 1.2

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Goedecke et al. (87)	Cross-sectional	Black SA women (n=15, OB)	White SA (n=15 OB)	F	Black SA women (36±5) White SA women (36±2)	Two-step isotope labelled, HEC.	Non-diabetic	No ethnic differences in whole-body insulin sensitivity.
Van der Merwe et al. (4)	Cross-sectional	Black SA women (n=10, OB)	White SA women (n=10, OB)	F	Black obese SA women: 34.8 ± 0.8 years White obese SA women: 34.3 ± 0.8	HEC	Non-diabetic	Insulin sensitivity was lower in black SA women.
Reimann et al. (112)	Cross-sectional	Black SA women (n-65, AB) Black SA women (n-=21, WAB)	White SA women (n=56, AB) White SA women (n=34, WAB)	F	Black SA women AB: 27 (23,30) years WAB: 32 (28, 35) years White SA women AB: 25 (23, 30) years WAB: 29 (23, 33) years	OGTT	Non-diabetic	There were no ethnic differences in insulin sensitivity.
Osei et al. (7)	Cross-sectional	African-American (n=32)	European-American (n=30)	Both F and M	African-American F: 27.8 ± 3.3 years M: 32.4 ± 1.8 years European-American F: 27.9 ± 1.9 years M: 25.4 ± 1.6 years	IVGTT	Non-diabetic	Mean insulin sensitivity was 35% lower in African-Americans compared to European-Americans.
Haffner et al. (19)	Prospective	African-American (n=288) (From Los Angeles and Oakland)	European-American (n=229) (From Los Angeles and Oakland)	Both M and F	African-American Los Angeles : 54.2 ± 0.7 years Oakland: 54.6 ± 0.7 years European-American Los Angeles : 54.7 ± 0.8 years Oakland: 56.1 ± 0.73 years	IVGTT	NGT and IGT	African-American had lower insulin sensitivity than European-American.
Szczepaniak et al. (42)	Cross-sectional	African-American (n=20)	European-American (n=30)	Both F and M	African-American: 37±3 years White American: 43 ±2 years	IVGTT	Non-diabetic	Lower insulin sensitivity in African-Americans compared to white Americans.

Abbreviations: AB, Abdominal obesity; F, Female; HEC, Hyperinsulinaemic-euglycaemic clamp; IGT, impaired glucose tolerance; IVGTT, Intravenous glucose tolerance test; M, Male;NGT, Normal glucose tolerance; NW, Normal weight; OB, Obese; OGTT, Oral glucose tolerance tests; SA, South Africa; T2D, Type 2 diabetes; UK, United Kingdom; WAB, Without abdominal obesity

Continuation of Table 1.2

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Fosam et al. (61)	Cross-sectional	African-American (n=36)	European-American (n=47)	Both F and M	African-American: 39 ±11 years Non-Hispanic white: 39 ±13 years	MMTT	Non-diabetic	No ethnic difference in insulin sensitivity between the groups.
Goff et al. (6)	Cross-sectional	UK Afro-Caribbean (n=35)	UK white (n=417)	Both F and M	UK Afro-Caribbean F:42.6 ±7 years M: 44.9 ±9.7 years UK white European F: 51.8 ± 10.3 years M: 52.8 ± 10.6 years	IVGTT	Non-diabetic	When adjusted for age and BMI, Afro-Caribbean had lower insulin sensitivity than UK white European individuals.
Velasquez-Mieyer et al. (106)	Cross-sectional	African-American (n=16) (severely obese)	European-American (n=26) (severely obese)	Not specificed	African-American: 36 ± 2 years European-American: 38 ± 2 years	OGTT	NGT and IGT	Similar whole-body insulin sensitivity between the two ethnic groups.
HOMA-IR: as a proxy of fasting hepatic insulin sensitivity								
Kanaley et al. (105)	Cross-sectional	Black African post-menopausal women (n=21)	White European post-menopausal women (n=33)	F	Black African women: 52.3 ± 1.0 years White European women: 54.2 ± 1.6 years	Fasting parameters to estimate HOMA-IR	Non-diabetic	HOMA-IR similar between black African and white European post-menopausal women.
Reimann et al. (112)	Cross-sectional	Black SA women (n-65, AB) Black SA women (n-=21, WAB)	White SA women (n=56, AB) White SA women (n=34, WAB)	F	Black SA women AB: 27 (23,30) years WAB: 32 (28, 35) years White SA women AB: 25 (23, 30) years WAB: 29 (23, 33) years	Fasting insulin and glucose to estimate HOMA-IR.	Non-diabetic	There were no ethnic differences in HOMA-IR.
Keswell et al. (21)	Cross-sectional	Black SA (n=288)	White SA (n=197)	F	Black SA women: 22 (22–33) years White SA women: 32 (24–39] years	Fasting parameters to estimate HOMA-IR	Non-diabetic	No ethnic difference in HOMA-IR.

Abbreviations: AB, Abdominal obesity; F, Female; HOMA-IR, Homeostasis model assessment-insulin resistance; IGT, impaired glucose tolerance; IVGTT, Intravenous glucose tolerance test; M, Male; MMTT, Mixed meal tolerance tests; NGT, Normal glucose tolerance; NW, Normal weight; OB, Obese; OGTT, Oral glucose tolerance tests; SA, South Africa; UK, United Kingdom; WAB, Without abdominal obesity.

Continuation of Table 1.2

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Goff et al. (6)	Cross-sectional	UK Afro-Caribbean (n=35)	UK white European (n=417)	Both F and M	UK Afro-Caribbean F:42.6 ±7 years M: 44.9 ±9.7 years UK white European F: 51.8 ± 10.3 years M: 52.8 ± 10.6 years	Fasting parameters to estimate HOMA2-IR.	Non-diabetic	HOMA2-IR, adjusted for age and BMI was 20% greater in black African-Caribbean compared with white European women.
Guerrero et al (20)	Cross-sectional	African-American (n=1058)	European-American (n=719)	Both F and M	African-American F:46 (37-54) years M: 45 (38-54) years European-American F: 48 (38-55) years M: 44 (38-52) years	Fasting parameters to estimate HOMA-IR	Non-diabetic	HOMA-IR not different between the two ethnic groups.
Hepatic insulin sensitivity								
Chung et al. (103)	Cross-sectional	Black African pre-menopausal women (n=24)	White European pre-menopausal women (n=22)	F	Black African pre-menopausal women: 37 ± 1 years White European pre-menopausal women: 37 ± 1 years	Two-step isotope labelled, HEC.	Non-diabetic	Hepatic insulin sensitivity was similar between black African and white European women. However, at any given level of whole-body insulin sensitivity, black African pre-menopausal women had greater hepatic insulin sensitivity compared to their white European counterparts.
Goedecke et al. (87)	Cross-sectional	Black SA women (n=15, OB)	White SA (n=15 OB)	F	Black SA women: 36 ± 5 years White SA women: 36 ± 2 years	Two-step isotope labelled, HEC.	Non-diabetic	Hepatic insulin sensitivity was greater in black than in white SA women.

Abbreviations: F, Female; HEC, Hyperinsulinaemic-euglycaemic clamp; HOMA-IR, Homeostasis model assessment-insulin resistance; M, Male; OB, Obese; SA, South Africa; T2D, Type 2 diabetes; UK, United Kingdom.

1.7.2. Hyperinsulinaemia, described in the context of insulin secretion and clearance

Ethnic differences in the prevalence of hyperinsulinaemia between black African and white Europeans are well documented (Table 1.3). Notably, a large proportion of studies assessing hyperinsulinaemia between black Africans and white Europeans show that black Africans from SA, USA and UK are more likely to be hyperinsulinaemic than their white European counterparts (3,5,106,6,7,18,19,42,53,55,79). The hyperinsulinaemia in the adult studies of black African (i.e., both sexes) (7,61) was attributed to a reduction in insulin clearance instead of an increase in insulin secretion (Table 1.3). In contrast, the hyperinsulinaemia in black African women from SA and USA has been primarily attributed to increased beta-cell function (3,5,55,79). Reason for this, is that these studies did not measure insulin clearance, instead they estimated beta-cell function, which was based on the assessment of DI, insulin, C-peptide concentrations/insulin secretion and/or βCGS levels (3,5,55,79,113). Furthermore, another study measured beta-cell function using indices such as IGI, which does not differentiate between insulin secretion and clearance, thus making it difficult to compare the relative contribution of insulin secretion and clearance to hyperinsulinaemia in black African women (3). Nonetheless, in the few studies in which both insulin secretion and clearance have been measured, it was reported that both parameters were significant contributors to hyperinsulinaemia in African-American pre-menopausal women (53), whereas in a different study consisting of both pre- and post-menopausal African-American women, insulin clearance was reported as the only contributor to hyperinsulinaemia (18). Further exploration of insulin clearance revealed that hepatic rather than extra-hepatic insulin clearance was the main contributor to hyperinsulinaemia in African-American women (53).

Table 1.3: Ethnic comparison of hyperinsulinaemia, beta-cell function and insulin clearance between black and white populations

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Chandler-Laney et al. (5)	Cross-sectional	African-American (n=76) 7–12 years (n=33) 18–32 years (n=25) 40–70 years (n=18)	European-American (n=92) 7–12 years (n=29) 18–32 years (n=32) 40–70 years (n=31)	F	African- American Prepubertal: 10.3 ± 1.5 years Pre-menopausal: 25.9 ± 3.4 years Post-menopausal: 55.7 ± 4.2 years European American Prepubertal: 10.3 ± 1.5 years Pre-menopausal: 25.9 ± 3.4 years Post-menopausal: 55.7 ± 4.2 years	IVGTT	NGT	-African-Americans in all age groups had higher intravenous glucose tolerance, acute C-peptide release, first- phase and total beta-cell responsivity to glucose than European American women, which remained higher after adjustment for percentage body fat and IAAT. -Differences in acute C-peptide release, first-phase and total beta-cell responsivity to glucose also remained significant after adjustment for insulin sensitivity.
Goree et al. (55)	Cross-sectional	African-American women (n=76) Prepubertal (n=34) Pre- (n=27) and Post-menopausal (n=15)	European-American women (n=78) Prepubertal (n=14) Pre- (n=33) and Post-menopausal (n=21)	F	African American Prepubertal girls: 9.3±1.5 years Pre-menopausal: 24.8 ± 3.3 years Post-menopausal: 56.6± 5.1 years European American Prepubertal: 9.6±1.6 years Pre-menopausal: 25.7 ± 3.5 years Post-menopausal: 55.7± 4.2 years	IVGTT	Non-diabetic	-Fasting insulin levels and DI were higher in African-American women than European-American women. -AIRg was also higher among African-American women compared to European-American women, and remained higher after adjusting for insulin sensitivity.

Abbreviations: AIRg, Acute insulin response to glucose; DI, Disposition Index; F, Female; IAAT Intra-abdominal adipose tissue; IVGTT, Intravenous glucose tolerance test; NGT, Normal glucose tolerance.

Continuation of Table 1.3

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Piccinini et al. (53)	Cross-sectional	African-American women (n=18)	European-American (n=29)	F	African-American: 25 (4) years European-American: 26(4) years	IVGTT	NGT	-First 20mins of IVGTT, plasma insulin levels were 110% higher in African-Americans vs European-Americans. -Insulin secretion after glucose administration was also higher in African-Americans. -In the fasted state, hepatic insulin first-pass extraction was two- thirds lower in African-Americans. In contrast, extrahepatic insulin clearance was similar between the two ethnic groups.
Smith et al. (79)	Cross-sectional	African-American women (n=34)	European-American women (n=83)	F	African-American women: 39 ±1.9 years European-American women: 44 ±0.9 years	OGTT IVGTT	Non-diabetic	-AIRg was higher in African-American women, and remained higher after adjustment for age and body fat.

Abbreviations: AIRg, Acute insulin response to glucose; F, Female; IVGTT, Intravenous glucose tolerance test; M, Male; MMTT, Mixed meal tolerance tests; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance tests.

Continuation of Table 1.3

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Chung et al. (18)	Cross-sectional	Black African women (n=71) Pre-menopausal (n=52) Post-menopausal (n=19)	White European women (=48) Pre-menopausal (n=35) Post-menopausal (n=13)	F	Black women: 42.3 ± 9 years Pre-menopausal: 39 ± 7 years Post-menopausal: 53 ± 4 years White European women: 43.3±2 years Pre-menopausal: 38 ± 7 years Post-menopausal: 57± 4 years	OGTT IVGTT MMTT	NGT and IGT	-Black women had greater insulin concentrations at 20 to 40 minutes vs white women during the MMTT, regardless of the menopausal status. -Insulin response was greater in black African women than in white European women in all three tests. -DI was greater in black African women vs white European women, in particular in black African pre-menopausal women during the IVGTT. -No ethnic difference in basal and total β-cell insulin secretion rates during the MMTT (Results not reported for the OGTT and IVGTT). - Beta-cell responsivity to changes in glucose concentration during the MMTT was greater in black African women (Results not reported for the OGTT and IVGTT). -Lower basal and total hepatic insulin clearance in black African women during the IVGTT and MMTT (Results not reported for the OGTT).

Abbreviations: DI, Disposition Index; F, Female; IGT, Impaired glucose tolerance; IGT, Insulinogenic index: IVGTT, Intravenous glucose tolerance test; MMTT, Mixed meal tolerance tests; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance tests.

Continuation of Table 1.3

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Goedecke et al. (3)	Cross-sectional	Black SA women (n=29) 13 NW and 16 OB	White SA (n=28) 14 NW and 14 OB)	F	Black SA women NW: 24 ± 2 years OB: 28 ± 1 years White SA women NW: 26 ± 2 years OB: 31 ±2 years	OGTT IVGTT	Non-diabetic	OGTT -Fasting serum insulin levels were higher in black vs white SA women -IGI was higher in black SA women vs white women. -No differences in DI between the groups. IVGTT -Insulin levels greater in black vs white SA women after glucose injection. -Matched by BMI and body fat, AIRg was higher in black than white SA women and remained higher in black even after adjusting for VAT. -DI was similar between the two ethnic groups.
Shires et al (114)	Cross-sectional	OB black SA women (n=10)	OB black SA white SA women (n=8)	F	OB black SA black SA women: 39.4 ± 1.7 years OB black SA white SA women: 34.0 ± 2.7 years	OGTT followed by intravenous tolbutamide and glucagon stimulation	Non-diabetic	-Serum insulin and C-peptide levels were significantly lower in OB black SA women at basal state and at 30 mins and 60 min after OGTT and tolbutamide and-glucagon administration. -Hepatic insulin clearance was similar between the ethnic groups at basal and postprandial states.

Abbreviations: AIRg, Acute insulin response to glucose; BMI, Body mass index: DI, Disposition Index; F, Female; IVGTT, Intravenous glucose tolerance test; MMTT, Mixed meal tolerance tests; NW, Normal weight; OB, Obese; OGTT, Oral glucose tolerance tests; SA, South Africa; VAT, Visceral adipose tissue: WAB, Without abdominal obesity.

Continuation of Table 1.3

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Reimann et al. (112)	Cross-sectional	Black SA women (n-65, AB) Black SA women (n-=21, WAB)	White SA women (n-=56, AB) White SA women (n-=34, WAB)	F	Black SA women AB: 27 (23,30) years WAB: 32(28, 35) years White SA women AB: 25 (23, 30) years WAB: 29 (23, 33) years	OGTT	Non-diabetic	-No ethnic differences in basal C-peptide levels between women with AB. -However, postprandial C-peptide levels were lower in black SA women with AB than white women with AB. - Postprandial insulin clearance was lower black SA women with AB vs white women with AB. -Fasting C-peptide were significantly higher in black women WAB compared to their white counterparts. -Basal insulin clearance was higher in black SA WAB vs white counterparts.
Punyadeera et al. (113)	Cross-sectional	Black SA (n=17) (lean and obese)	White SA (n=17) (lean and obese)	F	Black SA women Lean: 31.9 ± 3.0 years OB: 42.5 ± 2.6 years White SA women Lean: 30.5 ± 1.6 years OB: 42.0 ± 2.5 years	MMTT	NGT	- Insulin level at 30 minutes was higher in OB black women compared to OB white women. -No difference in total insulin AUC between the two ethnic groups.

Abbreviations: AB, Abdominal obesity; AUC, Area under the curve; F, Female; IGT, Impaired glucose tolerance; MMTT, Mixed meal tolerance tests; NGT, Normal glucose tolerance; NW, Normal weight; OB, Obese; OGTT, Oral glucose tolerance tests; SA, South Africa; T2D, Type 2 diabetes; WAB, Without abdominal obesity.

Continuation of Table 1.3

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Van der Merwe et al. (4)	Cross-sectional	Black SA women (n=10, OB)	White SA women (n=10, OB)	F	Black obese SA women: 34.8 ± 0.8 years White obese SA women: 34.3 ± 0.8	HEC	Non-diabetic	-Lower fasting insulin levels in OB black SA women compared to their white counterparts. -No ethnic differences in mean insulin levels between the groups. -C-peptide concentrations were lower in OB black women vs white women.
Haffner et al. (19)	Prospective	African-American (n=288) (From Los Angeles and Oakland)	European-American (n=229) (From Los Angeles and Oakland)	Both M and F	African American Los Angeles: 54.2 ± 0.7 years Oakland: 54.6 ± 0.7 years European-American Los Angeles: 54.7 ± 0.8 years Oakland: 56.1 ± 0.73 years	OGTT on the first day. IVGTT (on the second day)	NGT and IGT	-African-Americans had higher fasting insulin levels, 2-h insulin concentrations and AIR than European-American, even after adjusting for age, BMI, body fat distribution, and behavioural factors.
Szczepaniak et al. (42)	Cross-sectional	African-American (n=20)	European-American (n=30)	Both F and M	African-American: 37 ± 3 years European-American: 43 ± 2 years	IVGTT	Non-diabetic	-AIRg and DI were higher in African-Americans vs European-Americans.

Abbreviations: AIRg, Acute insulin response to glucose; BMI, Body mass index: DI, Disposition Index; F, Female; HEC, Hyperinsulinaemic-euglycaemic clamp; IGT, Impaired glucose tolerance; IVGTT, Intravenous glucose tolerance test; M, Male; NGT, Normal glucose tolerance; OB, Obese; OGTT, Oral glucose tolerance tests; SA, South Africa.

Continuation of Table 1.3

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Osei et al. (7)	Cross-sectional	African-American (n=32)	European-American (n=30)	Both F and M	African-American F: 27.8 ± 3.3 years M: 32.4 ± 1.8 years European-American F: 27.9 ± 1.9 years M: 25.4 ± 1.6 years	OGTT IVGTT	Non-diabetic	OGTT -Fasting serum insulin levels and mean insulin response (at 30, 60, 120 and 180 mins) were significantly higher in African-American than in European-Americans. -Fasting C-peptide and AUC C-peptide were not different between the two ethnic groups. -The mean basal hepatic insulin extraction was 33% lower in the African-American than in European-Americans. IVGTT -Stimulated insulin levels were significantly greater (2-3 fold) in African-American compared to European-American. -Post-stimulation C-peptide levels were similar between the two ethnic groups. -Postprandial hepatic insulin clearance was lower in African-America vs European-American.

Abbreviations: AUC, Area under the curve; IVGTT, Intravenous glucose tolerance test; M, Male; OGTT, Oral glucose tolerance tests.

Continuation of Table 1.3

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Fosam et al. (61)	Cross-sectional	African-American (n=36)	European-American (n=47)	Both F and M	African-American: 39 ± 11 years European-American: 39 ± 13 years	MMTT	Non-diabetic	-Higher fasting and mean postprandial insulin levels in African-American vs European-American. -Fasting and mean postprandial C-peptide levels were similar between African-American and European-American. -Fasting and total insulin secretion rates were similar between the groups. But, early insulin secretion was significantly higher in African-Americans. - Beta-cell glucose sensitivity was also higher in African-Americans -Insulin clearance in the basal and postprandial states was lower in African-American -Postprandial insulin clearance remained lower in African-Americans even after adjusting for BMI and insulin sensitivity.

Abbreviations: BMI, Body mass index; F, Female; M, Male; MMTT, Mixed meal tolerance tests.

Continuation of Table 1.3

Reference	Study design	Study population (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Blood Tests	Glucose tolerance	Findings
Velasquez-Mieyer et al. (106)	Cross-sectional	African-American (n=16) (severely obese)	European-American (n=26) (severely obese)	Not specified	African-American: 36 ± 2 years European-American: 38 ± 2 years	OGTT	NGT and IGT	-With similar whole-body insulin sensitivity, African-American had significantly higher insulin response and AUC insulin at 30 mins compared to European-American. -Postprandial insulin clearance was lower in African-American vs European-American.
Goff et al. (6)	Cross-sectional	UK Afro-Caribbean (n=35)	UK white European (n=417)	Both F and M	UK Afro-Caribbean F:42.6 ±7 years M: 44.9 ±9.7 years UK white European F: 51.8 ± 10.3 years M: 52.8 ± 10.6 years	IVGTT	Non-diabetic	When adjusted for age and BMI, AIRg was higher in UK Afro-Caribbean compared to the UK white European participants.

Abbreviations: AIRg, Acute insulin response to glucose; AUC, Area under the curve; F, Female; IGT, Impaired glucose tolerance; IGT, Insulinogenic index; IVGTT, Intravenous glucose tolerance test; M, Male; MMTT, Mixed meal tolerance tests; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance tests; UK, United Kingdom.

The exact reasons for the discrepancies in the contribution of insulin secretion and clearance to hyperinsulinemia in black Africans are unknown. It is postulated that they may be due to differences in age, level of IR, glucose tolerance, adiposity status and ectopic fat deposition, as well differences in genetic and lifestyle factors between the study populations. Also, the inconsistencies between the studies might also be due different methods or techniques applied by the studies. These factors, which are closely interlinked, have been implicated in hyperinsulinaemia via their interaction with insulin secretion and clearance (5,9,19,41,42,56–58,61,115). To elaborate, insulin clearance has been shown to be a major contributor to hyperinsulinaemia in majority of the studies in black Africans including studies conducted in children (7,8,18,53,54,61,106,116–118). In contrast, the contribution of beta-cell insulin secretion to hyperinsulinaemia varies between studies, in particular amongst black African adults (7,18,53,61). This suggests that the contribution of insulin secretion to hyperinsulinaemia may be related to factors such as age and/or age-related factors including increase in adiposity as well as lifestyle factors (e.g., unhealthy diet or physical inactivity). One of the few studies to explore sex differences in insulin sensitivity and insulin response in black SA women and men reported that black SA women with NGT had lower insulin sensitivity compared to black men, which was associated with increased beta-cell function in order to maintain normoglycaemia (9). Interestingly, with advancing age, insulin sensitivity was unaffected, while beta-cell function declined similarly in both black SA men and women.

In addition to the effects of age, the discrepancies in the contribution of insulin secretion and clearance to hyperinsulinaemia in black Africans might also be attributed to glucose tolerance status. A study in African-American pre-menopausal women with NGT reported that both insulin secretion and clearance were significant contributors to hyperinsulinaemia (53). In contrast, insulin clearance was the only contributor to hyperinsulinaemia in a study that combined NGT and IGT black African women (18), which also corroborates some of the findings reported in studies including other black African adults from USA and/or West Africa, that included participants with both NGT and IGT (or was classified as non-diabetic) (7,18,53,61). It is also important to note that the women tested by Piccinini et al. (53) were overweight and younger (e.g., African-American, (mean (standard deviation, SD) 25(4) years of age), while those tested by

Chung et al. (18) were mostly obese and older (e.g., black African women, 42.3 ± 9 years of age) (Table 1.3).

Moreover, it is also important to highlight that the contribution of insulin secretion and clearance to hyperinsulinemia might also vary according to adiposity status. Polonsky et al. (56) showed that insulin secretion was a major contributor to hyperinsulinemia in non-diabetic subjects with normal weight and obesity. Although, insulin clearance was not significantly different between the two groups, it appeared to be lower in obese individuals (56). It is postulated that the lack of significant difference in insulin clearance between two groups might be attributed to the fact that total adiposity might be a weaker correlate of insulin clearance than measures such as VAT and liver fat accumulation. Accordingly, liver fat accumulation is often inversely associated with hepatic insulin clearance, and hepatic, muscle and adipose IR (41,43,110). However, it is important to note that these associations have been inconsistent in both black Africans and white Europeans (41,43,87,103,110), as discussed in section 1.8.2. For these reasons, the pathophysiology of hyperinsulinaemia, especially in black Africans requires further exploration.

It is also proposed that insulin secretion and clearance may also vary according to the degree of IR (e.g., high and intermediate insulin sensitivity and IR). Accordingly, two multi-ethnic studies revealed that insulin secretion was higher in obese individuals, but a significantly lower insulin clearance in obesity was limited to those with impaired insulin sensitivity (i.e., intermediate) or IR (57,58). Jung et al. (58) showed that obese individuals with either intermediate insulin sensitivity or IR adapt to changes in insulin sensitivity by reducing insulin clearance before any significant changes to insulin secretion (i.e., “additional” increase). This was based on the findings that obese individuals with intermediate insulin sensitivity had lower insulin clearance but similar insulin secretion compared to the group with high insulin sensitivity (58). Interestingly, those with IR had both decreased insulin clearance and increased insulin secretion (i.e., assuming a more advanced state of insulin secretion than that typically found only in obesity). With these findings, one might postulate that the reduction in insulin clearance

during obese-insulin resistant states might serve as a protective mechanism to “rest or preserve” and even delay beta-cell failure, and the subsequent onset of T2D.

Although insulin sensitivity or IR have been linked to hyperinsulinaemia via increased insulin secretion and reduced insulin clearance (3,5,9,18,56–58), it is important to note that hyperinsulinaemia may not merely be a compensatory response to impaired insulin sensitivity or IR or even obesity. This is based on the findings that black Africans remain hyperinsulinaemic even after adjusting for insulin sensitivity (5,55,118) including total and central adiposity (3,6,8,19,116–118). This suggests that IR and obesity may not be the initial drivers of hyperinsulinaemia in black Africans, rather hyperinsulinaemia may cause IR, in particular in those with obesity. In line with this, it has been proposed that hyperinsulinaemia might be a primary defect that causes obesity or IR (29,32,119,120) as presented in Figure 1.3. Others have also proposed that lifestyle (i.e., dietary intake) and genetic factors might be initial drivers for the development of hyperinsulinaemia and its involvement in T2D, more especially in black Africans (31,64,80). In line with this, a high fat-to-carbohydrate ratio (117) or carbohydrate intake (64) have been linked to hyperinsulinaemia via its association with hepatic insulin clearance and hepatic IR, with these associations being stronger in African-American than in European-American children (117). It is postulated that a high carbohydrate diet enhances hepatic de novo lipogenesis, which in turn increases liver fat accumulation, which subsequently impairs hepatic insulin sensitivity and reduces hepatic insulin clearance, resulting in hyperinsulinaemia (64). Furthermore, others have proposed that hyperinsulinaemia in black Africans might be genetically determined (80), in particular by genes regulating hepatic insulin clearance such as insulin degrading enzyme (IDE) and carcinoembryonic antigen–related cell adhesion molecule 1 (CEACAM1) (59–61). However, genetic studies investigating hepatic insulin clearance in relation to hyperinsulinaemia are very scarce in black Africans. A recent study examining IDE and CEACAM1 in age, sex and BMI matched African-American and European-American cadavers, revealed similar IDE and CEACAM1 protein levels between the two ethnic groups (61). However, IDE activity was lower in African-American cadavers compared to European-American cadavers (61). Although genetic studies are limited in the black Africans, several studies in non-African populations have also implicated IDE and CEACAM1 in metabolic disorders that

include obesity, fatty liver disease and T2D (59,60). Collectively, these studies suggest that the contribution of hepatic insulin clearance to the development of hyperinsulinaemia and its role in metabolic disorders such as T2D might be linked to genetic predisposition, in particular in black Africans. Since hyperinsulinaemia is a complex and multifactorial condition, it is difficult to make a strong hypothesis based on the contribution of lifestyle and genetic factors, without taking into account the effects of other risk factors such as obesity and IR, which are also closely linked to lifestyle and genetic factors. It is also important to highlight that explorations of these associations are also limited in the majority of studies that are currently available, in particular studies in black Africans from Africa.

Furthermore, studies investigating the contribution of both insulin secretion and clearance to hyperinsulinaemia in black African adults, in particular black African women are very scarce. There are only two studies (112,114) that have examined both insulin secretion and clearance in black SA women. These studies have reported contradictory findings to those reported above in which insulin levels and/or C-peptide levels were lower in black SA women compared to white SA women (112,114). It is important to note that one study was conducted in a small sample more than three decades ago, and thus might not reflect the metabolic status of black SA women today (114). Indeed, changes in urbanisation and its effect on lifestyle and environmental factors over the past three decades have been implicated in non-communicable diseases (NCDs) in black SA women (11–16,89,121,122). With only two studies with conflicting results, the pathophysiology of hyperinsulinaemia is still poorly understood in black SA women. Therefore, the relative contribution of insulin secretion and clearance to hyperinsulinaemia, and their associations with obesity and IR, major prominent features of T2D in black SA women requires investigation.

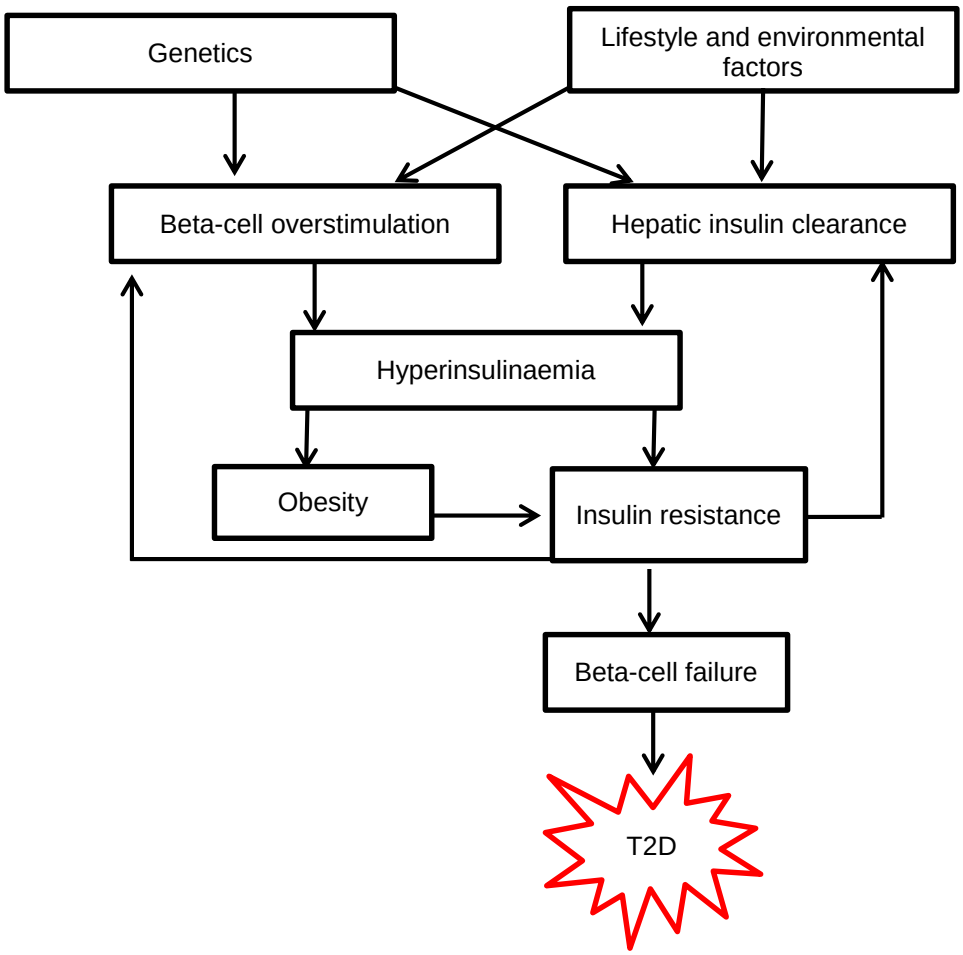


Figure 1.3: Alternative model for the development of T2D (As proposed by several authors (29,31,32,64,119,120)).

1.8. Determinants of insulin sensitivity/IR and hyperinsulinaemia: focus on black SA women

Although the exact causes of T2D in black SA women are not known, several risk factors have been described. These include non-modifiable risk factors such as age, genetic susceptibility, ethnicity and sex. Moreover, urbanisation, which is often accompanied by unhealthy diets, physical inactivity and increased sedentary behaviours have also been linked to the development of obesity and T2D in black SA women (11–16,89,121,122). Socio-demographic (e.g., SES) and socio-cultural factors have also been implicated in T2D pathophysiology through their effect on lifestyle factors (i.e., diet

and physical activity), eating behaviours and body perceptions (11–16,89,121,122). For a more detailed description of the risk factors implicated in the development of T2D in African women, in particular black SA women, see previous reviews (121,122).

1.8.1. Body fat and fat distribution

The alarming increase in the prevalence of NCDs, in particular in developing countries such as SA, have been largely attributed to the rapid increase in obesity (1,10,89,111,123). Accordingly, 87% of T2D cases in SA adults were linked to excess body weight (89). Furthermore, SA women were disproportionately more affected than men by excess weight-associated morbidity and mortality related to NCDs (89). Thus, it is not surprising that black SA women who have the highest levels of obesity (40.9%) compared to white women (30.6%) and their male counterparts (8.7%) (111), have the greatest risk and prevalence of T2D (9,10).

Although, obesity is closely associated with T2D risk, it is important to note that it is the accumulation of central obesity, in particular VAT or intra- abdominal adiposity (IAAT), rather than total obesity or any proxy measures of adiposity such as BMI, waist circumference (WC) or waist-to-hip ratio (WHR) that determines the risk for IR and T2D (17,21,40,44,99,105,124–126). This might be partly due to the fact that VAT releases its lipid intermediates and pro-inflammatory cytokines into the hepatic portal circulation, which in turn increase liver fat accumulation and interferes with the metabolic functions of the liver including the ability of the liver to respond to insulin (84,90,96,99) (Figure 1.2). In contrast, peripheral (gluteo-femoral) SAT has greater capacity to expand and store excess FFAs than VAT (39,90,96), which in turn reduces the risk of fat accumulation in the visceral depot and other ectopic sites such as the liver. This partly explains why peripheral adiposity is associated with high insulin sensitivity and reduced T2D risk (97,98,124,127).

High precision methods of measuring adiposity such as the computed tomography (CT) and magnetic resonance imaging (MRI) are considered gold standards for the assessment of VAT (128–130). However, these techniques have disadvantages, which

include the high doses of radiation exposure in CT scanning. Furthermore, both techniques are time consuming and expensive, which restricts their use in routine research and clinical setting, more especially in under-resourced settings. For these reasons, dual-energy X-ray absorptiometry (DXA) have been proposed as an alternative technique for the assessment of body fat and its distribution, including VAT (128–130). Most importantly, DXA is relatively cheap and the radiation exposure associated with a DXA is very low (equivalent to about 1 day of natural background radiation) (128). Moreover, DXA-derived VAT has been shown to correlate with VAT estimated using the MRI and CT (128–130). However, it is important to highlight that DXA has also been reported to be unreliable in estimating VAT at extremely low and high levels compared to the MRI and CT methods (129,130). Despite its limitations, DXA has been shown to accurately measure VAT as well as CT, among obese participants, which makes it a suitable technique for individuals with high prevalence of obesity such as the black SA women (111,129).

Despite black Africans being at higher risk for IR and T2D, the majority of studies from SA and USA have shown that black Africans have lower VAT compared to their white European counterparts (3,5,18,20,21,42,131) (Table 1.4). Indeed, several studies conducted in pre-menopausal women show that for the same level of body fat, black African pre-menopausal women from SA and USA accumulate less VAT compared to white European counterparts (3,4,127,5,18,21,22,44,103,105,113) (Table 1.4). Moreover, with increasing WC black African women also accumulate less VAT than white European women (22). Notably, Chandler-Laney et al. (5) showed that the increase in IAAT in African-American girls and women was smaller between the pre-pubertal and pre-menopausal stages but greater between pre- and post-menopausal stages when compared to white European-America women. This suggests that ageing or age-related factors such as the menopause-transition might be prominent factors driving VAT accumulation in black African women.

Accordingly, the transition from pre-menopause to post-menopause is characterised by a significant decrease in sex hormones, mainly oestrogen, which is associated with a redistribution of fat from the peripheral region to the central region, with a significant

increase in VAT (44,105,132,133). Therefore, it is postulated that the significant differences in VAT between the black African and white European pre-menopausal women are driven by the smaller increase in VAT in black African women (and girls) between the pre-pubertal and pre-menopausal stages (5). In contrast, if black African women accumulate more VAT than white European women between the pre- and post-menopausal stages, the odds of observing significant differences in VAT between the two ethnic groups, in the post-menopausal stage are lower. To elaborate, while there were significant differences in VAT between black African and white European women in the entire cohort (18), further analyses by menopausal status revealed that VAT was not significantly different between black African and white European post-menopausal women (18). Rather, the ethnic differences in VAT were due to a significantly lower VAT in black African pre-menopausal women (18). Similarly, Lovejoy et al. (125) did not report significant differences in VAT between middle-aged African-American and European-American women. Notably, these two studies (18,125) contradict those reported by other authors in post-menopausal women, where black African post-menopausal women had significantly lower VAT than their white European counterparts (20,44,105). The exact reasons for these disparities are unknown. Nonetheless, it is postulated that the disparities in VAT between black African and white European post-menopausal women (18,20,44,105,125) might be due to differences in age, adiposity, age-related increase in adiposity, menopause status, sex hormones, intrinsic differences in adipose tissue metabolism, as well as lifestyle and behavioural factors. Notably, these risk factors have been associated with increased VAT accumulation (44,105,127,132,134,135).

Fewer studies comparing VAT between black African and white European women have been conducted in post-menopausal women (18,44,105,125) compared to pre-menopausal women (3–5,18,21,22,103,113,127) (Table 1.4). Further, the majority of studies in black African women from SA and USA are cross-sectional and are mostly conducted in pre-menopausal women, and yet those in post-menopausal stage, whom are at highest risk for T2D are understudied. Despite these inconsistencies in VAT accumulation between black African and white European post-menopausal women, VAT accumulation has been closely associated with unfavourable lipid profiles, insulin levels

and IR, irrespective of ethnicity (21,44,105) (Table 1.4). Accordingly, DXA-derived measures of central (i.e., trunk and VAT) and peripheral adiposity were positively and inversely associated with IR in both black and white SA pre-menopausal women, respectively (21). However, the association between VAT and IR was stronger in black than in white SA women (21). These findings are in accordance to those reported by Chung et al. (103), in which VAT was strongly associated with hepatic insulin sensitivity in black African women compared to their white European counterparts (Table 1.4). Furthermore, a study from the Netherlands showed that VAT accumulation was a predictor of future T2D risk in white Europeans, with the association being stronger in women (109). Moreover, a 7-year prospective multi-ethnic cohort study of obese participants from the USA reported that VAT was independently associated with incident prediabetes and T2D (108). With the dearth of studies using precise and accurate measures of body fat and its distribution such as DXA, it remains unclear if VAT and peripheral adiposity or other measures of body fat and fat distribution are determinants of T2D in black Africans, in particular from Africa. Therefore, more studies, in particular longitudinal study designs, are needed to examine the effects body fat and fat distribution have on the development of T2D in black African women, more especially in black African post-menopausal women.

As the menopause-transition is associated with a shift in adipose tissue, characterised by a reduction in peripheral adiposity and an increase in central adiposity, it is important to consider the relationship between these two adipose tissue depots. While central adiposity, in particular VAT is associated with increased T2D risk, peripheral adiposity is associated with reduced T2D risk (97,98,127). Compared to white women, black SA women have greater peripheral (gluteal-femoral) fat accumulation and gluteal SAT adipogenic and lipogenic gene expression (17,87,98,136), which is associated with greater insulin sensitivity (30,98,127). However, with obesity or excessive fat accumulation, black SA women have significantly reduced expression of these genes in the gluteal depot (136), which might increase the risk of VAT accumulation, and subsequent development of IR and T2D. Accordingly, in the only longitudinal study to examine the role of body fat and fat distribution in the development of T2D in SA women, a significant increase in fat mass (FM) (approximately 4.3 kg) over a 5.5 year

period was linked to a relative decrease in peripheral FM and an increase in central FM in black SA pre-menopausal women (137). Furthermore, baseline trunk: leg ratio was associated with the reduction in insulin sensitivity at follow-up (137). Therefore, in addition to the effects of menopause, it is also postulated that the reduction in adipogenic and lipogenic gene expression in the gluteal depot as a consequence of obesity might predispose middle-aged black women to increased central obesity (i.e., VAT), and subsequently increased T2D risk. Thus, more studies are needed to examine if increases in body fat and fat distribution over time are associated with future T2D risk in black SA post-menopausal women, whom have the highest prevalence of obesity, greater T2D risk and prevalence, and yet remain the most understudied group.

Table 1.4: Ethnic comparison of visceral adipose tissue between black African and white European individuals

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Fat distribution test	Findings
Chandler-Laney et al. (5)	Cross-sectional	African-American (n=76) 7–12 years (n=33) 18–32 years (n=25) 40–70 years (n=18)	European-American (n=92) 7–12 years (n=29) 18–32 years (n=32) 40–70 years (n=31)	F	African- American Pre-pubertal: 10.3 ± 1.5 years Pre-menopausal: 25.9 ± 3.4 years Post-menopausal: 55.7 ± 4.2 years European American Pre-pubertal: 10.3 ± 1.5 years Pre-menopausal: 25.9 ± 3.4 years Post-menopausal: 55.7 ± 4.2 years	CT scan	IAAT was lower in African-American women than in European American women.
Chung et al. (18)	Cross-sectional	Black African women (n=71) Pre-menopausal (n=52) Post-menopausal (n=19)	White European women (=48) Pre-menopausal (n=35) Post-menopausal (n=13)	F	Black African women: 42.3 ± 9 years Pre-menopausal: 39 ± 7 years Post-menopausal: 53 ± 4 years White European women: 43.3±2 years Pre-menopausal: 38 ± 7 years Post-menopausal: 57± 4 years	MRI	VAT was lower in black than in white women. The ethnic difference in VAT was largely due to a significantly lower VAT in black African pre-menopausal women compared to white European pre-menopausal women.

Abbreviations: CT, Computed tomography, scan; F, Female; HDL-C, High-density lipoprotein; IAAT Intra-abdominal adipose tissue; MRI, Magnetic resonance image; VAT, Visceral adipose tissue.

Continuation of Table 1.4

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Fat distribution test	Findings
Chung et al. (103)	Cross-sectional	Black African pre-menopausal women (n=24)	White European pre-menopausal women (n=22)	F	Black African pre-menopausal women: 37 ± 1 years White European pre-menopausal women: 37 ± 1 years	^{1}H-MRS	Black African women had less VAT compared to white European women. VAT was correlated with hepatic insulin sensitivity in both groups. However, the correlation was stronger in black African women (r= -0.77, p<0.01 vs r=-0.67, p=0.02).
Lovejoy et al. (125)	Cross-sectional	African-American (n=55)	European-American (n=103)	F	African-American: 46.7± 0.24 years White American (47.7 ± 0.21)	DXA	VAT was not significantly different between the two ethnic groups, but tended to be lower in middle-aged African-American women. In both ethnicities, VAT was strongly associated with fasting glucose and insulin, as well as serum lipids, independent of body fat.

Abbreviations: DXA, Dual-energy X-ray absorptiometry; F, Female; NW, ^{1}H-MRS, Proton magnetic resonance spectroscopy; VAT, Visceral adipose tissue.

Continuation of Table 1.4

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Fat distribution test	Findings
Kanaley et al. (105)	Cross-sectional	Black African post-menopausal women (n=21)	White European post-menopausal women (n=33)	F	Black women: 52.3 ± 1.0 years White women: 54.2 ± 1.6 years	MRI	VAT and %VAT were lower in African-American post-menopausal women compared to their white European counterparts. Irrespective of ethnicity, VAT was associated with insulin, IR and lipid profiles.
Demerath et al. (44)	Cross-sectional	African-American (n=111)	European-American (n=617)	F	African-American: 46.3 ± 14.9 years European-American: 48.1 ± 15.5 years	MRI	% VAT was lower in African-American women than in European-American women. %VAT were associated with age, with this association being stronger in European-American women (interaction of ethnicity by age, p=0.03).
Goedecke et al. (3)	Cross-sectional	Black SA women (n=29) 13 NW and 16 OB	White SA (n=28) 14 NW and 14 OB)	F	Black SA women NW: 24 ± 2 years OB: 28 ± 1 years White SA women NW: 26 ± 2 years OB: 31 ±2 years	DXA	Matched for BMI and body fat, OB black SA women had lower VAT than OB white SA women.
Van der Merwe et al. (4)	Cross-sectional	Black SA women (n=10, OB)	White SA women (n=10, OB)	F	Black obese SA women: 34.8 ± 0.8 years White obese SA women: 34.3 ± 0.8)	CT scan	VAT was lower in black SA women than in white SA women.

Abbreviations: DXA, Dual-energy X-ray absorptiometry; F, Female; MRI, Magnetic resonance image; NW, Normal weight; OB, Obese; SA, South Africa; VAT, Visceral adipose tissue.

Continuation of Table 1.4

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Fat distribution test	Findings
Sumner et al. (22)	Cross-sectional	Black women (n=1261) African-American (n=607) Black SA (n=186) West African (445) Black African living in the states (n=23)	White SA women (n=148)	F	18-50 years	CT scan	Black women had lower VAT vs white women. With increasing WC black African women had lower increase in VAT compared to white European women.
Keswell et al. (21)	Cross-sectional	Black SA (n=288)	White SA (n=197)	F	Black SA women: 22 (22–33) years White SA women: 32 (24–39] years	DXA	VAT was lower in black SA women compared to white SA women. VAT was associated with insulin and IR in both groups, but the association between VAT and IR was stronger in black SA women ($p < 0.01$ vs $p < 0.05$ in white SA women). In white SA women only, a higher VAT was associated with lipid profiles (i.e., TG, HDL-C and LDL-C).
Punyadeera et al. (113)	Cross-sectional	Black SA (n=17) 8 (lean) and 9 (OB)	White SA (n=17) 8 (lean) and 9 (OB)	F	Black SA women Lean: 31.9 ± 3.0 years OB: 42.5 ± 2.6 years White SA women Lean: 30.5 ± 1.6 years OB: 42.0 ± 2.5 years	CT scan	VAT was lower in obese black SA women vs obese white SA women.

Abbreviations: CT, Computed tomography, scan; F, Female; OB, Obese; SA, South Africa; VAT, Visceral adipose tissue; WC, Waist circumference.

Continuation of Table 1.4

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Fat distribution test	Findings
Szczepaniak et al. (42)	Cross-sectional	African-American (n=20)	European-American (n=30)	Both F and M	African-American: 37±3 years European-American: 43 ±2 years	MRI	Lower VAT in black vs white populations.
Guerrero et al. (20)	Cross-sectional	African-American (n=1058)	European-American (n=719)	Both F and M	African-American: F: 46 (37-54) years M: 45 (38-54) years European-American: F: 48 (38-55) years M: 44 (38-52) years	^{1}H-MRS	African-American had lower VAT than European-American, which remained lower after adjustment for total adiposity. No ethnic difference in VAT between African-American women and European-American women. African-American men had lower VAT than European-American men.

Abbreviations: F, Female; HDL-C, High-density lipoprotein; M, Male; MRI, Magnetic resonance image; ^{1}H-MRS, Proton magnetic resonance spectroscopy; VAT, Visceral adipose tissue.

1.8.2. Ectopic fat deposition: liver fat accumulation and NAFLD prevalence

Ectopic fat accumulation in non-adipose tissues such as the liver, muscle and pancreas is another prominent feature purported to underlie the development of T2D. Non-alcoholic fatty liver disease (NAFLD) and high liver fat (also known as hepatic fat or intrahepatic lipid content, IHL) are associated with adverse metabolic outcomes such as reduced hepatic insulin clearance, and hepatic, muscle and adipose IR (43,83,110,138,139). In contrast, low liver fat is associated with favourable outcomes such as improved insulin sensitivity (87,103). Compared to populations of European descent, the prevalence of NAFLD is significantly lower in black Africans (41) (Table 1.5). Similar to VAT and NAFLD prevalence, the majority of studies assessing liver fat between black African and white European individuals from USA, SA and UK show that black Africans have lower liver fat compared to their white European counterparts (18,41,87,103). However, not all the studies reported significant differences between the two ethnic groups (18,87). Liver fat tended to be lower in black SA pre-menopausal women but was not significantly different to that of white SA women (87). However, in black SA women only, it was associated with greater hepatic insulin sensitivity, characterised by enhanced suppression of EGP (87). Similarly, black African pre-menopausal women from a study conducted in USA were shown to have significantly lower liver fat and rates of gluconeogenesis than white European women (103). Furthermore, it was reported that at any given level of whole-body insulin sensitivity, black African pre-menopausal women had greater hepatic insulin sensitivity than white European pre-menopausal women (103). These findings suggest that black African pre-menopausal women may be more sensitive to the effects of liver fat accumulation on hepatic insulin sensitivity than white European women (87,103). However, longitudinal studies are needed to confirm these associations.

While liver fat was negatively correlated with the suppression of EGP in black SA women only (87), in a study conducted in USA, liver fat was negatively correlated with hepatic insulin sensitivity in both black African and white European pre-menopausal women (103). These findings corroborate those of another USA study reported by Trico et al. (41) in a multi-ethnic cohort of adolescents. In addition to the discrepancies in the

relationship between liver fat accumulation and insulin sensitivity, inconsistent findings have also been reported in the association of liver fat accumulation with insulin clearance. For example, a significant correlation between liver fat and hepatic insulin clearance was reported in white European women, but not in black African women (18). findings are also in accordance with those reported by Utzschneider et al. (110), where liver fat was not correlated with hepatic insulin clearance in a multi-ethnic cohort study. Rather, Utzschneider et al. (110) reported that hepatic insulin clearance was strongly associated with muscle and adipose tissue insulin sensitivity.

Based on these findings, it is evident that the interaction between liver fat and insulin sensitivity as well as insulin clearance is a complex phenomenon that is not fully understood. Since the discrepancies in the association between liver fat and markers of T2D risk are inconsistent across ethnicities, it is postulated that ethnicity may play a minor role in the detrimental effects of liver fat accumulation in T2D pathophysiology. Indeed, in the multi-ethnic study conducted in obese adolescents, liver fat correlated with hepatic insulin clearance and insulin sensitivity, even after adjusting for age, sex, ethnicity, adiposity, waist-hip ratio, pubertal status and plasma glucose levels (41). Therefore, factors such as lifestyle or behavioural and gene-environmental factors, through their effects on liver fat accumulation and liver fat-related processes such as lipolysis or circulating FFAs, might have a more prominent role in the involvement of liver fat accumulation in T2D pathophysiology. Therefore, there is an urgent need to elucidate the pathophysiology of T2D in black African women, in the context of liver fat accumulation and its association with markers of T2D risk.

Of great concern is that studies exploring liver fat accumulation in relation to T2D risk are very scarce in black Africans from Africa, particularly in SA women whom have the highest obesity and T2D prevalence as well as T2D risk (3,4,9,10,111). Indeed, the majority of the studies are conducted in white Europeans and/or African-Americans, whose lifestyle differs from those living in developing countries such as SA. The limited studies might also be due to the fact that precise and accurate methods for assessing liver fat such as MRI and ^{1}H-MRS are costly and not practical for large epidemiological studies, especially for under-resourced settings. Hence, indices such as the fatty liver

index (FLI) have been developed to estimate liver fat accumulation (140). The FLI has been validated against ^{1}H-MRS and has been shown to accurately identify those with and without fatty liver disease (141). Furthermore, FLI has also been shown to predict the development of NAFLD and other metabolic disorders in various samples of non-African individuals (142–144). The FLI is yet to be explored and validated in an African population. Studies investigating liver fat accumulation in relation to T2D risk, either using accurate methods or proxy indices are needed to elucidate the pathophysiology of T2D in black Africans, in particular middle-aged black SA women.

Table 1.5: Ethnic comparisons of liver fat accumulation and NAFLD prevalence between black African and white European participants

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Fat distribution test	Findings
Chung et al. (18)	Cross-sectional	Black African women (n=71) Pre-menopausal (n=52) Post-menopausal (n=19)	White European women (=48) Pre-menopausal (n=35) Post-menopausal (n=13)	F	Black women: 42.3 ± 9 years Pre-menopausal: 39 ± 7 years Post-menopausal: 53 ± 4 years White European women: 43.3±2 years Pre-menopausal: 38 ± 7 years Post-menopausal: 57± 4 years	$^{+}$H-MRS	In the entire cohort, liver fat was not statistically significant to white women, but tended to be lower in black African women. However, black African pre-menopausal had lower liver fat than white European counterparts. Liver fat was correlated with MMTT early insulin response, irrespective of ethnicity or menopausal status. Liver fat was significantly correlated basal insulin clearance in white European women only.
Chung et al. (103)	Cross-sectional	Black African pre-menopausal women (n=24)	White European pre-menopausal women (n=22)	F	Black African pre-menopausal women: 37 ± 1 years White European pre-menopausal women: 37 ± 1 years	$^{+}$H-MRS	Black African women had significantly lower liver fat than white European women. Regardless of ethnicity, liver fat was significantly correlated with hepatic insulin sensitivity. This association was weaker in black African women (r=-0.56, p=0.02 vs r=-70, p<0.001). Fractional and absolute gluconeogenesis was lower in black African women.

Reference	Study design	Ethnicity (n)	Comparison population (n)	Gender (M/F)	Mean age (range)	Fat distribution test	Findings
Goedecke et al. (87)	Cross-sectional	Black SA women (n=15 OB)	White SA (n=15 OB)	F	Black SA OB women: 36 ± 5 years White SA OB women: 36 ± 2 years	DXA	Liver fat tended to be lower in black SA women but not statistically significant to white SA women. In black SA women only, liver fat was associated with hepatic insulin sensitivity (% suppression of EGP, r=-0.57, p<0.05).
Guerrero et al. (20)	Cross-sectional	African-American (n=1058)	European-American (n=719)	Both F and M	African-American: F: 46 (37-54) years M: 45 (38-54) years European-American: F: 48 (38-55) years M: 44 (38-52) years	^{1}H-MRS	African-American had lower liver fat than European-American, which remained lower after adjustment for total adiposity. Liver fat was similar between African-American women and European-American women. African-American men had lower liver fat compared to European-American men. NAFLD prevalence was lower in African-Americans compared to European-Americans.

Abbreviations: DXA, Dual-energy X-ray absorptiometry; EGP, endoegenous glucose production; F, Female; $^{+}$H-MRS, Proton magnetic resonance spectroscopy; M, Male; NAFLD, Non-alcoholic fatty liver disease; OB, Obese; SA, South Africa; VAT, Visceral adipose tissue; WC, Waist circumference.

1.8.3. Age, gender and genetic factors

It is well documented that the risk for developing T2D increases with age, regardless of ethnicity (1,10). Accordingly, the prevalence of T2D in urban black SA women increases with age, from 10.1% between 35-44 years to 24.3% between 55-64 years (10). However, it is important to consider that the increasing risk for T2D with advancing age might not be related to the effects of aging, rather due to age-related increase in obesity, in particular central obesity, and age-related decline in beta-cell function, sex hormones and in physical activity (5,9,44,105,132,133). For example, in a sample of black SA women and men it was reported that beta-cell function declines with increasing age, with this decline being similar in both women and men (9).

Studies investigating T2D pathophysiology between black African men and women are very scarce. However, the few studies that have explored sex differences in T2D risk have shown that black African men often present with favourable metabolic outcomes such as higher insulin sensitivity, lower insulin response and lower prevalence of obesity compared to black African women (6,9,10,111). However, with aging black SA men might be equally or at higher risk of developing T2D through a complex T2D pathophysiology that differs from that observed in black SA women. Indeed, black SA men show a similar age related decline in beta-cell function as black SA women (9). Furthermore, the association between body fat and centralization of body fat and insulin sensitivity is stronger in middle-aged black SA men than in women (Kufe et al., unpublished). Moreover, total adiposity is negatively associated with DI only in black SA men (Kufe et al., unpublished). This suggests that age-related increase in adiposity in black SA men may predispose them to early beta-cell failure and place them at higher risk of developing T2D compared to middle-aged black SA women. The T2D pathophysiology in black African women is well documented in comparison to black African men, and includes factors such as age-related decline in beta-cell function, decrease in sex hormones as well as physical activity, and age-related increases in adiposity(3,5,9,44,105,132,133). These factors might gradually lead to VAT and liver accumulation, and subsequently increase the risk for T2D (3,5,9,44,105,132,133).

Although beyond the scope of this thesis, it is important to consider that the increased T2D risk in black Africans might be genetically determined, in particular by genes regulating adipose tissue function and hepatic insulin clearance (59–61). In support of this, several genetic variants linked to favourable adipose function characterised by peripheral SAT expansion and storage of excess fat were associated with favourable metabolic profiles such as low liver fat and VAT/SAT ratio including reduced T2D risk in white Europeans (37,145,146). Although genetic studies linking adipose tissue dysfunction to T2D risk are limited in Africans, a study comparing the expression of adipogenic and lipogenic genes between obese black and white SA women, revealed that these genes were significantly lower in the gluteal depot of obese black SA women compared to obese white SA women (136). Furthermore, these genes were positively correlated with insulin sensitivity in obese black SA women, which is also in accordance with the results reported by Gower and Fowler (30). These findings suggest that black African women from SA and USA might be carriers of the favourable adipose tissue variants. However, with increasing fat accumulation these variants might be detrimentally affected, predisposing black African women to ectopic fat deposition, in particular increased liver fat accumulation and a subsequent increase for T2D risk. Future studies are required to test whether the positive association between peripheral adiposity and insulin sensitivity is genetically determined, and whether this genetic predisposition is linked to reduced T2D risk in black African women. Furthermore, the findings that hepatic insulin clearance is a major determinant of hyperinsulinaemia in black Africans suggests that hepatic insulin clearance might be a conserved genetic trait as previously suggested by other authors (31,80). Therefore, future studies are needed to investigate genes regulating hepatic insulin clearance such as IDE and CEACAM1 to add to the limited knowledge about the contribution of hepatic insulin clearance to hyperinsulinaemia and T2D prevalence in black Africans.

1.8.4. Socio-cultural, socio-demographic and lifestyle factors

1.8.4.1. Socio-cultural and demographic factors

Socio-demographic and socio-cultural factors have also be linked to T2D pathophysiology in black SA women through their effect on lifestyle behaviours and body perception (11–16,89,121,122). Black women are more likely to be overweight or obese and less physically active than men, whereas the prevalence of tobacco use and alcohol consumption is higher in men than women (11,111). Furthermore, in many black SA communities being overweight or obese is sometimes associated with signs of prosperity, good health, fertility and happiness (13,14,16). This might also explain why some individuals perceive leanness as a sign of being sick or suffering from diseases such as HIV, which is still stigmatized in many black communities in SA (13,14,16). These findings might also explain why black girls and women often show preference towards being overweight (13,14,16).

In addition to the contribution of socio-cultural factors, SES status is another important factor in the development of T2D, in particular in the black SA population. South Africa has a high burden of unemployment, with those disproportionally affected being the young population (18-35 years), in particular women (147). Thus, it is not surprising that in the recent poverty report (2014/2015) published by Statistics SA (147) it was reported that 49.2% of the 35.1 million adult population in 2015 (18 years and older) were living below the upper-bound poverty line. The upper-bound poverty line refers to the amount of money that an individual needs in order to meet required minimum daily energy intake plus the average amount calculated from non-food items (i.e., total amount is R992 per person per month) (147). Women experienced higher levels of poverty compared to their male counterparts, across all age groups (147). Poverty is intricately linked to food insecurity, thus might predispose black SA women to T2D risk through unhealthy diets or food behaviours. However, no study has investigated this association. In contrast, high SES in the black SA population has also been linked to increased obesity, T2D and CVD risk (12,13,15,148,149). Indeed, high SES, described as increased wealth (i.e., owning assets such as a motor vehicle or a bigger household) or a high level of education, is often linked to increased consumption of meat products, bigger portion sizes and more frequent intake of fast foods (12,13,15). Notably, consumption of unhealthy diets is more prevalent in urban areas than in rural areas. Accordingly, urban black SA women who followed a typical Western diet, characterised by high fat and

sugar intake, were more susceptible to developing T2D and cardiovascular diseases (CVD) than their rural counterparts who still followed a traditional diet; high intake of fibre, fruit, vegetables and low intake of red meat and processed meat (11,150–152). Interestingly, it was reported that both urban and rural groups experienced an inadequate intake of micro-nutrients such as fibre, elements (e.g., calcium and potassium) and vitamin C, which have been associated with reduced CVD risk (150). This suggests that consumption of a "healthier diet" (e.g., in this case, the traditional diet in the rural group) might not be sufficient to protect against disease risk if the diet lacks essential nutrients. These results also suggest that advanced dietary tools which capture the quantity of the entire diet, and not just single macro- and micronutrients might be beneficial in understanding the role of dietary intake in the pathophysiology of metabolic diseases such as T2D.

Recent studies have revealed that unhealthy diets may be involved in the pathogenesis of NCDs by inducing low-grade inflammation, while healthier diets characterised by low inflammatory profile such as the Mediterranean diet, which are high in fruits, vegetables and fish might protect against NCDs (153–156). Consequently, several dietary patterns or tools such as the dietary inflammatory index (DII®) have been established to examine the inflammatory profile of a diet in order to understand the role of diet in the development of metabolic disorders. Briefly, the DII is a newly-developed literature based tool that measures the inflammatory profile of the diet (156). For the detailed description of the DII, please see Chapter 4, The DII®.

The DII has been validated with several inflammatory markers, such as CRP and interleukins (157), and has also been used in a variety of populations, using different dietary assessment tools such as the 24-hour food recall (158), food diary (159) and Food Frequency Questionnaire (FFQ) (154,160–162). Furthermore, the DII has also been associated with markers of glycaemia, namely, fasting glucose, HbA1c, post-load glucose and IR in non-African populations (154,160–162). Further, it has been shown that individuals with high DII scores have high levels of obesity, in particular central obesity, and increased T2D risk, compared to those with low DII scores (154,161,163). With these findings, it is proposed that the consumption of an unhealthy, pro-

inflammatory diet might predispose black SA women, in particular middle-aged black SA women to obesity, in particular central obesity (i.e., increased VAT accumulation), which might increase the risk of IR and T2D. Black SA pre-menopausal women have been shown to have higher abdominal and gluteal SAT pro-inflammatory gene expression than white SA pre-menopausal women (98). However, the inverse association between SAT inflammatory gene expression profile and insulin sensitivity was stronger in white than in black SA pre-menopausal women (98). Although, the association between the DII and insulin sensitivity has not been investigated in an African population, it is proposed that the DII might be linked to increased T2D risk via insulin sensitivity. This is based on the fact that obesity and/or obesity-related inflammation is closely associated with overnutrition, which means that the effects of DII might be mediated through pro-inflammatory profile and increased adiposity, in particular central adiposity. Therefore, studies are required to explore the role of diet-induced inflammation in black Africans, in particular from Africa, whose lifestyle, behavioural and environmental factors might differ from other populations (154,161,163). This knowledge will assist in elucidating the pathophysiology of T2D in black Africans, by providing more insights on the role of unhealthy diets in the development of T2D, in particular in black SA women with high T2D risk.

1.8.4.2. Lifestyle factors

Prevention of metabolic disorders requires a balance between dietary energy intake and energy expenditure, which is why insufficient physical activity has also been implicated in the rapid increase of NCDs in black SA women. Lack of access or affordability to safe recreational facilities for physical activity participation are some of the factors contributing to the low levels of physical activity in black SA women (147). Despite the reported benefits associated with physical activity, in particular leisure physical activity, black SA women have low levels of leisure time physical activity (164,165). Physical activity data from the Global Physical Activity Questionnaire (GPAQ) showed that although black SA women do not typically participate in leisure physical activity, the majority of the women meet WHO physical activity guidelines (e.g., 150 minutes of moderate-intensity activity per week) through work- and transport-related physical

activity, which is often undertaken at low-moderate intensities (e.g., walking) (17,164,165). Accordingly, black SA women who met the WHO guidelines had more favourable cardio-metabolic outcomes such as lower BMI, body fat, central adiposity and IR compared to those who did not meet the guidelines (165). However, it was reported that after a 5.5 year follow-up period, there was a significant increase in body fat in both those who met and did not meet WHO guidelines (165). Notably, these findings have been replicated with objective measures of physical activity (i.e., ActiGraph accelerometers) and sedentary behaviours (i.e., activPAL devices) (166,167). In the study by Dickie et al (166) it was reported that 51.3% of the women met the WHO physical activity guidelines through walking or stepping (i.e., light physical activity) (166). However, 59.4% of the waking hours was spent in sedentary behaviours, followed by 37% of the day which was spent in light activity and the least amount of the time (3.5%) was spent performing moderate-to-vigorous activities (166). Of great concern is that these women were shown to have very low levels of cardiorespiratory fitness, which was below the fifth percentile for females between 30-39 years of age (168). Notably, light physical activity was inversely associated with trunk FM, while cardiorespiratory fitness was inversely associated with both adiposity (i.e., total and central adiposity) and IR (166). Furthermore, the association between cardiorespiratory fitness and IR, remained significant even after adjustment for fat mass, but not VAT. In line with these findings, it was recently reported that a 12-week exercise intervention consisting of aerobic and resistance exercise in pre-menopausal black SA women improved insulin sensitivity and cardio respiratory fitness, and decreased BMI and gynoid FM (167). However, there was no change in insulin response, insulin secretion or clearance as well as in VAT following the 12-week exercise intervention (167). Together, these studies suggest that although physical activity might have great benefits for reducing obesity, they may not be sufficient to prevent VAT accumulation and protect against IR, and the subsequent development of T2D, more especially over a short period. These results imply that promotion of leisure physical activity in black African women is needed in order to curb the increasing obesity prevalence, and its role in the development of metabolic disorders such as T2D. These findings also suggest that longer intervention training programmes or a combination of both exercise training and dietary intervention programmes might be required in order to yield optimal metabolic health outcomes in black SA women.

1.9. A brief overview of metabolomics in T2D

Due to the fact that T2D is a multi-factorial disease that involves several mechanisms, global metabolomics analyses has been used as a complementary technique to generate novel insights and hypotheses into the pathophysiology of T2D in African women. Briefly, metabolomics is by definition a hypothesis-generating tool that aims to measure all small molecules (the metabolome) in a specific tissue, which consists of a snap-shot of all metabolic reactions (169–172). One of the many advantages of metabolomics is that the metabolome is close to the phenotype since measures of metabolites are downstream of the genome, transcriptome and proteome and highly related to changes in lifestyle and environmental factors (171–173).

The measurement of metabolites and their response to a stimulus or disease makes metabolomics a suitable tool for the identification of novel risk biomarkers or biomarker patterns for highlighting putative affected pathways, which can be used for understanding, diagnosis and risk assessment of metabolic diseases such as T2D (174–177). The most commonly used analytical instruments for the measurements of metabolites and/or metabolic pathways include the nuclear magnetic resonance (NMR) and mass spectroscopy (MS) (169,171,173). Compared to NMR, MS is highly sensitive and its sensitivity can also be improved by combining it with other analytical techniques such as the gas and liquid chromatography (GC and LC) (169,173,176). Due to its sensitivity, MS techniques include more instrumental variation compared to NMR. To this end, instrument monitoring by means of internal standards and quality control samples are crucial components to obtain reliable MS data. Furthermore, NMR is non-destructive compared to MS as it require little or no sample separation, thus maintains sample integrity (169,171,173).

Even though metabolomics is untargeted by its nature, it is often divided into non-targeted and targeted metabolomics (173,176,178,179). Non-targeted approaches refer to the comprehensive analysis of all metabolites in a tissue, e.g., glucose, fatty acids, larger lipids, amino acids, bile acids, drug metabolites and unknown putative metabolites (171,173,175–177,179). Targeted metabolomics is somewhat contradictory since this

approach does not try to identify as many metabolites as possible in a tissue, but instead focuses on a pre-defined list of compounds (173,178,180). After acquiring data, various data processing techniques need to be applied to quantify and identify putative metabolites and often normalised using isotopic labelled internal standards (169,173). Next steps include sample comparison analyses to elucidate metabolites or metabolite patterns that can discriminate between different conditions, e.g., NGT, IGT and T2D, or describing a relevant end point, e.g., insulin sensitivity (169,171,173). Since metabolomics data are hypothesis generating by nature and include many correlated variables, multivariate analyses such as principal component analyses (PCA) and partial least squares analyses (PLS) are often applied to extract discriminating metabolite patterns (173,176,178,179,181). Of note, metabolomics studies, targeted or non-targeted are always limited to the available compound information which are mainly found in the existing compound libraries (171–173). For the purpose of this review, a brief overview of the metabolites and/or metabolic pathways implicated in T2D will be discussed. Detailed reviews described elsewhere (171,172,182).

1.9.1. Branched chain amino acids and lipids

Branched chain amino acids (BCAAs, i.e., leucine, isoleucine, and valine,) and their catabolic intermediates such as branched-chain keto acids (BCKAs) (e.g., ketoleucine, ketoisoleucine and ketovaline) and BCAA-carnitines (e.g., C3- and C5-acylcarnitine) have shown to both predict and mediate IR and T2D (175,176,178,179,183–185). For example, Wang et al. (175) followed 2422 normoglycaemic participants from the Framingham Offspring Study Cohort over a 12-year period and found that elevated levels of BCAA and aromatic amino acids (e.g., tyrosine and phenylalanine) were associated with increased risk for future T2D (175). A later study by Wang-Sattler et al. (186) revealed that changes in BCAAs and aromatic acids occur at a later stage in the development of T2D and have stronger predictive power for high risk individuals (i.e., obese and IR subjects). Rather, it was reported that glycine (an amino acid) and lysophosphatidylcholine (LPC, a lipid subtype) were the key candidate metabolites for predicting the risk for developing IGT and T2D (186). Furthermore, in one of the limited metabolomics studies to investigate the association of BCAA and insulin clearance

(183), it was reported that high BCAA levels were inversely associated with insulin clearance and insulin sensitivity, as well as being positively associated with fasting insulin levels in a multi-ethnic cohort consisting of European-Americans and Hispanics and African Americans (183). Interestingly, when these associations were stratified by ethnicity, they remained significant in European-Americans and Hispanics participants, but not in black Africans (183). In contrast, a recent study in young, non-obese and NGT individuals did not report a significant association between BCAAs and hyperinsulinaemia (187). This might partly be due to the fact that the tested sample was at low risk for T2D, in terms of age, glucose tolerance and adiposity status as well as low levels of IR. However, when the sample was stratified by hyperinsulinaemia, the fourth quartile group, having the highest hyperinsulinaemia, had the highest measures of beta-cell function (i.e., insulin secretion and βCGS) (187) and the lowest insulin sensitivity and insulin clearance levels compared to the rest of the groups. Moreover, fourth quartile group had higher amino acids (e.g., phenylalanine, asparate, glutamate and glutamine) compared to the other groups (187). Notably, these amino acids have been linked to obesity, IR and T2D risk (174–176). These findings suggest that there is a close interaction between hyperinsulinaemia, insulin sensitivity and BCAA metabolism. Thus, more studies are required to replicate these findings, in particular in those at greatest risk for T2D such as black African women.

Although the exact mechanisms linking BCAA accumulation to obesity, IR and T2D are not fully understood, it has been proposed that impaired BCAA-oxidation in liver might drive the increased circulating BCAA (188–190). Indeed, several studies have shown increased activation of key enzymes involved in the first steps of BCAA metabolism (mainly liver), which might explain the increased BCAA levels in peripheral tissues (188–190). This is based on the finding that deletion of the branched-chain amino transferase gene prevents accumulation of BCAAs in the peripheral tissues and is associated with favourable metabolic outcomes such as improved glycaemic control, insulin sensitivity and lipid profile (188). A putative link is that, due to impaired liver oxidation, BCAA oxidation is shifted towards the skeletal muscle and competes with the oxidation of lipids leading to the accumulation of lipid intermediates (191). Indeed, increased accumulation of lipid intermediates from excessive fat intake or adipose tissue lipolysis or lipogenesis,

is an important link between obesity, IR and T2D development (192,193). For instance, elevated FFAs serve as precursors for several lipid metabolic pathways, that further lead to greater production and deposition of lipid molecules (e.g., diacylglycerol, glycerophospholipids, acylcarnitines and sphingolipids) in non-adipose tissues (85,177,192,194,195), which might interfere with insulin signalling mechanisms (38,192,196). For example, diacylglycerol levels serve as a key substrate for the formation of glycerophospholipids. Glycerophospholipids include molecules such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which are the most abundant phospholipids making up the majority of the components of cellular membranes (197). Notably, impairments in phospholipid metabolism, including their ratios (e.g., PE: PC ratio) have shown to impair lipid mobilization and infer fatty liver disease (198–200). Also, high concentrations of lysophosphatidylcholine (LPC) (e.g., LPC C18:1 and C18:2) and/or lysophosphatidylethanolamine (LPE), which are derived from their phospholipids precursors (i.e., PC and PE) are associated with decreased IGT risk and 69% reduction in T2D risk, whereas low levels in LPC and/or LPE are linked to increased metabolic disease risk in white Europeans (178,186,201).

Moreover, when excess FFAs (e.g., long chain FFAs) are delivered to non-adipose tissues for metabolism, they are first converted to acyl-CoA in order to be transported by carnitine across the mitochondrial membrane for beta-oxidation and further metabolism in the tricarboxylic acid (TCA) cycle (177,195,202). Therefore, alteration in long-chain acylcarnitines have been used to reflect impairments in fat oxidation which may link to altered insulin signalling and/or glucose uptake, mitochondrial overload with an impaired TCA cycle (195,202). It is also important to note that lipids and carbohydrates compete for oxidation under physiological conditions such as during fasting and feeding. In a healthy organism, this competition or shift between lipid and carbohydrate oxidation is efficiently regulated. However, in metabolic disorders such as obesity and T2D**,** lipid and carbohydrate oxidation are impaired, leading to a phenomenon known as metabolic inflexibility. In addition to lipid dysregulation, metabolic inflexibility is also characterised by alterations in carbohydrate or sugar metabolites, in particular metabolites that regulate several biological pathways such as glycolysis, gluconeogenesis, the pentose phosphate pathway and the TCA cycle (144,149,151). Indeed, most of these

metabolites are captured in metabolomics analysis. For example, hexose sugars (i.e., glucose, fructose and inositol), lactic acid, glycerol, mannose, sorbitol and xylose levels are increased, whereas glycerol-3-phosphate are decreased in obesity and T2D (174,178,179). Further, a factor consisting of hexose sugars along with diacyl-phosphatidylcholines, BCAAs, aromatic amino acids, and propionylcarnitine was significantly associated with increased T2D risk in white European cohorts (178).

1.9.2. Bile acids

Bile acids, are another metabolite class captured by metabolomics analyses that have been implicated in obesity, IR and the development of T2D (203–205). Briefly, the human liver produces primary bile acids, namely, cholic acid (CA) and chenodeoxycholic acid (CDCA), which are derived from cholesterol. The production of primary bile acids involve multiple hepatic enzymes such as the cholesterol 7α -hydroxylase (CYP7A1), sterol 12α-hydroxylase (CYP8B1) and sterol 27α-hydroxylase (CYP27A1) and 25-hydroxy-cholesterol-7α-hydroxylase (CYP7B1) which convert bile acid intermediates to bile salts through two pathways (206–208), as presented in Figure 1.4. These two pathways include the classical or neutral and alternative or acidic pathways, with the former being responsible for the majority of bile acid production (206–209). The classic bile acid synthesis is regulated first by the rate-limiting enzyme, CYP7A1, whereas the alternative/acidic pathway is initiated by the CYP27A1 and followed by CYP7B1 (206–209) (Figure 1.4). Notably, both pathways produce primary bile acids (i.e., CA and/or CDCA) that are by-products of cholesterol (Figure 1.4). However, CDCA and CA are both produced by the classical pathway, whereas the alternative pathway mainly generates CDCA (206–209). The ratio between these two primary bile acids is determined by the CYP8B1, and also regulates the production of CA synthesis from CDCA (206–209).

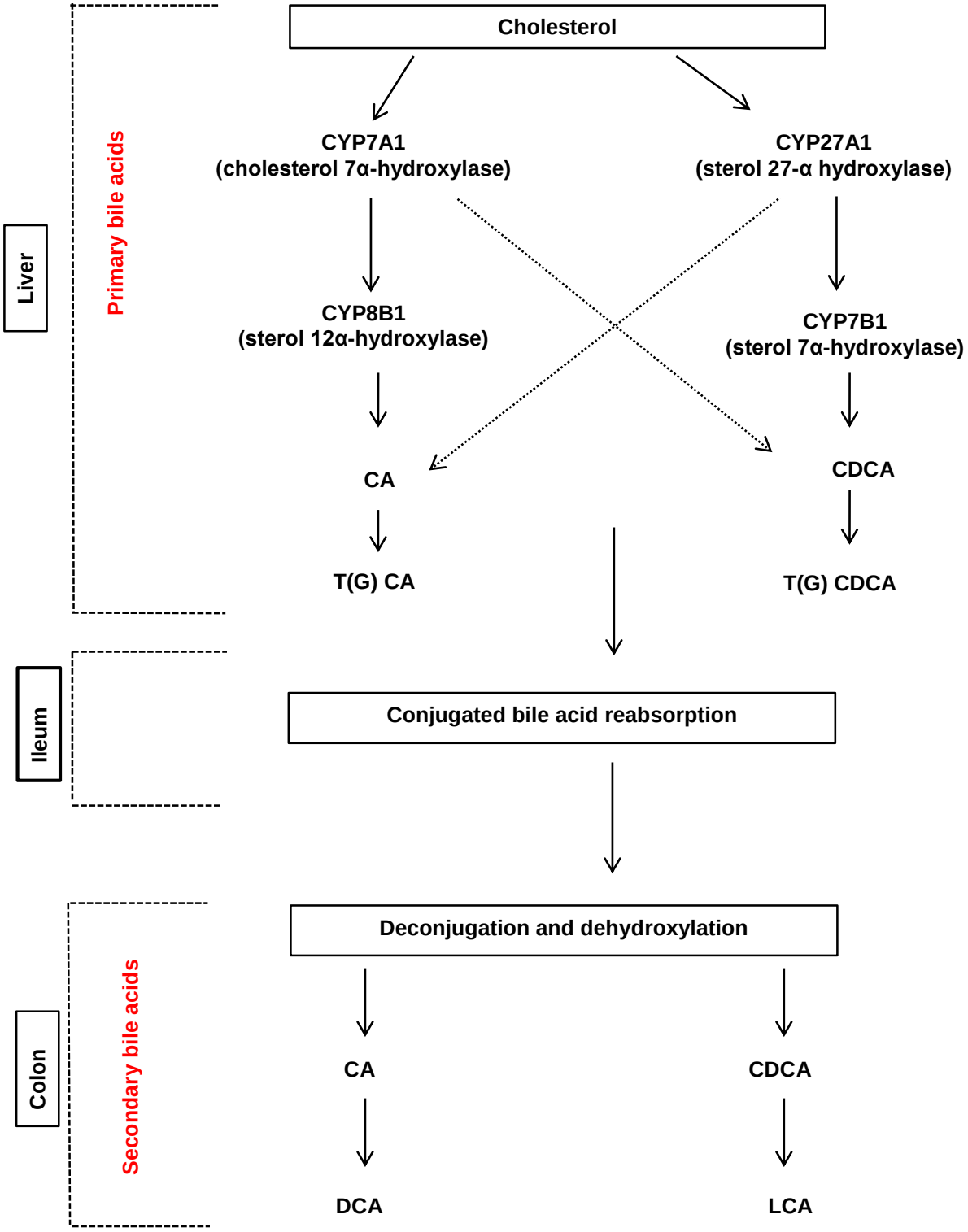


Figure 1.4: A schematic diagram of the bile acid synthetic pathway (Adapted from Haeusler et al. (204) and Wahlstrom et al.(209)). Abbreviations: CA, Cholic acid; CDCA, Chenodeoxycholic acid; DCA, Deoxycholic acid;G, Glycine; LCA, Lithocholic acid; T, Taurine.

The newly synthesised bile acids become conjugated to amino acids, glycine or taurine, then are secreted by hepatocytes, and subsequently stored in the gallbladder (206–208) (Figure 1.4). After a meal, bile acids are immediately released into the intestinal lumen, where primary bile acids can be deconjugated and dehydroxylated by the bacteria of the gut microflora to form secondary bile acids, deoxycholic acid (DCA), lithocholic acid (LCA), which are derived from CDCA and CA, respectively (209,210) (Figure 1.4). Humans also produce ursodeoxycholic acid (UDCA) as a second bile acid (209). Notably, bile acids are actively reabsorbed in the intestine and transported back to the liver via the portal vein for reconjugation and resecretion together with newly synthesised bile acids, but some remain in the circulation (206–209). Notably, the gut microbiome plays an important role in bile acid synthesis and biotransformation, thus controls circulating bile acid pool size and bile acid composition. The bile acid pool, which is also known as the total bile acids circulating in the gut-liver axis/enterohepatic circulation, is made up of bile acids from the liver, intestine (ileum, cecum and colon) and gallbladder (211). The human bile acid pool consists of CA, CDCA, and DCA in the ratio of 40, 40 and 20%, respectively. With the ratio of the glycine to taurine-conjugated bile acids being 3 to 1 (211).

Bile acids assist in the absorption of lipid substrates, serve as signalling molecules, and regulate glucose homeostasis through the activation of two prominent receptors, namely, bile-acid-activated nuclear receptor, farnesoid X receptor (FXR) and G-protein-coupled receptor, TGR5 (208,212–214). Bile acid activation of FXR and TGR5 in animal and human studies has been shown to play an important role in controlling glucose homeostasis in multiple organs and tissues such as intestine, liver, adipose tissue and muscles (208,212–214). Through these organs and tissues, bile acid FXR activation has been implicated in several metabolic processes that include regulation of microbiota and incretin secretion, hepatic EGP, as well as insulin signalling (208,212–216). On the other hand, activation of TGR5 by bile acid or TGR5 agonist has been associated with improved glucose homeostasis via increased GLP-1 production and beta-cell insulin secretion (217,218).

The literature on bile acid metabolism and its role in the pathophysiology of T2D is not fully understood, but is currently under investigation (203,204,219,220). Nonetheless, alterations in circulating total bile acid pool and/or bile acid composition have been reported in obesity and in T2D (203,204,219,220). In the fasted state, total bile acids have been shown to be similar between normal weight and obese participants (219). However, in the postprandial state, an increase in bile acids was reported in both groups, with this increase being lower in obese participants (219). Interestingly, in a different study, obese individuals were reported to have higher markers of bile acid synthesis compared to non-obese participants, which were also inversely associated with insulin sensitivity (204). It was further noted that insulin infusion during the HEC procedure caused a significant reduction in circulating bile acids in non-obese individuals, however, this response was blunted in obese subjects (204). Consequently, the authors proposed that this effect was unlikely to be caused by the deactivation of bile acid synthesis since newly produced bile acids only contribute an insignificant fraction to the bile acid pool. This notion was supported by the findings that in both obese and non-obese groups, there were not significant changes in markers of bile acid synthesis during the clamp. Therefore, it was postulated that insulin reduces bile acid levels by altering the transportation of bile acids into or out of the plasma. Most importantly, it was believed that bile acid transport system was dysfunctional in obese individuals. In line with this, the bile acid levels were significantly higher in the T2D group compared to the non-T2D group. Moreover, a higher bile acid ratio (i.e., 12a-hydroxylated to non– 12a-hydroxylated bile acid) was inversely associated with insulin sensitivity, independent of age, sex and BMI and glucose (203).

1.9.3. Metabolites and prediction of T2D

Majority of metabolomics studies have been performed in populations from developed countries (175,178,186), whereas only few studies have been undertaken in African American women (177,179). To our knowledge, there are no studies that have examined the metabolite profile linked to T2D in black African women, whose lifestyle differs from those living in developed countries. Therefore, future studies are needed to determine whether the findings reported in predominately white Europeans can be

replicated in other ethnic groups or individuals living in other geographical regions with different lifestyle, behavioural and environmental factors. Of note, multiple studies have shown that the prediction scores of prediabetes and T2D are significantly improved when metabolites are included in the models containing T2D classical clinical parameters such as age, BMI, physical activity, fasting glucose and insulin (186). For example, metabolite levels were found to predict T2D risk better than the Diabetes Risk Score; a score comprised of diabetes risk factors such as diet, lifestyle and anthropometry in white European participants (178,221). Based on these findings, it can be argued that a large number of individuals who may be at risk for T2D may not be identified when only clinical parameters are used to screen individuals for T2D risk. This suggests that the identification of metabolites in combination with traditional clinical parameters have the potential to be more effective in predicting T2D risk compared to when only classical clinical parameters are used (e.g., blood glucose measurements, family history of the disease, BMI). This is of relevance for black Africans whom traditional risk factors such as WC, BMI and VAT may not be strong indicators of T2D risk. Thus, a comprehensive analysis of the metabolite and/or metabolic pathways linked to T2D risk will enable us to gain better insights on the biological mechanisms underlying the development of T2D in black Africans from Africa. This knowledge will assist in the early detection of T2D, diagnosis and management of the disease.

It is also important to highlight that metabolomics has some limitations. One of these limitations is that it is not clear if the identified metabolites and/or metabolic pathways cause the metabolic diseases or are the direct consequence of the metabolic disorders under investigation. Hence, future research on cell culture models and animal models where the affected metabolite and/or metabolic pathways can be up or downregulated needs to be explored. Furthermore, some of the metabolites linked to T2D risk might be unknown, which motivate the usage of untargeted metabolomics to not limit number of metabolites to those available in the research database libraries (171–173). Current metabolic studies use expensive instruments to cover the large-scale measure of metabolites with different chemical properties, which might not be feasible and cost-effective for under-funded research settings, in particular in Africa. Moreover, analysis of metabolomics data require complicated data processing, thus requires an expert or

trained personnel in the field.

1.10. Summary, gaps in literature and motivation for research

In summary, black Africans, in particular black African women, present with a complex T2D pathophysiology that is characterised by lower VAT and liver fat, and greater peripheral adiposity, but paradoxically present with hyperinsulinaemia and higher IR and T2D risk, compared to their white European counterparts (3,5,18,44,53,55,79,105,127). Irrespective of ethnicity, majority of research implicate VAT accumulation with increased T2D risk via IR (21,103,105,125,222), with some studies reporting stronger associations between the two factors in black Africans (21,103,222). In contrast, the associations of liver fat accumulation with markers of T2D risk such as insulin sensitivity and insulin clearance are weak or absent and often inconsistent in both black Africans and white Europeans (18,41,43,87,222). Notably, these inconsistent findings might be partly explained by the fact that the existing literature is limited and is mostly based on findings conducted in pre-menopausal women using cross-sectional study designs (18,20,87,103). It is also worth mentioning that even though the detrimental contribution of VAT to T2D pathophysiology are well characterised in terms of its effect on IR, studies assessing the relationship between VAT and measures of insulin secretion and clearance, which are also prominent features of T2D, are very scarce. Collectively, these inconsistent findings and limited literature makes it difficult to fully elucidate the contribution of VAT and liver fat accumulation in the development of T2D, as measured by IR insulin secretion and clearance, in particular in black African women from Africa. Indeed, there is a lack of studies characterising body fat and fat distribution in relation to T2D following menopause in African women outside the USA. No studies have examined whether body fat and fat distribution predicts T2D risk in middle-aged or older black SA women, despite being the group with the highest risk for T2D (10).

Although obesity and IR are prominent features in the pathophysiology of T2D in black Africans, it is important to highlight that these risk factors may only play a minor role in the development of T2D in this group. This is based on the fact that some studies have

demonstrated that even after adjusting for total or central adiposity, black Africans remain hyperinsulinaemic and their insulin sensitivity also remains lower compared to their white European counterparts (3,5,6,8,19,116–118). Furthermore, hyperinsulinaemia is also present even after adjusting for insulin sensitivity (5,55,118). These findings, suggest that hyperinsulinaemia may not necessarily be a compensatory response to obesity or IR. Rather, hyperinsulinaemia may cause obesity or IR and be an initial defect in the development of T2D, as proposed by various authors (29,32,119,120). In line with this, others have also suggested that hyperinsulinaemia might be initially driven by lifestyle (i.e., carbohydrate diet) and genetic factors (e.g., IDE activity) via their associations with insulin secretion and clearance, more especially in black Africans (31,64,80). Future longitudinal and dietary intervention studies are needed to elucidate the pathophysiology of hyperinsulinaemia and its role in the development of T2D. Of great importance is that even cross-sectional studies exploring the associations between insulin sensitivity, adiposity and liver fat accumulation, with insulin secretion and clearance, are lacking in black Africans. Indeed, while there is extensive literature on hyperinsulinaemia in the context of beta-cell insulin secretion, few studies have examined the relative contribution of both insulin secretion and clearance to hyperinsulinaemia in the adult black African population, especially in black African women (18,53,112,114). There are only two studies that have investigated the contribution of insulin secretion and clearance to hyperinsulinaemia in black SA women (112,114). Not only are these studies insufficient, they have also reported contradictory results from those reported in the majority of black Africans, especially from USA (7,8,18,53,61,80,116–118), where insulin levels and/or C-peptide levels were reported to be lower instead of being higher than in white Europeans. It is also possible that the inconsistent findings might be due to the different methodology or techniques used to measure insulin secretion and clearance such as intravenous vs oral, assessment of beta-cell function using insulin vs C-peptide levels, thus making it difficult to explore the true effects of insulin secretion and clearance on hyperinsulinaemia in black African women. With these conflicting findings, the pathophysiology of hyperinsulinaemia in black SA women is poorly understood and requires further investigation. Hence, more studies with larger sample sizes and in higher risk individuals such as the middle-aged and older black SA women are needed to provide more insights on the contribution of insulin secretion and clearance to hyperinsulinaemia. Of interest, the use of a

mathematic model of beta-cell function to study hyperinsulinaemia in an African population has not been explored. The mathematic model of beta-cell function allows us to gain better insights on the relationship between beta-cell function and prevailing glucose concentrations (74,75), and is a better approach than indices such as IGI, which does not differentiate the contribution of insulin secretion and clearance to insulin response.

It is also important to highlight that the pathophysiology of T2D varies within the African population, as evidenced by disparities or inconsistencies in insulin sensitivity, beta-cell insulin secretion and clearance, as well as VAT and liver fat accumulation in the studies conducted in SA, USA and UK (18,41,43,44,87,103,105,125). These discrepancies suggest that the black Africans are a very diverse ethnic group and that findings reported in some black African populations (e.g., African-Americans) may not necessarily be replicated in other black Africans, in particular those living in different geographical locations. Indeed, the majority of the studies are conducted in African-Americans who have a very distinct genetic admixture (223–225). Accordingly, it was estimated that the proportion of African-Ancestry in African-American individuals varies from 1-99% (223–225). Hence, it is important to consider that the pathophysiology of metabolic diseases might vary within the same ethnicity due to genetic variations, and region-specific factors such as lifestyle factors. Accordingly, this thesis has been undertaken to study the development of T2D in the black Africans from Africa, middle-aged black SA women.

It is also postulated that the disparities in T2D pathophysiology in the black population might also be explained by differences in biological processes and/or metabolic pathways (e.g., BCAA metabolism) that have not yet been examined or fully understood due to the dearth of studies in the African population. Indeed, the majority of the metabolomics studies have been performed in populations from developed countries (175,178,186), whereas only few studies have been undertaken in black African women (177,179). To our knowledge, there are no studies that have examined the metabolite profile linked to T2D in black African women from Africa, whose lifestyle differs from those living in developed countries. While lifestyle factors (i.e., diet and physical activity)

have contributed to the rapid increase in the prevalence of NCDs including T2D (11,12,15,148–152), there are very few studies that have objectively measured diet and physical activity, and investigated their roles in the development of T2D in a black population, in particular from Africa (166,167). Of interest is that while unhealthy diets have been linked to the increased prevalence of NCDs in urban black SA women (11,12,15,148–152), the majority of these studies used single macro- and micronutrients to examine the effects of diet in T2D risk. This approach has some limitations since these single macro- and micronutrients are rarely consumed alone. Hence, the use of advanced dietary tools such as the DII, which capture the quantity of the entire diet, might provide better insights on the role of habitual dietary intake in the development of T2D in black SA women. This is of great importance since urban black SA women have the highest obesity levels, which means that diet and/or obesity-related inflammation might be implicated in the pathophysiology of T2D in this group. Accordingly, urban black SA women were shown to have a high inflammatory profile, which was also linked to insulin sensitivity (98). No study has investigated the role of diet-induced inflammation in relation to pro-inflammatory profile, insulin sensitivity and adiposity, and whether these associations increase T2D risk in urban black SA women.

In conclusion, an understanding of the pathophysiology of T2D in black Africans, with a focus on body fat and its distribution, insulin sensitivity, and on the contribution of insulin secretion and clearance to hyperinsulinaemia, as well as identifying the metabolites and/or metabolic pathways associated with T2D risk, will assist in elucidating the underlying biological mechanisms implicated in the development of T2D in black Africans, in particular black African women from Africa. This knowledge will also be beneficial in the early detection of T2D, to ensure more effective management within a health system that is severely under-resourced.

1.11. Aim and objectives

Overall Aim

The primary aims of this thesis were to examine T2D risk factors, in particular lifestyle (i.e., dietary intake), body fat and fat distribution, associated with insulin sensitivity, secretion and clearance; and to elucidate the relative contribution of insulin secretion and clearance to hyperinsulinaemia in order to provide better insights regarding the pathophysiology of T2D in middle-aged black SA women. Furthermore, this study also aimed to identify metabolites and/or metabolic pathways that predict risk for future T2D. These aims were addressed in four separate results chapters (chapters 2-5), in which three of the four chapters have been published. The chapters are described below:

Chapter two

Chapter two includes the first study of the thesis, which tests the hypothesis that obesity, in particular central obesity, will be associated with lower insulin sensitivity and impaired glucose metabolism (IGM) (defined as IFG and IGT) and T2D, while peripheral adiposity will be protective against the development of IGM and T2D, over a 13-year follow-up period. Therefore, the aims of the study were to explore the association between body fat and fat distribution (i.e., central and peripheral adiposity) and the risk of developing T2D, 13 years later; and to investigate the independent associations between baseline and/or change in body fat and fat distribution, and measures of glycaemia at 13-year follow-up period in middle-aged urban black SA women.

Chapter three

The third study of the thesis tests the hypothesis that hyperinsulinaemia in black Africans is associated with increased beta-cell insulin secretion and reduced insulin clearance, with the latter being more closely associated with hyperinsulinaemia. It is also hypothesised that insulin secretion and clearance will be significantly associated with insulin sensitivity, measures of adiposity, in particular central and peripheral fat, as well

as liver fat. Therefore, the aims of this study were to use a mathematical model of beta-cell function and clearance to examine the relative contribution of insulin secretion and clearance to basal and postprandial hyperinsulinaemia, and further explore the associations with insulin sensitivity, adiposity and a marker of liver fat in middle-aged black SA women.

Chapter four

The second study of the thesis tests the hypothesis that unhealthy diets, characterised by high intakes of fat and sugar, and low intakes of fruit and vegetables, will result in a pro-inflammatory diet that will be associated with increased T2D risk. Therefore, in middle-aged urban black SA women, the aim of this study was to determine whether dietary-induced inflammation, measured by the DII, is associated with markers of T2D risk, and if this association is mediated by adiposity and/or inflammatory markers.

Chapter five

The last study of the thesis examined the hypothesis that a metabolic pattern consisting of BCAAs and their catabolic by-products, aromatic acids, lipid-subtypes in particular glycerophospholipids and acylcarnitines, and bile acids will predict the development of T2D 13 years before its onset. Furthermore, it is also hypothesized that these metabolites will be significantly altered in participants with T2D compared to those with NGT. Thus, the aims of this chapter were to identify metabolites and/or metabolic pathways that predict the development of T2D in African women and to examine if the identified metabolic profiles are significantly different by the glycaemic categories (NGT, IGT and T2D).

CHAPTER TWO

Fat redistribution and accumulation of visceral adipose tissue predicts type 2 diabetes risk in middle-aged black South African women: a 13-year longitudinal study

Data from this chapter have been published in Mtintsilana A, Micklesfield LK, Chorell E, *et al* Fat redistribution and accumulation of visceral adipose tissue predicts type 2 diabetes risk in middle-aged black South African women: a 13-year longitudinal study. *Nutrition and Diabetes* 2019; **9**:1-12.

Author Contributions: Designed or conceptualised the study, A.M., L.K.M., T.O., and J.H.G.; Conducted the research, performed biochemical and statistical analyses and wrote the first draft of manuscript, A.M.; Principal investigators and acquired funding for the study, L.K.M., E.C., T.O., and J.H.G.; Supervised the study and the writing of the manuscript, L.K.M., and J.H.G.; Critically reviewed and commented on the manuscript, L.K.M., E.C., T.O., and J.H.G.

2.1 Introduction

The rapid world-wide increase in obesity levels has led to a rise in the prevalence of non-communicable diseases such as T2D and CVDs, in particular in low-and middle-income countries such as SA (2,226). The prevalence of obesity in SA is high, particularly in black women (40.9%) (111), and accounts for 87% of T2D risk (89). According to Statistics SA, diabetes mellitus (mainly T2D) was the second cause of death in SA and the leading cause of death among SA women, accounting for 7.2% of all deaths in women in 2016 (2). Notably, the pathophysiology of T2D differs between black and white women (4,17,87,227,228). Insulin resistance, a major risk factor for T2D, is more pronounced in black SA women compared to their white counterparts even when matched for body fat and WC, linked to a greater insulin response to maintain normoglycaemia (4,17,87,228). It is the accumulation of central body fat, in particular VAT, that mainly determines the risk for IR and T2D (21,137,229). Estimation of central body fat via measurement of WC thus does not adequately discriminate between VAT and SAT (99). Interestingly, black SA women are more sensitive to the effects of VAT on hepatic insulin sensitivity than white SA women (17,87). This could possibly, at least partly, explain the fact that with increasing WC, black women accumulate less VAT, despite being more insulin resistant than their white counterparts (4,17,87). Notably, these findings are based on cross-sectional studies, mainly conducted in pre-menopausal women (4,17,87). There is therefore a great need for prospective studies to delineate the possible predictive ability of VAT accumulation, using methods with adequate precision, in the development of T2D among African populations. In particular, studies of middle-aged or older black SA women, who are at high risk of developing T2D, are of main interest in this aspect (10). This period coincides with menopausal transition in women, and is often characterised by relative redistribution of fat from the peripheral to the central region, and a significant increase in VAT (132,133,230). This shift in adipose tissue has been associated with T2D and hypertension risk in pre- and post-menopausal black SA women, respectively (137,231). There is a lack of studies characterizing body fat and fat distribution following menopause in Africans (105,133,231,232) and to our knowledge none have examined whether body fat and fat distribution, including central body fat depots, predict T2D risk in middle-aged or older black SA women. For these reasons, this study in a middle-aged population of urban

black SA women aimed to: i) explore the association between body fat and fat distribution and the risk of developing T2D, 13 years later; and ii) investigate the independent associations between baseline and/or change in body fat and fat distribution, and measures of glycaemia at 13-year follow-up period.

2.2 Material and methods

2.2.1 Study population

Baseline data collection was completed between 2002 and 2003 and follow-up took place between 2015 and 2016 (approximately 13 years later). In brief, at baseline, 2174 caregivers of the Birth-to-Twenty plus (Bt20+) cohort were invited to visit a data collection facility at either Chris Hani Baragwanath Hospital in Soweto or the University of the Witwatersrand Medical School in Johannesburg (233). However, 1251 were eligible for inclusion in the study and underwent extensive testing that included interviews, whole-body composition assessment by DXA, and blood sampling (233) (Figure 2.1). Of these participants, few (n=476) had blood analyte data at baseline. At follow-up, contactable participants (n=323) were invited to participate in the follow-up study if they fulfilled the following inclusion criteria: (1) Same female caregiver tested at baseline; (2) less than 65 years of age; (3) had a baseline DXA scan; (4) had normal fasting glucose (<6.1 mmol/l) at baseline; (5) HIV negative on testing. One hundred and seventy nine participants were excluded owing to the strict inclusion criteria and to reasons stated in Figure 2.1. Of those tested (n=144), blood samples were not obtained from two participants who were then excluded from the statistical analyses. The final sample size for this study was n=142 (Figure 2.1).

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, M010556 (for Bt20+ cohort) and M150530 (see Appendix C for Ethics clearance certificate for the current study). The procedures and risks associated with the study were explained to the participants and all participants signed the consent form prior to participation in the study. All testing procedures were performed at the South African Medical Research Council/University of the

Witwatersrand Developmental Pathways for Health Research Unit at the Chris Hani Baragwanath Hospital in Soweto Johannesburg.

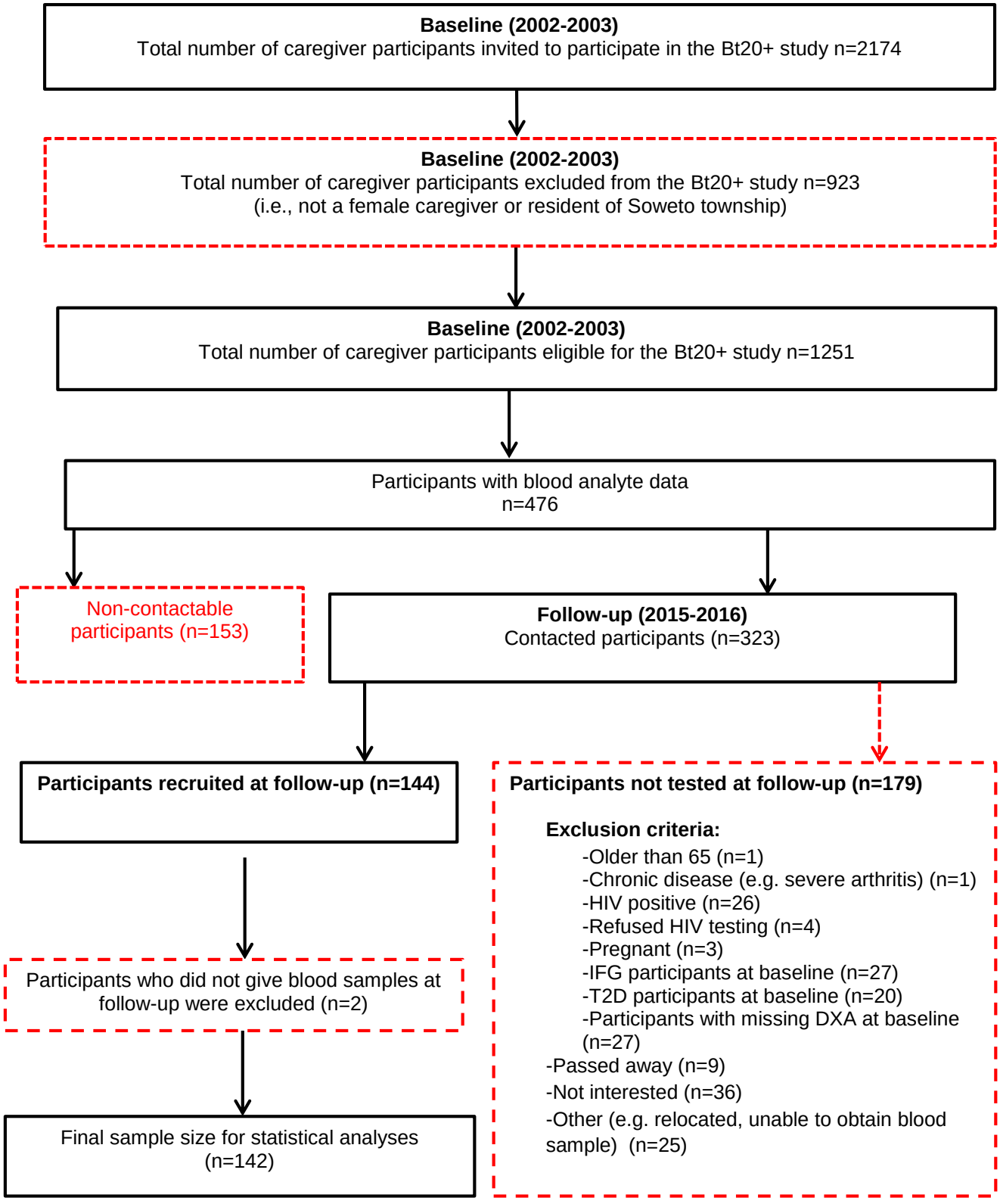


Figure 2.1: Sample selection flow chart of the female caregivers (e.g. mother, grandmother, aunt) of the Birth to Twenty (Bt20+) plus cohort

2.2.2 Body composition

Weight and height, in lightweight clothing without shoes, were measured at baseline and follow-up, using a standard scale and stadiometer, respectively. Waist (level of umbilicus) and hip (largest gluteal area) circumferences were measured in triplicate and the mean used for statistical analyses. Whole body composition was measured using DXA (Hologic Discovery-W (S/N 71201), Bedford, MA, USA) at both time points, and included subtotal (whole body minus head) FM and fat–free soft tissue mass. Regional body fat, namely trunk, arm, and leg FM (expressed in kg and as a percentage of sub-total FM, %FM) were also measured using DXA cut-off lines positioned at standard anatomical positions, as defined in the software (software version 13.4.2:7). In addition, abdominal VAT and SAT were estimated using algorithms included in the DXA, which have been shown to perform as well as clinical computed tomography (128). Baseline and follow-up scans were analysed using the same software (software version 13.4.2:7). Body composition of participants exceeding the scan field limits were calculated using the arm-replacement method (234). The coefficients of variations during the course of the study were 1% and <2% for fat-free soft tissue mass and total FM, respectively.

2.2.3 Menopausal status

Self-reported menopausal status was determined by questionnaire at follow-up only (Appendix D). Pre-menopause was defined as having a regular menstrual cycle, while peri-menopausal was characterised by menstrual irregularity or having missed 3 months of consecutive menstrual periods. Post-menopause was defined as cessation of the menstrual cycle for more than 12 months.

2.2.4 Blood sampling and biochemical analysis at follow-up

At baseline, fasting blood samples were drawn for the analysis of plasma glucose concentrations (233). At follow-up, blood samples were drawn in the fasted state (average 12 h) for the subsequent determination of HbA1c (glycated haemoglobin),

plasma glucose and serum insulin concentrations. Thereafter, all non-T2D participants (based on self-reported status and/or use of T2D medication) completed an OGTT. In brief, participants ingested 75g of anhydrous glucose dissolved in 250ml water. Following the glucose ingestion, blood samples were taken at 30, 60, 90 and 120 min. The samples were centrifuged at 3000 rpm for 10 min at 4°C, the plasma was stored at -20°C for subsequent analysis of glucose concentrations, and the serum was stored at -80°C for the analysis of insulin concentrations. Plasma glucose concentrations were analysed on the Randox RX Daytona Chemistry Analyser using enzymatic methods (Catalog number, GL8318) (Randox Laboratories Ltd., London, UK). Serum insulin assays were analysed on the Immulite® 1000 Immunoassay System (Catalog number, 10381429) (Siemens Chemiluminescent Healthcare GmbH, Henkestr, Germany). HbA1c levels were measured on whole blood samples using the D-10™ Hemoglobin Analyser (Catalog number, 2200101) (Bio-Rad Laboratories, Inc., California, USA).

At baseline, participants were classified as having NGT if their fasting glucose data was < 6.1 mmol/l (24). At follow-up, participants were divided into three glycaemic categories using the OGTT results; NGT (fasting glucose < 6.1 mmol/l and 2-h post glucose load < 7.8 mmol/l), IFG (fasting glucose ≥ 6.1 and < 7.0 mmol/L and 2-h post glucose load < 7.8 mmol/l), IGT (fasting glucose levels < 7.0 mmol/l and 2-h post glucose load ≥ 7.8 mmol/l and <11.1 mmol/l) and T2D (fasting glucose ≥ 7.0 mmol/l and/or 2-h post glucose load ≥ 11.1 mmol/l) (24). Based on these categories, 64.1% participants remained NGT over the 13-year follow-up period, 24.7% and 11.3% progressed to IFG/IGT and T2D, respectively. The IFG/IGT and T2D participants were combined into one group, called the impaired glucose metabolism (IGM) and T2D group (IGM/T2D; n=51) owing to limited sample size and the fact that they had a similar adiposity phenotype; in particular the IFG/IGT group had a closer body composition profile to the T2D than the NGT group (Appendix B, Table 2S1).

IR was calculated from fasting glucose and insulin using the HOMA2-IR calculator v2.2.3 (http://www.dtu.ox.ac.uk/homacalculator/) (68). Fasting and OGTT glucose and insulin data (0, 30, 60, 90 and 120) were used to calculate insulin sensitivity using the Matsuda Index web calculator (http://mmatsuda.diabetes-smc.jp/english.html) (69). Participants

with missing glucose and insulin data from the OGTT (n=13), and diabetic participants (i.e., those already diagnosed post-baseline, n=8) were excluded from these parameters.

2.3 Statistical analysis

Shapiro-Wilk test was used to assess the distribution of continuous variables. Normally distributed data are presented as mean ± standard deviation (SD) while skewed data are presented as median and interquartile range (IQR). Change in sub-total and regional FM (expressed as percentage of subtotal FM, %FM) between baseline and follow-up in the whole sample were compared using paired t-tests, whereas nonparametric variables were compared using the Wilcoxon signed rank test. Differences in body composition between baseline and follow-up were compared between the NGT and IGM/T2D groups, as well as between the three groups (NGT, IFG/IGT and T2D) using two-way repeated measures analysis of variance (ANOVA). For the ANOVA, skewed data was transformed into normally distributed data and Levene's test was used to test for equal variance between groups. Logistic regression analysis was performed to determine the odds ratios (ORs) associated with the risk of developing IGM/T2D (outcome variable, n=51) compared with remaining NGT (reference group, n=91) at follow-up. Moreover, multinomial logistic regression analyses were used to determine the relative risk ratios associated with progressing to IFG/IGT and developing T2D, compared with remaining NGT after 13 years. In addition, robust regression analyses were used to assess the independent contribution of baseline or change in body composition, to measures of glycaemia at follow-up, while adjusting for baseline age and fat mass. To determine whether central and peripheral FM variables were independent predictors of T2D risk, DXA-derived measures of central (VAT) and peripheral (leg) FM were included in the robust and logistic regression. Statistical significance was set at $p< 0.05$. All data were analysed using Stata (Version 13.1, Statcorp, College Station, Texas).

2.4 Results

2.4.1 Changes in body composition over the 13-year follow-up period

Characteristics of the study population at baseline and follow-up are summarised in Table 2.1. The average follow-up period was 13 (12-14) years and the median (IQR) age of the participants at follow-up was 54 (49-59) years. At follow-up, 27.5% of the participants were pre-menopausal, 9.9% peri-menopausal and 62.7% post-menopausal (data not shown). All measures of body composition increased significantly between baseline and follow-up ($p<0.0001$). Body weight and BMI increased by an average of 9.3%. Measures of central obesity increased to a greater extent than lower-body FM, with trunk FM increasing on average by 25%, whereas leg FM increased by 12.3%. Within the abdominal region, VAT increased by 41% compared to 16% in SAT.

Table 2.1: Participant characteristics at baseline and follow-up (n=144)

	Baseline	Follow-up	Absolute change	% Change
Age (years)	42 (37-48)	54 (49-59)	11 (10- 12)	27 (23 - 32)*
Anthropometry				
Weight (kg)	77.4 ± 14.7	84.4 ± 17.3	7.0 ± 9.0	9.3 ± 11.6*
BMI (kg/m^2)	30.9 ± 5.8	33.9 ± 6.8	3.0 ± 3.5	9.3 ± 11.6*
Normal-weight / overweight / obese (%)	20.4 / 25.4 / 54.2	8.5 / 21.1 / 70.4*		
WC (cm)	88.2 ± 11.5	99.7 ± 12.7	11.5 ± 7.9	13.4± 9.9*
Hip circumference (cm)	114.7 ± 12.8	120.5 ± 13.8	5.9 ± 8.5	5.3 ± 7.1*
WHR	0.77 (0.72-0.82)	0.82 (0.79-0.88)	0.05(0.02-0.09)	6.35 (3.38-11.59)*
Body composition and body fat distribution				
Fat-free soft tissue mass (kg)$^{\#}$	36.2 (32.1-39.3)	36.5 (33.0-39.8)	0.5 (-1.4-2.3)	1.6 (-3.7-6.9)$^{\#}$
Body fat mass (kg)	33.4 ± 10.3	39.2 ± 12.2	5.8 ± 6.3	19.6 ± 22.6*
Body fat (%)	46.7 (42.3 - 50.4)	50.4 (45.9-54.1)	3.4 (1.7-5.7)	
Trunk FM (kg)	15.0 ± 5.2	18.3 ± 6.0	3.2 ± 3.5	25.2 ± 31.1*
Trunk (%FM)	44.6 ± 4.7	46.4 ± 5.6	1.8 ± 3.4	
Arm FM (kg)	3.8 (2.9-4.7)	4.6 (3.5-5.7)	0.8 (0.3-1.3)	26.0 (8.3-37.6)*
Arm (%FM)	11.2 (10.2-12.4)	11.6 (10.6-13.2)	0.5 (-0.1-1.1)	
Leg FM (kg)	14.1 (11.2-17.3)	15.6 (12.1-19.8)	1.8 ± 2.6	12.3 (1.0-25.4)*
Leg (%FM)	44.0 ± 5.5	41.8 ± 6.1	-2.2 ± 3.3	
VAT (cm^2)	120 (81 -163)	162 (122 -204)	41 (15-67)	41 (14-72)*
SAT (cm^2)	426 (285-536)	505 (382 -608)	70 (23-131)	16 (5-36)*

Data presented as means ± SD or median (25th-75th percentiles). BMI, body mass index; %FM (expressed as a percentage of sub-total fat mass); VAT, Visceral adipose tissue area; SAT, Subcutaneous adipose tissue area; WC, Waist circumference; WHR, Waist-to-hip ratio. *p<0.0001 and $^{\#}$p<0.05.

2.4.2 Differences in body composition between NGT and IGM/T2D groups at baseline and follow-up

Differences in body composition between the NGT and IGM/T2D groups at baseline and at follow-up, as well as change between baseline and follow-up, are presented in Table 2.2. Whereas weight, BMI, hip circumference, fat-free soft tissue mass, body fat mass and % body fat were not different between the two groups at baseline or follow-up, central adiposity measures, namely WC, WHR, trunk %FM and VAT, as well as arm %FM were significantly higher in the IGM/T2D compared to the NGT group at baseline and follow-up ($p<0.05$). In contrast, leg %FM was significantly lower in the IGM/T2D group than the NGT group at both time points. We found similar findings when the three glycaemic groups (i.e., NGT, IFG/IGT and T2D) were assessed independently; both T2D and IFG/IGT groups had higher trunk FM, VAT and lower leg FM than the NGT group (Appendix B, Table 2S1). Furthermore, the T2D group had higher waist and hip circumference, WHR, and arm FM than the NGT group, but these differences were not found between the IFG/IGT and NGT groups. When comparing the T2D and IFG/IGT groups, the T2D group had higher WHR and trunk FM (only significant at baseline) and lower leg FM than the IFG/IGT group (Appendix B, Table 2S1).

In both groups (i.e., NGT and IGM/T2D), all anthropometric measurements, subtotal FM and DXA-derived measures of central FM (trunk %FM, VAT and SAT) and arm % FM increased significantly between baseline and follow-up, (all $p<0.0001$), whereas lower body peripheral adiposity (leg %FM) decreased over time (all $p<0.0001$). Notably, menopausal status did not differ between groups with 39.8% vs 21.6% of the NGT vs. IGM/T2D groups being self-reported pre-menopausal, 9.9% vs 9.8 peri-menopausal and 59.3% vs 68.6% post-menopausal, respectively ($p=0.503$).

Table 2.2: **Body composition characteristics between the NGT and IGM/T2D groups at baseline and follow-up**

	NGT group (n=91)		IGM/T2D group (n=51)		ANOVA p-value		
	Baseline	**Follow up**	**Baseline**	**Follow-up**	**Group**	**Time**	**Group* Time**
Age (years)	41 (36-47)	53 (48-59)*	45 (39-49)	56 (51-60)*	0.049	<0.0001	0.160
Anthropometry							
Weight (kg)	75.6 (64.5-89.6)	83.0 (70.8-96.4)*	77.6 (69.5-87.5)	79.4 (72.6-100.2)*	0.383	<0.0001	0.363
BMI (kg/m^2)	30.8 (25.1-35.6)	33.4 (28.6-38.6)*	31.2 (27.3-34.8)	32.6 (28.8-38.1)*	0.459	<0.0001	0.304
WC (cm)	86.1 ± 10.7	98.0 ± 12.5*	92.0 ± 12.0^{aa}	102.7 ± 12.5^{a}*	0.007	<0.0001	0.269
Hip circumference (cm)	114.5 (105.6-123.0)	120.2 (110.5-129.0)*	112.5 (106.0-122.0)	116.4 (109.0-128.9)*	0.636	<0.0001	0.874
WHR	0.75 (0.70-0.80)	0.82 (0.77-0.85)*	0.81 (0.76-0.86)^{aa}	0.85 (0.81-0.90)^{aa}*	<0.0001	<0.0001	0.426
Body composition and body fat distribution							
Fat-free soft tissue mass (kg)	35.9 (31.4-39.2)	36.7 (32.8-39.8)	36.5 (33.4-40.2)	36.4 (33.4-40.4)	0.099	0.154	0.209
Body fat mass (kg)	31.7 (24.4 -40.2)	38.7 (29.5-48.3)*	32.9 (28.1-40.6)	35.7(31.6-47.8)*	0.571	<0.0001	0.280
Body fat (%)	46.7 (40.5-51.1)	51.1 (45.3-54.5) *	46.8 (43.2-49.7)	50.1 (45.9-53.5) *	0.655	<0.0001	0.586
Trunk %FM	43.2 ± 4.3	44.6 ± 5.2*	47.2 ± 4.2^{aa}	49.5 ± 4.8^{aa}*	<0.0001	<0.0001	0.151
Arm %FM	10.9 (9.9-11.9)	11.5 (10.5-12.5) *	11.7 (10.8-13.1)^{aa}	12.3 (10.7-14.0)^{a}	0.002	<0.0001	0.172
Leg %FM	45.6 (42.3-49.6)	42.9 (40.2-47.5)*	41.0 (37.3-44.1)^{aa}	38.5 (35.4-41.7)^{aa}*	<0.0001	<0.0001	0.244
VAT (cm^2)	102 (71 -153)	151 (104 -193)*	136 (103-171)^{aa}	174 (150-230)^{aa}*	<0.001	<0.0001	0.601
SAT (cm^2)	410 (274 -539)	520 (376-614)*	445 (322-535)	496 (382-608)*	0.558	<0.0001	0.135

Data presented as means ± SD or median (25th-75th percentiles). The NGT group (n=91) includes participants that remained NGT at follow-up. The IGM/T2D group (n=51) consist of participants that developed IFG/IGT and T2D over the 13 year follow-up period. BMI, body mass index; %FM (expressed as a percentage of sub-total fat mass); VAT, Visceral adipose tissue area; SAT, Subcutaneous adipose tissue area; WC, Waist circumference; WHR, Waist-to-hip ratio.

^{a}p<0.05, ^{aa}p<0.01 IGM/T2D group vs NGT group at baseline and/or follow-up.

*p<0.0001 Baseline vs follow-up within a defined group

2.4.3 DXA-derived body composition measures as predictors of IGM/T2D risk at follow-up

The ORs associated with developing IGM/T2D compared to remaining NGT at follow-up, determined by baseline and change in DXA-derived body composition measures, adjusted for the potential effects of age and body fat mass at baseline, are presented in Table 2.3. The baseline and change in body fat mass were not significantly associated with the risk of IGM/T2D at follow-up. In contrast, greater trunk FM at baseline was significantly associated with approximately 2-fold greater risk (OR: 1.92, 95% Confidence Interval (95% CI): 1.41-2.61) of developing IGM/T2D risk 13 years later, independent of baseline fat mass and age ($p<0.05$). Furthermore, baseline arm FM (upper body peripheral adiposity) was associated with greater odds of developing IGM/T2D at follow-up ($p<0.05$). Conversely, greater leg FM at baseline was associated with 45% reduced risk (OR: 0.55, 0.41-0.73) of developing IGM/T2D at follow-up, after adjusting for fat mass and age at baseline ($p <0.05$). Both baseline and change in VAT were independently associated increased risk (OR: 1.25, 1.10-1.42 and 1.12, 1.03-1.23, respectively) of developing IGM/T2D ($p<0.05$), whereas there were no associations with SAT. When both VAT and leg FM were included in the same model, baseline VAT showed a tendency for increased IGM/T2D risk, whereas the change in VAT was associated with increased risk of developing IGM/T2D, whereas baseline leg FM was independently associated with decreased IGM/T2D risk ($p <0.05$).

Similar findings were reported when the multinomial logistic regression was performed to assess the relative risk ratios associated with baseline and change in body fat and fat distribution measures in the progression of NGT to IFG/IGT and T2D, compared to remaining NGT at follow-up (Appendix B, Table 2S2).

When menopausal status at follow-up was included as a covariate in the models, baseline DXA-derived measures of central and peripheral adiposity including the change in VAT remained predictors of IGM/T2D risk at follow-up (Appendix B, Table 2S3). Menopausal status was not a contributor to IGM/T2D risk at follow-up (Appendix B, Table 2S3).

Table 2.3: **Body fat and fat distribution measures as predictors of IGM/T2D risk at follow-up**

Variables	Odds ratio	95% CI	p-value	Model p-value
FM (kg)				
Baseline fat mass (kg)	1.01	0.97-1.04	0.622	0.170
Change fat mass (kg)	0.99	0.93-1.04	0.605	
Trunk FM (kg)				
Baseline trunk FM (kg)	1.95	1.43-2.67	<0.0001	<0.0001
Change trunk FM (kg)	1.08	0.97-1.21	0.162	
Arm FM (kg)				
Baseline arm FM (kg)	2.73	1.39-5.38	0.004	0.004
Change arm FM (kg)	0.98	0.64-1.51	0.941	
Leg FM (kg)				
Baseline leg FM (kg)	0.55	0.41-0.73	<0.0001	<0.0001
Change leg FM (kg)	1.02	0.86-1.21	0.809	
VAT (10 cm^2)				
Baseline VAT (10 cm^2)	1.25	1.10-1.42	<0.0001	<0.001
Change VAT (10 cm^2)	1.12	1.03-1.23	0.010	
SAT (10 cm^2)				
Baseline SAT (10 cm^2)	1.02	0.96-1.08	0.573	0.201
Change SAT (10 cm^2)	0.98	0.94-1.03	0.437	
VAT (10 cm^2) and Leg FM (kg)				
Baseline VAT (10 cm^2)	1.14	0.98-1.32	0.082	<0.0001
Change VAT (10 cm^2)	1.13	1.01-1.27	0.037	
Baseline leg FM (kg)	0.65	0.47-0.90	0.008	
Change leg FM (kg)	0.91	0.74-1.11	0.362	

Data are presented as odds ratios, 95% confidence interval (CI) and p-values adjusted for age. Each model includes baseline body fat and fat distribution measures, the change in the body fat and fat distribution measures as predictor variables. Outcome variables include two groups categorised as either “0”, the NGT group (i.e. NGT participants, reference group (n=91)) or “1”, the IGM/T2D group (i.e. IFG/IGT and T2D participants, the exposure group (n=51)). All the body fat and fat distribution models were adjusted for the potential effects of age at baseline, and regional FM, VAT and SAT models were also adjusted for baseline body fat mass. FM, Fat mass; VAT, Visceral adipose tissue area; SAT, Subcutaneous adipose tissue area.

2.4.4 Associations between DXA-derived body composition measures, and markers of glycaemia at follow-up

The associations reported above were repeated using the continuous measures of glycaemia at follow-up, namely, HbA1c, HOMA2-IR and Matsuda Index (Table 2.4). Baseline body fat, trunk FM, leg FM and VAT, but not SAT and arm FM, were

associated with HbA1c (only body fat mass and trunk), HOMA2-IR and Matsuda Index at follow-up ($p<0.05$). In addition, only the change in VAT, and not trunk FM, was associated with HOMA2-IR (positive association) and both measures of central adiposity were negatively associated with Matsuda Index. In contrast, only the change in arm and not leg FM was associated with Matsuda Index at follow-up ($p<0.05$). Body fat mass accounted for a significant but small (4-7%) proportion of the variance in measures of glycaemia at follow-up, whereas measures of central and peripheral adiposity explained a greater proportion of the variance (8-18 % and 9-12%, respectively). When VAT and leg FM were combined in the same model, only baseline and change in VAT were associated with HOMA2-IR and Matsuda Index, accounting for similar variance to the individual models. These associations were independent of menopausal status at follow-up (Appendix B, Table 2S4).

Table 2.4: Regression coefficients from robust multiple linear models for the prediction of HbA1c, fasting insulin resistance (HOMA2-IR) and OGTT-derived insulin sensitivity (Matsuda Index) at follow-up

	HbA1c				Insulin resistance (HOMA2-IR)				Insulin sensitivity (Matsuda Index)			
	β	**95% CI**	**p-value**	**R^2**	**β**	**95% CI**	**p-value**	**R^2**	**β**	**95% CI**	**p-value**	**R^2**
Body fat mass (kg)												
Baseline fat mass (kg)	0.01	0.00-0.01	0.008	0.07*	0.03	0.01-0.05	0.002	0.07*	-0.07	-0.12- -0.02	0.005	0.04*
Change fat mass (kg)	0.00	-0.01-0.01	0.868		0.02	-0.01-0.05	0.280		-0.06	-0.15-0.02	0.155	
Trunk FM (kg)												
Baseline trunk FM (kg)	0.06	0.02-0.10	0.008	0.11*	0.25	0.12-0.38	<0.0001	0.15*	-0.63	-0.97- -0.29	<0.0001	0.10*
Change trunk FM (kg)	0.01	-0.01-0.03	0.332		0.05	-0.01-0.10	0.083		-0.15	-0.29- -0.01	0.034	
Arm FM (kg)												
Baseline arm FM (kg)	-0.02	-0.14-0.09	0.662	0.08	0.10	-0.25-0.44	0.583	0.08*	-0.38	-1.37-0.611	0.448	0.07*
Change arm FM (kg)	0.05	-0.03-0.12	0.203		0.15	-0.08-0.37	0.206		-0.72	-1.37- -0.07	0.031	
Leg FM (kg)												
Baseline leg FM (kg)	-0.04	-0.08-0.00	0.065	0.09*	-0.19	-0.31- -0.07	0.002	0.12*	0.47	0.15-0.78	0.004	0.07*
Change leg FM (kg)	-0.00	-0.03-0.03	0.983		0.04	-0.04-0.12	0.367		-0.10	-0.30-0.10	0.331	
VAT area (10 cm^2)												
Baseline VAT (10 cm^2)	0.02	-0.00-0.03	0.093	0.08*	0.11	0.06-0.16	<0.0001	0.18*	-0.23	-0.38- -0.09	0.001	0.11*
Change VAT (10 cm^2)	0.01	-0.01-0.02	0.454		0.08	0.04-0.12	<0.0001		-0.20	-0.31- -0.09	0.001	
SAT area (10 cm^2)												
Baseline SAT (10 cm^2)	0.00	-0.01-0.01	0.385	0.08*	0.01	-0.02-0.05	0.372	0.08*	-0.08	-0.18-0.01	0.093	0.09*
Change SAT (10 cm^2)	0.00	-0.00-0.01	0.356		0.02	-0.01-0.04	0.186		-0.09	-0.16- -0.02	0.013	
VAT are (10 cm^2) and Leg FM (kg)												
Baseline VAT (10 cm^2)	0.01	-0.01-0.03	0.353	0.10*	0.09	0.04-0.15	0.002	0.18*	-0.19	-0.36- -0.02	0.027	0.12*
Change VAT (10 cm^2)	0.00	-0.01-0.02	0.593		0.08	0.03-0.12	0.002		-0.16	-0.22- -0.54	0.007	
Baseline leg FM (kg)	-0.03	-0.07-0.02	0.269		-0.07	-0.20-0.06	0.306		0.16	-0.22-0.54	0.409	
Change leg FM (kg)	-0.00	-0.03-0.03	0.784		-0.03	-0.11-0.06	0.556		0.02	-0.21-0.24	0.863	

Data are presented as β-coefficients, 95% confidence intervals, R^2 for each model and p-values. Each model includes baseline body fat and fat distribution measures, the change in the body fat and fat distribution measures as predictor variables and HbA1c, fasting insulin resistance (HOMA2-IR) and OGTT-derived insulin sensitivity (Matsuda Index) measures at follow-up as the outcome variables. All the body fat and fat distribution models were adjusted for the potential effects of age at baseline, and regional FM, VAT and SAT models were also adjusted for baseline body fat mass. FM, Fat mass. VAT, Visceral adipose tissue area; SAT, Subcutaneous adipose tissue area. *P value for the model <0.05.

2.5 Discussion

We show for the first time that both the baseline and change in VAT predicted later development of IGM/T2D in middle-aged black SA women 13 years later. Moreover, only baseline, and not the change, in DXA-derived measures of central (e.g. trunk) and lower-body peripheral (leg) adiposity predicted IGM/T2D at follow-up. Specifically, baseline trunk FM was associated with a two-fold increased IGM/T2D risk, while leg FM was associated with ± 50% reduced risk of developing IGM/T2D.

The finding that both baseline and change in VAT predicted IGM/T2D risk in black African women is notable as previous studies in SA and the USA have shown that for the same BMI or WC, black women have less VAT and more abdominal and gluteal SAT (4,98,136,227,228) than their white counterparts, despite being more insulin resistant (4,228). Further, a number of studies showed that abdominal SAT, rather than VAT, was the major determinant of IR in black South African and African American women (17,227). However, all these studies were cross-sectional and included only pre-menopausal women (17,227). In this first longitudinal study, we show that both baseline VAT and the change in VAT over the 13-year follow-up period, and not SAT, were the major determinants of IGM/T2D. Put into context, we showed that a 10 cm^2 increase in VAT was associated with a 12% increase risk for IGM/T2D over a period of 13 years, suggesting that the average increase of 41 cm^2 in VAT in this study population over the 13 years increases the risk of developing IGM/T2D by ~ 50%. In line with this, we recently showed that obese black women were more sensitive to the effects of VAT on hepatic insulin sensitivity compared to obese white women (87). Based on these findings it is clear that VAT, despite lower levels than reported in white women, is also a major determinant of IGM/T2D in black women. Putative underlying mechanisms behind VAT accumulation and development of IR and T2D includes via its hyperlipolytic activity, pro-inflammatory profile, and due to its anatomical position, higher FFA influx directly into the portal circulation, resulting in hepatic IR (96,99,235,236).

Another important finding was that greater baseline trunk and arm FM and lower leg FM predicted IGM/T2D in black women. Indeed, we consistently showed higher central and

lower peripheral adiposity in the IGM/T2D group compared to the NGT group at baseline and follow-up. Similarly, when the three groups were analysed independently, the T2D and IFG/IGT groups had higher trunk FM, VAT and lower leg FM than the NGT group. Whether the three glycaemic groups are analysed independently or the IFG/IGT and T2D combined (i.e., IGM/T2D), the 'take-home" message is the same; having high central and low peripheral obesity at baseline predisposes individuals to the risk of developing T2D later in life.

Although the effects of trunk FM may be mediated via VAT, the protective effects of lower body peripheral adiposity against IGM/T2D risk may be explained by lower-body SAT acting as a "metabolic sink" that traps excess FFAs, thus inducing a favourable metabolic profile (96,97,236). Notably, the increase in FM over the 13-year follow-up period was characterised by a relative redistribution of fat from the gluteo-femoral region to abdominal region, with a subsequent increase in VAT. This was previously shown in a study including pre-menopausal black SA women, with the centralisation of fat being associated with reduced insulin sensitivity after a 5-year follow-up period (137). Previously, we have shown downregulation of adipogenic and lipogenic genes in the gluteal depot of obese black SA women (136), which may explain the relative redistribution of fat from the periphery to the central regions. Importantly, by using DXA we showed that VAT increased to a greater extent than SAT over time, which corroborates previous findings in pre-menopausal women (137), and may explain why both baseline and change in VAT were associated with reduced insulin sensitivity and predicted IGM/T2D, whereas the protective effects of leg FM were diminished in the presence of VAT. These findings suggest that peripheral and central adiposity are interlinked in the pathophysiology of T2D and that any disruption to their distribution exacerbates the chances of developing T2D.

The findings of our study of middle-aged women contrast to earlier data from pre-menopausal black women, in which VAT was not a major predictor of IR (137). It has been proposed that older women are at greater risk of developing T2D than younger women due to the effects of menopause (132,133,230). The transition from pre-menopause to post-menopause is characterised by a significant decrease in sex

hormones, mainly oestrogen (105,133), which may contribute to an increase in central obesity, in particular VAT (132,133,230). We found that menopausal status was a contributor to IR and insulin sensitivity at follow-up (Appendix B, Table 2S4). However, even after adjusting for menopausal status at follow-up, VAT was independently associated with markers of glycaemia and T2D risk. These findings suggest that other factors than menopause per se might be responsible for the accumulation of VAT in post-menopausal women. Indeed, several cross-sectional studies showed that VAT was associated with lifestyle factors such as smoking, diet and physical activity (44,133,134). Furthermore, frequent participation in sports activities was associated with almost 30% reduction in VAT in older (> 55 years) as well as in younger (< 40 years) women (44). Therefore, it is possible that a combination of physiological and lifestyle factors might be responsible for the increased central obesity, in particular VAT, over the 13 years, thereby predisposing this population to develop IGM/T2D.

This study has some strengths and weaknesses. Sample size was limited owing to failure to contact and recruit many of the participants at follow-up. The IGM and T2D participants were combined into one group (IGM/T2D) even though the two groups have different phenotypes (24). Notably, the body composition profile of the IGM group was closer to the body composition profile of the T2D group, suggesting that the IGM group were already at a high risk of developing T2D. Glucose and insulin were collected at a single time-point. We used surrogate measures of hepatic IR (homeostatic model assessment-IR) and peripheral insulin sensitivity (Matsuda Index), instead of the HEC. However, these measures have been shown to be good proxies of IR and insulin sensitivity (70,237). Furthermore, we did not adjust for lifestyles factors and sex hormones at baseline or the change in these factors over time. Moreover, menopausal status was only collected at follow-up. This study focussed on black SA women (and not men), being a high risk group for development of T2D and its complications (2,111).

2.6 Conclusion

Greater central fat mass, in particular VAT, and lower leg fat mass predicted the development of IGM/T2D in middle-aged black SA women 13 years later. These findings highlight the detrimental effects of fat distribution and VAT accumulation on T2D risk in black middle-aged women. Future studies are required to understand why black women are more sensitive to its effects on insulin sensitivity and T2D risk than white women (87). Prevention of central obesity is a key factor in reducing the development of T2D among middle-aged urban black SA women.

CHAPTER THREE

Hyperinsulinaemia in middle-aged black South African women: contribution of insulin secretion and clearance

3.1 Introduction

Type 2 diabetes is a multifactorial disease with a complex pathophysiology that appears to differ by ethnicity (1,238,239). Compared to Europeans, African populations present with higher levels of obesity and IR, which are often accompanied by hyperinsulinaemia (3–5). Hyperinsulinaemia is a biological condition that is characterised by greater insulin response to glucose, often exceeding that which is required to maintain normoglycaemia (18,52,57,58). It is caused by increased pancreatic beta-cell insulin secretion and/or reduced insulin clearance by the liver, and to a lesser extent by extra-hepatic tissues such as kidneys, muscle and adipose tissue (18,52,57,58). Classically, hyperinsulinaemia is viewed as a compensatory response to metabolic disorders such as obesity and/or IR (3,7,9,56–58). However, others have shown that hyperinsulinaemia is an independent predictor of T2D (81,82). Based on these findings, it is evident that the exact biological mechanisms underlying hyperinsulinaemia and its subsequent role in the development of T2D are not fully understood, but is an area of active research (9,18,57,58).

Regardless of age and sex, black Africans from the USA, Europe (i.e., UK) and Africa (i.e., West Africa and SA) have a hyperinsulinaemic response to glucose compared to their white European counterparts (3,5,18,53,80,116–118). Interestingly, the contribution of insulin secretion and clearance to hyperinsulinaemia appears to be inconsistent within participants of African Ancestry, with different results reported in studies on children (54,116–118), men (80) and women (18,53). Hyperinsulinaemia in African-American compared to European-American children has been linked to a combination of increased insulin secretion and reduced insulin clearance (116–118). In contrast, the hyperinsulinaemia in the adult study of black African male participants from West Africa and USA (80) was attributed to a reduction in insulin clearance rather than an increase in insulin secretion, while the hyperinsulinaemia in black African women from SA and USA has been primarily attributed to increased beta-cell function (3,5,9,18). However, in some of these studies C-peptide was not measured or in cases where C-peptide was measured, insulin clearance was not examined (3,5,9), thus the relative contribution of insulin secretion and clearance could not be determined. This is due to the fact that peripheral insulin levels reflect the amount of insulin secreted by the beta-cells and that

cleared by the hepatocytes (49,50). Unlike insulin, C-peptide does not undergo extraction, hence it is a preferred marker of endogenous pre-hepatic insulin secretion (49,50). Notably, is that there is a lack of studies investigating the contribution of insulin secretion and clearance to hyperinsulinaemia in black African women, in particular black African women from Africa (18,53). This is of great importance as black African women from SA and USA present with a complex T2D risk phenotype that differs to white European women, and is characterised by greater hepatic insulin sensitivity, lower VAT and liver fat accumulation, and greater peripheral adiposity, but paradoxically lower peripheral insulin sensitivity, higher insulin response and greater T2D risk and prevalence (3–5,18,53,87,136). Further, the biological mechanisms underlying hyperinsulinaemia in black Africans are poorly understood. Factors such as insulin sensitivity, body fat and fat distribution, liver fat accumulation, hyperlipidaemia and genetic factors, have been linked to hyperinsulinaemia through their association with insulin secretion and clearance in various studies conducted in USA and UK (41,43,57,58,61,83,110). These associations have not been explored in an African population, whose pathophysiology might differ to that of black Africans from the USA, due to genetic and region-specific factors such as lifestyle and environment factors. Investigating the associations of insulin sensitivity, body fat and fat distribution including liver fat accumulation with measures of insulin secretion and clearance may provide better insights into the role of hyperinsulinaemia in the pathophysiology of T2D in this high-risk population. Therefore, the aims of this study were to investigate the relative contribution of insulin secretion and clearance to hyperinsulinaemia in middle-aged black SA women, and to explore the associations of insulin secretion and clearance with insulin sensitivity, adiposity and FLI.

3.2 Materials and methods

3.2.1 Study design

This cross-sectional study was conducted between 2015 and 2016 and included 188 black female caregivers of the original BT20+ cohort, living in Soweto, Johannesburg (240,241). Inclusion criteria were as follows: [1] Female caregiver of the original cohort;

[2] provided stored serum samples and blood analyte data (criteria for the larger study (242)); [3] less than 65 years of age; [4] HIV negative on testing; [5] not currently pregnant or lactating.

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, M010556 (for Bt20+ cohort) and M150530 (see Appendix C for Ethics clearance certificate for the current study). The procedures and risks associated with the study were explained to the participants and they all signed the consent form prior to participation in the study. All testing procedures were performed at the South African Medical Research Council (MRC)/University of the Witwatersrand Developmental Pathways for Health Research Unit (DPHRU) at the Chris Hani Baragwanath Hospital in Soweto Johannesburg.

3.2.2 Body composition

The assessment of basic anthropometry (i.e., weight, height, and waist and hip circumference) including BMI, as well DXA-derived body fat and fat distribution has been previously described (241). In brief, weight and height were measured in participants wearing only lightweight clothing and without shoes, and were used to calculate BMI (weight (kg)/height (m^2)). BMI was classified according to WHO criteria; underweight (<18.5 kg/m2), normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), or obese (≥30 kg/m^2) (243). Furthermore, whole body composition was measured using DXA (Hologic Discovery-W (S/N 71201), Bedford, MA, USA; software version 13.4.2:7), and included subtotal FM (whole body minus head) and leg FM (expressed in kg and as a percentage of sub-total FM, %FM). Abdominal VAT and SAT were estimated from DXA (128).

3.2.3 Socio-demographic and behavioural factors

Participants completed a socio-demographic questionnaire in their language of choice (i.e., English, IsiXhosa, IsiZulu, SeSotho, Sepedi, SeTswana, TshiVenda, XiTsonga, SiSwati), in which measures of SES (i.e., housing density, asset index, household food

insecurity access scale, marital status, employment and education), behavioural factors, including smoking and alcohol intake were collected (244) (Appendix D).

3.2.4 Blood sampling and biochemical analysis

Blood samples were drawn at least 10 hours after an overnight fast for the subsequent determination of plasma glucose, serum insulin, C-peptide and lipid concentrations, as well as liver enzymes. Thereafter, all non-T2D participants (based on self-reported status and/or use of T2D medication) completed a 75g standard OGTT. Following the glucose ingestion, blood samples were taken at 10, 20 30, 60, 90 and 120 min and centrifuged at 3000 rpm for 10 min at 4 ◦C. The plasma was stored at −20◦ C for subsequent analysis of glucose concentrations, and the serum was stored at −80◦ C for the analysis of insulin and C-peptide concentrations. Plasma glucose concentrations and lipids were analysed on the Randox RX Daytona Chemistry Analyser using enzymatic methods (Randox Laboratories Ltd., London, UK). Serum insulin and C-peptide assays were analysed on the Immulite® 1000 Immunoassay System (Siemens Chemiluminescent Healthcare GmbH, Henkestr, Germany). Fasting serum was also used for the analysis of liver enzymes, namely, gamma-glutamyltransferase (GGT) and alanineamino- transferase (ALT). Liver enzymes were measured on a chemistry analyser using enzymatic and kinetic rate methods (Beckman Coulter Inc, Brea, California, USA). The FLI was calculated using GGT, BMI, WC and TGs in a simple algorithm (140). Briefly, a FLI less than 30 excludes the possibility of a fatty liver, whereas a FLI greater or equal to 60 is indicative of a fatty liver (140).

Participants were classified into three glycaemic categories; NGT (fasting glucose < 6.1 mmol/l and 2-h post glucose load < 7.8 mmol/l), IGM which included IFG (fasting glucose ≥ 6.1 and < 7.0 mmol/L and 2-h post glucose load < 7.8 mmol/l) and IGT (fasting glucose levels < 7.0 mmol/l and 2-h post glucose load ≥ 7.8 mmol/l and < 11.1 mmol/l), and T2D (fasting glucose ≥ 7.0 mmol/l and/or 2-h post glucose load ≥ 11.1 mmol/l) (24). Out of 188 participants, 68.6% were categorised with NGT, while 24.5% were classified with IGM and 6.9% were newly diagnosed with T2D.

3.2.5 Beta-cell function and insulin clearance parameters

Mathematical modelling of glucose and C-peptide concentrations during the OGTT was used to calculate beta-cell parameters (i.e., insulin secretion rates and β-CGS), based on a previously developed model by Mari et al. (74,75). Briefly, the Mari model aims to describe the relationship between insulin secretion and glucose concentration, as a sum of two components (74,75). The first component reflects the ability of beta-cells to secrete insulin in response to changes in plasma glucose concentrations, through a dose-response function. The dose-response function is represented by the β-CGS (the slope) parameter (74,75). Moreover, dose-response function is regulated by the potentiation factor, which takes into account the influence of physiological factors such as the incretin hormones, neural control and changes into plasma glucose concentrations after an oral ingestion (74,75). The second component of the model is regulated by the rate sensitivity, which refers to the magnitude of the beta-cell response to any given rate of change in plasma glucose.

Insulin secretion rates, at basal state and over the 2-hour OGTT were calculated by deconvolution of C-peptide concentration. The total insulin secretion rates were calculated as the incremental area under the curve (AUC), using the trapezoidal rule. Insulin secretion rates were adjusted for body surface area and age to account for glucose concentration variability between participants. Beta-cell glucose sensitivity was estimated by determining the mean slope of the insulin secretion rates over the observed glucose concentration range during the OGTT.

Insulin clearance was calculated as the ratio of insulin secretion to insulin levels at baseline and as $AUCinsulinsecretion_{120}/AUCinsulin_{120}$ during the entire 120 min OGTT. Hyperinsulinaemia was characterised as basal insulin levels and postprandial insulin response. Notably, postprandial insulin response was calculated as the ratio of $AUCinsulin_{120}/AUCglucose_{120}$ for the total OGTT. Insulin sensitivity was calculated using the Matsuda Index, as previously described (69).

3.3 Statistical analysis

The Shapiro-Wilk test was used to assess the distribution of continuous variables. Normally distributed data are presented as mean ± standard deviation (SD), skewed variables are represented as median (25th-75th percentile), and categorical data are presented as frequency (%). Differences in anthropometric, DXA-derived body fat and fat distribution, and biochemistry data including beta-cell parameters (i.e., insulin secretion and β-CGS) and insulin clearance, were compared between three glycaemic groups (i.e., NGT, IGM and T2D), using linear and robust linear regression analyses, as appropriate. Categorical variables were compared between the groups using the Chi-Square test or the Fisher exact test. Furthermore, robust linear regression analyses were used to assess the contribution of insulin secretion rate (model 1) and insulin clearance (model 2) to measures of hyperinsulinaemia: basal insulin levels and postprandial insulin response (i.e., $AUCins_{120}/AUCgluc_{120}$), after adjusting for age and glycaemic status. Due to collinearity between insulin secretion and clearance, the two variables could not be included in the same model. Robust linear regression analyses were also used to examine the associations of insulin sensitivity, body fat and fat distribution (i.e., VAT and leg %FM) and FLI, to measures of insulin secretion and clearance, in separate models. All the model analyses were adjusted for the potential effects of age and glycaemic status (i.e., NGT, IGM and T2D). All the incremental AUC were calculated using the trapezoidal rule. Data were analysed using Stata® (Version 13.1, Statcorp, College Station, TX, USA). A test was considered statistically significant if $p \leq 0.05$.

3.4 Results

3.4.1 Socio-demographic and behavioural factors by glycaemic groups

Participants' demographic and behavioural factors according to the glycaemic groups (NGT, n=129; IGM, n=46; T2D, n=13) are depicted in Table 3.1. Age was not different between any of the groups. Also, there were no significant differences in SES and behavioural factors (i.e., smoking and alcohol intake) between the three glycaemic groups (Table 3.1).

Table 3.1: Socio-demographic and behavioural factors of middle-aged black South African women by glycaemic groups

	NGT (n=129)	IGM (n=46)	T2D (n=13)
Age (years)	53 (48-58)	56 (50-60)	57 (53-59)
Housing density (persons/room)	0.9 (0.7-1.3)	1.0 (0.8-1.4)	0.8 (0.7-1.3)
Asset index (out of 12 items)	9 (7-11)	10 (7-11)	9 (7-11)
Household Food insecurity score	4 (0-9)	4 (0-9)	2 (0-8)
Marital status, n (%)			
Married	66 (51.2)	28 (60.9)	6 (46.2)
Unmarried	61 (48.8)	18 (39.1)	7 (53.8)
Highest level of education, n (%)			
Primary education	16 (12.4)	9 (19.6)	3 (23.1)
Some secondary education	65 (50.4)	22 (43.5)	4 (30.8)
Secondary completed (grade 12)	29 (22.5)	9 (19.6)	2 (15.4)
Tertiary education	19 (14.7)	8 (17.4)	4 (30.8)
Employment, n (%)			
Employment	72 (55.8)	21 (45.7)	8 (61.5)
Unemployed	57 (44.2)	25 (54.3)	5 (38.5)
Behavioral and lifestyle factors			
Smoking status, n (%)			
Never smoked	122 (94.6)	46 (100)	13 (100)
Current smoker	7 (5.4)		
Alcohol intake, n (%)			
Yes	35 (27.1)	10 (21.7)	3 (23.1)
No/Never	94 (72.9)	37 (78.3)	10 (76.9)

Data presented as means ± SD or median (25th-75th percentiles). HDL, High-density lipoproteins; LDL, Low-density lipoproteins; IGM, Impaired glucose metabolism; NGT, Normal glucose tolerance; T2D, Type 2 diabetes.

3.4.2 Basic anthropometry, body fat and fat distribution and biomarker characteristics according to glycaemic groups

Characteristics of the participants, based on glycaemic categories (NGT, IGM and T2D) are presented in Table 3.2. Both the IGM and T2D groups had higher WC than the NGT groups (P≤ 0.05). While body fat (expressed in kg and as a percentage) was not different between the glycaemic groups, both the IGM and T2D groups had lower leg (%FM) and higher VAT than the NGT group. The T2D group also had higher VAT compared to the IGM group (P≤ 0.05). Abdominal SAT was not significantly different between the three groups.

There no significant differences in HDL, LDL-cholesterol and total cholesterol between the three glycaemic groups (Table 3.2). In contrast, the IGM and T2D groups had higher fasting triglycerides than the NGT group (P≤ 0.05). Liver enzyme differences included ALT concentration which was significantly higher in the IGM compared to the NGT group, and GGT concentration which was also higher in the IGM and T2D groups than the NGT group (P≤ 0.05). The FLI was higher in the IGM and T2D groups compared to the NGT group (P≤ 0.05). Furthermore, the prevalence of FLI >60 (i.e., indicative of a fatty liver) in the entire cohort was 39% (data not shown). When classified according to the glycaemic groups, the percentage of participants with an FLI >60 in the NGT, IGM and T2D groups was 18.2%, 30% and 38.5%, respectively (Table 3.2).

Differences in the glucose, insulin and C-peptide response to the OGTT (Figure 3.1 A-C), and the corresponding modelled insulin secretion rates and β-CGS (Figure 3.2 A and B) between the 3 glycaemic groups, are presented in Figure 3.1 and Table 3.2. Basal glucose, insulin and C-peptide levels, and insulin secretion rate, were higher in the IGM and T2D groups compared to the NGT group (Table 3.2) (P≤ 0.05). The T2D and IGM groups also had lower postprandial insulin response during the 120minutes of the OGTT than the NGT group. Total insulin secretion rate was higher in the IGM group compared to the NGT group (P≤ 0.05). Both the IGM and T2D groups had lower beta-cell glucose sensitivity (β-CGS) than the NGT group (P≤ 0.05). The IGM and T2D groups had lower insulin sensitivity (Matsuda Index) than the NGT group (P≤ 0.05). Basal and total insulin clearance rates were not significantly

different between the three glycaemic groups (Table 3.2).

Table 3.2: Basic anthropometry, body fat and fat distribution and biomarker characteristics of middle-aged black South African women by glycaemic groups (n=188)

	NGT (n=129)	IGM (n=46)	T2D (n=13)
Anthropometry			
Height (m)	1.58 ± 0.06	1.58 ± 0.05	1.57 ± 0.06
Weight (kg)	84.5 ± 19.2	87.9 ± 16.2	96.5 ± 26.7
BMI (kg/m^2)	33.9 ± 7.5	35.3 ± 6.5	38.7 ± 8.6
BMI categories, n (%)			
Normal weight	17 (13.2)	1 (2.2)	
Overweight	23 (17.8)	7 (15.2)	1 (7.7)
Obese	89 (69.0)	40 (82.6)	12 (92.3)
WC (cm)	98.7 ± 13.5	102.6 ± 11.1^z	108.2 ± 14.7^x
Hip circumference (cm)	121.0 ± 14.8	122.4 ± 13.5	127.4 ± 17.1
WHR	0.82 ± 0.07	0.84 ± 0.05^z	0.85 ± 0.05
Body fat and fat distribution			
Body fat mass (kg)	39.0 (29.1-48.3)	40.3 (33.8-47.6)	40.0 (34.5-56.7)
Body fat (%)	50.7(45.6-54.1)	51.2 (46.8-53.3)	53.9 (45.6-54.2)
Leg FM (kg)	16.2 (12.4-20.0)	16.1 (12.7-20.0)	17.4 (12.1-21.0)
Leg (%FM)	43.5 ± 6.2	39.5 ± 4.3^z	37.4 ± 5.4^x
VAT area (cm^2)	152 ± 61	185 ± 54^z	227 ± 71^{xy}
SAT area (cm^2)	502 ± 168	515 ± 137	541 ± 139
Biochemistry data			
Lipid			
HDL-cholesterol (mmol/l)	1.3 (1.1-1.5)	1.1 (1.1.1-3)	1.1 (1.0-1.2)
LDL-cholesterol (mmol/l)	2.9 ± 0.9	3.0 ± 0.9	2.9 ± 0.9
Total cholesterol (mmol/l)	4.6 ± 3.9	4.7 ± 1.0	4.9 ± 1.1
Triglycerides (mmol/l)	0.8 (0.7-1.1)	1.0 (0.8-1.3)^z	1.4 (1.0-2.9)^x
Liver enzymes and Fatty Liver Index			
ALT (IU/L)	14 (12-17)	17 (13-23)^z	15 (13-18)
GGT (IU/L)	17 (13-23)	25 (18-34)^z	27 (17-30)^x
FLI	25 (7-50)	34 (22-63)^z	50 (39-94)^x
FLI <30, n (%)	64 (52.9)	17(47.5)	2 (15.4)
FLI >60, n (%)	22 (18.2)	12 (30.0)	5 (38.5)
Glucose metabolism, insulin secretion and clearance biomarkers			
Basal glucose (mmol/L)	4.7 (4.2-5.2)	5.3 (4.6-5.8)^z	7.2 (6.0-7.4)^{xy}
2-hour glucose (mmol/L)	6.0 (4.9-6.8)	8.8 (8.1-9.5)^z	12.6 (11.7-13.4)^{xy}

Basal C-peptide (pmol/L)	603.9 (452.9-782.1)	846.5 (636.9-1019.7)^{z}	1072.5 (643.5-1683.0)^{x}
Basal insulin (pmol/L)	61.5 (35.4-104.2)	98.3 (47.9-155.6)^{z}	124.3 (88.2-256.3)^{x}
$AUCinsu_{120}/AUCgluc_{120}$ (pmol/L)/(mmol/L)	331.5 (172.2-567.1)	170.5 (113.9-247.0)^{z}	81.0 (63.5-109.4)^{x}
Basal insulin secretion rate (pmol)/(min m^2)	75.6 (54.4-95.5)	108.6 (78.0-121.4)^{z}	141.7 (76.1-185.2)^{x}
Total insulin secretion rate (nmol m^2)	49.8 (38.6-65.9)	62.5 (49.2-83.6)^{z}	54.0 (48.3-59.9)
Beta-cell glucose sensitivity, β-CGS (pmol/(min m^2 mM))	157.6 (82.2-264.4)	111.0 (67.6-152.5)^{z}	50.6 (44.0-74.7)^{x}
Insulin sensitivity (Matsuda Index)	4.4 (2.6-6.1)	2.2 (1.6-4.6)^{z}	1.6 (1.0-2.1)^{x}
Basal insulin clearance (L min m^2)	1.2 (0.9-1.7)	1.2 (0.8-1.6)	0.8 (0.7-1.1)
Total insulin clearance (L min m^2)	0.9 (0.7-1.1)	0.9 (0.7-1.1)	0.7 (0.7-0.9)

Data presented as means ± SD or median (25th-75th percentiles). ALT, alanine aminotransferase, AUC, area under the curve; BMI, Body mass index; FLI, Fatty liver index; FM, Fat mass; GGT, Gamma-glutamyl transferase, IGT, Impaired glucose tolerance; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance test; SAT, Subcutaneous adipose tissue; T2D, Type 2 diabetes; VAT, Visceral adipose tissue; WC; Waist circumference; WHR, waist-to-hip ratio.

^{x}T2D vs NGT group (p≤0.05); ^{y}T2D vs IGM group (p≤0.05); ^{z}IGM vs NGT group (p≤0.05)

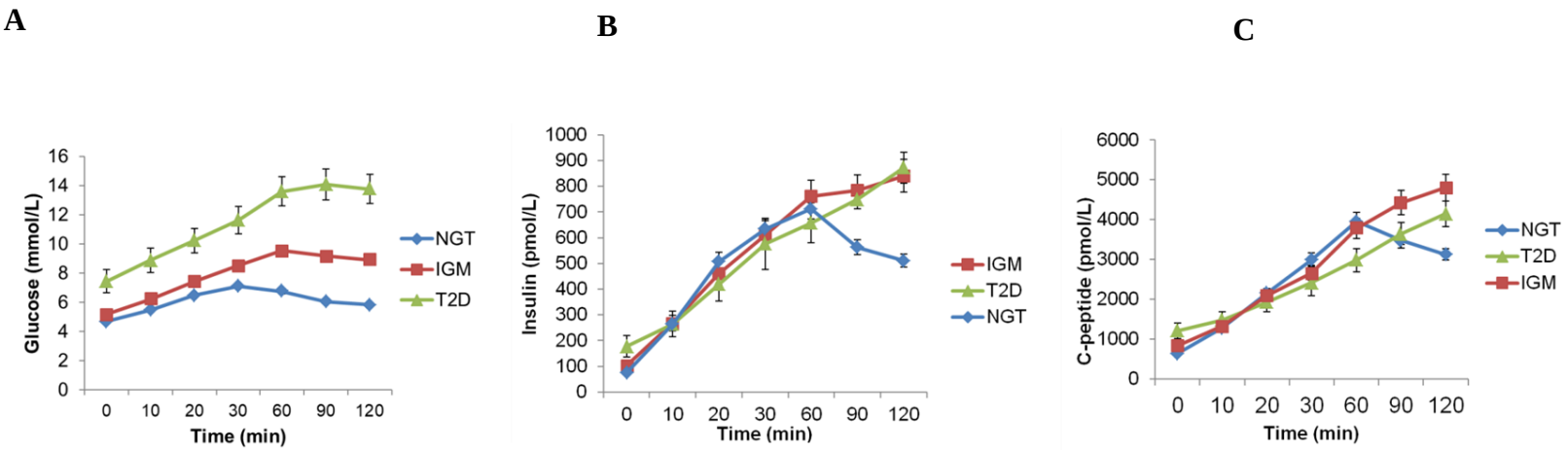


Figure 3.1: Represents plasma glucose **(A**), insulin **(B)** and C-peptide **(C)** serum concentrations during an oral glucose tolerance tests for the three glycaemic groups; Normal glucose tolerance (NGT) group, Impaired glucose metabolism (IGM) and type 2 diabetes (T2D) group. IGM, Impaired glucose metabolism; IS, Insulin sensitivity; NGT, Normal glucose tolerance; T2D, Type 2 diabetes. Data are mean ± SEM.

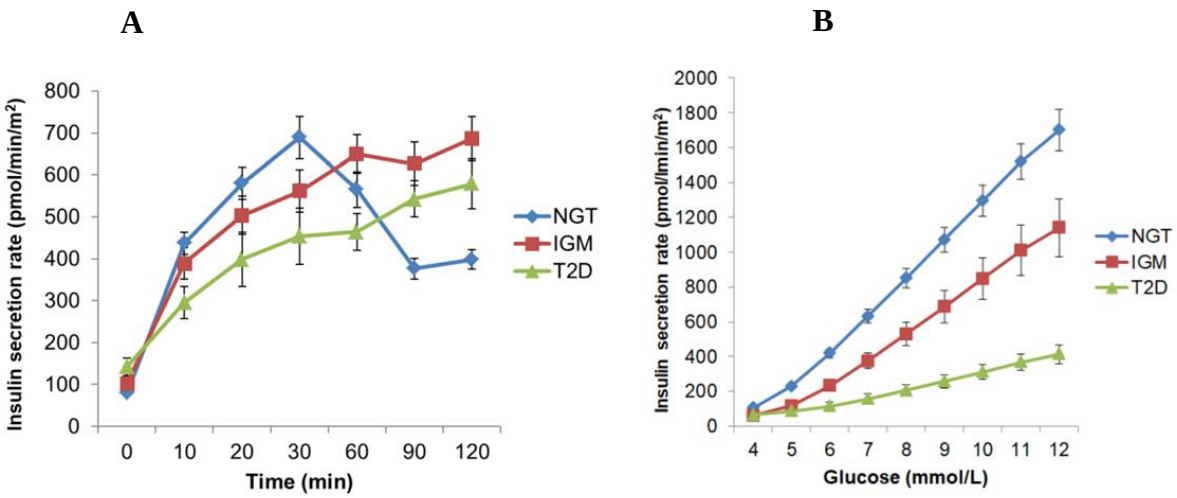


Figure 3.2: Represents insulin secretion rate **(A)** and β-CGS (presented as the slope mean slope of the insulin secretion rates over the observed glucose concentration range during the OGTT) **(B)**, calculated from the mathematical model during an oral glucose tolerance test (OGTT) for the normal glucose tolerance (NGT) group, impaired glucose metabolism (IGM) and type 2 diabetes (T2D) group. β-CGS, beta-cell glucose-sensitivity; IGM, Impaired glucose metabolism; IS, Insulin sensitivity; NGT, Normal glucose tolerance; T2D, Type 2 diabetes. Data are mean ± SEM.

3.4.3 Contribution of insulin secretion and clearance to basal and postprandial hyperinsulinaemia

The associations between insulin secretion and clearance (basal and total), and basal and postprandial hyperinsulinaemia (i.e., $AUCinsu_{120}/AUCgluc_{120}$), adjusted for age and glycaemic status, are presented in Table 3.3. Insulin secretion was positively, while insulin clearance was negatively associated with basal and postprandial measures of hyperinsulinaemia. In the basal state, insulin secretion explained a greater proportion of the variance in the outcome than the insulin clearance measure (Table 3.3). In contrast, during the 120 minutes of the OGTT, total insulin secretion and clearance accounted for a similar proportion of the variance, 12 and 15%, respectively in total postprandial insulin response.

Table 3.3: Contribution of insulin secretion and clearance to basal and postprandial hyperinsulinaemia ($AUCins_{120}/AUCglu_{120}$), explained by insulin secretion and clearance

	Basal insulin (pmol/L)			
	β	**95% CI**	**p-value**	**R^2**
Model 1:				
Basal insulin secretion rate (pmol)/(min m^2)	1.36	1.22-1.50	**<0.0001**	0.47*
Model 2:				
Basal insulin clearance (L min m^2)	-31.06	-36.70 - -25.42	**<0.0001**	0.37*
	$AUCinsu_{120}/AUCgluc_{120}$			
	β	**95% CI**	**p-value**	**R^2**
Model 1:				
Total insulin secretion rate (nmol m^2)	1.25	0.42-2.08	**0.003**	0.12*
Model 2:				
Total insulin clearance (L min m^2)	-167.70	-246.35 - -89.04	**<0.0001**	0.15*

Data are presented as β-coefficients, 95% confidence intervals (CI), R^2 for each model and p-values. Each model includes measures of hyperinsulinaemia in the basal state and over the total 120 minutes ($AUCins_{120}/AUCglu_{120}$) of the OGTT as the outcome variables, with insulin secretion and clearance (at basal state and total) as the independent variables. The models were adjusted for the potential effects of age and glycaemic groups (i.e., NGT, IGM and T2D). *P value for the model <0.05. AUC, Area under the curve; IGM, Impaired glucose metabolism; IS, Insulin sensitivity; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance test; T2D, Type 2 diabetes

3.4.4 Associations between insulin sensitivity, body fat and fat distribution, and FLI, and insulin secretion and clearance

The associations between measures insulin sensitivity (Matsuda Index), body fat and fat distribution (VAT and leg %FM), and FLI, and beta-cell function (i.e., insulin secretion and β-CGS) and insulin clearance, adjusted for age and glycaemic status, are presented in Table 3.4 (β-CGS data presented in Appendix B, Table 3S1). Both insulin sensitivity and leg %FM were inversely associated with basal and total insulin secretion. Total adiposity was only associated with basal insulin secretion rate, while FLI and VAT were positively associated with basal and total insulin section rate. Insulin sensitivity explained more of the variance in insulin secretion rate at both time points than any of the other factors. Moreover, insulin sensitivity was the only factor significantly associated with β-CGS (β=-7.58, p=0.001) (Appendix B, Table 3S1).

Insulin sensitivity was positively, and total adiposity, FLI and VAT were negatively associated with insulin clearance at baseline and 120 minutes. Leg %FM was not significantly associated with any of the measures of insulin clearance. Similar to insulin secretion, insulin sensitivity accounted for a higher proportion of the variance in the measures of insulin clearance compared to the measures of total and peripheral body fat, and FLI. Notably, FLI and VAT explained a similar proportion of the variance to measures of insulin secretion and clearance (Table 3.4).

Table 3.4: Associations between insulin sensitivity, body fat and fat distribution, and FLI, and insulin secretion and clearance (n=188)

	Basal insulin secretion rate (pmol)/(min m^2)				Basal insulin clearance (L min m^2)			
	β	**95% CI**	**p-value**	**R^2**	**β**	**95% CI**	**p-value**	**R^2**
Insulin sensitivity	-5.93	-7.06- -4.81	**<0.0001**	0.38*	0.20	0.18-0.22	**<0.0001**	0.26*
Body fat mass (kg)	0.78	0.44-1.12	**<0.0001**	0.17*	-0.01	-0.01- -0.003	**<0.0001**	0.08*
FLI	0.51	0.35-0.66	**<0.0001**	0.23*	-0.01	-0.01- -0.004	**<0.0001**	0.07*
VAT area (10 cm^2)	2.44	1.71-3.17	**<0.0001**	0.23*	-0.03	-0.04- -0.02	**<0.0001**	0.07*
Leg %FM	-1.95	-2.75- -1.15	**<0.0001**	0.18*	0.01	0.00-0.03	0.036	0.02#
	Total insulin secretion rate (nmol m^2)				**Total insulin clearance (L min m^2)**			
Insulin sensitivity	-2.87	-3.70- -2.05	**<0.0001**	0.18*	0.05	0.04-0.06	**<0.0001**	0.21*
Body fat mass (kg)	-0.02	-0.25-0.21	0.846	0.04*	-0.01	-0.01- -0.004	**<0.0001**	0.10*
FLI	0.13	0.02-0.24	**0.018**	0.07*	-0.003	-0.004- -0.002	**<0.0001**	0.09*
VAT area (10 cm^2)	0.81	0.32-1.30	**0.001**	0.08*	-0.01	-0.02- -0.01	**<0.0001**	0.07*
Leg %FM	-1.08	-1.60- -0.55	**<0.0001**	0.09*	-0.00	-0.01-0.00	0.317	0.02#

Data are presented as β-coefficients, 95% confidence intervals, R^2 for each model and p-values. Each model includes measures of insulin secretion and clearance (basal state and total) as the outcome variables, with insulin sensitivity (Matsuda index) and adiposity as independent variables. The models were adjusted for the potential effects of age and glycaemic groups (i.e., NGT, IGM and T2D). FLI, fatty liver index; FM, fat mass; VAT, visceral adipose tissue. *P value for the model <0.05. #P value for the model not significant (P>0.05). FLI, Fatty liver index; FM, Fat mass; IGM, Impaired glucose metabolism; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance test; T2D, Type 2 diabetes; VAT, Visceral adipose tissue.

3.5 Discussion

To our knowledge, this is the first study to investigate the relative contribution of insulin secretion and clearance to hyperinsulinaemia, and their associations with insulin sensitivity, adiposity and FLI (i.e., a proxy of liver fat) in a black SA population. We have shown that both insulin secretion and clearance were associated with hyperinsulinaemia, and while insulin secretion explained more of the variance at baseline and during the first phase insulin response, both insulin secretion and clearance contributed similarly to total hyperinsulinaemia during the 2-hour OGTT. We also found that there was a stronger association between insulin sensitivity and measures of insulin secretion and clearance compared to measures of body composition and the FLI. Another novel finding was that peripheral adiposity was only associated with measures of insulin secretion and not insulin clearance, while total and central adiposity measures, as well as FLI were associated with both insulin secretion and clearance.

Our findings that both insulin secretion and clearance contribute to hyperinsulinaemia are in accordance with a study in African-American pre-menopausal women with NGT who underwent an FSIGT (53), and studies in non-diabetic African-American adolescents, using testing methods such as the OGTT and/or hyperglycaemic clamp (116–118). However, our findings are in contrast to studies in other black African adults from USA and West Africa, in which hyperinsulinaemia was attributed to insulin clearance, rather than insulin secretion (7,18,61,80). However, in our study the contribution of insulin secretion and clearance was examined in one group (i.e., black SA women), whereas in these other studies the results reported were generated by comparing two ethnicities (7,18,61,80,116–118), with insulin clearance being a significant contributor to hyperinsulinaemia in black Africans. Despite this, inconsistencies between studies are notable. Reasons for these discrepancies are not fully understood but may be due to differences in glucose tolerance status, variation in IR and adiposity, as well as genetic and lifestyle factors between studies (41,58,61,64,83,110). The inconsistent findings could also be attributed to different methodologies or techniques (i.e., OGTT vs IVGTT, and insulin vs C-peptide levels)

used to measure insulin secretion and clearance. For example, studies which included participants with both NGT and IGT (7,18,61,80), insulin clearance was the only significant contributor to hyperinsulinaemia in black Africans. In contrast, studies in which only NGT participants were recruited (53), both parameters were significant contributors to hyperinsulinaemia. Glucose tolerance rarely exists on its own, as it is closely linked to insulin sensitivity and adiposity, which in turn, are associated with insulin secretion and clearance, as evidenced by the current and previous findings (41,57,58,61,64,83,110). The findings that insulin sensitivity had stronger associations with measures of insulin secretion and clearance compared to measures of body composition and the FLI needs to be interpreted with caution as all these risk factors are closely inter-linked and the nature of the relationship between IR and hyperinsulinaemia is still not fully understood, and is currently under investigation (58,119,120). Collectively, these findings suggest that hyperinsulinaemia is a complex and multifactorial condition.

Although adiposity and FLI had weaker associations with insulin secretion and clearance than insulin sensitivity, these results still highlight adiposity and FLI as important risk factors in the pathophysiology of hyperinsulinaemia and T2D risk. Obesity and ectopic fat deposition have been associated with increased T2D risk, while peripheral adiposity (i.e., leg %FM) has been linked to reduced disease risk in studies from SA and other countries (17,21,43,105,127,241). Although obesity has strong associations with IR and T2D risk, it is important to note that it is the accumulation of central obesity, in particular VAT, rather than total adiposity that mainly determines the risk for IR and T2D (17,21,105,241). Similar to VAT, liver fat accumulation has also been implicated in increased T2D risk via its association with hepatic insulin clearance and IR (41,43). Furthermore, it has been proposed that increased liver fat accumulation leads to the production and deposition of toxic lipid intermediates (e.g., FFAs and TG-VLDL), which in turn stimulate beta-cells and subsequently increase insulin secretion (42,43,83,86). However, gradual increase in FFA levels in the pancreas may inhibit insulin secretion and induce beta-cell apoptosis, leading to beta-cell failure and overt T2D (42,43,83,86). Interestingly, it has been shown that when matched for body fat, black African women accumulate less VAT and/or liver fat compared to white European women

(3,5,18,21,87,103,105). Despite the ethnic differences in VAT, VAT accumulation has been associated with adverse lipid profiles, insulin and IR (21,105). In contrast, the associations between liver fat and markers of T2D risk have been inconsistent (18,41,43,87,103,245). For example, liver fat was not associated with insulin clearance in either black men of West African origin or white European men (43), which corroborates findings reported by Utzschneider et al. (110) in a multi-ethnic cohort study, including both men and women participants. In contrast, in the current study, we showed significant associations between FLI and measures of insulin secretion and clearance, independent of age and glucose tolerance status. Although we used a proxy of liver fat, our findings are in accordance with the findings reported by Trico et al. (41), using liver fat that was measured by MRI. In this multi-ethnic cohort study, liver fat was inversely associated with insulin clearance and positively associated with hepatic IR, even after adjusting for age, plasma glucose concentrations, ethnicity and adiposity (41). Based on these inconsistent findings, it is evident that the role of liver fat accumulation in T2D risk is a complex phenomenon that requires further investigation. Of note, the current findings that VAT and FLI were associated with measures of insulin secretion and clearance imply that black SA women might be sensitive to the effects of VAT and liver fat accumulation, despite having lower VAT and liver fat accumulation compared to white European women (3,5,18,21,87,103,105). Future studies, in particular longitudinal study design are needed to verify the associations between VAT and liver fat accumulation, and insulin secretion and clearance in black African women.

The finding that peripheral fat was associated with measures of insulin secretion, and not clearance is notable. It is postulated that the non-significant association between leg %FM and measures of insulin clearance might be due to the fact that we were unable to distinguish between hepatic and extra-hepatic insulin clearance since we measured insulin clearance using peripheral C-peptide concentrations, which reflects both hepatic and extrahepatic insulin clearance (49,50,52). This is of great importance as hepatic rather than extra-hepatic insulin clearance has been shown to be a major contributor to hyperinsulinaemia in black Africans, specifically in African-American adolescents and women (18,53,80). The significant inverse association between peripheral adiposity and insulin secretion might be indirectly linked to its ability to act as a metabolic sink that

traps excess FFAs (97). To elaborate, previous research has shown that black SA women have greater peripheral adiposity and gluteal adipogenic and lipogenic gene expression compared to white SA women, which is associated with improved insulin sensitivity and reduced T2D risk (127,241). However, with increasing adiposity, the expression of these genes are lower in the gluteal depot of black SA women compared to white SA women and are associated with a relative redistribution of fat to the central depots (127), which increases the risk for IR and T2D. Together, these results support a close interaction between leg %FM, insulin sensitivity and beta-cell function. Since we did not measure FFA levels, future studies are needed to assess the role of FFA levels in hyperinsulinaemia and explore these associations in detail in black African women.

Although our current findings suggest that higher insulin secretion and lower insulin clearance, which are correlated with insulin sensitivity, adiposity and FLI, contribute to hyperinsulinaemia, it is important to note that this is a cross-sectional study and our results do not infer causality. Most importantly, the relationship between IR and hyperinsulinaemia is a very complex phenomenon that is still not fully understood. For instance, black Africans remain hyperinsulinaemic even after adjusting for adiposity (i.e., total and central) and insulin sensitivity (5,118). Therefore, hyperinsulinaemia may not merely be a compensatory response to obesity and IR. This means that factors other than adiposity and IR such as genetic susceptibility are likely to be initial drivers of hyperinsulinaemia and to have a more prominent role in the development of hyperinsulinaemia and T2D, particularly in Africans, as previously proposed (31,80). For example, a recent study in age, sex and BMI matched African-American and European-American cadavers, reported similar IDE and CEACAM 1 levels between the two ethnic groups (61). However, insulin degrading enzyme activity was lower in African-American cadavers compared to European-American cadavers (61). However, studies investigating these factors are very scarce, especially in black Africans (61,64) and further studies are required to investigate these factors in more detail.

The strength of the study was that we used a mathematical model of beta-cell function to investigate the pathophysiology of hyperinsulinaemia in a black sample from Africa. This model is a more reliable measure of beta-cell function than the IGI, which has been

previously used in the black SA population (9) as it uses C-peptide instead of insulin concentrations to better estimate endogenous pre-hepatic insulin secretion (74,75). Thus, it is able to quantify insulin secretion and clearance as distinct processes, thus enabling us to provide a better analysis of the relationship between insulin secretion and clearance, and glucose concentrations (74,75). Furthermore, we also used DXA to accurately measure body fat and fat distribution in order to better understand the association between body fat distribution and measures of insulin secretion and clearance. This is of particular relevance for these women who have the highest prevalence of obesity in SA, but have less VAT and more peripheral adiposity and higher T2D prevalence compared to their white European counterparts (3,17,21,87). This study has some limitations. We used an algorithm to estimate liver fat (140), instead of using an objective measure such as liver biopsies, MRI or $^{+}$H-MRS. Nonetheless, the FLI has been validated against ^{1}H-MRS and shown to identify those with and without fatty liver disease (141). Considering that black Africans often have a lower prevalence of fatty liver than their white European counterparts (18,41,87,103), a low prevalence of FLI was expected for this cohort. Moreover, the FLI performed as expected, with increase in FLI>60 from NGT to IGM to T2D. Nonetheless, the FLI still needs to be validated in black Africans. We also acknowledge the limitations of the study, which include a small sample size. The use of the OGTT, which does not differentiate between hepatic and extra-hepatic insulin clearance, which have been shown to be independently regulated (52). This is of relevance for this population, since it has been shown that hepatic insulin clearance is a major contributor to hyperinsulinaemia in Africans (52–54).

3.6 Conclusion

In conclusion, our results suggest that both insulin secretion and clearance contribute to hyperinsulinaemia in middle-aged black SA women. Overall, insulin secretion and clearance contribute similarly to total hyperinsulinaemia; however, their contribution in the basal state and during the first 30 minutes of the OGTT is mainly accounted for by insulin secretion. Our findings suggest that progression to IGM and T2D is associated with a reduced compensatory hyperinsulinaemia, mainly due to a decrease in insulin

secretion rather than insulin clearance. The exact mechanisms underlying these phenomena are not fully understood, but may be largely attributed to insulin sensitivity and to a lesser extent adiposity and FLI. Whether hyperinsulinaemia is a cause or consequence of IR requires further research, with more focus on genetic, longitudinal and intervention studies (e.g., diet) to explore the role of hyperinsulinaemia in the development of T2D in black African women.

CHAPTER FOUR

Adiposity Mediates the Association between the Dietary Inflammatory Index and Markers of Type 2 Diabetes Risk in Middle-Aged Black South African Women

Data from this chapter have been published in Mtintsilana A, Micklesfield LK, Chorell E, *et al* Adiposity mediates the association between the dietary inflammatory index and markers of type 2 diabetes risk in middle-aged Black South African women. *Nutrients* 2019; **11**: 1-19.

Author Contributions: Designed or conceptualised the study, A.M., L.K.M., A.P.K., and J.H.G.; Conducted the research, performed biochemical and statistical analyses and wrote the first draft of manuscript, A.M.; Principal investigators and acquired funding for the study, L.K.M., E.C., T.O., and J.H.G.; Supervised the study and the writing of the manuscript, L.K.M., and J.H.G.; Performed the E-DII score analyses, N.S.; Are part of the researchers from the University of South Carolina that developed the DII, N.S. and J.R.H.; Critically reviewed and commented on the manuscript, L.K.M., E.C., T.O., N.S., J.R.H., A.P.K., and J.H.G.

4.1 Introduction

The rapid increase in the prevalence of NCDs, such as T2D, has become a major health concern, particularly in developing countries, such as SA (2,226,246). South Africa had a diabetes prevalence of 5.5% in 2017 (246). In 2016, diabetes was the second leading cause of mortality in SA after tuberculosis, accounting for 5.5% of all deaths in both males and females (2). When classified by sex, diabetes-attributed mortality was worse in females (7.2%) than in males (4.0%), and ranked first and fifth as the cause of death in females and males in 2016, respectively (2). The prevalence of obesity and IR, major risk factors for T2D, is higher in black SA women than in white SA women (3,4,111). Of great concern is that urban black SA women are at even greater risk of NCDs compared to their rural counterparts (10,152). Urbanisation, which is often accompanied by unhealthy diets and low levels of physical activity, has been identified as the main driver of obesity in developing countries (11,150–152). Urban black SA women who follow a typical Western diet characterised by a high intake of fat, sugar and processed meat, and low intake of fruit and vegetables, are more susceptible to NCDs than rural black women who still follow a traditional diet, which is high in fibre, fruit, vegetables and low in animal products (150–152). The relationship between unhealthy diets and NCDs can be attributed to numerous factors, including diet-induced inflammation (155,163). Indeed, several studies suggest that unhealthy diets may be involved in the pathogenesis of NCDs through their effects on increasing low-grade inflammation (155,156,163), while healthier diets, such as the Mediterranean diet, which are high in fruits, vegetables and fish, are associated with lower levels of inflammation (155,163,247). Low-grade inflammation is characterised by high circulating levels of pro-inflammatory cytokines, such as CRP, TNF-α, interleukins (e.g., IL-6), and infiltration of macrophages in insulin-dependent tissues, which may lead to IR and, subsequently, to T2D (248,249).

The DII, is a literature-derived dietary tool that measures the inflammatory potential of an individual's diet (156). Participants with higher DII (i.e., positive values) scores are categorized as having a more pro-inflammatory diet and those with lower DII (i.e., negative values) score represent a more anti-inflammatory diet (156). The DII has been validated with several inflammatory markers, such as CRP and interleukins (157); and

has recently been associated with markers of glycaemia, namely, fasting glucose, HbA1c, post-load glucose and IR in non-African populations (154,160,161). Moreover, participants with higher DII scores were at greater risk of developing prediabetes and T2D compared to participants with lower DII scores (154,161). Notably, the association between DII and markers of T2D has not been studied in a black African population, whose lifestyle (i.e., diet and SES) differs from white European and Hispanic populations. SES (both low and high) and sociocultural factors have thus, through their effects on unhealthy diets and eating behaviours, been associated with NCD risk in black SA communities (13,121). Furthermore, the black SA population still experiences inadequate micronutrient intake (150,151), in particular micronutrients with putative anti-inflammatory properties, such as vitamin A, niacin, iron and zinc. In contrast, energy intake has not undergone any major change (150,151), suggesting that the quality of the diet, rather than the quantity of food consumed, and changes in energy expenditure may explain the high obesity levels, and, in turn, the susceptibility to T2D risk in urban black SA women. Use of the DII may contribute to our understanding of the role of diet-induced inflammation in the pathophysiology of T2D, while linking it to markers of low-grade inflammation and obesity. Indeed, the DII has been associated with markers of low-grade inflammation and measures of adiposity (i.e., BMI) and these risk factors have also been suggested to mediate the association between DII and markers of T2D in non-African populations (160).

The aim of this study was to determine whether dietary-induced inflammation, measured by the DII, is associated with markers of T2D risk, and if this association is mediated by adiposity and/or inflammatory markers, in middle-aged and older black SA women.

4.2 Materials and methods

4.2.1 Study design

This cross-sectional study was conducted between 2015 and 2016 and included 190 black female caregivers of the original BT20+ cohort, living in Soweto, Johannesburg (233). The female caregivers were invited to participate if they fulfilled the following

inclusion criteria: (1) Female caregiver of the original cohort; (2) provided stored serum samples and blood analyte data (criteria for the larger study); (3) less than 65 years of age; (4) HIV negative on testing; (5) not currently pregnant or lactating. Of those tested (n = 221), nine women were excluded from the statistical analyses, due to unreliable energy intake data (i.e., excessive energy intake (≥30,000 kJ), n = 4), lack of FFQ data (n = 2) and lack of blood samples (n = 3). Furthermore, 22 diabetic participants (based on self-report and/or use of T2D medication) were excluded from the analyses, resulting in a final sample size of 190 participants.

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, M010556 (for Bt20+ cohort) and M150530 (see Appendix C for Ethics clearance certificate for the current study). The procedures and risks associated with the study were explained to the participants and they all signed the consent form prior to participation in the study. All testing procedures were performed at the South African Medical Research Council (MRC)/University of the Witwatersrand (WITS) Developmental Pathways for Health Research Unit (DPHRU) at the Chris Hani Baragwanath Hospital in Soweto Johannesburg.

4.2.2 Body composition

Weight and height were measured in subjects wearing only lightweight clothing and without shoes, using a standard scale and stadiometer. Waist (level of umbilicus) and hip (largest gluteal area) circumferences were measured in triplicate and the average was used for statistical analyses. The International Diabetes Federation WC threshold (women: 80 cm) was used to calculate abdominal obesity (expressed as a percentage) (250). BMI was calculated as weight (kg)/height $(m)^2$ and classified according to WHO criteria; underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), or obese (≥30 kg/m^2) (243). Whole body composition was measured using dual energy x-ray absorptiometry (DXA; Hologic Discovery-W (S/N 71201), Bedford, MA, USA; software version 13.4.2:7), and included subtotal (whole body minus head) FM (expressed in kg and as a percentage of sub-total FM, %FM). Abdominal VAT was estimated using algorithms included in the DXA, and has been shown to correlate with

clinical CT (128). Body composition of participants exceeding the scan field limits was calculated using the arm-replacement (234). The coefficient of variations were 1% and <2% for fat-free soft tissue mass and total fat mass, respectively.

4.2.3 Socio-demographic assessment

A demographic questionnaire, written in English, was administered in the participant's language of choice (i.e., English, IsiXhosa, IsiZulu, SeSotho, Sepedi, SeTswana, TshiVenda, XiTsonga, SiSwati) and included measures of SES, including housing density, asset index, household food insecurity, marital status, educational assessment, and employment (Appendix D). Housing density was defined as the number of persons per room living in the household. Asset index was based on 12 items (e.g., motor vehicle, washing machine, TV) reflecting individual and household wealth. Food insecurity was assessed using a validated nine question Household Food Insecurity Access Scale (251). The responses to the nine-questionnaire scale are summed to create a food security score, with a maximum score of 27. The higher the score the more food insecurity the household experienced, whereas the lower the score the less food insecurity experienced in the past four weeks (251). Education was categorized by grades passed. Participants were categorized as being married or not, employed or unemployed. Moreover, questions on lifestyle factors, including smoking and alcohol intake were recorded. Physical activity and sedentary behavior were measured using ActiGraph GT3X-Plus triaxial accelerometers (ActiGraphGTX3+, ActiGraph LLC, Pensacola, Florida) and activPAL devices (activPAL3c, PAL Technologies Ltd., Glasgow, UK), respectively, as described previously (252).

4.2.4 The DII®

The DII scores for all the participants in the current study were calculated from the dietary intake data collected using a seven-day food frequency questionnaire (253,254). The development and validation of the DII have been described in detail elsewhere (156,157). The DII is a literature-derived, validated tool used to measure the inflammatory potential of the diet based on extensive literature search performed to

identify peer-reviewed primary research articles published through 2010 that reported the association between various dietary factors and six inflammatory markers (IL-1β, IL-4, IL-6, IL-10, TNF-α, and CRP). A total of 1943 qualifying articles were reviewed, indexed, and scored to derive the component-specific inflammatory effect score for 45 dietary factors (i.e., components of DII), comprising macronutrients and micronutrients, as well as some bioactive components (156). These FFQ-derived food and nutrient intake data were first adjusted for total energy (i.e., per 1000 kilocalories) and standardized by creating a z score for each component using mean and standard deviation data from a global energy-adjusted dietary database comprised of dietary intake from 11 populations living in different regions of the world. To reduce the effect of right-skewing the z scores for each DII component were converted to proportions (i.e., values from 0 to 1) and then each proportion was centred by doubling and subtracting 1. From this, the energy-adjusted standardized dietary intake (expressed as centred proportions) was then multiplied by the corresponding literature-derived inflammatory effect score for each DII component. Individual scores from each DII component were then summed to determine the overall E-DII score for each individual (156). A higher (i.e., more positive) score indicates a more pro-inflammatory diet and a lower score (i.e., more negative) represents a more anti-inflammatory diet. The DII scores can range between 7.98 (maximally pro-inflammatory) and -8.87 (maximally anti-inflammatory) (156). Steps involved in the DII calculations are described in Figure 4.1.

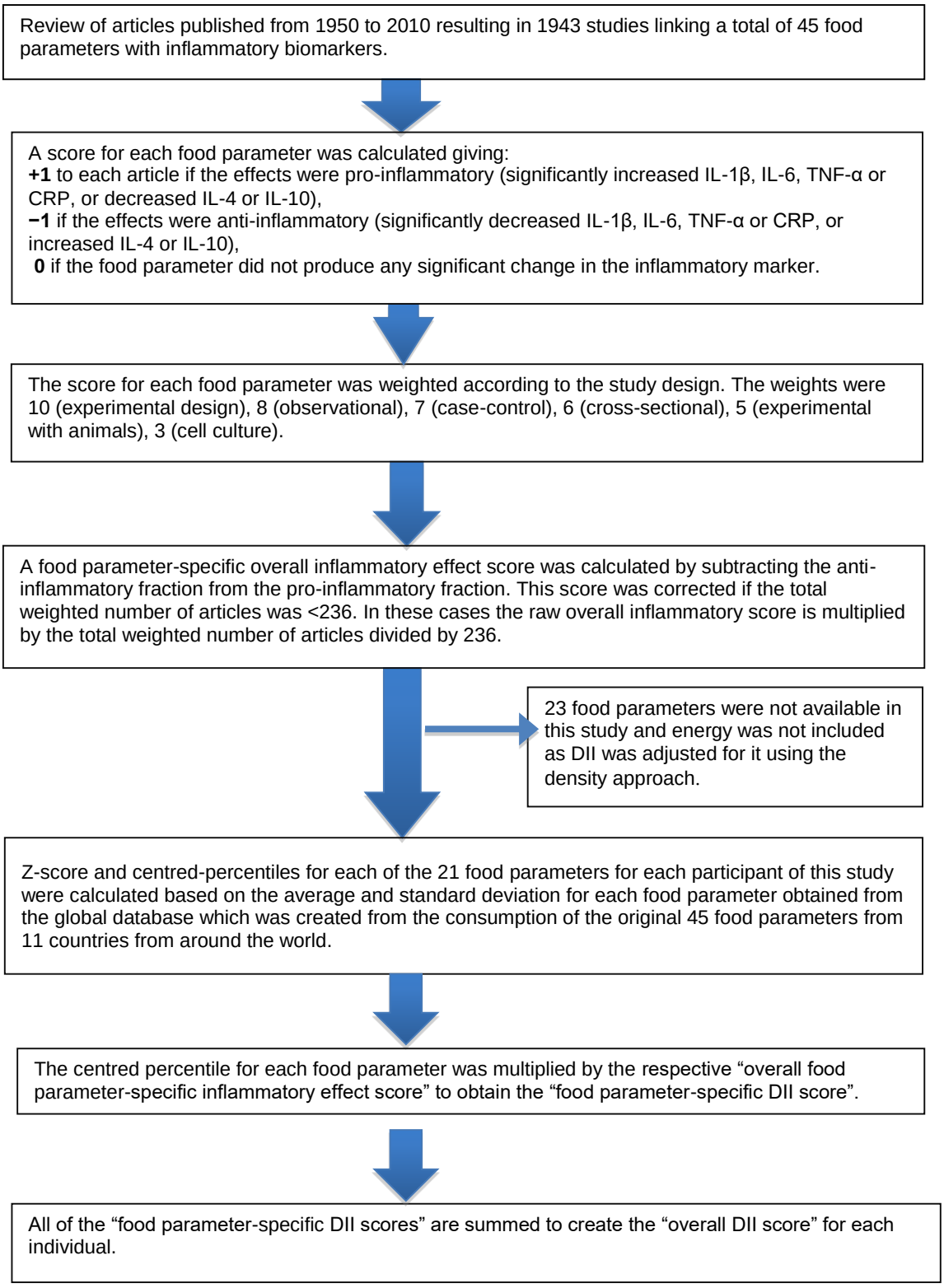


Figure 4.1: Sequence of steps in creating the energy-adjusted- DII (dietary inflammatory index) in middle-aged black South African women.

For the current study, a total of 21 out of 45 food parameters were available in our dietary intake database, and were thus used for the analysis of the DII score. The 21 food parameters included protein, fat, saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid , carbohydrate, cholesterol, alcohol, fibre, iron, magnesium, Zinc, vitamin A (retinol equivalent), thiamine, riboflavin, niacin, vitamin B6, vitamin B12, vitamin C, vitamin D and vitamin E. Participants with E-DII scores greater than 0 were classified as the positive E-DII group (reflecting pro-inflammatory diet; *n* = 83) and those with E-DII scores less than 0 were classified as negative E-DII group (indicating anti-inflammatory diet; *n* = 107). There were no participants with E-DII score equal to 0.

4.2.5 Biochemical analysis

Blood samples were drawn in the fasted state (12 hours, overnight) for the subsequent determination of glycated haemoglobin (HbA1c), plasma glucose and serum insulin concentrations. Thereafter, all participants completed a standard oral glucose tolerance test (OGTT). In brief, participants ingested 75g of anhydrous glucose dissolved in 250 mL water, within 5 minutes. Following the glucose ingestion, blood samples were taken at 30, 60, 90 and 120 min. The samples were centrifuged at 3000 rpm for 10 min at 4 °C, the plasma was stored at −20 °C for subsequent analysis of glucose concentrations, and the serum was stored at −80 °C for the analysis of insulin concentrations and inflammatory markers. Plasma glucose concentrations were analysed on the Randox RX Daytona Chemistry Analyser using enzymatic methods (Randox Laboratories Ltd., London, UK). Serum insulin assays were analysed on the Immulite® 1000 Immunoassay System (Siemens Chemiluminescent Healthcare GmbH, Henkestr, Germany). HbA1c levels were measured on whole blood samples using the D-10™ Hemoglobin Analyser (Bio-Rad Laboratories, Inc. Hercules, California, USA). Inflammatory cytokines, namely, IL-6, IL-1ra, IL-8, IL-10, IL15, monocyte chemotactic protein (MCP)1, interferon (IFN) gamma, and TNF-α were measured using Milliplex MAP MAG Human Cytokine kit (Merck, Johannesburg, South Africa) and xMAP technology (Luminex, Austin, Texas) according to the manufacturer's instructions. Out of the eight cytokines measured, only TNF-α, 1L-8 and MCP-1 were detectable in all the samples, while the rest were below the detectable range, and therefore not included in the analyses.

Insulin resistance was calculated from fasting glucose and insulin using the HOMA2-IR calculator v2.2.3 (68). Fasting and OGTT glucose and insulin data (0, 30, 60, 90 and 120) were used to calculate insulin sensitivity using the Matsuda Index web calculator [(69)]. Participants with missing glucose and insulin data from the OGTT (*n* = 17) were excluded from this parameter.

4.3 Statistical analysis

The Shapiro-Wilk test was used to assess the distribution of continuous variables. Normally distributed data are presented as mean ± standard deviation (SD), skewed variables are represented as medians and 25th–75th percentiles, and categorical data are presented as frequency (%). Moreover, Levene's robust test was used to test for equal variance between groups. Differences in measures of SES, lifestyle/behavioral factors, body composition and markers of T2D risk between the positive and negative E-DII groups were examined using the Student's t test and the Wilcoxon rank-sum (Mann-Whitney), or the Chi square test or Fisher exact test as appropriate. To determine whether the E-DII was associated with markers of T2D risk (fasting glucose and insulin, HbA1c, HOMA2-IR, two-hour glucose and OGTT-derived insulin sensitivity), or whether the association was mediated by adiposity (WC, BMI, VAT and body fat mass) and/or inflammatory markers (TNF-α, IL-8 and MCP-1), a simple mediation analysis was completed (Figure 4.2a,b) (255). In each model, an independent, a dependent and a mediator variable were included in order to calculate the unstandardized regression coefficients and generate three outputs, namely, total, direct and indirect effects [(255)]. Path c, represents the simple total effect of E-DII on markers of T2D without adjusting for mediators (Figure 4.2a). Path α represents the regression coefficient between the independent variable (i.e., E-DII) and the mediator variable (e.g., VAT) (Figure 4.2a). The regression coefficient of Path β represents the effect of the mediator variable on the markers of T2D (Figure 4.2b). The product of regression coefficients α and β (αβ) represents the mediated effect (indirect effect) of E-DII on markers of T2D through the mediator variable (Figure 4.2b). Path c' represents the direct effect of E-DII on markers of T2D after controlling for the effect of the mediator (Figure 4.2b).

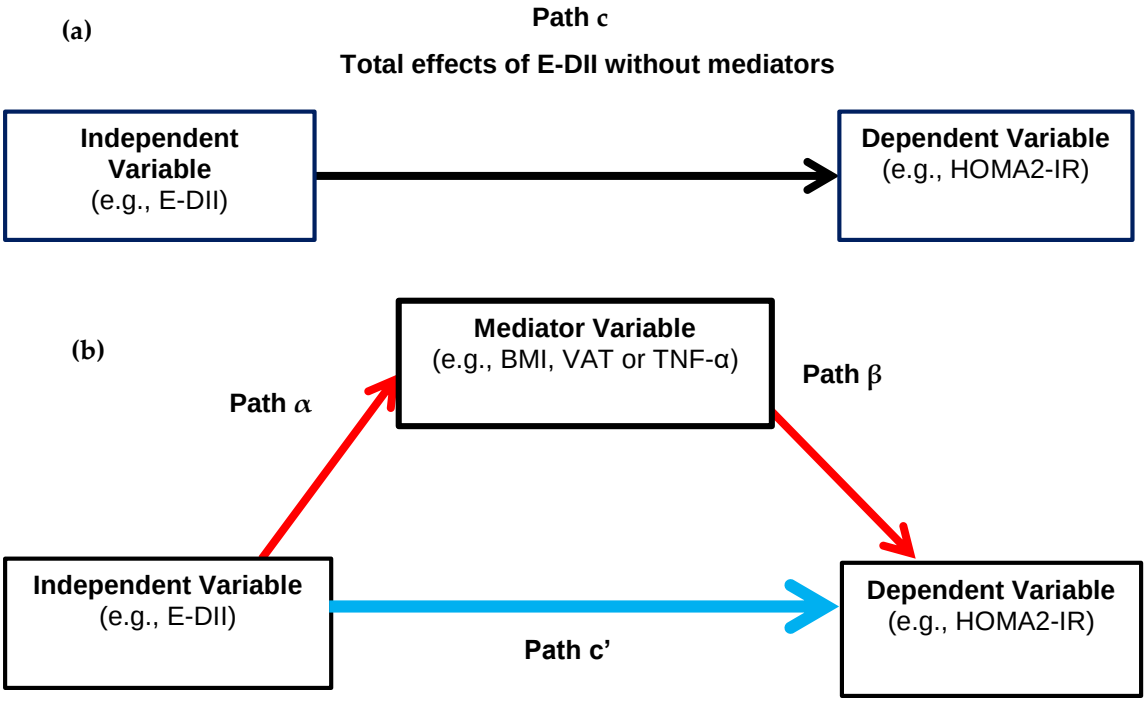


Figure 4.2: Represent a single mediator model used to test the association between the energy-adjusted-dietary inflammatory index (E-DII) (i.e., independent variable**)** and markers of type 2 diabetes (T2D) (i.e., dependent variable), body composition and inflammatory cytokines as mediators**: (a)** Path c, represents the simple total effect of E-DII on markers of T2D, without adjusting for mediators; (**b**) Represents the direct (Path c') and indirect effects (product of Paths α and β, αβ) of E-DII on markers of T2D after controlling for the effect of the mediator. BMI, Body mass index; E-DII, Energy-adjusted-dietary inflammatory index; HOMA2-IR, Homeostasis Model Assessment- insulin resistance; TNF-α, Tumour necrosis factor alpha; VAT, Visceral adipose tissue.

4.4 Results

4.4.1 Participants' characteristics according to E-DII score

Characteristics of the participants are summarised according to negative (n=107) and positive (n=83) E-DII scores, and presented in Table 4.1 The median (25th–75th percentiles) age of the total sample was 53 (48–59) years. The E-DII ranged from +1.92 (most pro-inflammatory) to −2.51 (most anti-inflammatory). Participants were grouped based on whether they had a positive (*n* = 83; 43.7%) or negative (*n* = 107; 56.3%) E-DII score (Table 4.1).

Table 4.1: Body composition and clinical characteristics of the participants according to E-DII scores.

	Overall	**Negative E-DII Group (*n* = 107)**	**Positive E-DII Group (*n* = 83)**	***p* Value**
E-DII score (minimum-maximum value)	−2.51–1.92	−2.51–0.01	0.01–1.92	
E-DII score	−0.2 ± 1.0	−0.9 ± 0.6	0.6 ± 0.5	
Age (years)	53 (48–59)	54 (49–59)	54 (49–60)	0.87
Anthropometry				
Height (m)	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.93
Weight (kg)	84.7 (73.6–96.8)	84.0 (70.4–94.2)	87.2 (76.4–100.2)	0.15
BMI (kg/m^2)	33.5 (29.8–38.8)	33.5 (29.0–37.2)	34.2 (30.2–40.1)	0.23
BMI categories, *n* (%)				
Normal weight	18 (9.5)	10 (9.4)	8 (9.6)	0.46
Overweight	30 (15.8)	20 (18.7)	10 (12.1)	
Obese	142 (74.7)	77 (72.0)	65 (78.3)	
WC (cm)	100.1 ± 13.0	98.3 ± 12.3	102.4 ± 13.7	0.03
WC-abdominal obesity, *n* (%)				
WC (≥80 cm)	177 (93.2)	100 (93.5)	77 (92.8)	0.85
WC (<80 cm)	13 (6.8)	7 (6.5)	6 (7.2)	
Hip circumference (cm)	121.8 ± 14.5	121.0 ± 14.3	122.9± 14.7	0.21
WHR	0.82 ± 0.06	0.81 ± 0.06	0.83 ± 0.07	0.04

Body composition and body fat distribution				
Body Fat mass (kg)	39.6 (31.5 –48.2)	38.7 (29.8–46.6)	40.9 (33.1–50.8)	0.12
Body fat (%)	50.7 (46.2–54.0)	50.1 (44.8–53.8)	51.5 (47.0–54.6)	0.09
VAT area (cm^2)	165 ± 62	155 ± 59	178 ± 64	0.01
SAT area (cm^2)	507 ± 157	495 ± 154	521 ± 161	0.27
Clinical biomarkers				
Fasting glucose (mmol/L)	4.9 (4.3–5.5)	4.8 (4.3–5.4)	5.1 (4.3–5.7)	0.07
Fasting insulin (mUI/mL)	10.4 (5.7–18.0)	9.8 (5.7–15.7)	11.7 (5.9–18.9)	0.27
Two-hour glucose (mmol/L)	6.7 (5.3–8.1)	6.3 (5.3–7.8)	7.2 (6.0–8.6)	0.02
HbA1c (%)	5.5 (5.1–5.7)	5.4 (5.1–5.7)	5.5 (5.2–5.8)	0.48
HOMA2-IR	1.5 (0.8–2.6)	1.4 (0.8–2.2)	1.7 (0.8–2.7)	0.23
Matsuda Index (OGTT-derived insulin sensitivity)	3.6 (2.1–6.0)	3.9 (2.4–6.1)	2.9 (1.9–5.6)	0.10
TNF-α (pg/mL)	11.0 (8.0–15.1)	9.9 (7.8–14.2)	12.1 (8.7–16.7)	0.03
IL-8 (pg/mL)	5.4 (2.4–11.4)	4.8 (2.6–8.9)	6.3 (2.2–12.6)	0.16
MCP-1 (pg/mL)	436.2(325.2–627.5)	449.9 (321.2–664.7)	422.6 (330.1–595.7)	0.71

Data presented as means ± SD or median (25th–75th percentiles). BMI, Body mass index; DII, Dietary inflammatory index; HbA1c, Glycated haemoglobin; HOMA2-IR, Homeostasis Model Assessment- insulin resistance; IL-8; Interleukin-8, MCP-1, Monocyte chemotactic protein 1; OGTT, Oral glucose tolerance test. SAT, subcutaneous adipose tissue; TNF-α, Tumour necrosis factor alpha; VAT, Visceral adipose tissue; WC, Waist circumference; WHR, Waist-to-hip ratio.

While BMI and total body fat did not differ between groups, WC, WHR and VAT were higher in the positive E-DII group compared to the negative E-DII group. Furthermore, participants with a positive E-DII score had higher two-hour glucose and TNF-α levels than those with a negative E-DII score ($p < 0.05$). There were no significant differences in any of the fasting or OGTT-derived measures of T2D risk, and inflammatory markers (IL-8 and MCP-1) did not differ between the groups (Table 4.1).

Comparisons of measures of SES and lifestyle/behavioral factors, and nutrient intakes between the two E-DII score groups are presented in Appendix B, Tables 4S1 and 4S2, respectively. There were no differences in any of the measures of SES and lifestyle/behavioral factors, including physical activity and sedentary behaviours between the two E-DII score groups (Appendix B,

Tables 4S1). A median score of 9 out of 12 possible household items (e.g., motor vehicle and washing machine) was reported for the participants. Furthermore, the participants scored an average of 4 (range: 0–9) on the Household Food Insecurity Access Scale, indicating that the participant/household experienced less food insecurity for the measured period. Energy and carbohydrate intake (except when carbohydrate is expressed as a percentage of energy intake), alcohol and cholesterol levels did not differ between groups (Appendix B, Table 4S2). In contrast, protein was lower, but total fat, SFA, MUFA and PUFA (both in grams and percentage of energy intake) were higher in the positive E-DII score group than the negative E-DII score group (all $p < 0.05$) (Appendix B, Table 4S2). Vitamins A, B12 and D, and riboflavin did not differ between the groups, whereas fibre intake and the rest of the minerals (e.g., iron, magnesium and zinc) and vitamins (e.g., vitamin B6, C and E) were lower in the positive DII score compared to the negative DII score group ($p < 0.05$) (Appendix B, Table 4S2).

4.4.2 Associations between E-DII and markers of T2D risk in black SA women

The unstandardized regression coefficients of the total effect of E-DII on markers of T2D risk without (Path c) and with body composition and inflammatory cytokines as mediators (Path c') are presented in Table 4.2 and Table 4.3, respectively. All the models were adjusted for the potential effects of age. E-DII was positively associated with all markers of T2D risk, namely, fasting glucose and insulin, HbA1c, HOMA2-IR, two-hour glucose levels and Matsuda Index (i.e., OGTT-derived insulin sensitivity) (all $p < 0.05$).

Table 4.2: Direct and indirect effects of E-DII on markers of T2D risk with body composition as mediators in black South African (SA) women.

Variables	Total Effect (c)		Direct Effect (c')		Indirect Effect (αβ)		Proportion of Mediation
Mediators and Outcomes	**Estimate (95%CI)**	***p*-Value**	**Estimate (95%CI)**	***p*-value**	**95%CI**	***p*-value**	**%**
Fasting glucose (mmol/L)	0.17 (0.01–0.34)	0.04					
via WC (cm)			0.12 (−0.07–0.32)	0.21	0.04 (−0.03–0.12)	0.25	23.5

via BMI (kg/m^2)			0.13 (−0.05–0.32)	0.16	0.04 (−0.03–0.10)	0.28	23.5
via VAT (cm^2)			0.07 (−0.10–0.24)	0.42	0.10 (0.00–0.20)	0.04	58.8
via body fat mass (kg)			0.14 (−0.05–0.33)	0.14	0.03 (−0.03–0.08)	0.371	17.6
Fasting insulin (mUI/mL)	1.70 (0.45–2.96)	0.008					
via WC (cm)			0.38 (−0.87–1.63)	0.55	1.32 (0.40–2.25)	0.005	77.6
via BMI (kg/m^2)			0.71 (−0.51–1.93)	0.26	0.99 (0.20–1.78)	0.01	58.2
via VAT (cm^2)			0.08 (−1.19–1.35)	0.90	1.62 (0.60–2.64)	0.002	95.3
via body fat mass (kg)			0.89 (−0.30–2.09)	0.14	0.81 (0.11–1.51)	0.02	47.6
HbA1c (%)	0.10 (0.01–0.18)	0.02					
via WC (cm)			0.07 (−0.02–0.15)	0.14	0.03 (−0.00–0.06)	0.07	30.0
via BMI (kg/m^2)			0.08 (−0.01–0.16)	0.08	0.02 (−0.01–0.05)	0.13	20.0
via VAT (cm^2)			0.05 (−0.02–0.14)	0.18	0.04 (0.00–0.08)	0.04	40.0
via body fat mass (kg)			0.08 (−0.00–0.17)	0.06	0.01 (−0.01–0.04)	0.19	10.0
HOMA2-IR	0.25 (0.07–0.44)	0.008					
via WC (cm)			0.05 (−0.15–0.26)	0.59	0.20 (0.05–0.35)	0.009	80.0
via BMI (kg/m^2)			0.10 (−0.09–0.30)	0.30	0.15 (0.02–0.28)	0.02	60.0
via VAT (cm^2)			0.00 (−0.20–0.21)	0.97	0.25 (0.08–0.41)	0.003	100.0
via body fat mass (kg)			0.13 (−0.05–0.32)	0.16	0.12 (0.01–0.23)	0.03	48.0
Two-hour glucose (mmol/L)	0.48 (0.10–0.86)	0.01					
via WC (cm)			0.34 (−0.08–0.76)	0.11	0.14 (−0.01–0.028)	0.06	29.2
via BMI (kg/m^2)			0.39 (−0.02–0.81)	0.06	0.09 (−0.04–0.21)	0.17	18.8
via VAT (cm^2)			0.21 (−0.15–0.56)	0.26	0.27 (0.08–0.47)	0.006	56.3
via body fat mass (kg)			0.42 (0.00–0.83)	0.05	0.06 (−0.04–0.17)	0.27	12.5
Matsuda Index *	0.05 (0.02–0.09)	0.003					
via WC (cm)			0.01 (−0.04–0.06)	0.62	0.04 (0.00–0.08)	0.03	80.0
via BMI (kg/m^2)			0.02 (−0.02–0.07)	0.31	0.03 (−0.00–0.06)	0.05	60.0
via VAT (cm^2)			−0.01 (−0.06–0.05)	0.85	0.05 (0.01–0.10)	0.01	100.0
via body fat mass (kg)			0.03 (−0.01–0.07)	0.16	0.02 (−0.00–0.05)	0.08	40.0

Regression coefficients c, α, β and c' are shown in Figure 4.2a,b. All estimates were adjusted for the potential effects of age. BMI, Body mass index; DII, Dietary inflammatory index; HbA1c, Glycated haemoglobin; HOMA2-IR, Homeostasis Model Assessment-insulin resistance; VAT, Visceral adipose tissue. * Matsuda index was modelled as an inverse variable in order to calculate the proportion of mediation effect, thus the regression coefficients are positive instead of negative.

Table 4.3: Total, direct and indirect effects of E-DII on markers of T2D risk with inflammatory cytokines as mediators in black SA women.

Variables	Total Effect (c)		Direct Effect (c')		Indirect Effect (αβ)	
Mediators and Outcomes	**Estimate (95%CI)**	***p*-Value**	**Estimate (95%CI)**	***p*-Value**	**Estimate (95%CI)**	***p*-Value**
Fasting glucose (mmol/L)	0.17 (0.01–0.34)	0.04				
via TNF-α (pg/mL)			0.16 (−0.01–0.33)	0.07	0.01 (−0.01–0.04)	0.28
via IL-8 (pg/mL)			0.17 (0.01–0.34)	0.04	−0.00 (−0.01–0.01)	0.71
via MCP-1 (pg/mL)			0.16 (0.01–0.32)	0.04	0.01 (−0.01–0.03)	0.43
Fasting insulin (mUI/mL)	1.70 (0.45–2.96)	0.008				
via TNF-α (pg/mL)			1.55 (0.32–2.78)	0.01	0.15 (−0.06–0.36)	0.17
via IL-8 (pg/mL)			1.72 (0.47–2.97)	0.007	−0.02 (−0.14–0.11)	0.77
via MCP-1 (pg/mL)			1.66 (0.43–2.88)	0.008	0.04 (−0.13–0.21)	0.63
HbA1c (%)	0.10 (0.01–0.18)	0.02				
via TNF-α (pg/mL)			0.11 (0.02–0.19)	0.01	−0.01 (−0.02–0.00)	0.15
via IL-8 (pg/mL)			0.10 (0.02–0.18)	0.02	−0.00 (−0.01–0.00)	0.49
via MCP-1 (pg/mL)			0.10 (0.02–0.18)	0.02	−0.00 (−0.01–0.01)	0.92
HOMA2-IR	0.25 (0.07–0.44)	0.008				
via TNF-α (pg/mL)			0.23 (0.05–0.41)	0.02	0.02 (−0.01–0.06)	0.15
via IL-8 (pg/mL)			0.26 (0.07–0.44)	0.007	−0.00 (−0.02–0.02)	0.75
via MCP-1 (pg/mL)			0.25 (0.07–0.43)	0.007	0.01 (−0.02–0.03)	0.59
Two-hour glucose (mmol/L)	0.48 (0.10–0.86)	0.01				
via TNF-α (pg/mL)			0.46 (0.07–0.85)	0.02	0.02 (−0.02–0.07)	0.31
via IL-8 (pg/mL)			0.49 (0.11–0.87)	0.01	−0.01 (−0.04–0.01)	0.42
via MCP-1 (pg/mL)			0.46 (0.08–0.83)	0.02	0.03 (−0.04–0.09)	0.41
Matsuda Index *	0.05 (0.02–0.09)	0.003				
via TNF-α (pg/mL)			0.05 (0.01–0.08)	0.007	0.00 (−0.00–0.01)	0.23
via IL-8 (pg/mL)			0.05 (0.02–0.09)	0.003	−0.00 (−0.00–0.00)	0.61
via MCP-1 (pg/mL)			0.05 (0.02–0.09)	0.003	0.00 (−0.00–0.01)	0.67

Regression coefficients α, β and c' are shown in Figure 4.2a,b. All estimates were adjusted for the potential effects of age. DII, Dietary inflammatory index; HbA1c, Glycated haemoglobin; HOMA2-IR, Homeostasis Model Assessment- insulin resistance; IL-8; Interleukin-8, MCP-1, Monocyte chemotactic protein 1; TNF-α, Tumour necrosis factor alpha. * Matsuda index was modelled as an inverse variable in order to calculate the proportion of mediation effect, thus the regression coefficients are positive instead of negative.

The association between the proposed mediators and E-DII (path α) are presented in Table 4.4, while the associations between the potential mediators and the outcome, markers of T2D risk (path β), are presented in Table 4.5. Path α showed that E-DII had a significant association with all body composition measures, whereas only TNF-α associated with E-DII among the pro-inflammatory cytokines (Table 4.4). Analysis of Path β, showed that all the body composition measures were significantly associated with fasting insulin, HOMA2-IR and Matsuda Index Table 5). Furthermore, both WC and VAT (measures of central obesity) were positively associated with HbA1c and two-hour glucose load, with only VAT significantly associated with fasting glucose. In contrast, none of the inflammatory cytokines were significantly associated with markers of T2D risk (Table 4.5).

Table 4.4: Regression coefficients (95% CIs) for the association between E-DII and measures of body composition and inflammatory cytokines in black SA women.

Mediator Variables	Estimate	95%CI	*p*-Value
WC (cm)	3.27	1.39–5.16	0.001
BMI (kg/m^2)	1.54	0.52–2.55	0.003
VAT area (cm^2)	16.88	8.30–25.46	<0.0001
Body fat mass (kg)	2.49	0.65–4.32	0.008
TNF-α (pg/mL)	0.98	0.13–1.82	0.02
IL-8 (pg/mL)	0.98	−1.09–3.01	0.36
MCP-1 (pg/mL)	25.73	−17.47–68.92	0.24

Estimates for the association between E-DII and body composition and inflammatory cytokines represent the regression coefficients for Path α Figure 4.2b. All estimates were adjusted for the potential effects of age. BMI, Body mass index; DII, Dietary inflammatory index; IL-8; Interleukin-8, MCP-1, Monocyte chemotactic protein 1; TNF-α, Tumour necrosis factor alpha; VAT, Visceral adipose tissu

Table 4.5. Regression coefficients (95% CIs) for the associations between body composition and inflammatory cytokines and markers of T2D risk in black SA women.

	Fasting Glucose			Fasting Insulin			HbA1c		
	Estimate	**95%CI**	***p*-Value**	**Estimate**	**95%CI**	***p*-Value**	**Estimate**	**95%CI**	***p*-Value**
WC (cm)	0.01	−0.01–0.03	0.22	0.40	0.23–0.57	<0.0001	0.01	0.00–0.02	0.04
BMI (kg/m^2)	0.02	−0.02–0.06	0.25	0.65	0.34–0.95	<0.0001	0.01	−0.00–0.03	0.09
VAT area (cm^2)	0.01	0.00–0.01	**0.02**	0.10	0.06–0.13	<0.0001	0.00	0.00–0.00	0.02
Body fat mass (kg)	0.01	−0.01–0.03	0.33	0.33	0.17–0.48	<0.0001	0.01	−0.00–0.01	0.14
TNF-α (pg/mL)	0.01	−0.01–0.03	0.20	0.15	−0.04–0.34	0.11	−0.01	−0.02–0.00	0.06
IL-8 (pg/mL)	−0.00	−0.01–0.01	0.63	−0.02	−0.13–0.09	0.73	−0.00	−0.01–0.00	0.14
MCP-1 (pg/mL)	0.00	−0.00–0.00	0.29	0.00	−0.00–0.01	0.56	−0.00	−0.00–0.00	0.92
	HOMA2-IR			**Two-Hour Glucose**			**Matsuda Index ***		
	Estimate	**95%CI**	***p*-Value**	**Estimate**	**95%CI**	***p*-Value**	**Estimate**	**95%CI**	***p*-Value**
WC (cm)	0.06	0.03–0.09	<0.0001	0.04	0.01–0.08	0.03	0.01	0.00–0.02	0.008
BMI (kg/m^2)	0.10	0.04–0.15	<0.0001	0.06	−0.01–0.12	0.10	0.02	0.00–0.03	0.02
VAT area (cm^2)	0.01	0.01–0.02	<0.0001	0.02	0.01–0.02	<0.0001	0.00	0.00–0.00	0.001
Body fat mass (kg)	0.05	0.02–0.08	<0.0001	0.03	−0.01–0.06	0.20	0.01	0.00–0.01	0.03
TNF-α (pg/mL)	0.02	−0.00–0.05	0.10	0.03	−0.02–0.07	0.26	0.00	−0.00–0.01	0.13
IL-8 (pg/mL)	−0.00	−0.02–0.01	0.70	−0.01	−0.03–0.01	0.19	−0.00	−0.00–0.00	0.39
MCP-1 (pg/mL)	0.00	−0.00–0.00	0.49	0.00	−0.00–0.00	0.16	0.00	−0.00–0.00	0.58

Regression coefficients correspond to Path β in Figure 4.2b. All estimates were adjusted for the potential effects of age. BMI, body mass index; DII, dietary inflammatory index; HbA1c, glycated haemoglobin; HOMA2-IR, Homeostasis Model Assessment- insulin resistance; IL-8; Interleukin-8, MCP-1, Monocyte chemotactic protein 1; TNF-α, Tumour necrosis factor alpha; VAT, Visceral adipose tissue. * Matsuda index was modelled as an inverse variable in order to calculate the proportion of mediation effect, thus the regression coefficients are positive instead of negative.

4.4.3 Associations between E-DII and markers of T2D Risk in back SA women with body composition as mediators

The direct (Path c') and indirect effects (product of Paths α and β, αβ) of E-DII on markers of T2D risk, through body composition, including the proportion of mediation by the body composition measures also are presented in Table 4.2. A consistent finding was that VAT was a significant mediator of the association between E-DII and all markers of T2D risk, including fasting glucose and insulin, HbA1c, HOMA2-IR, two-hour glucose and Matsuda Index (all $p < 0.05$). Both WC and BMI mediated the association between E-DII and fasting insulin, HOMA2-IR and Matsuda Index (all $p < 0.05$). Body fat mass mediated the association between E-DII and fasting insulin and HOMA2-IR (all $p < 0.05$). The proportion of the effect mediated by measures of central obesity (i.e., VAT and WC) was higher (23.5–100%) than the proportion mediated by measures of total obesity, including BMI and body fat mass (10–60%). Notably, VAT mediated most of the association between E-DII and markers of T2D risk, in particular for measures of insulin resistance (HOMA2-IR) and sensitivity (Matsuda Index) (proportion of mediation: 100%). Furthermore, the proportion of the mediation effect for HOMA2-IR and Matsuda Index was higher (40–100%) than for measures of glucose tolerance (e.g., fasting and two-hour glucose load). For instance, the mediating effects on the association between E-DII and HOMA2-IR were: For VAT (100.0%), WC (80.0%), BMI (60.0%) and body fat mass (48.0%) (Table 4.2).

Direct effects (Path c') between E-DII and T2D markers were not significant after adjusting for WC, BMI and VAT (Table 4.2). Similar findings were reported for body fat mass, except for the association between E-DII and two-hour glucose load.

4.4.4 Direct and Indirect effects of E-DII on markers of T2D in black SA women with inflammatory cytokines as mediators

The associations between E-DII and markers of T2D before and after adjustment for the effect of inflammatory cytokines on T2D risk are shown in Table 4.3. The three inflammatory cytokines did not have any significant mediating effect on the association

between E-DII and markers of T2D risk (Table 4.3). After adjusting for the inflammatory markers, E-DII was directly associated with fasting glucose (except for TNF-α) and insulin, HbA1c, HOMA2-IR, two-hour glucose load and Matsuda Index (Table 4.3) ($p < 0.05$).

4.5 Discussion

This is the first study, to our knowledge, to suggest a link between diet-associated inflammation, as assessed by E-DII, and markers of T2D in a sample of middle-aged black SA women. Notably, we show that this association is mediated by adiposity, in particular central obesity (VAT and WC). Accordingly, measures of central adiposity were higher in participants with a positive E-DII score, reflecting a pro-inflammatory diet, compared to those with a negative E-DII score, which reflected an anti-inflammatory diet. While circulating TNF-α concentrations were higher in the positive E-DII group than the negative E-DII group, circulating inflammatory markers did not mediate the association between E-DII and markers of T2D in middle-aged black SA women.

Typical components of a Western diet, including high intakes of fat and red meat, and low intakes of vitamin A-rich fruit and vegetables, are linked to inflammation and a higher E-DII score (155,163) and have been associated with T2D and CVD risk in urban black SA populations (11,150,151). These results suggest that pro-inflammatory foods are part of the habitual diet of urban black SA populations, and thus implicated in the pathophysiology of T2D in this population. Although the exact biological mechanisms implicated in the association between diet-induced inflammation and the development of T2D are not fully understood, we propose that excessive intake of pro-inflammatory foods may enhance fat accumulation, increase total and central obesity, in particular VAT, and subsequently induce an inflammatory profile, which is often reported in an obese state (248,249,256). To substantiate this notion, the direct associations between E-DII and markers of T2D, after adjusting for measures of body composition, in particular VAT, were no longer significant. In contrast, the direct associations between E-DII and markers of T2D remained significant, after adjusting for the inflammatory cytokines. This suggests that a pro-inflammatory diet has a close association with

measures of obesity, in particular for pro-inflammatory adipose tissues, such as VAT, and that the presence of obesity is more detrimental than that of low-grade inflammation in the development of T2D risk in this population.

Furthermore, the proposed mechanism for the development of an obese and/or inflammatory state in the presence of a pro-inflammatory diet is supported by the significantly higher measures of central adiposity and TNF-α levels in the positive E-DII score group compared to the negative E-DII group. Accordingly, obesity, in particular central obesity-related inflammation, has been implicated in the development of T2D (248,249,257). In line with these findings, we reported that VAT mediated most of the association between E-DII and markers of T2D in comparison to other measures of adiposity. The higher proportion of mediation by measures of central obesity, in particular VAT, compared to measures of total adiposity (BMI as a proxy) might be explained by VAT having a higher inflammatory profile and higher rates of lipolysis than other adipose tissue sites (96,99,258). Further, the anatomical position of VAT relative to the liver, leading to delivery of excess FFAs and pro-inflammatory cytokines (e.g., TNF-α) via the hepatic portal system directly to the liver will impair hepatic insulin signaling (96,99,258). Indeed, ectopic fat accumulation in the liver has been shown to impair many biological functions of the liver, and has been associated with hepatic IR, increased hepatic glucose production and reduced insulin-stimulated hepatic glucose uptake, thus increased risk for T2D (99,257,258).

Our finding that the association between diet-induced inflammation (assessed by the E-DII) and the risk of developing T2D is mediated by adiposity is notable, as only one study, to our knowledge, has reported both adiposity and low-grade inflammation as mediators in the association between diet-induced inflammation and markers of T2D in a European population (160). However, the two studies are not easily comparable as two different versions of the DII were used; we used a new version of the DII with the updated scoring algorithm. Nonetheless, in our study, low-grade inflammation did not mediate the association between E-DII and markers of T2D. This can be explained by the non-significant association between inflammatory cytokines and markers of T2D risk, thus making a mediation effect “impossible”. Moreover, we used three separate

inflammatory cytokines, while a total/summary score of six inflammatory cytokines, including CRP, IL-6, 1L-8 and TNF-α was used as a marker of low-grade inflammation in the previous study (160).

Interestingly, out of the three inflammatory cytokines used in our analyses, only TNF-α was significantly different between the positive and negative E-DII score groups, and it also was associated with the E-DII score. TNF-α is the most highly secreted cytokine during obesity and was the first inflammatory cytokine linking obesity-induced inflammation to IR (259,260). This suggests that TNF-α is a more prominent biomarker of obesity-induced inflammation than 1L-8 and MCP-1, in particular for this population, with high levels of obesity. However, the non-significant association between TNF-α and markers of T2D risk suggests that TNF-α (and other inflammatory cytokines) may not be directly associated with markers of T2D risk in this population or their effects might be diminished in the presence of more detrimental risk factors, such as obesity. Accordingly, it was reported that, despite a higher inflammatory profile at the genetic, adipose and circulating level, inflammation may not be the main driver of the higher T2D risk in black populations (98). However, inflammation acts via paracrine and autocrine effects, limiting our ability to measure their specific effects. Nonetheless, results from our study and others suggest that other risk factors, such as body fat and its distribution, SES and lifestyle factors, including physical activity, as well as their interactions may be relatively more important risk factors for T2D in this population.

In our study the E-DII score ranged from −2.51 to +1.92, which is narrower than that reported in a previous study of T2D (−5.49 to + 4.12) (154). The differences in the E-DII range between our study and the previous study may be explained by numerous factors, including differences in sample size, age, gender representation, type and number of food-parameters used in the calculation of the DII score and quantity of the food-parameters consumed. We also propose that the narrow range in the E-DII score might be explained by the homogeneity of the sample, in terms of SES and socio-cultural background (i.e., eating similar foods and calorically similar amounts), thus resulting in a low variety of the diet, especially when taking into account energy intake. Furthermore, it is also important to note that SES factors were not significantly different between the

positive and negative E-DII score groups, implying that other external factors (e.g., socio-cultural factors) might be contributing to the pro- and anti-inflammatory profile of the diet. Furthermore, those individuals consuming a pro-inflammatory diet are at high risk of developing T2D, in particular those with central obesity.

This is the first study to investigate diet-associated inflammation in relation to T2D risk, in a high-risk and understudied African population, whose lifestyle differs from other populations. Importantly, we used DXA to provide a more accurate and objective measure of whole-body composition, including estimates of VAT. Despite its strengths, our study has potential limitations. We did not compare the characteristics of the study participants to non-participants. However, we believe our sample is still a representative of the Bt20+ caregiver cohort and middle-aged women from Soweto or middle-aged black SA women. Among SA women, the national prevalence of overweight and obesity between the range of 35-44, 45-54 and 55-65 years of age is 77.5%, 81.9% and 81.3%, respectively (111). Moreover, black SA women have higher obesity levels (40.9%) than white SA women (30.6%) and are at higher risk of developing T2D compared to their white counterparts (111).This is a cross-sectional study, thus our results do not imply causality. Also, only three out of the eight possible inflammatory cytokines were detected in all the samples and used in the analysis, thus reducing the chance of finding significant results. Furthermore, the dietary intake was measured at a single time point, thus might not reflect the long-term or seasonal changes in dietary intake and lifestyle activities of the participants. In an attempt to control and reduce the confounding effects of total energy intake between the participants, the DII was adjusted per 1000 calories. Another potential weakness of the study is the use of only 21 food parameters out of 45 for the analysis of the DII score. However, the majority of earlier studies have not used all 45 food parameters (154,161,163). For example, both Vahid et al. (161) and Denova-Gutierrez et al. (154) who have shown positive associations between DII and prediabetes or T2D risk used 27 food items out of the possible 45 food parameters. Nonetheless, positive associations between DII and inflammation have been reported even with fewer food parameters (154,161,163), supporting the robustness of the DII in investigating the association between diet-induced inflammation and inflammatory-related diseases. Furthermore, E-DII takes into account the entire diet of the individual

and measures its inflammatory potential (156), making it a better tool compared to other dietary tools that only consider dietary patterns/food-groups and single-nutrients. Nonetheless, the DII score was calibrated against a global dietary database that did not include an African population (156); which may not capture the true inflammatory profile of an African diet. Future refinements of the DII might include expansion of the global comparative database, which may help to capture even greater dietary diversity between populations.

4.6 Conclusions

In conclusion, our findings suggest that a pro-inflammatory diet may exacerbate the effects of obesity, in particular central obesity, and that a pro-inflammatory diet may increase the risk of developing T2D. Therefore, the promotion of healthy eating might prevent or reduce central obesity and obesity-induced inflammation, and subsequently lower the risk of developing T2D in black middle-aged black SA women.

CHAPTER FIVE

Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women: a prospective cohort study

Data from this chapter have been published in Zeng Y, Mtintsilana A, Goedecke JH, *et al* Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women. *Metabolism, Clinical and Experimental* 2019; **95**: 57-64.

Author Contributions: Designed or conceptualised the study, E.C., L.K.M., T.O., and J.H.G.; Responsible for data analysis and wrote the first draft of manuscript, Y.Z.; and E.C.; Responsible for sample collection, involved in data analyses and reviewed and edited the manuscript, A.M.; Principal investigators and acquired funding for the study, L.K.M., E.C., T.O., and J.H.G.; Critically reviewed and commented on the manuscript, L.K.M., T.O., and J.H.G.

5.1 Introduction

The global burden of T2D continues to rise in conjunction with obesity, related to a sedentary lifestyle and energy-dense diets (261). Within SSA, SA has the highest global projected increase in diabetes risk for the next 25 years (262). A recent population-based study in urban-dwelling black SA found that the age-standardised prevalence of T2D was 13%. Among those individuals, 40% were aged 65–74 years, and women were at higher risk than men (10). Type 2 diabetes is a progressive disease characterised by increased insulin resistance (IR), impaired β-cell function, and eventually β-cell failure (261). Prior to disease onset, hyperinsulinemia, as a result of increased insulin secretion and reduced hepatic clearance, develops to compensate for IR and to maintain euglyceamia (263). This compensation may last for decades. Chronic factors, including glucolipotoxicity, inflammation, endoplasmic reticulum stress, and oxidative stress may jointly contribute to exhaust this compensatory mechanism, but the mechanisms are unclear (94). Several studies have shown that T2D remission is possible either via lifestyle changes or bariatric surgery, but the success rates are highly dependent on disease duration (264,265). Thus the need to detect early signs/markers of IR and T2D is important to prevent long-term health complications, notably cardio- vascular diseases, with related health costs (266). Importantly, studies in SA and the USA have shown that factors typically associated with IR and T2D risk in white European populations, including circulating TGs, HDL-cholesterol, ectopic fat accumulation, and adipose tissue inflammation, are not significant correlates in black African populations (87,98,267). These findings reinforce the fact that obesity-related disorders involve a complex interaction between genetic background, environmental and lifestyle factors. To understand this complex interaction, it is of major interest to explore the biochemical balance downstream of the genome, where metabolomics and the comprehensive analyses of metabolites provide a direct link between biochemical pathways and phenotypic changes. Previous studies have shown that elevated BCAA and metabolites related to their catabolism, including BCKA or C3- and C5-carnitines,may predict, and potentially mediate, IR and T2D development (185,268). Notably, these findings were demonstrated in different white European populations (175,178). Further, low levels of glycine and LPC (C18:2) was shown to predict the risk for developing IGT in the KORA- and EPIC-cohorts from white Europeans (186). Despite the high prevalence of T2D in

urban black SA women, metabolic pathways putatively involved in its pathophysiology have been scarcely studied in this population. Our hypothesis was that specific metabolite patterns or pathways can predict the development of T2D in black SA women >10 years prior to diagnosis.

5.2 Material and methods

5.2.1 Study design and population

The study participants included black SA women who were the caregivers of BT20+ cohort (233). Briefly, between 2002 and 2003 (baseline), 2174 caregivers of the BT20+ cohort were invited to participate in the study. Of those invited, 1251 accepted and underwent blood sampling and general anthropometric measurements. From these, 476 individuals had complete blood analyte data and stored serum samples at baseline (Appendix B, Figure 5S1). Approximately 13 years later (follow-up), between 2015 and 2016, contactable participants were asked to participate in the current follow-up study if they fulfilled the inclusion criteria: (1) an available stored serum sample; (2) normal fasting glucose at baseline; (3) a negative HIV test at follow-up; and (4) <65 years of age. Participants were excluded if they had a chronic or infectious disease, or were pregnant or lactating. At follow-up, all recruited women (n = 144) underwent clinical measurements. Those without known T2D underwent an OGTT. WHO diabetes diagnostic criteria were used to diagnose IGT and type 2 diabetes (269). We included all women that had NGT at baseline (based on the 2002–2003 data) and had developed (at follow-up) either (i) type 2 diabetes (n = 20, NGT-T2D group) or (ii) IGT (n = 27, NGT-IGT group). These were matched with a third group that remained NGT (n = 28, NGT-NGT group). Further, we calculated a principal component model and used a statistical experimental design to match for multiple variables in order to minimize group differences in baseline measures of fasting glucose, age, BMI, and WC, and also transitions in BMI and WC from base- line to the 13-year follow-up (270). A flow chart that schematically illustrates the study design and exclusion criteria is presented in Appendix B, Figure 5S1. All participants provided written informed consent. The study was approved by the Human Research Ethics Committee (Medical) of the University of

the Witwatersrand, M010556 (for Bt20+ cohort) and M150530 (see Appendix C for Ethics clearance certificate for the current study), in accordance with the Declaration of Helsinki. Procedures and associated risks were explained to the participants who then signed a written consent prior to participation. Sample collection was carried out at the South African Medical Research Council (MRC)/University of the Witwatersrand (WITS) Developmental Pathways for Health Research Unit at the Chris Hani Baragwanath Hospital in Soweto Johannesburg. Metabolomics analyses were conducted on serum samples from all 75 women, sampled at baseline and follow-up.

5.2.2 Clinical measurements

At baseline and follow-up, basic anthropometric measurements, including weight, height, WC, hip circumference, and BMI, were collected after an overnight fast. At follow-up, participants underwent a 75-g OGTT after an overnight fast to determine glucose tolerance based on WHO criteria (269). Serum glucose, total cholesterol, TGs and HDL cholesterol were analysed on the Randox RX Daytona Chemistry Analyser (Randox Laboratories Ltd., UK). Serum insulin and C-peptide assays were analysed on the Immulite® 1000 Immunoassay System (Siemens Chemiluminescent Technology, Germany). HbA1c levels were measured on whole blood using the D-10™ Hemoglobin Analyser (Bio-Rad Laboratories, Inc. USA). Serum samples were stored at −80 °C until metabolomics analyses.

5.2.3 Sample preparation and metabolomics analyses

In view of known methodological biases, all samples were prepared and analysed in a specific order (271) keeping samples from the same subject in close connection, but with a randomized internal order and batches were balanced according to the diagnostic groups. Quality control samples (QC, pooled from all samples) were included in each batch to assist with the identification of metabolites, to monitor instrument stability, exclude background features, and minimize methodological biases that could interfere with interpretations of results. Sample preparation was performed as described previously (272). The multi-platform metabolomics analyses included gas

chromatography coupled with time-of-flight mass spectrometry (GC-TOF/MS) and liquid chromatography coupled with time-of-flight mass spectrometry (LC-TOF/MS, operating in positive and negative ion modes). Detailed sample preparation, the use of internal standards, analysis protocols, and subsequent data processing methods are provided in the Appendix B, research design and material 5S2.

5.3 Statistical analysis

First, we inspected the dataset using PCA to detect groupings, outliers, and trends. To minimize the influence of confounders related to storage, we did not compare the metabolite patterns between the two samplings, i.e., baseline and follow-up. Instead, we calculated separate OPLS-discriminant analysis models (OPLS-DA) at baseline and follow-up to compare the NGT-NGT and the NGT-T2D groups. This allowed us to elucidate similarities and differences in metabolite profiles before and after disease development, respectively. Next, the metabolite profiles of the NGT-IGT group were predicted into existing OPLS-models to enable comparisons of metabolite profile among all diagnostic groups. The obtained models were validated, based on analysis of variance of the cross-validated OPLS scores (CV-ANOVA) to test for significance (273). Metabolites were considered significant when fulfilling the statistical significance criteria using post-hoc linear regression on loadings calculated from the validated OPLS-models (274). A non-parametric Wilcoxon-Mann-Whitney rank sum test and Spearman rank correlation on a 95% confidence level was used to explore relationship between putative metabolites, diagnosis and clinical endpoints. The above analyses were performed with SIMCA 14.0.0 software (Umetrics, Umeå, Sweden) and MATLAB R2016a (The MathWorks, Natick, MA, USA).

5.4 Results

5.4.1 Subject characteristics

All clinical characteristics and anthropometric data are presented in Table 5.1. The groups did not differ in age, weight, BMI, or hip circumference at baseline or follow-up.

WC, WHR and fasting glucose concentrations were significantly higher in the NGT-T2D group than the other two groups at baseline. Within-group changes in anthropometrical variables over the 13-year follow-up period were significant. Within the NGT-IGT and NGT-NGT groups, WC (+14%, P=0.0004 and +11%, P=0.02) and WHR (+7%, P=0.001 and+7%, P=0.004) increased, respectively. Only the WHR (+6.5%, P= 0.04) significantly increased in the NGT-T2D group over the 13-year follow-up period. At follow-up, the NGT-T2D group had significantly higher HbA1c and fasting glucose, insulin, HOMA-IR, and triglycerides concentrations, compared to the other two groups. The NGT-IGT group had significantly higher fasting glucose, C-peptide, and 120-min glucose compared to the NGT-NGT group at follow-up (Table 5.1).

Table 5.1: Characteristics of participants in the three diagnostic groups, at baseline and at 13-year follow-up. All participants exhibited normal glucose tolerance (NGT) at baseline.

Participant groups	NGT-NGT (n=28)	NGT-IGT (n=27)	NGT-T2D (n=20)
Baseline (2002-2003)			
Age (years)	43 (39-48)	44 (38-49)	45 (41-46)
Fasting glucose (mmol/L)	4.8 (4.4-5.1) *	4.6 (4.4-5.0) #	5.4 (5.0-5.9) * #
Body composition			
Weight (kg)	76.3 (62.1-88.0)	78.8 (68.0-85.0)	81.1 (73.3-106.0)
BMI (kg/m^2)	31.6 (26.1-35.4)	31.8 (27.0-34.0)	33.9 (29.8-39.1)
WC (cm)	87.0 (77.4-96.5) *	88.2 (81.0-96.0) #	96.5 (90.8-110.0) * #
Hip circumference (cm)	113.0 (105.0-123.0)	112.0 (104.5-122.0)	117.0 (108.0-125.4)
WHR	0.76 (0.71-0.80) * §	0.80 (0.76-0.82) # §	0.86 (0.80-0.88) * #
13-year follow-up			
Age (years)	56 (52-61)	58 (51-62)	58 (54-59)
Body composition			
Weight (kg)	81.4 (67.2-89.8)	83.1 (72.9-101.1)	82.9 (72.5-99.2)
BMI (kg/m^2)	33.4 (27.7-37.3)	33.9 (28.9-37.9)	32.4 (30.0-38.9)
WC (cm)	97.8 (85.4-106.5)	100.0 (93.5-110.4)	102.6 (95.4-117.7)
Hip circumference (cm)	117.4 (111.1-127.1)	121.0 (110.8-128.0)	117.5 (107.0-130.8)
WHR	0.82 (0.76-0.80) * §	0.85 (0.80-0.90) §	0.90 (0.84-0.90) *
Glucose tolerance, insulin resistance			
Fasting glucose (mmol/L)	4.7 (4.1-5.1) * §	5.2 (4.4-5.6) # §	7.5 (6.3-9.3) * #
120 min glucose (mmol/L)	5.7 (4.8-7.0) * §	8.9 (8.3-9.5) # §	11.8 (11.5-15.4) * #
Fasting insulin (mIU/L)	9.7 (3.7-15.8) *	11.4 (5.6-18.3) #	18.4 (11.5-22.6) * #
Fasting C-peptide (ng/mL)	1.8 (1.1-2.5) * §	2.5 (1.6-2.8) §	2.0 (1.7-3.9) *
HbA1c (mmol/mol)	36.6 (33.3-38.8) *	36.6 (32.2-39.8) #	51.9 (42.1-67.2) * #
HbA1c (%)	5.5 (5.2-5.7) *	5.5 (5.1-5.8) #	6.9 (6.0-8.3) * #
HOMA-IR	1.8 (0.8-3.7) *	2.8 (1.0-3.7) #	6.1 (3.3-10.4) * #
Serum lipid profile			
HDL cholesterol (mmol/L)	1.3 (1.1-1.5) *	1.2 (1.1-1.4)	1.1 (1.0-1.2) *
Total cholesterol (mmol/L)	4.6 (4.1-5.6)	4.7 (4.0-5.1)	4.7 (4.1-5.1)
Triglycerides (mmol/L)	0.8 (0.7-1.1) *	1.0 (0.8-1.2) #	1.3 (1.0-1.2) * #

Data presented as median (25th-75th percentiles). *p <0.05 for NGT-T2D vs. NGT-NGT; #p <0.05 for NGT-T2D vs. NGT-IGT; §p <0.05 for NGT-IGT vs. NGT-NGT; all comparisons were based on non-parametric Wilcoxon-Mann-Whitney rank sum tests. BMI, body mass index; HbA1c, Glycated haemoglobin; HDL, High-density lipoprotein; HOMA-IR, Homeostatic model-insulin resistance; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2D, type 2 diabetes; WC; Waist circumference; WHR, waist-to-hip ratio.

5.4.2 Metabolomics analysis

The combination of GC-TOF/MS and LC-TOF/MS, provided a comprehensive coverage of serum metabolites with different chemical properties. After removing the noisy peaks, we detected 1076 putative metabolites. Among these, 252 were identified and classified into amino acids, carbohydrates, fatty acids, lipids, acylcarnitines, organic acids etc. A list of the identified and classified metabolites is shown in Appendix B, Table 5S1. Additional details regarding data pre-processing, data inspection with PCA, and efforts to identify unknowns are shown in Appendix B, Table 5S1.

5.4.3 Metabolite profiles that describe the development and risk of T2D in black SA women

With OPLS modeling, we constructed two separate models, one for baseline samples and one for follow-up samples. These models highlight the metabolite profile that significantly discriminates between the NGT-T2D and NGT-NGT groups, calculated from all 1076 putative metabolites. At baseline, when all women were NGT, the metabolite profile comprised 28 annotated and 81 non-annotated metabolites could significantly discriminate between the NGT-T2D and NGT-NGT groups (CV-ANOVA, $P < 0.05$, Figure 5.1A). At follow-up, the metabolite profile was amplified, including 70 annotated and 66 non-annotated metabolites that differed significantly between the NGT-T2D and NGT- NGT groups (OPLS-DA, CV-ANOVA, $P < 0.001$, Figure 5.1B). Only annotated metabolites are shown in figures. Information on the non-annotated compounds are shown in Appendix B, Table 5S2. Several phospholipid metabolites differed at baseline between the NGT-T2D and the NGT-NGT group. Specifically, we detected a higher (LPC(18:2):LPE (C18:2)) ratio, and lower levels of glycerophospholipid precursors (i.e., glycerol-2-phoshpate and glycerol-3-phosphate), conjugated C16:0 andC18:0-fatty acids (namely 2-hydroxypalmitic acid (C16-OH), palmitic acid methyl ester (C16:0-ME), and octadecanoic acid methyl ester (C18:0-ME)), and lower levels of the omega-6 fatty acid, eicosadienoic acid (C20:2n6). At baseline, we could also detect lower circulating TCA cycle intermediates (i.e., citric acid and isocitric acid) in the NGT-T2D group along with lower alpha-aminobutyric acid, beta-alanine, a platelet aggregation factor (PAF, C16:2),

and LPE(C18:2), compared to the NGT-NGT group. At baseline, the NGT-T2D group had lower levels of two bile acid species, CDCA (a primary bile acid) and UDCA (a secondary bile acid), compared to the NGT-NGT group. Compared to the NGT-NGT group, both baseline and follow-up metabolite profiles of the NGT-T2D group had significantly higher circulating levels of leucine, ketoleucine (the BCKA of leucine), and a C5-carnitine, fructosyl-leucine/isoleucine (a glycated-BCAA; it was not possible to separate glycated-leucine from isoleucine). In addition, we detected consistently higher lactic acid and lower glycerol-3-posphate levels in the NGT-T2D group, compared to the NGT-NGT group, at both baseline and follow-up.

The NGT-IGT group had a metabolite profile that was in-between that of the NGT-T2D and NGT-NGT groups at both baseline and follow-up (Figures 5.1C and D). This is also shown in the raw data for the relative concentrations of BCAAs and their catabolic intermediates (Figure 5.2). Similar to the NGT-T2D group, leucine catabolic metabolites (ketoleucine, P = 0.03) were higher in the NGT-IGT group compared to the NGT-NGT group at baseline (Figure 5.2A), with slightly higher valine catabolic intermediate levels in the NGT-IGT group compared to the NGT-NGT group at follow-up (Figure 5.2B). At follow-up, the NGT-T2D group had higher circulating fatty acids and lower levels of specific LPCs, containing fatty acids C14:0, C16:1, C17:0, C18:1, C18:2, and C20:5, compared to the NGT-NGT group. The bile acid pool size and composition were also altered as type 2 diabetes developed. The NGT-T2D group had higher levels of secondary and conjugated bile acids (i.e., DCA and glycodeoxycholate) at follow-up compared to the NGT-NGT group. At follow-up the NGT-T2D group had higher levels of all BCAAs, including their related BCKAs and metabolites closely related to their catabolism (e.g., ketovaline, 3-hydroxyisobutyrate and a C5-carnitine), compared to the NGT-NGT group (Figures 5.1B and 5.2B). Several long-chain acylcarnitines, such as C16:1, C18:2, C18:3, and C14:1, were lower in the NGT-T2D group than in the NGT-NGT group at follow-up. As expected, several circulating carbohydrates (e.g., inositol, galactitol, galactose, laminaribiose, maltose, mannitol, and mannose) were higher in the NGT-T2D group than in the NGT-NGT group at follow- up (Figure 5.1B). The NGT-IGT group had a metabolite profile that was in-between that of the NGT-T2D and NGT-NGT groups at both baseline and follow-up (Figures 5.1C and D).

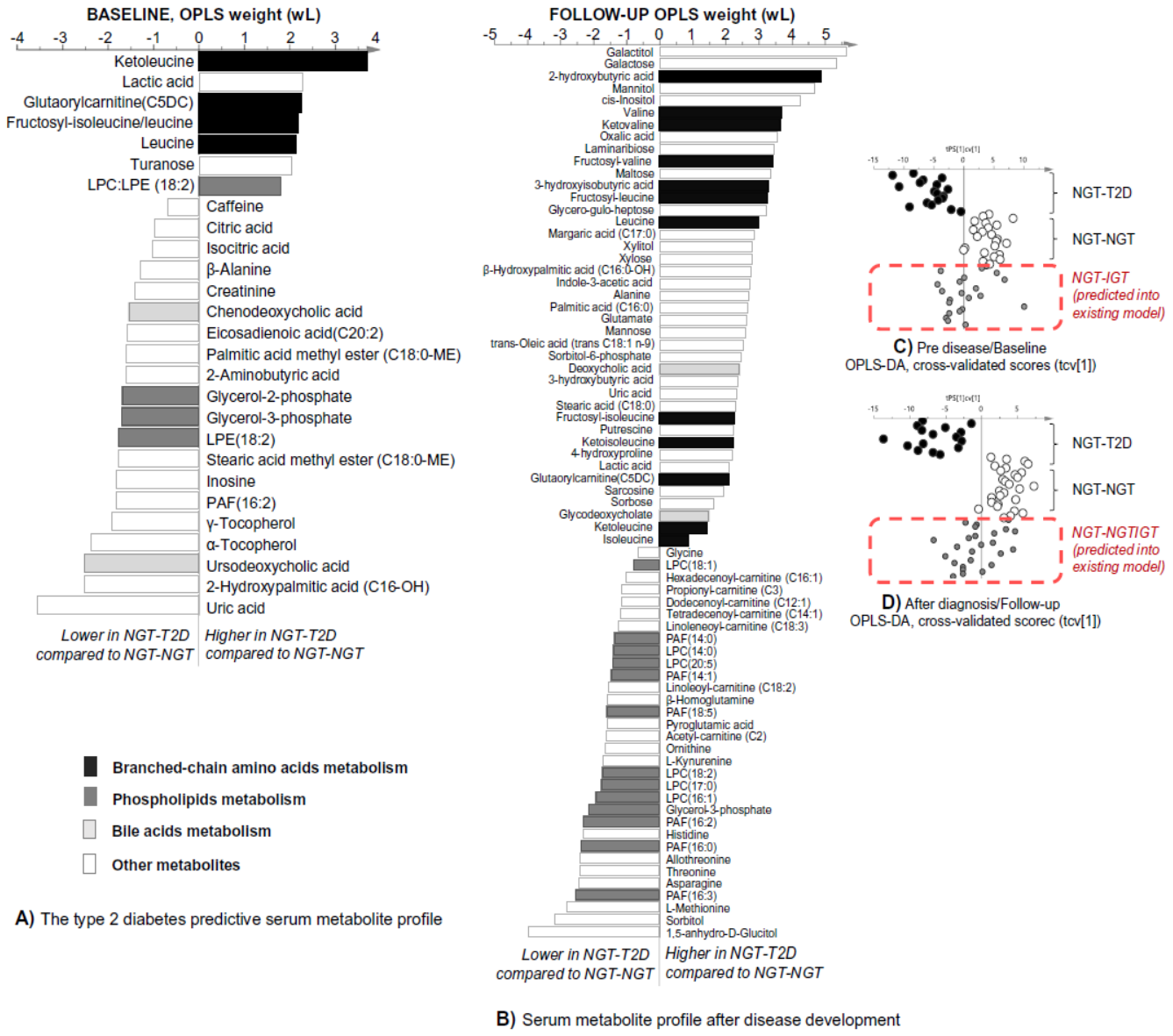


Figure 5.1: Two separate multivariate models (OPLS-DA) that describe circulating type 2 diabetes metabolite profiles in black South African women, before and after disease diagnosis. (A) At baseline: the metabolite profile (OPLS model weights, w [1]) that could discriminate between normal glucose tolerance (NGT) that later developed into type 2 diabetes (NGT-T2D) and NGT that remained unchanged (NGT-NGT) during follow-up. (B) At 13-year follow-up: the metabolite profile (OPLS weights, w [1]) that could discriminate between NGT-T2D and NGTNGT. (C) Baseline: Cross-validated (CV) OPLS-DA scores (tcv [1]) that describe subject variability prior to disease diagnosis. (D) At 13-year follow-up: Subject variability at follow-up (CV OPLS-DA scores, tcv [1]) shows the difference between NGT-T2D and NGT-NGT groups. Data for women classified as impaired glucose tolerant (IGT) were predicted into existing models to enable comparison of the complete metabolite profile. Only metabolites that were significantly altered are shown, according to the latent significant criteria on a 95% confidence level. Shading

indicates metabolites related to branched-chain amino acids (black), phospholipids (dark gray), bile acid (light gray) metabolism, or unclassified metabolites (white). All identified putative metabolites are listed in Appendix B, Table 5S1.

This is also shown in the raw data for the relative concentrations of BCAAs and their catabolic intermediates (Figure 5.2). Similar to the NGT-T2D group, leucine catabolic metabolites (ketoleucine, P = 0.03) were higher in the NGT-IGT group compared to the NGT-NGT group at baseline (Figure 5.2A), with slightly higher valine catabolic intermediate levels in the NGT-IGT group compared to the NGT-NGT group at follow-up (Figure 5.2B). At follow-up, the NGT-T2D group had higher circulating fatty acids and lower levels of specific LPCs, containing fatty acids C14:0, C16:1, C17:0, C18:1, C18:2, and C20:5, compared to the NGT-NGT group. The bile acid pool size and composition were also altered as type 2 diabetes developed. The NGT-T2D group had higher levels of secondary and conjugated bile acids (i.e., DCA and glycodeoxycholate) at follow-up compared to the NGT-NGT group. At follow-up the NGT-T2D group had higher levels of all BCAAs, including their related BCKAs and metabolites closely related to their catabolism (e.g., ketovaline, 3-hydroxyisobutyrate and a C5-carnitine), compared to the NGT-NGT group (Figures 5.1B and 5.2B). Several long-chain acylcarnitines, such as C16:1, C18:2, C18:3, and C14:1, were lower in the NGT-T2D group than in the NGT-NGT group at follow-up. As expected, several circulating carbohydrates (e.g., inositol, galactitol, galactose, laminaribiose, maltose, mannitol, and mannose) were higher in the NGT-T2D group than in the NGT-NGT group at follow- up (Figure 5.1B).

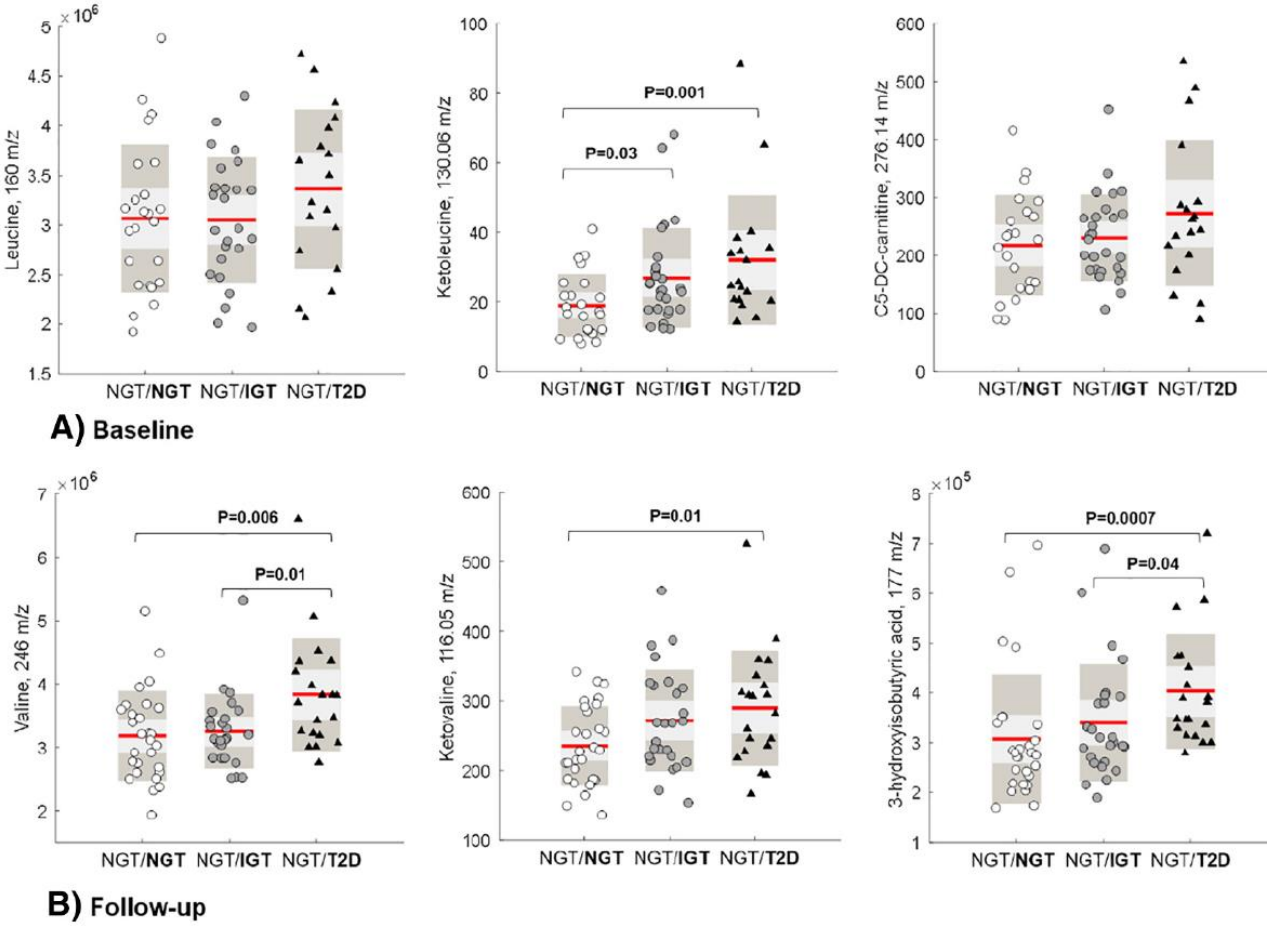


Figure 5.2: Branched-chain amino acid catabolism before (baseline) and after type 2 diabetes diagnosis (13-year follow-up). Relative concentrations of serum branched-chain amino acids (BCAAs: leucine, isoleucine, and valine) and their catabolic intermediates (ketoleucine, ketovaline, C5-carnitine, and 3-hydroxyisobutyric acid) are shown for NGT-T2D compared to NGT-NGT and NGT-IGT groups, at (A) baseline: prior to type 2 diabetes diagnosis and (B) 13-year follow-up: after diagnosis of type 2 diabetes or other conditions, as indicated. (A and B) The NGT-IGT group shows a BCAA pattern in-between the NGT-T2D and NGT-NGT profiles. The unique mass channel for each metabolite, used for quantification, is indicated on the y-axis. Red lines indicate the mean values, and data points are layered over the 95% confidence interval (white box) and the standard deviation (gray boxes). P-values were calculated by Wilcoxon-Mann-Whitney (WMW) rank sum test to compare the concentration differences of these highlighted metabolites between diagnosis groups.

A heatmap that displays baseline metabolite correlations to clinical endpoints at follow-up is shown in Figure 5.3. In line with the presented metabolite profiles, we found that the baseline LPC(C18:2):LPE(C18:2) ratio, their precursors (glycerol-2 and 3-phosphate) along with leucine and its catabolic intermediates were positively correlated with HOMA-IR, insulin, Hb1Ac and C-peptide at follow-up. The heatmap also highlights a negative correlation between CDCA (a primary bile acid) and UDCA (a secondary bile acid) and HOMA-IR, insulin, Hb1Ac and C-peptide at follow-up. To address the potential con- founding effect of anti-diabetic medications, we excluded all women that were on anti-diabetic medications at follow-up from the multivariate model (n = 7). This resulted in a similar albeit less pronounced metabolite pattern suggesting minor confounding of anti-diabetic medications (Appendix B, Figure 5S3).

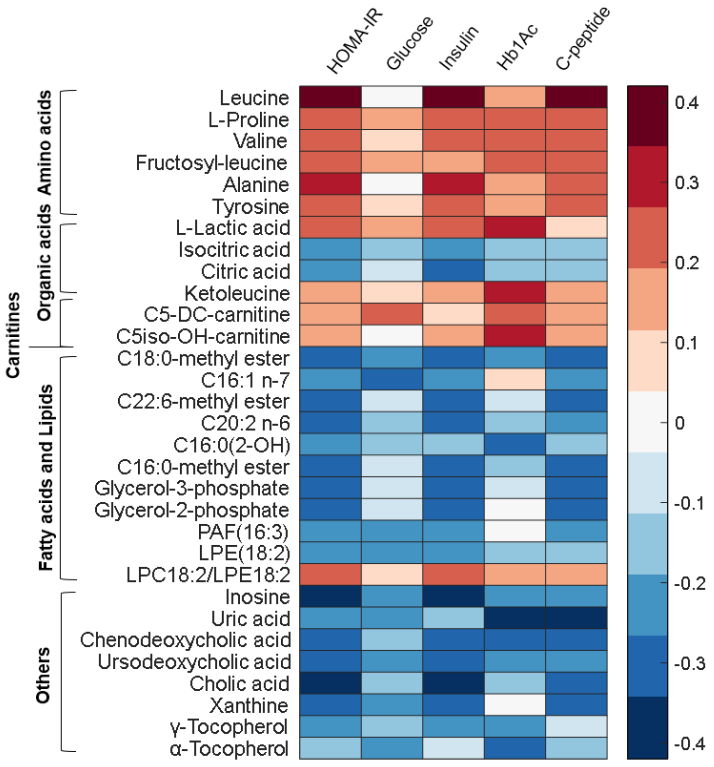


Figure 5.3. Heatmap of Spearman correlation coefficients between baseline metabolites and 13-year follow-up fasting clinical end points. Only annotated metabolites with average correlation to the clinical parameters above 0.15 are presented. Metabolites are sorted first by the compound class and then by their average correlations with clinical end points. The color code red indicates positive correlation, blue indicates negative correlation and white indicates no correlation with the specific parameters.

5.5 Discussion

Our multi-platform mass spectrometry-based metabolomics analysis revealed a distinct circulating metabolite profile that predicted the development of T2D in black SA women and presented novel and valuable information about an under-studied population with a high prevalence of T2D. Black SA women that developed T2D over a 13-year follow-up period had higher baseline LPC(C18:2):LPE(C18:2) ratio, a different bile acid metabolite profile, and higher levels of leucine along with its catabolic intermediates (i.e., ketoleucine and C5-carnitine), compared to women that remained NGT over the same period. Interestingly, the metabolites linked to the same pathways were altered between diagnostic groups at both baseline and follow-up with some similarities between the NGT-IGT and NGT-T2D groups. Metabolites related to phospholipid metabolism formed a part of the metabolic profile that predicted the development of T2D in this population. Prior to diagnosis, the NGT-T2D group had lower LPE (C18:2), a higher LPC(C18:2):LPE(C18:2) ratio, and lower phospholipid precursors (glycerol-2- and 3-phosphates), compared to the NGT-NGT group. In contrast, manifest T2D was associated with lower LPC(C18:2) levels, suggesting a shift in phospholipid metabolism as T2D develops. Lower LPC(C18:2) levels were markers for both prediabetes and T2D in the KORA- and EPIC-cohorts, which comprised white European/non-African individuals (186). More recently, data from the PREDIMED study showed that one-year changes in lysophospholipid levels were inversely related to T2D risk in a Mediterranean population including participants with high cardiovascular risk and many with established IGT (275). Lysophospholipids, such as LPC and LPE, play an important role in glucose-mediated insulin secretion and insulin sensitivity in peripheral tissues. Indeed, LPCs enhance glucose-dependent insulin secretion via an orphan G-protein coupled receptor (GPR), such as the GPR119 receptor, in pancreatic beta-cells, but their specificity remains to be determined (276). These lysophospholipid species may therefore act as lipokines that, via increased insulin secretion, induce beta-cell failure. Of note, previous research has shown that black SA women, compared to white SA women, hypersecrete insulin to maintain normoglycaemia (3,277). But with increasing age, insulin secretion decreases and is associated with IGT and T2D (9). However, lysophospholipid-related GPRs are also found in other tissues, such as skeletal muscle, where lysophospholipid-activation impairs fat and glucose oxidation and may thus induce metabolic inflexibility

and thus infer tissue dysfunction and potentially IR (278). Our finding of altered lysophospholipid metabolism could thus be a sign of both altered glucose-mediated insulin secretion and decreased metabolic flexibility associated with IR in skeletal muscle.

Similar to the lysophospholipids, we observed an altered bile acid profile between the glycaemic groups, both at baseline and at follow- up. The primary function of bile acids is to aid fat absorption, but they can also influence metabolic flexibility by stimulating glucagon-like peptide-1 and insulin secretion (279). After synthesis from cholesterol in the liver, primary bile acids are effectively re-absorbed and recycled in the distal ileum. However, when they are not re-absorbed, bile acid species can interact and mediate a wide-range of metabolic effects. Currently, the mechanisms underlying many of these effects remain unknown. We found that the levels of both a primary bile acid, CDCA, which is easily recycled, and a secondary bile acid, UDCA, which possesses anti-inflammatory properties (280), were lower at baseline in the NGT-T2D group, compared to the NGT-NGT group. At follow-up, we found higher levels of the secondary bile acid, DCA. This bile acid has been shown to modulate cell death by disrupting the mitochondrial outer membrane (281). However, UDCA has been shown to counteract/inhibit the apoptotic effect of DCA (282), which suggests that the NGT-T2D group had lower levels of “good” bile acids before T2D diagnosis and higher levels of “bad” bile acids after the diagnosis. In line with this, previous studies have shown that both the size and composition of the bile acid pool are altered in rodents and humans with T2D (213,283), potentially via impairments in insulin and glucose signalling. Numerous studies have shown that elevated BCAAs (leucine, isoleucine, and valine) predict the development of T2D, and it has been suggested that BCAAs might be involved in the pathogenesis of this disease (171,185). This may be due to a reduced activity of branched chain keto-acid dehydrogenase (BCKDH), the rate-limiting step in BCAA catabolism, which is markedly lower in T2D (171). Notably, our study is the first to show that changes in leucine catabolism precede alterations in valine and isoleucine catabolism >10 years before the onset of T2D. Although BCAAs are structurally similar, their catabolic pathways diverge after the BCKDH-step, which produces BCAA-specific CoA-intermediates with different metabolic fates. Leucine is exclusively ketogenic,

because it is directly degraded into acetyl-CoA, and it cannot be converted into glucose. Thus, this may make leucine and its intermediates prone to accumulate when circulating glucose levels are higher. The accumulation of BCAAs and their BCKAs, such as leucine and ketoleucine, have been shown to inhibit the nutrient sensing pyruvate dehydrogenase complex (PDH), which controls glucose oxidation (284). Indeed, inhibition of the PDH complex can result in the accumulation of toxic intermediates that confer tissue dysfunction via mitochondrial dysfunction/overload, which may ultimately lead to T2D (285). Leucine is also a potent activator of the mammalian target of rapamycin complex 1 signalling pathway, which enhances allosteric activation of PDH, altering TCA cycle activity, and potentially inducing metabolic inflexibility (171). Thus, elevated leucine metabolites could be a key mediator in the development of metabolic inflexibility and/or part of a feed-back mechanism of mitochondrial overload/dysfunction. This hypothesis is strengthened by our observation of elevated C5-carnitine levels in the NGT-T2D group at baseline. Our findings may indicate a “defensive response” since acylcarnitine-efflux has been suggested to serve as a detoxification process to prevent CoA-trapping and allow for CoA-dependent metabolic processes to continue, including the TCA cycle and β-oxidation (286). Based on our findings we suggest that a series of alterations in the discussed metabolic pathways occur during the development of T2D in overweight and obese black SA women. These are summarised in Figure 5.4 and include: 1) Inhibition of lysophospholipid synthesis; 2) Altered composition of the bile acid pool; 3) A reduced BCAA catabolism. Analyses of metabolite patterns in specific pathways that are associated with insulin resistance and β-cell failure, can therefore be useful in increasing the understanding of the pathophysiology, as well as predicting and monitoring the development of prediabetes and T2D in this high-risk population.

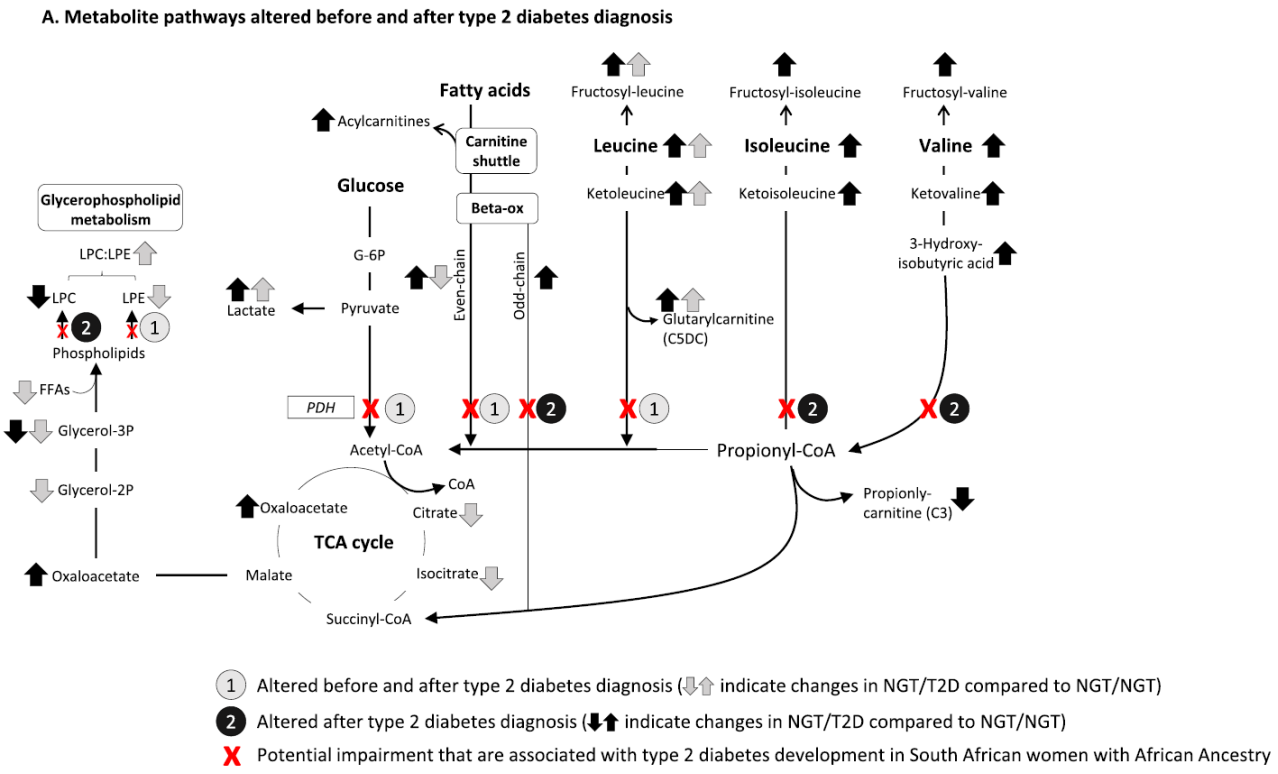


Figure 5.4: Metabolite pathways altered before and after type 2 diabetes development in black South African women. Leucine metabolites (leucine, ketoleucine, fructosyl-leucine) accumulate in the circulation of women that are normal glucose tolerant (NGT), but who develop type 2 diabetes over the study period (NGT-T2D), compared to those that will remain NGT (NGTNGT). Lactate also increases in the NGT-T2D group, presumably due to increased metabolic inflexibility, defined as inhibited glucose oxidation at the pyruvate dehydrogenase complex (PDH) (279). The lower levels of glycerophospholipid precursors, i.e. glycerol 2- and 3-phosphate, in the NGT-T2D group suggests altered glycerophospholipid biosynthesis in the pre type 2 diabetes state. This hypothesis is supported by the lower lyso-phospholipids in NGT-T2D compared to NGT-NGT. Impairments in branched-chain amino acid (BCAA) metabolism and glycerophospholipid metabolism form a putative link with impaired/lowered glucose oxidation, and thus, TCA intermediates, like malate, accumulate. Via the malate-aspartate shuttle, malate can be converted into oxaloacetate and glycerol-2-phosphate, which are consumed in glycerophospholipid synthesis, including the synthesis of lysophospholipids, like LPC and LPE. After disease development, all three BCAAs (leucine, isoleucine, and valine) and their catabolic intermediates are elevated in the NGT-T2D group compared to the NGT-NGT group, which suggests that this pathway is further impaired as disease progresses.

This study has some limitations including the low number of women that developed IGT and T2D during the study period, which affected the study power. However, since the same individuals were sampled at both occasions, the inter-individual variability was reduced, with increased study power. Differences in storage time did not allow for comparison of metabolite concentrations between baseline and follow-up, we therefore interpreted the two sampling occasions separately. Of note, even though all women had normal fasting glucose levels at baseline, baseline fasting glucose levels were higher in the NGT-T2D group compared to the NGT-NGT and NGT-IGT groups. Nonetheless we found distinct alterations in specific metabolite pathways that can provide additional information over and above that obtained from classical risk factors such as fasting glucose concentrations. The circulating metabolite profile provides a global snapshot of the metabolome; future studies should investigate the dynamics of the highlighted pathways in relevant tissues such as the muscle and liver, relative to changes in hepatic and peripheral insulin sensitivity for a broader understanding of the underlying mechanisms. Further studies are warranted on phospholipid metabolism and the flux within the bile acid pool that, compared to BCAA metabolism, are less explored in relation to the pathophysiology of T2D. It should be noted that our analyses did not capture the complete lipids metabolism, e.g., phosphatidylcholines/ethanolamines, ceramides and diacylglycerols, which are of interest and should be incorporated in future studies. Of note, we did not detect any consistent or prominent change in circulating fatty acids (precursors of ceramide metabolites) between baseline and follow-up. Importantly, circulating fatty acids as well as many other metabolites are highly associated with dietary intake. Future studies should therefore incorporate objective measures of dietary intake, including red blood cell lipid contents.

5.6 Conclusion

This is to the best of our knowledge the first longitudinal metabolomics study that has been conducted in overweight and obese black African women, a group at high risk of developing T2D. Our results may serve as a basis for further exploration of the pathophysiology with ensuing targeted prevention and treatment of T2D in this specific population.

CHAPTER SIX

Concluding chapter

6.1 Overview of research question and aim of the thesis

The rising burden of T2D is being primarily borne by low- and middle-income countries such as SA (1). Irrespective of geographical location, black Africans, especially women, have higher risk and T2D prevalence than white Europeans (3,5,7). Notably, the pathophysiology of T2D differs between the two ethnicities (3,18,30,87,103), with black African women having less VAT and liver fat accumulation, and more peripheral (i.e., gluteo-femoral) adiposity compared to their white European counterparts (3,5,18,21,87,105), as described in Figure 6.1. Despite these favourable metabolic phenotypes, black African women still present with higher IR, which is associated with greater insulin response to glucose (i.e., hyperinsulinaemia), often exceeding that which is needed to maintain normoglycaemia, compared to white European women (3,5,18,55,111) (Figure 6.1). The exact causes and biological mechanisms underlying these phenomena are poorly understood. This is due to the paucity of research, particularly in black African women from Africa, as well as the fact that the majority of studies have been limited to small cross-sectional studies of pre-menopausal women.

This thesis aimed to identify risk factors for T2D and elucidate some of the biological mechanisms implicated in the pathophysiology of T2D in middle-aged urban black SA women. This aim was based on the three complementary theories, namely spillover or adipose tissue expandability theory, portal theory and the twin-cycle theory, which describe how adipose tissue dysfunction leads to the development of T2D (39,84,90,100,101,287). Notably, these theories are discussed in detail under section 1.6. In brief, it is proposed that obesity or excessive fat accumulation, caused by non-modifiable and/or modifiable factors, reduces or results in the loss of SAT's capacity to store excess fat and suppress lipolysis (39,90,91). This in turn leads to the influx of FFAs into the visceral depot and in non-adipose tissues such as muscles and liver, and subsequently impairs the metabolic functions of these tissues including sensitivity to insulin (39,90,91) (Figure 6.1). Since VAT has a higher lipolytic activity and inflammatory profile than SAT, its lipid and pro-inflammatory products are directly released into the hepatic portal system (84,96,99,235). These circulating products get deposited into the liver, and subsequently cause hepatic IR and reduction in insulin clearance, and further

lead to the production of toxic lipid intermediates (e.g., TG-VLDL) in metabolic tissues including the pancreatic beta-cells (Figure 6.1). Gradual accumulation of FFAs and/or other lipid intermediates create a lipotoxic environment which aggravates these metabolic defects, in particular hyperglycaemia, IR and hyperinsulinaemia via reduced insulin clearance and increased insulin secretion (95,192,288). Collectively, these biological defects lead to beta-cell failure and subsequently result in the development of T2D (Figure 6.1).

Therefore, in an attempt to further elucidate the pathophysiology of T2D in middle-aged black SA women, the aim of this thesis was addressed in four chapters consisting of longitudinal and cross-sectional studies which were generated for this thesis. The summary of objectives, hypotheses and key findings of these chapters are described in Table 6.1.

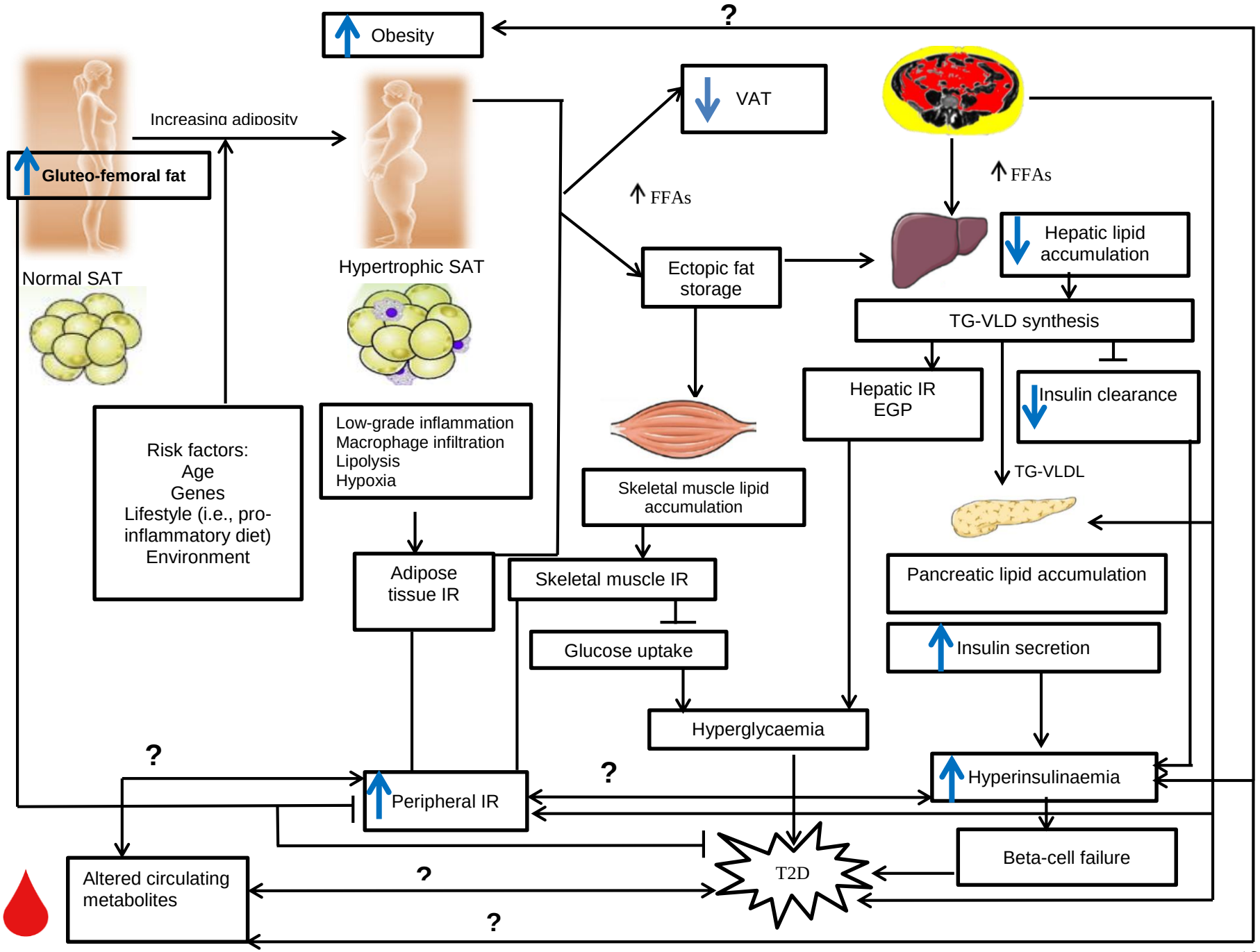
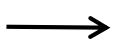

Obesity
?
Increasing adiposity
Gluteo-femoral fat
Normal SAT
Hypertrophic SAT
VAT
FFAs
FFAs
Ectopic fat storage
Hepatic lipid accumulation
TG-VLD synthesis
Hepatic IR EGP
Insulin clearance
TG-VLDL
Risk factors:
Age
Genes
Lifestyle (i.e., pro-inflammatory diet)
Environment
Low-grade inflammation
Macrophage infiltration
Lipolysis
Hypoxia
Skeletal muscle lipid accumulation
Adipose tissue IR
Skeletal muscle IR
Glucose uptake
Hyperglycaemia
Pancreatic lipid accumulation
Insulin secretion
Hyperinsulinaemia
Peripheral IR
Beta-cell failure
T2D
Altered circulating metabolites
?
?
?
?

Figure 6.1: A schematic diagram describing the pathophysiology of T2D in black African women. Abbreviations: EGP, Endogenous glucose production; FFA, Free fatty acids; IR, Insulin resistance; SAT, Subcutaneous adipose tissue; T2D, Type 2 diabetes; TG, Triglycerides; VAT, Visceral adipose tissue; VLDL, Very-low density lipoprotein.



Solid arrows indicate a positive association.



Solid line represents an inverse association.



Arrows indicate higher or lower in comparison to white European women.

?

Question marks suggest that the relationship between the two factors is not fully understood (e.g., peripheral IR vs hyperinsulinaemia)

6.2 Summary of the objectives, hypotheses and key findings

Table 6.1: Summary of the objectives, hypotheses and key findings

Chapter/ Study number	Objectives	Hypotheses	Summary of key findings
Chapter 2/Study 1 (Longitudinal study)	-To explore if baseline and/or change in body fat and fat distribution was associated with measures of glycaemia and the risk of developing IGM /T2D 13 years later.	-At baseline, obese women with obesity, in particular high central obesity and low peripheral adiposity will have low insulin sensitivity and high risk of developing IGM/T2D at follow-up.	-Over the 13-year follow-up period, body fat increased by 5.8 ± 6.3kg. -The increase in body fat over the 13 years was associated with a relative increase in central adiposity (i.e., trunk %FM and VAT) and a decrease in peripheral adiposity (leg %FM). -Independent of age, baseline trunk FM and VAT, and the change in VAT were positively associated with IGM/T2D risk at follow-up. -Furthermore, both baseline and change in trunk FM and VAT were inversely associated with insulin sensitivity, 13 years later. -In contrast, baseline leg FM was positively associated with insulin sensitivity and inversely associated with IGM/T2D risk at the 13-year follow-up period, respectively.
Chapter 3/Study 2 (Cross-sectional	-To examine the relative contributions of insulin	-In middle-aged and older black SA women, both insulin secretion and	-Both insulin secretion and clearance were significantly associated with hyperinsulinaemia in

study)	secretion and clearance to basal and postprandial hyperinsulinaemia. -Further, to explore the associations between insulin sensitivity, adiposity and a proxy of liver fat (i.e., FLI), and insulin secretion and clearance.	clearance are significantly associated with basal and postprandial hyperinsulinaemia. However, insulin clearance will explain a higher proportion of the variance in hyperinsulinaemia than insulin secretion. -Insulin sensitivity, measures of adiposity and FLI are significantly associated with insulin secretion and clearance.	the basal and postprandial states. However, insulin secretion accounted for more variance in the basal state, whereas total insulin secretion and clearance contributed similarly to total hyperinsulinaemia during the OGTT. - Insulin sensitivity, adiposity (i.e., total and VAT) and FLI (a proxy for liver fat) were associated with both insulin secretion and clearance parameters. - Peripheral adiposity was only significantly associated with measures of insulin secretion. - Insulin sensitivity accounted for a greater amount of the variance in both insulin secretion (8-38%) and clearance (13-26%) compared to measures of adiposity and FLI.
Chapter 4/Study 3 (Cross-sectional study)	-To determine whether dietary-induced inflammation, measured by the dietary inflammatory index (DII), was associated with markers of T2D risk, and if this association was mediated by adiposity and/or inflammatory markers.	-A pro-inflammatory diet is associated with markers of T2D and this association is mediated by adiposity and low-grade inflammation.	-Energy-adjusted DII was associated with markers of T2D risk, namely, fasting glucose and insulin, HbA1c, HOMA2-IR, two-hour glucose and insulin sensitivity and this association was mediated by measures of adiposity but not inflammatory markers. -Measures of central adiposity, in particular VAT, had proportionally higher (23.5–100%) mediation effects than total adiposity measures (10–60%).
Chapter 5/Study 4	-To identify circulating	-A circulating metabolic pattern	-A metabolite profile characterised by BCAA and

(Longitudinal study)	metabolite patterns that predict the development of T2D in black African women, and to examine if these metabolite profiles were significantly different between the three glycaemic groups (i.e., NGT, IGT and T2D).	consisting of BCAAs (i.e., isoleucine, leucine or valine), lipid-subtypes (e.g., lysophosphatidylcholines and long-chain acylcarnitines) and bile acids will be associated with the development of T2D 13 years later.	their catabolic metabolites, lysophospholipid and bile acid metabolites predicted the development of T2D in middle-aged black SA women. -At baseline, participants that developed T2D (NGT-T2D) had lower proliferation-related bile acids and higher phospholipid, BCAA, and their catabolic intermediates, compared to those who remained NGT (NGT-NGT). At follow-up, these metabolite patterns remained significantly altered between the two groups.

Abbreviations: BCAA, Branched-chain amino acids; DII, Dietary inflammatory index; FLI, Fatty liver index; FM, Fat mass; IGM, Impaired glucose metabolism; IGT, Impaired glucose tolerance; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance tests; SA, South Africa; T2D, Type 2 diabetes; VAT, Visceral adipose tissue.

6.3 Summary and implications of the study findings

Although there are ethnic differences in the pathophysiology of T2D, there are some common risk factors such as high central adiposity, in particular VAT, which is significantly associated with IR and T2D risk (17,21,99,105), while peripheral adiposity is inversely associated with IR and T2D risk (30,97,127,289) (Figure 6.1). With the dearth of studies using precise and accurate measures of body fat and fat distribution such as DXA, it was not known if VAT and peripheral adiposity or other measures of body fat and fat distribution were predictors of T2D in black Africans, in particular from Africa, as shown in white Europeans (108,109). The current findings from the first longitudinal study have added to literature by showing that VAT accumulation is a significant determinant of IR and the development of T2D, whereas peripheral adiposity protects against the development of IR and T2D in African women (Figure 6.1).

It is well documented that hyperinsulinaemia is mainly determined by increased insulin secretion and/or reduced insulin clearance, which are believed to act as compensatory responses to obesity and/or IR (3,5,7,9,18,56–58) (Figure 6.1). However, studies examining the contribution of both insulin secretion and clearance in black African women, in particular from Africa are very scarce (18,53,112,114). Only two studies have examined the contribution of insulin secretion and clearance to hyperinsulinaemia in black SA women (112,114). However, one of these studies was conducted more thirty years ago in a small sample of black (n=10) and white (n=8) SA women (114). Furthermore, both studies had contradictory results to those reported in majority of black Africans, with insulin levels and/or C-peptide levels being lower in black SA women compared to white SA women (112,114). With insufficient and contradictory findings, the pathophysiology of hyperinsulinaemia in black SA women required further investigation. Using a cross-sectional study design (study 2), I showed that both insulin secretion and clearance were significant contributors to hyperinsulinaemia in middle-aged black SA women. In contrast, insulin clearance rather than insulin secretion was the major contributor to hyperinsulinaemia in the majority of studies in other black African adults from the USA and West Africa (7,18,61,80). The discrepancies between my findings and the findings reported in previous studies could be attributed to several factors. These factors include the contribution of insulin secretion and clearance was explored in a

single group in the current study, while previous studies compared two ethnic groups (i.e., black African vs white European) (7,18,61,80,116–118). Although it is well recognised that black Africans have a hyperinsulinaemic response to glucose compared to white Europeans (3,5,18,53,80,116–118), the contribution of insulin secretion and clearance to hyperinsulinaemia in black Africans is not fully understood. This might be partly due different study populations, in terms of age, level of IR, glucose tolerance, adiposity status and ectopic fat deposition, as well as variations in genetic and lifestyle factors (41,58,61,64,83,110). The methods or techniques (e.g., intravenous vs oral) applied also vary. Furthermore, the assessment of hyperinsulinaemia, in particular in studies of black African women, has been examined in terms of beta-cell function (3,5,55,79,113), with few studies exploring insulin clearance (18,53). Collectively, these discrepancies make it difficult to delineate the pathophysiology of hyperinsulinaemia in black African women, and suggest that future studies recruit a homogeneous sample, or participants need to be classified according to their phenotypes (i.e., adiposity, glucose tolerance, insulin sensitivity). Moreover, studies should try to use both oral and intravenous techniques. Most importantly, they should also measure C-peptide levels to enable quantification of both insulin secretion and clearance. Together, these studies highlight the complexity of hyperinsulinaemia in black African women. Thus, in an attempt to gain insights in the pathophysiology of hyperinsulinaemia, in particular in black SA women, I explored the associations between insulin sensitivity, body fat and its distribution, VAT and FLI, and insulin secretion and clearance. These associations have been reported in various studies conducted in USA and Europe (41,58,83,110) (Figure 6.1) and needed to be explored in an African population. In study 2 (Chapter 3), I also showed that body fat, VAT, FLI (i.e., as a proxy of liver fat) and insulin sensitivity were associated with insulin secretion and clearance, with insulin sensitivity having stronger associations than the other factors. With these findings, it is postulated that the contribution of insulin secretion and clearance to hyperinsulinaemia may be largely attributed to insulin sensitivity and to a lesser extent adiposity and FLI. However, it is important to note that insulin sensitivity, adiposity and FLI are inter-linked (Figure 6.1). Furthermore, it is not clear if IR (or obesity) is a cause or direct consequences of hyperinsulinaemia (Figure 6.1). Hence further investigation, in particular longitudinal studies are required to confirm these associations. Future studies, in particular dietary intervention and longitudinal studies are required to provide better insights into the risk

factors and biological mechanisms of insulin secretion and clearance, and the subsequent development of hyperinsulinaemia in an African population.

The prevalence of obesity and T2D in black SA women has been attributed to the consumption of an unhealthy diet (150–152). However, these studies relied on the assessment of dietary patterns or single-nutrients to explore the association between dietary intake and T2D risk (150–152). This approach has some limitations, which include the fact that single nutrients are rarely consumed alone. Hence, dietary tools have been established, such as DII, which is an estimate of total diet-induced inflammation, to examine its association with metabolic disorders such as obesity and T2D (156–158). While DII has been linked to obesity and T2D risk in non-African populations (154,161,163), it was not known if these associations could be replicated in an African sample, whose lifestyle, and environmental factors differs from these other populations. Accordingly, in study 3, I showed that E-DII was associated with fasting glucose and insulin, HbA1c, HOMA2-IR, two-hour glucose and insulin sensitivity. These associations were mediated by measures of adiposity, in particular central adiposity. These findings show that a pro-inflammatory diet, common in our sample of middle-aged black SA women, is implicated in obesity, in particular central obesity, resulting in a higher risk for T2D. The use of the DII enabled us to gain a greater understanding of the effect of diet on the pathophysiology of T2D in a high-risk and understudied sample. Therefore, promotion of healthy eating behaviours might assist in addressing the increasing prevalence of obesity and T2D in black SA women.

Based on the above findings, it is evident that black African women present with a complex T2D pathophysiology that is not fully understood. Hence, a metabolomics approach was utilised in my fourth study (chapter five) to identify biomarkers that may assist in risk assessment and provide better insights on the underlying metabolic processes involved in the development of T2D in black African women. Accordingly, this thesis identified a metabolite profile that predicted future T2D in middle-aged black SA women, characterised by alterations in BCAA metabolites and their catabolic intermediates, phospholipid and bile acid metabolism. The observed alterations in BCAA metabolism during T2D development in this study suggests an increase in leucine and

its catabolic by-products occur in the early phase (<13 years prior to diagnosis) followed by increases in valine and isoleucine catabolism that occur at a later stage in the development of T2D. In contrast, it was shown that alterations in lysophospholipid and bile acid metabolisms occur before the development of T2D and continue to change as the disease progresses. It is important to highlight that metabolomics has some limitations, which are stated under the strengths and limitations of the thesis section. One of these limitations is that it cannot confirm a cause-effect relationship between the identified metabolites and/or metabolic pathways, and the development of T2D in this sample (Figure 6.1). Nonetheless, this study has advanced our knowledge about the biological mechanisms underlying the development of T2D in Africa women.

It is also important to highlight that the risk factors and biological mechanisms investigated in this thesis only provide a snapshot of a complex and multi-factorial pathophysiology of T2D in African women, as presented in Figure 6.1. Notably, black Africans are a very diverse group, as evidenced by the distinct genetic admixture in black Africans from the USA (223–225) and within the African continent (290) including the disparities or inconsistencies in some of the findings (e.g., insulin sensitivity, contribution of insulin secretion and clearance to hyperinsulinaemia, associations of liver fat and insulin clearance) between studies conducted in SA, USA and UK (43,53,87). This means that current findings may not reflect or represent the pathophysiology of T2D in all black Africans, more especially when taking into account genetic variations, and region-specific factors such as nutrition and physical activity. Nonetheless, the results generated from this study have advanced our understanding about the development of T2D in middle-aged and older black SA women, a high-risk group that is understudied. The study findings also have the potential to assist in the early detection, prevention and management of T2D in black African women, specifically middle-aged black SA women.

6.4 Strengths and limitations of the thesis

Notably, a convenient sample of women was recruited from Soweto instead of a random sample. Reasons for this were that the participants are the caregivers of the Bt20+ cohort, on whom we have extensive longitudinal data (240). Due to the strict inclusion

criteria and the difficulty contacting and recruiting participants at follow-up, the sample size for the longitudinal analyses was limited, resulting in few cases of IGM and/or T2D. Nonetheless it provided the first longitudinal cohort of black African women for which we could investigate the risk factors and/or biological mechanisms implicated in the development of T2D in this understudied group.

Chapter two of this thesis is the first longitudinal study to accurately measure DXA-derived body fat and its distribution, and examine its role in the development of T2D in an African population. The use of DXA is of particular relevance for these women who have the highest prevalence of obesity in SA, but have less VAT and more peripheral adiposity compared to their white European counterparts (3,17,21,87). The assessment of body fat and its distribution enabled us to gain a better understanding of the association between body fat and regional adiposity, and insulin sensitivity, secretion and clearance, as well as the DII. Despite the strength of measuring body composition with DXA, MRI and CT scans are preferred measures of VAT and liver fat accumulation (108,128–130). Although DXA-derived VAT has been validated against VAT measured with these instruments, it has been also been shown that DXA may under- and over-estimate VAT at very high and very low levels, respectively, compared to MRI or CT (108,128–130). However, with increasing BMI, more especially in the obese category, it has been noted that the variance in VAT between DXA and CT was the same (129). This implies that DXA might be appropriate in measuring VAT in individuals with a high prevalence of obesity such as the current sample.

There are many factors implicated in the pathophysiology of T2D which were not measured in the present study, particularly at baseline. These include factors such as sex hormones, physical activity, and genetic factors, and therefore I was unable to further explore the progression of T2D over the 13-year period. Prior to this study, no longitudinal study in Africa had explored the changes in body fat and fat distribution over the menopause transition, which is associated with redistribution of fat and an increase in VAT (44,105,132,133). Although I did not objectively measure sex hormones, particularly oestrogen, it is important to note that the development of T2D in this sample occurred over the menopausal period which may have exacerbated the increase in VAT

with ageing.

In study 2 (chapter three) we also used a mathematical model of beta-cell function in our sample of black SA women. This model is a more reliable measure of beta-cell function than the insulinogenic index, which has been previously used in the black SA population (9), as it uses C-peptide instead of insulin concentrations to estimate endogenous pre-hepatic insulin secretion (74,75). Thus, it is able to quantify insulin secretion and clearance as distinct processes, thus enabling us to provide a better analysis of the relationship between insulin secretion and clearance, and glucose concentrations, and their contribution to hyperinsulinaemia (74,75). Moreover, an algorithm, the FLI, was used to estimate liver fat (140). Although the FLI has not been validated in an African population, this parameter has been validated against ^{1}H-MRS and been shown to identify those with and without fatty liver disease (141). Compared to the gold standard methods such as ^{1}H-MRS, the FLI is more practical and cost-effective for large epidemiological studies and under-resourced settings, in particular in developing countries such as SA. For these reasons, I was able to use the FLI in an African population to explore its association with insulin secretion and clearance, thus providing a possible link between liver fat accumulation and hyperinsulinaemia in black Africans from Africa.

Surrogate measures of hepatic IR (HOMA-IR) and peripheral insulin sensitivity (Matsuda Index) were used instead of the HEC. These measures have been shown to be good proxies of IR and insulin sensitivity measured using the HEC (69,70). Although there is no gold standard test for measuring beta-cell function, the intravenous tests (e.g., IVGTT) and clamp procedures (i.e., HC) are often used (5,7,18,291). However, these tests are not feasible and are less physiological compared to the oral tests, as they do not induce or mimic the biological processes stimulated following the oral consumption of a glucose load or meal, such as the release of incretins or the effect of dietary composition, which have been shown to regulate insulin secretion and clearance (62–67). However, it is acknowledged that the OGTT including the intravenous tests and clamp procedures relies on glucose as the only nutrient stimulant, which MMTT a better representative of a habitual/ standardised diet. The OGTT also does not differentiate

between hepatic and extra-hepatic insulin clearance. These are distinct processes that are independently regulated (52). Their assessment is of relevance for this population, since it has been shown that hepatic insulin clearance is a major contributor to hyperinsulinaemia in Africans (52–54).

Due to the lack of accurate assessment of lifestyle factors, such as dietary intake using a validated questionnaire at baseline, I was unable to examine the association between diet and the development of T2D 13-years later. Although self-reported dietary assessment has limitation, at follow-up, a 7-day FFQ, which has been validated in a SA population was chosen as the dietary tool to investigate the association between diet and T2D (253,254). The FFQ data was used to calculate the DII, making this study the first one to examine the association between DII and T2D risk in an African population. This was also the first study to explore the association between DII and DXA-derived body fat and fat distribution. Indeed, previous studies used basic anthropometric measurements (e.g., BMI and WC) to assess the relationship between DII and adiposity (154,160–162). Therefore, as the first study to use the DII in combination with DXA-derived body fat and distribution, I showed that E-DII was associated with markers of T2D risk, and that this association was mediated by measures of adiposity but not inflammation. The findings that markers of inflammatory markers did not mediate the association between E-DII and T2D risk might be attributed to the fact that only three (i.e., TNF-α, 1L-8 and MCP-1) out of the eight possible inflammatory cytokines were detected in all the samples. It is important to emphasize that besides the group having high obesity levels, the participants did not have any inflammatory-related diseases, and approximately 12-13% of the entire sample had T2D, and these participants were excluded in study 3 (chapter four). Therefore, it is believed that the sensitivity of the assay was not sufficient to measure these inflammatory cytokines in this sample. The results were verified by the manufacture, and they confirmed that this was not a technical error. Nonetheless, out of these three inflammatory cytokines, only TNF-α was significantly associated with E-DII, but it was not associated with markers of T2D. Despite the advantages of using the DII to measure the inflammatory profile of the diet, it is important to note that the DII was calibrated against a global dietary database that did not include a habitual diet of an African population (14). Furthermore, some of the

variables (e.g., diet intake) and OGTT-derived data measured at a single time point, thus might not reflect the long-term lifestyle activities and the metabolic status of the participants.

This study was also the first to utilise a metabolomics approach to identify metabolites and/or metabolic pathways linked to the development of T2D in African women. A metabolite profile characterised by alterations in BCAA metabolites and their catabolic intermediates, phospholipid and bile acid metabolism, predicted future T2D 13 years later in our sample of middle-aged black SA women. It is important to note that metabolomics has some limitations, and these include the fact that it is not clear if the identified metabolites and/or metabolic pathways cause the metabolic diseases or are the direct consequence of the metabolic disorders under investigation. It is also not known which tissues the altered metabolites occurred since the identified metabolites and/or metabolic pathways were measured in circulation. Due to differences in storage, the changes in the metabolites over time could not be explored, rather the metabolite patterns between the NGT-T2D and NGT-NGT groups was examined separately at baseline and follow-up.

It is also important to highlight that insufficient physical activity might have been a contributing factor to excess fat accumulation, in particular VAT, and its subsequent development into T2D in this sample. However, it is postulated that physical inactivity might have a lesser role to play in the development of T2D compared to unhealthy diets. To elaborate, black SA women do not typically participate in leisure physical activity, but do meet the WHO physical activity guidelines, through moderate to vigorous intensity activity which is undertaken through work- and transport-related activity (165). However, assessment of physical activity in black SA women in cross-sectional and longitudinal analyses has shown minimal effects in protecting against obesity, IR and hyperinsulinaemia, and subsequently T2D risk (165,167). In accordance with these findings, the cross-sectional comparison of objectively measured physical activity and sedentary data between the NGT and IGM/T2D groups was similar in the current study. Based on these findings, it is postulated that promotion of healthy eating behaviours in combination with leisure physical activity participation (i.e., through moderate-to-

vigorous intensity) as lifestyle activities might have promising results in combating the risk of developing obesity and T2D in this population.

6.5 Future research and recommendations

Future studies should identify the risk factors and/or biological mechanisms that predict the development of VAT and liver fat accumulation in African women, including the factors that link VAT and liver fat accumulation in the development of T2D in this group. This knowledge will also assist in understanding how black Africans are more predisposed to T2D risk, when they paradoxically present with lower VAT and liver fat compared to their white European counterparts (3,5,18,21,87,105). Accordingly, future studies should also examine whether black Africans, in particular black African women, are more sensitive to the effects of VAT and liver fat accumulation. If this is the case, what are the driving factors underlying this phenomenon? It is recommended that assessment of lifestyle factors (e.g., dietary intake), through intervention and longitudinal studies might provide better insights on how changes in these risk factors lead to VAT and liver fat accumulation, and subsequently increase the risk of developing IR, hyperinsulinaemia and T2D. For example, future studies could examine if a very low-calorie diet decreases VAT and liver fat as well pancreatic fat, leading to the recovery of beta-cell function in T2D individuals, as previously shown in The Diabetes Remission Clinical Trial (265,292).

While I was able to investigate the relative contribution of insulin secretion and clearance to hyperinsulinaemia in this sample using a cross-sectional design, it is important to highlight that longitudinal studies are needed to confirm these associations and further delineate the risk factors and/or biological mechanisms linking insulin secretion and clearance to the development of hyperinsulinaemia in black African women. Further, longitudinal studies can examine if VAT and liver fat, as well as body fat and other measures of fat distribution, predict insulin clearance as well as beta-cell insulin secretion in the African population. Although, IR and hyperinsulinaemia are closely related, the nature of their relationship is not fully understood. Thus, exploration of the relationship between IR and hyperinsulinaemia requires investigation in order to

determine whether IR is the cause or the direct consequence of hyperinsulinaemia in this group, or if association varies within the black African population. Furthermore, studies should investigate which factors, for example, adipose tissue dysfunction, IR, and genetic and lifestyle factors, are the main drivers of hyperinsulinaemia and T2D in black Africans, and whether these risk factors are region or population specific.

The DII needs to incorporate the diversity of the African diet in order to truly capture effects of an inflammatory diet on the development of obesity and T2D in an African population. Most importantly, future studies should also measure more inflammatory cytokines, preferably using assays that have higher sensitivity than the one used in the present study. This might also help determine whether or not inflammatory markers are significant mediators of the association between E-DII and markers of T2D. Moreover, the validation of the FLI is required to assess the reliability and accuracy of FLI in estimating liver fat and the risk of NAFLD in an African population. This is of great importance since black Africans present with lower liver fat and NAFLD prevalence than their white European counterparts (18,20,41,43,103). The validation of the FLI will be beneficial for under-resourced research and clinical settings in Africa that might not afford to utilise the precise techniques for liver assessment such as the MRI and $^{+}$H-MRS.

Future research needs to incorporate genetic, lifestyle factors in metabolic analyses in order to provide better insights into the risk factors underlying changes in metabolic and/or metabolic pathways. It is also believed that analysis of metabolite and/or metabolic pathways in the metabolic tissues in which the perturbations are occurring, will have great benefits in elucidating the pathophysiology of T2D in black Africans. In addition to insulin sensitivity, future studies are recommended to expand on the association between metabolite and/or metabolic pathways and other markers of T2D such as insulin secretion and clearance. While there is extensive literature on BCAA metabolism and its link in the development of T2D, other metabolite and/or metabolic pathways such as bile acid metabolism are poorly understood, and thus need to be explored in detail. This is of relevance since bile acid metabolism is closely linked to liver function (208,209,213), which might in turn provide better insights on the

pathophysiology of hyperinsulinaemia, in particular via insulin clearance. Considering the high prevalence of obesity in this sample, future studies should also incorporate lipidomics to provide better insights on the lipid metabolites implicated in the development of T2D. Moreover, analysis of the metabolites and/or metabolic pathways in the relevant tissues requires investigation. Future research on cell culture and animal models where the affected metabolite and/or metabolic pathways are manipulated might assist in addressing the raised concerns; whether the identified metabolites and/or metabolic pathways are the cause or direct consequence of the metabolic disorders.

This study focused on black SA women, being a high risk group for development of T2D (2,10). While SA women, especially black women are at greater risk for T2D compared to their male counterparts, it is important to note that the pathophysiology of T2D in SA men is understudied. Furthermore, with the findings that behavioural factors such as smoking and alcohol intake are rapidly increasing among young men (11), the pathophysiology of T2D in black SA men requires urgent investigation. Notably, SA also has an additional challenge of infectious diseases, particularly HIV. Although there has been a significant decline in HIV-related deaths in SA over the years, SA women are still at higher risk of acquiring HIV than men, with new infections being reported in young women (2). Of relevance, black SA women receiving antiretroviral drugs were shown to have an unfavourable adipose tissue phenotype (i.e., increased central obesity and reduced peripheral adiposity (293), which has been implicated in increased T2D risk and hypertension (137,231). There is a great need for future studies to provide insights on the biological mechanisms implicated in the dual burden of T2D and infectious diseases and the associated effects of treatment. Investigation of the biological mechanisms implicated in the development of T2D in African populations such as adipose tissue dysfunction, IR, hyperinsulinaemia, and metabolite and/or metabolic pathways are also needed to be explored by sex, menopausal status and HIV status.

6.6 Conclusion

In conclusion, this thesis has provided novel insights into the complex pathophysiology of T2D in black Africans, in particular black African women in the post-menopausal

stage, who are understudied compared to their pre-menopausal counterparts. The take-home message from this thesis is that prevention of obesity, in particular central obesity is crucial in preventing or reducing the high risk and T2D prevalence in black African women. In the long-term, the knowledge generated from this thesis has significant benefits in formulating public health policies and practices to assist in addressing the rapid increase in the prevalence of obesity and T2D in SA, in particular in black SA women. As a first initiative, it is recommended that government should work together with urban black SA communities to promote the importance of healthy eating in order to prevent obesity, in particular central obesity, and lower risk of developing IR, hyperinsulinaemia and T2D.

REFERENCES

Reference list

1. IDF. IDF Diabetes Atlas 9th Edition. 9th ed. Suvi Karuranga, Belma Malanda, Pouya Saeedi PS, editor. International Diabetes Federation; 2019. 1–64 p.
2. Statistics South Africa. Mortality and causes of death in South Africa, 2016: Findings from death notification. Statistical release. Pretoria, South Africa: Statistics South Africa; 2018. 1–135 p.
3. Goedecke JH, Dave JA, Faulenbach M V, Utzschneider KM, Lambert E V, West S, et al. Insulin Response in Relation to Insulin Sensitivity-An appropriate Beta-cell response in black South African women. Diabetes Care. 2009;32(5):860–5.
4. van der Merwe M, Crowther N, Schlaphoff G, Gray I, Joffe B, Lonnroth P. Evidence for insulin resistance in black women from South Africa. Int J Obes. 2000;24:1340–6.
5. Chandler-Laney P, Phadke R, Granger W, Fernández J, Muñoz J, Man C, et al. Age-related changes in insulin sensitivity and β-cell function among European American and African American women. Obes (Silver Spring). 2011;19(3):528–35.
6. Goff LM, Griffin BA, Lovegrove JA, Sanders TA, Jebb SA, Bluck LJ, et al. Ethnic differences in beta-cell function, dietary intake and expression of the metabolic syndrome among UK adults of South Asian, black African-Caribbean and white-European origin at high risk of metabolic syndrome. Diabetes Vasc Dis Res. 2013;10(4):315–23.
7. Osei K, Schuster DP. Ethnic Differences in Secretion, Sensitivity, and Hepatic Extraction of Insulin in Black and White Americans. Diabet Med. 1994;11:755–62.
8. Uwaifo GI, Nguyen TT, Keil MF, Russell DL, Nicholson JC, Bonat SH, et al. Differences in insulin secretion and sensitivity of Caucasian and African American prepubertal children. J Pediatr. 2002;140(6):673–80.
9. Goedecke JH, George C, Veras K, Peer N, Lombard C, Victor H, et al. Sex differences in insulin sensitivity and insulin response with increasing age in black South African men and women. Diabetes Res Clin Pract [Internet]. 2016;122:207–14. Available from: http://dx.doi.org/10.1016/j.diabres.2016.11.005
10. Peer N, Steyn K, Lombard C, Lambert E V., Vythilingum B, Levitt NS. Rising Diabetes Prevalence among Urban-Dwelling Black South Africans. PLoS One. 2012;7(9):1–9.
11. Peer N, Bradshaw D, Laubscher R, Steyn N, Steyn K. Urban-Rural and gender differences in tobacco and alcohol use, diet and physical activity among young black South Africans between 1998 and 2003. Glob Health Action. 2013;6(1):1–10.
12. Vorster H. The emergence of cardiovascular disease during urbanisation of Africans. Public Health Nutr [Internet]. 2002;5(1a):239–43. Available from:

http://www.journals.cambridge.org/abstract_S1368980002000332
13. Puoane T, Matwa P, Bradley H, Hughes G. Socio-cultural factors influencing food consumption patterns in the black African population in an urban township in South Africa. Hum Ecol. 2006;14:89–93.
14. Okop KJ, Mukumbang FC, Mathole T, Levitt N, Puoane T. Perceptions of body size, obesity threat and the willingness to lose weight among black South African adults: a qualitative study. BMC Public Health [Internet]. 2016;1–13. Available from: http://dx.doi.org/10.1186/s12889-016-3028-7
15. Nienaber-Rousseau C, Sotunde OF, Ukegbu PO, Myburgh PH, Wright HH, Havemann-Nel L, et al. Socio-Demographic and Lifestyle Factors Predict 5-Year Changes in Adiposity among a Group of Black South African Adults. Int J Environ Res Public Health. 2017;14(9).
16. Draper CE, Davidowitz KJ, Goedecke JH. Perceptions relating to body size, weight loss and weight-loss interventions in black South African women: a qualitative study. Public Health Nutr [Internet]. 2016;19(03):548–56. Available from: http://www.journals.cambridge.org/abstract_S1368980015001688
17. Goedecke JH, Levitt NS, Lambert E V., Utzschneider KM, Faulenbach M V., Dave JA, et al. Differential effects of abdominal adipose tissue distribution on insulin sensitivity in black and white South African women. Obesity. 2009;17(8):1506–12.
18. Chung ST, Galvan-De La Cruz M, Aldana PC, Mabundo LS, DuBose CW, Onuzuruike AU, et al. Postprandial Insulin Response and Clearance Among Black and White Women: The Federal Women's Study. J Clin Endocrinol Metab. 2019;104(1):181–92.
19. Haffner SM, D'Agostino R, Saad MF, Rewers M, Mykkänen L, Selby J, et al. Increased insulin resistance and insulin secretion in nondiabetic African-Americans and Hispanics compared with non-Hispanic whites: The Insulin Resistance Atherosclerosis Study. Diabetes. 1996;45(6):742–8.
20. Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: An insulin resistance paradox? Hepatology. 2009;49(3):791–801.
21. Keswell D, Tootla M, Goedecke J. Associations between body fat distribution, insulin resistance and dyslipidaemia in black and white South African women. Cardiovasc J Afr [Internet]. 2016;27(3):177–83. Available from: http://cvja.co.za/onlinejournal/vol27/vol27_issue3/#55/z
22. Sumner AE, Micklesfield LK, Ricks M, Tambay A V., Avila NA, Thomas F, et al. Waist circumference, BMI, and visceral adipose tissue in white women and women of African descent. Obesity [Internet]. 2011;19(3):671–4. Available from: http://dx.doi.org/10.1038/oby.2010.201/nature06264

23. Erzse A, Stacey N, Chola L, Tugendhaft A, Freeman M, Hofman K. The direct medical cost of type 2 diabetes mellitus in South Africa: a cost of illness study. Glob Health Action [Internet]. 2019;12(1). Available from: https://doi.org/10.1080/16549716.2019.1636611
24. World Health Organisation. Screening for type 2 diabetes. WHO. 2003;161(7):797–8.
25. American Diabetes Association. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020;43(January):S14–31.
26. World Health Organisation. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva, Switzerland; 2011.
27. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J. 2020;41(2):255–323.
28. SEMDSA. Management of Type 2 Diabetes Mellitus. JEMDSA. 2017;22(1):S1–196.
29. Thomas DD, Corkey BE, Istfan NW, Apovian CM. Hyperinsulinemia: An Early Indicator of Metabolic Dysfunction. J Endocr Soc. 2019;3(9):1727–47.
30. Gower BA, Fowler LA. Obesity in African-Americans: The role of physiology. J Intern Med. 2020;288(3):295–304.
31. Bergman RN, Piccinini F, Kabir M, Kolka CM, Ader M. Hypothesis: Role of Reduced Hepatic Insulin Clearance in the Pathogenesis of Type 2 Diabetes. Diabetes. 2019;68(9):1709–16.
32. Esser N, Utzschneider KM, Kahn SE. Early beta cell dysfunction vs insulin hypersecretion as the primary event in the pathogenesis of dysglycaemia. Diabetologia. 2020;63(10):2007–21.
33. Bano G. Glucose homeostasis, obesity and diabetes. Best Pract Res Clin Obstet Gynaecol. 2013;44:1–12.
34. Kruger DF, Martin CL, Sadler CE. New insights into glucose regulation. Diabetes Educ. 2006;32(2):221–8.
35. Thomsen SK, Gloyn AL. The pancreatic β cell: Recent insights from human genetics. Trends Endocrinol Metab. 2014;25(8):425–34.
36. Grarup N, Sandholt CH, Hansen T, Pedersen O. Genetic susceptibility to type 2 diabetes and obesity: From genome-wide association studies to rare variants and beyond. Diabetologia. 2014;57(8):1528–41.
37. Yaghootkar H, Whitcher B, Bell JD, Thomas EL. Ethnic differences in adiposity and diabetes risk – insights from genetic studies. J Intern Med. 2020;1–13.
38. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. Int J Mol Sci.

2019;20(2358):1–23.
39. Tan CY, Vidal-puig A. Adipose tissue expandability : the metabolic problems of obesity may arise from the inability to become more obese. Biochem Soc Trans. 2008;36:935–40.
40. Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasan RS, Irlbeck T, et al. Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the framingham heart study. Obesity. 2010;18(11):2191–8.
41. Tricò D, Galderisi A, Mari A, Polidori D, Galuppo B, Pierpont B, et al. Intrahepatic fat, irrespective of ethnicity, is associated with reduced endogenous insulin clearance and hepatic insulin resistance in obese youths: a cross-sectional and longitudinal study from the Yale Pediatric NAFLD cohort. Diabetes, Obes Metab. 2020;22(9):1–11.
42. Szczepaniak LS, Victor RG, Mathur R, Nelson MD, Szczepaniak EW, Tyer N, et al. Pancreatic steatosis and its relationship to β-cell dysfunction in humans: Racial and ethnic variations. Diabetes Care. 2012;35(11):2377–83.
43. Hakim O, Bello O, Bonadonna RC, Mohandas C, Shojaee-Moradie F, Jackson N, et al. Ethnic differences in intrahepatic lipid and its association with hepatic insulin sensitivity and insulin clearance between men of black and white ethnicity with early type 2 diabetes. Diabetes, Obes Metab. 2019;21(9):1–6.
44. Demerath W, Rogers N, Reed D, Lee M, Choh A, Siervpgel R, et al. Significant associations of age, menopausal status and lifestyle factors with visceral adiposity in African-American and European-American women. Ann Hum Biol. 2011;38(3):247–56.
45. Yerramalla MS, Fayosse A, Dugravot A, Tabak AG, Kivimäki M, Singh-Manoux A, et al. Association of moderate and vigorous physical activity with incidence of type 2 diabetes and subsequent mortality: 27 year follow-up of the Whitehall II study. Diabetologia. 2019;537–48.
46. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, et al. Quantification of the relationship between insulin sensitivity and β- cell function in human subjects: Evidence for a hyperbolic function. Diabetes. 1993;42(11):1663–72.
47. Lorenzo C, Wagenknecht LE, Rewers MJ, Karter AJ, Bergman RN, Hanley AJG, et al. Disposition index, glucose effectiveness, and conversion to type 2 diabetes: The insulin resistance atherosclerosis study (IRAS). Diabetes Care. 2010;33(9):2098–103.
48. Utzschneider K, Prigeon R, Faulenbach M, Tong J, Carr D, Boyko E, et al. Oral Disposition Index Predicts the Development of Future Diabetes Above and Beyond fasting and 2-h Glucose Leveles. Diabetes Care. 2009;32(2):335–41.
49. Field JB. Extraction of Insulin by Liver. Annu Rev Med. 1973;24(1):309–14.
50. Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. Vol. 217,

J cell Biol. 2018. p. 2273–89.

51. Eaton RP, Allen RC, Schade DS. Hepatic removal of insulin in normal man: Dose response to endogenous insulin secretion. J Clin Endocrinol Metab. 1983;56(6):1294–300.
52. Polidori DC, Bergman RN, Chung ST, Sumner AE. Hepatic and extrahepatic insulin clearance are differentially regulated: Results from a novel model-based. Diabetes. 2016;65(6):1556–64.
53. Piccinini F, Polidori DC, Gower BA, Bergman RN. Hepatic but Not Extrahepatic Insulin Clearance Is Lower in African American Than in European American Women. Diabetes. 2017;66(10):2564–70.
54. Piccinini F, Polidori DC, Gower BA, Fernandez JR, Bergman RN. Dissection of hepatic versus extra-hepatic insulin clearance: Ethnic differences in childhood. Diabetes Obes Metab. 2018;20(12):2869–75.
55. Goree LLT, Darnell BE, Oster RA, Brown MA, Gower BA. Associations of free fatty acids with insulin secretion and action among African-American and European-American girls and women. Obesity. 2010;18(2):247–53.
56. Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, Beebe C, et al. Quantitative study of insulin secretion and clearance in normal and obese subjects. J Clin Invest. 1988;81(2):435–41.
57. Kim MK, Reaven GM, Kim SH. Dissecting the relationship between obesity and hyperinsulinemia: Role of insulin secretion and insulin clearance. Obesity. 2017;25(2):378–83.
58. Jung SH, Jung CH, Reaven GM, Kim SH. Adapting to insulin resistance in obesity: role of insulin secretion and clearance. Diabetologia. 2018;61(3):681–7.
59. Lee W. The CEACAM1 expression is decreased in the liver of severely obese patients with or without diabetes. Diagn Pathol. 2011;6(1):1–6.
60. Rudovich N, Pivovarova O, Fisher E, Fischer-Rosinsky A, Spranger J, Möhlig M, et al. Polymorphisms within insulin-degrading enzyme (IDE) gene determine insulin metabolism and risk of type 2 diabetes. J Mol Med. 2009;87(11):1145–51.
61. Fosam A, Sikder S, Abel BS, Tella SH, Walter MF, Mari A, et al. Reduced Insulin Clearance and Insulin-Degrading Enzyme Activity Contribute to Hyperinsulinemia in African Americans. J Clin Endocrinol Metab. 2020;105:1–12.
62. Brandt A, Katschinski M, Arnold R, Polonsky KS, Göke B, Byrne MM. GLP-1-induced alterations in the glucose-stimulated insulin secretory dose-response curve. Am J Physiol Endocrinol Metab. 2001;281:E242–7.
63. Rudovich NN, Rochlitz HJ, Pfeiffer AFH. Reduced hepatic insulin extraction in response

to gastric inhibitory polypeptide compensates for reduced insulin secretion in normal-weight and normal glucose tolerant first-degree relatives of type 2 diabetic patients. Diabetes. 2004;53(9):2359–65.

64. Bojsen-møller KN, Lundsgaard A, Madsbad S, Kiens B, Holst JJ. Hepatic Insulin Clearance in Regulation of Systemic Insulin Concentrations — Role of Carbohydrate and Energy Availability. Diabetes. 2018;67(11):2129–36.
65. Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and β-cell function. Best Pract Res Clin Endocrinol Metab. 2003;17(3):305–22.
66. Cersosimo E, Solis-Herrera C, Trautmann ME, Malloy J, Triplitt CL. Assessment of Pancreatic beta-Cell Function: Review of Methods and Clinical Applications. Curr Diabetes Rev. 2014;10:2–42.
67. Ferrannini E, Mari A. β-Cell function in type 2 diabetes. Metabolism [Internet]. 2014;63(10):1217–27. Available from: http://dx.doi.org/10.1016/j.metabol.2014.05.012
68. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27(6):1487–95.
69. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic glucose clamp. Diabetes Care. 1999;22(9):1462–70.
70. Otten J, Ahrén B, Olsson T. Surrogate measures of insulin sensitivity vs the hyperinsulinaemic- euglycaemic clamp: A meta-analysis. Diabetologia. 2014;57(9):1781–8.
71. Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: Impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. Diabetes Care. 2013;36(4):845–53.
72. Thompson DS, Boyne MS, Osmond C, Ferguson TS, Tulloch-Reid MK, Wilks RJ, et al. Limitations of fasting indices in the measurement of insulin sensitivity in Afro-Caribbean adults. BMC Res Notes [Internet]. 2014;7(1):1–5. Available from: BMC Research Notes
73. Uwaifo G, Fallon E, Chin J, Elberg J, Parikh S, Yanovski J. Indices of insulin action, disposal, and secretion derived from fasting samples and clamps in normal glucose-toleranct Black and White children. Diabetes Care. 2002;25(11):2081–7.
74. Mari A, Tura A, Gastaldelli A, Ferrannini E. Assessing Insulin Secretion by Modeling in Mutliple-Meal Tests : Role of Potentiation. Diabetes. 2002;51(Supplement 1):S221–6.
75. Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E. Meal and oral glucose tests for assessment of β-cell function: modeling analysis in normal subjects. Am J Physiol Endocrinol Metab. 2002;283(6):E1159–66.

76. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels: Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes. 1992;41(3):368–77.
77. Mari A, Tura A, Pacini G, Kautzky-Willer A, Ferrannini E. Relationships between insulin secretion after intravenous and oral glucose administration in subjects with glucose tolerance ranging from normal to overt diabetes. Diabet Med. 2008;25(6):671–7.
78. Galderisi A, Polidori D, Weiss R, Giannini C, Pierpont B, Tricò D, et al. Lower insulin clearance parallels a reduced insulin sensitivity in obese youths and is associated with a decline in B-cell function over time. Diabetes. 2019;68(11):2074–84.
79. Smith LM, Yao-Borengasser A, Starks T, Tripputi M, Kern PA, Rasouli N. Insulin resistance in African-American and Caucasian women: Differences in lipotoxicity, adipokines, and gene expression in adipose tissue and muscle. J Clin Endocrinol Metab. 2010;95(9):4441–8.
80. Osei K, Schuster DP, Owusu SK, Amoah AGB. Race and ethnicity determine serum insulin and C-peptide concentrations and hepatic insulin extraction and insulin clearance: Comparative studies of three populations of West African ancestry and white Americans. Metabolism. 1997;46(1):53–8.
81. Dankner R, Chetrit A, Shanik MH, Raz I, Roth J. Basal-state hyperinsulinemia in healthy normoglycemic adults is predictive of type 2 diabetes over a 24-year follow-up: A preliminary report. Diabetes Care. 2009;32(8):1464–6.
82. Weyer C, Hanson RL, Tataranni PA, Bogardus C, Pratley RE. A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance: Evidence for a pathogenic role of relative hyperinsulinemia. Diabetes. 2000;49(12):2094–101.
83. Tricò D, Natali A, Mari A, Ferrannini E, Santoro N, Caprio S. Triglyceride-rich very low-density lipoproteins (VLDL) are independently associated with insulin secretion in a multiethnic cohort of adolescents. Diabetes, Obes Metab. 2018;20(12):2905–10.
84. Kabir M, Catalano KJ, Ananthnarayan S, Kim SP, Van Citters GW, Dea MK, et al. Molecular evidence supporting the portal theory: A causative link between visceral adiposity and hepatic insulin resistance. Am J Physiol Endocrinol Metab. 2005;288:E454–61.
85. Szendroedi J, Yoshimura T, Phielix E, Koliaki C, Marcucci M, Zhang D, et al. Role of diacylglycerol activation of PKCθ in lipid-induced muscle insulin resistance in humans. PNAS. 2014;111(26):9597–602.
86. Hakim O, Bonadonna RC, Mohandas C, Billoo Z, Sunderland A, Boselli L, et al. Associations between pancreatic lipids and β-cell function in black african and white european men with type 2 diabetes. J Clin Endocrinol Metab. 2019;104(4):1201–10.

87. Goedecke JH, Keswell D, Weinreich C, Fan J, Hauksson J, Victor H, et al. Ethnic differences in hepatic and systemic insulin sensitivity and their associated determinants in obese black and white South African women. Diabetologia. 2015;58(11):2647–52.
88. Lorenzo C, Wagenknecht LE, D'Agostino RB, Rewers MJ, Karter AJ, Haffner SM. Insulin resistance, β-cell dysfunction, and conversion to type 2 diabetes in a multiethnic population: The insulin resistance atherosclerosis study. Diabetes Care. 2010;33(1):67–72.
89. Joubert J, Norman R, Bradshaw D, Goedecke JH, Steyn NP, Puoane T. Estimating the burden of disease attributable to excess body weight in South Africa in 2000. SAMJ. 2007;97(8):683–90.
90. Gealekman O, Guseva N, Hartigan C, Apotheker S, Gorgoglione M, Tran K, et al. Depot-Specific Differences and Insufficient Subcutaneous Adipose Tissue Angiogenesis in Human Obesity. Circulation. 2011;123:186–94.
91. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered Fat Storage and Mobilization in the Pathogenesis of Insulin Resistance and Type 2 Diabetes. Endocr Rev. 2002;23(2):201–29.
92. Pereira SS, Alvarez-Leite JI. Low-Grade Inflammation, Obesity, and Diabetes. Curr Obes Rep. 2014;3(4):422–31.
93. León-Pedroza JI, González-Tapia LA, del Olmo-Gil E, Castellanos-Rodríguez D, Escobedo G, González-Chávez A. Low-grade systemic inflammation and the development of metabolic diseases: From the molecular evidence to the clinical practice. Cir Cir. 2015;83(6):543–51.
94. Muoio DM, Newgard CB. Mechanisms of disease: Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9(3):193–205.
95. Taylor R. Banting Memorial Lecture 2012 Reversing the twin cycles of Type 2 diabetes. Diabet Med. 2013;30(3):267–75.
96. Ibrahim MM. Subcutaneous and visceral adipose tissue: Structural and functional differences. Obes Rev. 2010;11(1):11–8.
97. Manolopoulos KN, Karpe F, Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. Int J Obes. 2010;34(6):949–59.
98. Evans J, Goedecke JH, Söderström I, Burén J, Alvehus M, Blomquist C, et al. Depot- and ethnic-specific differences in the relationship between adipose tissue inflammation and insulin sensitivity. Clin Endocrinol (Oxf). 2011;74(1):51–9.
99. Bonora E. Relationship between regional fat distribution and insulin resistance. Int J Obes. 2000;24(Suppl 2):S32–5.

100. McLaughlin T, Craig C, Liu LF, Perelman D, Allister C, Spielman D, et al. Adipose cell size and regional fat deposition as predictors of metabolic response to overfeeding in insulin-resistant and insulin-sensitive humans. Diabetes. 2016;65(5):1245–54.
101. Gustafson B, Gogg S, Hedjazifar S, Jenndahl L, Hammarstedt A, Smith U. Inflammation and impaired adipogenesis in hypertrophic obesity in man. Am J Physiol Endocrinol Metab 297. 2009;297(5):999–1003.
102. Kotzé-Hörstmann LM, Keswell D, Adams K, Dlamini T, Goedecke JH. Hypoxia and extra-cellular matrix gene expression in adipose tissue associates with reduced insulin sensitivity in black South African women. Endocrine. 2017;55(1):144–52.
103. Chung ST, Courville AB, Onuzuruike AU, Galvan-De La Cruz M, Lilian S M, DuBose CW, et al. Gluconeogenesis and risk for fasting hyperglycemia in Black and White women. JCI Insight. 2018;3(18):1–13.
104. Kabakambira JD, Baker RL, Briker SM, Courville AB, Mabundo LS, DuBose CW, et al. Do current guidelines for waist circumference apply to black Africans? Prediction of insulin resistance by waist circumference among Africans living in America. BMJ Glob Heal. 2018;3(5):1–11.
105. Kanaley JA, Giannopoulou I, Tillapaugh-Fay G, Nappi JS, Ploutz-Snyder LL. Racial differences in subcutaneous and visceral fat distribution in postmenopausal black and white women. Metabolism. 2003;52(2):186–91.
106. Velasquez-mieyer PA, Cowan PA, Umpierrez G, Lustig RH, Cashion AK, Burgen G. Racial differences in glucagon-like peptide-1 (GLP-1) concentrations and insulin dynamics during oral glucose tolerance test in obese subjects. Int J Obes Relat Metab Disord. 2003;27(11):1359–64.
107. Wu JHY, Marklund M, Imamura F, Tintle N, Ardisson Korat A V., de Goede J, et al. Omega-6 fatty acid biomarkers and incident type 2 diabetes: pooled analysis of individual-level data for 39 740 adults from 20 prospective cohort studies. Lancet Diabetes Endocrinol. 2017;5(12):965–74.
108. Neeland IJ, Ayers CR, Powell-wiley TM, Vega GL, Mcguire DK, Lemos JA De. Dysfunctional Adiposity and the Risk of Prediabetes and Type 2 Diabetes in Obese Adults. JAMA. 2012;308(11):1150–9.
109. Brahimaj A, Rivadeneira F, Muka T, Sijbrands EJG, Franco OH, Dehghan A, et al. Novel metabolic indices and incident type 2 diabetes among women and men: the Rotterdam Study. Diabetologia. 2019;62(9):1581–90.
110. Utzschneider KM, Kahn SE, Polidori DC. Hepatic insulin extraction in NAFLD is related to insulin resistance rather than liver fat content. J Clin Endocrinol Metab. 2018;
111. National Department of Health (NDoH), Statistics South Africa (Stats SA), South African

Medical Research (SAMRC), ICF. South African Demographic and Health Survey (SADHS) 2016: Key Indicators. Pretoria, South Africa, and Rockville, Maryland, USA: NDoH, Stats SA, SAMRC, and ICF; 2017. 1–59 p.

112. Reimann M, Schutte AE, Huisman HW, Schutte R, van Rooyen JM, Malan L, et al. Ethnic differences in C-peptide secretion but not in non-esterified fatty acid metabolism in pre-menopausal women with and without abdominal obesity. Diabetes Res Clin Pract. 2007;77(1):62–9.
113. Punyadeera C, Crowther NJ, Van Der Merwe MT, Toman M, Immelman AR, Schlaphoff GP, et al. Metabolic response to a mixed meal in obese and lean women from two South African populations. Obes Res. 2002;10(12):1207–16.
114. Shires R, Joffe BI, Seftel HC. Maximal pancreatic beta-cell stimulation and the counter-regulatory hormonal responses in South African black and white obese subjects. South African Med J. 1985;67(21):845–7.
115. Ladwa M, Hakim O, Amiel SA, Goff LM. A Systematic Review of Beta Cell Function in Adults of Black African Ethnicity. J Diabetes Res. 2019;2019.
116. Hannon TS, Bacha F, Lin Y, Arslanian SA. Hyperinsulinemia in African-American adolescents compared with their american white peers despite similar insulin sensitivity: A reflection of upregulated β-cell function? Diabetes Care. 2008;31(7):1445–7.
117. Arslanian SA, Saad R, Lewy V, Danadian K, Janosky J. Hyperinsulinemia in African-American Children. Diabetes. 2002;51(10):3014–9.
118. Gower BA, Granger WM, Franklin F, Shewchuk RM, Goran MI. Contribution of insulin secretion and clearance to glucose-induced insulin concentration in African-American and Caucasian children. J Clin Endocrinol Metab. 2002;87(5):2218–24.
119. Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y, Roth J. Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? Diabetes Care. 2008;31 Suppl 2.
120. Corkey BE. Banting lecture 2011: Hyperinsulinemia: Cause or consequence? Diabetes. 2012;61(1):4–13.
121. Micklesfield LK, Lambert E V, Hume DJ, Chantler S, Pienaar PR, Dickie K, et al. Socio-cultural, environmental and behavioural determinants of obesity in black South African women. Cardiovasc J Afr. 2013;24(9):369–75.
122. Goedecke JH, Mtintsilana A, Dlamini SN, Pascal Kengne A. Type 2 diabetes mellitus in African women. Diabetes Res Clin Pract. 2017;123:87–96.
123. World Health Organization. GLOBAL REPORT ON DIABETES. 2016;
124. Shay CM, Carnethon MR, Church TR, Hankinson AL, Chan C, Jacobs Jr DR, et al. Lower Extremity Fat Mass Is Associated With Insulin Resistance in Overweight and Obese

Individuals: The CARDIA Study. Obesity. 2011;19(11):2248–53.
125. Lovejoy JC, Smith SR, Rood JC. Comparison of regional fat distribution and health risk factors in middle-aged white and African American women: The Healthy Transitions Study. Obes Res. 2001;9(1):10–6.
126. Bouchard C. BMI, fat mass, abdominal adiposity and visceral fat: Where is the “beef”? Int J Obes. 2007;31(10):1552–3.
127. Goedecke JH, Evans J, Keswell D, Stimson RH, Livingstone DEW, Hayes P, et al. Reduced gluteal expression of adipogenic and lipogenic genes in black South African women is associated with obesity-related insulin resistance. J Clin Endocrinol Metab. 2011;96(12):2029–33.
128. Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. Obesity. 2012;20(5):1109–14.
129. Davidson FE, Matsha TE, Erasmus RT, Ismail S, Kengne AP, Goedecke JH. Comparison of single-slice CT and DXA-derived measures of central adiposity in South African women. Eur J Clin Nutr. 2020;74(9):1282–9.
130. Mohammad A, De Lucia Rolfe E, Sleigh A, Kivisild T, Behbehani K, Wareham NJ, et al. Validity of visceral adiposity estimates from DXA against MRI in Kuwaiti men and women. Nutr Diabetes. 2017;7(1):e238-5.
131. Liska D, Dufour S, Zern TL, Taksali S, Calí AMG, Dziura J, et al. Interethnic differences in muscle, liver and abdominal fat partitioning in obese adolescents. PLoS One. 2007;2(6):1–8.
132. Gambacciani M, De Simone L, Piaggesi L, Genazzani AR, Orlandi R, Ciaponi M, et al. Body Weight, Body Fat Distribution, and Hormonal Replacement Therapy in Early Postmenopausal Women. J Clin Endocrinol Metab. 1997;82(2):414–7.
133. Kanaley JA, Sames C, Swisher L, Swick AG, Ploutz-Snyder LL, Steppan CM, et al. Abdominal fat distribution in pre- and postmenopausal women: The impact of physical activity, age, and menopausal status. Metabolism. 2001;50(8):976–82.
134. Fujimoto WY, Jablonski KA, Bray GA, Kriska A, Haffner S, Hanson R, et al. Body Size and Shape Changes and the Risk of Diabetes in the Diabetes Prevention Program. Diabetes. 2007;56(6):1680–5.
135. Hairston KG, Vitolins MZ, Norris JM, Anderson AM, Hanley AJ, Wagenknecht LE. Lifestyle factors and 5-year abdominal fat accumulation in a minority cohort: The IRAS family study. Obesity. 2012;20(2):421–7.
136. Goedecke JH, Evans J, Keswell D, Stimson RH, Livingstone DEW, Hayes P, et al. Reduced gluteal expression of adipogenic and lipogenic genes in black South African

women is associated with obesity-related insulin resistance. J Clin Endocrinol Metab. 2011;96(12):2029–33.

137. Chantler S, Dickie K, Micklesfield LK, Goedecke JH. Longitudinal Changes in Body Fat and Its Distribution in Relation to Cardiometabolic Risk in Black South African Women. Metab Syndr Relat Disord. 2015;13(9):381–8.
138. Tricò D, Mari A, Ferrannini E, Tricò D, Natali A, Arslanian S, et al. Implications of Primary Insulin Hypersecretion in Nondiabetic Adults and Adolescents. JCI Insight. 2018;3(24):1–15.
139. Goff LM, Ladwa M, Hakim O, Bello O. Ethnic distinctions in the pathophysiology of type 2 diabetes: a focus on black African-Caribbean populations. Proc Nutr Soc. 2019;(April):1–10.
140. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006;6:1–7.
141. Cuthbertson DJ, Weickert MO, Lythgoe D, Sprung VS, Dobson R, Shoajee-moradie F, et al. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. Eur J Endocrinol. 2014;171(5):561–9.
142. Motamed N, Hossein A, Reza M, Maadi M. Fatty liver index (FLI) and prediction of new cases of non-alcoholic fatty liver disease : A population-based study of northern Iran. Clin Nutr [Internet]. 2020;39(2):468–74. Available from: https://doi.org/10.1016/j.clnu.2019.02.024
143. Olubamwo OO, Virtanen JK, Pihlajamaki J, Mantyselka P, Tuomainen T-P. Fatty liver index as a predictor of increased risk of cardiometabolic disease : finding from the Kuopio Ischaemic Heart Disease Risk Factor Study Cohort. BMJ Open. 2019;9:1–10.
144. Khang A, Lee H, Yi D, Kang Y, Son S. The fatty liver index , a simple and useful predictor of metabolic syndrome : analysis of the Korea National Health and Nutrition Examination Survey 2010 – 2011. Diabetes, Metab Syndr Obes Targets Ther. 2019;12:181–90.
145. Yaghootkar H, Lotta LA, Tyrrell J, Smit RAJ, Jones SE, Donnelly L, et al. Genetic Evidence for a Link Between Favorable Adiposity and Lower Risk of Type 2 Diabetes, Hypertension, and Heart Disease. Diabetes. 2016;65(August):2448–60.
146. Ji Y, Yiorkas AM, Frau F, Mook-kanamori D, Staiger H, Thomas EL, et al. Genome-Wide and Abdominal MRI Data Provide Evidence That a Genetically Determined Favorable Adiposity Phenotype Is Characterized by Lower Ectopic Liver Fat and Lower Risk of Type 2 Diabetes, Heart Disease, and Hypertension. Diabetes. 2019;68(January):207–19.
147. Statistics South Africa (Stats SA). Men, Women and Children: Findings of the Living

Conditions Survery. 2014/2015. Pretoria, South Africa; 2018.
148. Peer N, Lombard C, Steyn K, Levitt N. Differential associations of cardiovascular disease risk factors with relative wealth in urban-dwelling South Africans. J Public Health (Bangkok). 2016;38(3):e232–9.
149. Mchiza ZJ, Steyn NP, Hill I, Kruger A, Schönfeldt H, Nel J, et al. A review of dietary surveys in the adult South African population from 2000 to 2015. Nutrients. 2015;7(9):8227–50.
150. Dolman RC, Wentzel-Viljoen E, Jerling JC, Feskens EJ, Kruger A, Pieters M. The use of predefined diet quality scores in the context of CVD risk during urbanization in the South African Prospective Urban and Rural Epidemiological (PURE) study. Public Health Nutr. 2013;17(7):1–11.
151. Wentzel-Viljoen E, Lee S, Laubscher R, Vorster HH. Accelerated nutrition transition in the North West Province of South Africa: results from the Prospective Urban and Rural Epidemiology (PURE-NWP-SA) cohort study, 2005 to 2010. Public Health Nutr. 2018;(12):1–12.
152. Vorster HH, Kruger A, Wentzel-Viljoen E, Kruger HS, Margetts BM. Added sugar intake in South Africa: Findings from the Adult Prospective Urban and Rural Epidemiology cohort study. Am J Clin Nutr. 2014;99(6):1479–86.
153. Bodén S, Wennberg M, Van Guelpen B, Johansson I, Lindahl B, Andersson J, et al. Dietary inflammatory index and risk of first myocardial infarction; a prospective population-based study. Nutr J. 2017;16(1):1–10.
154. Denova-Gutiérrez E, Muñoz-Aguirre P, Shivappa N, Hébert JR, Tolentino-Mayo L, Batis C, et al. Dietary inflammatory index and type 2 diabetes mellitus in adults: The diabetes mellitus survey of Mexico City. Nutrients. 2018;10(4):1–15.
155. Montonen J, Boeing H, Fritsche A, Schleicher E, Joost HG, Schulze MB, et al. Consumption of red meat and whole-grain bread in relation to biomarkers of obesity, inflammation, glucose metabolism and oxidative stress. Eur J Nutr. 2013;52(1):337–45.
156. Shivappa N, Steck SE, Hurley TG, Hussey JR HJ. Dietary Inflammatory Index. Public Health Nutr. 2014;17(8):1689–96.
157. Wirth MD, Shivappa N, Davis L, Hurley TG, Ortaglia A, Drayton R, et al. Construct validation of the Dietary Inflammatory Index among African Americans. J Nutr Heal Aging. 2017;21(5):487–91.
158. Wirth M, Herbet J, Shivappa N, Hand G, Hurley T, Drenowatz C, et al. Anti-inflammatory Dietary Inflammatory Index scores are associated with healthier scores on other dietary indices Michael. Nutr Res. 2016;36(3):214–9.
159. Kim Y, Chen J, Wirth MD, Shivappa N, Hebert JR. Lower dietary inflammatory index

scores are associated with lower glycemic index scores among college students. Nutrients. 2018;10(2).

160. Van Woudenbergh GJ, Theofylaktopoulou D, Kuijsten A, Ferreira I, Van Greevenbroek MM, Van Der Kallen CJ, et al. Adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: The cohort study on diabetes and atherosclerosis maastricht (CODAM) and the hoorn study1-4. Am J Clin Nutr. 2013;98(6):1533–42.
161. Vahid F, Shivappa N, Karamati M, Naeini AJ, Hebert JR, Davoodi SH. Association between Dietary Inflammatory Index (DII) and risk of prediabetes: a case-control study. Appl Physiol Nutr Metab [Internet]. 2017;42(4):399–404. Available from: http://www.nrcresearchpress.com/doi/10.1139/apnm-2016-0395
162. Ramallal R, Toledo E, Martínez-González MA, Hernández-Hernández A, García-Arellano A, Shivappa N, et al. Dietary inflammatory index and incidence of cardiovascular disease in the SUN cohort. PLoS One. 2015;10(9):1–15.
163. Ruiz-Canela M, Zazpe I, Shivappa N, Hébert JR, Sánchez-Tainta A, Corella D, et al. Dietary inflammatory index and anthropometric measures of obesity in a population sample at high cardiovascular risk from the PREDIMED (PREvención con DIeta MEDiterránea) trial. Br J Nutr. 2015;113(6):984–95.
164. Gradidge PJ, Crowther NJ, Chirwa ED, Norris SA, Micklesfield LK. Patterns , levels and correlates of self-reported physical activity in urban black Soweto women. BMC Public Heal 2014,. 2014;14:1–10.
165. Dickie K, Micklesfield LK, Chantler S, Lambert E V, Goedecke JH. Meeting physical activity guidelines is associated with reduced risk for cardiovascular disease in black South African women; a 5.5-year follow-up study. BMC Public Health. 2014;14(498):1–11.
166. Dickie K, Micklesfield LK, Chantler S, Lambert E V., Goedecke JH. Cardiorespiratory Fitness and Light-Intensity Physical Activity Are Independently Associated with Reduced Cardiovascular Disease Risk in Urban Black South African Women: A Cross-Sectional Study. Metab Syndr Relat Disord. 2016;14(1):23–32.
167. Fortuin-de Smidt MC, Mendham AE, Hauksson J, Hakim O, Stefanovski D, Clamp L, et al. Effect of exercise training on insulin sensitivity, hyperinsulinemia and ectopic fat in black South African women: a randomized controlled trial. Eur J Endocrinol. 2020;183(1):51–61.
168. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, et al. Physical activity and public health: Updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc. 2007;39(8):1423–34.
169. Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB. Metabolomics by

numbers: Acquiring and understanding global metabolite data. Trends Biotechnol. 2004;22(5):245–52.

170. Bain JR, Muehlbauer MJ. Metabolomics reveals unexpected responses to oral glucose. Diabetes. 2013;62(8):2651–3.
171. Newgard CB. Metabolomics and Metabolic Diseases: Where Do We Stand? Cell Metab. 2017;25(1):43–56.
172. Lu J, Xie G, Jia W, Jia W. Metabolomics in human type 2 diabetes research. Front Med. 2013;7(1):4–13.
173. Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM, Newgard CB. Metabolomics applied to diabetes research: Moving from information to knowledge. Diabetes. 2009;58(11):2429–43.
174. Shaham O, Wei R, Wang TJ, Ricciardi C, Lewis GD, Vasan RS, et al. Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. Mol Syst Biol. 2008;4(214):1–9.
175. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nat Med. 2011;17(4):448–53.
176. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A Branched-Chain Amino Acid-Related Metabolic Signature that Differentiates Obese and Lean Humans and Contributes to Insulin Resistance. Cell Metab. 2009;9(4):311–26.
177. Adams SH, Hoppel CL, Lok KH, Zhao L, Wong SW, Minkler PE, et al. Plasma Acylcarnitine Profiles Suggest Incomplete Long-Chain Fatty Acid β-Oxidation and Altered Tricarboxylic Acid Cycle Activity in Type 2 Diabetic African-American Women. J Nutr. 2009;139(6):1073–81.
178. Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, Joost HG, et al. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes. 2013;62(2):639–48.
179. Fiehn O, Timothy Garvey W, Newman JW, Lok KH, Hoppel CL, Adams SH. Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African-American women. PLoS One. 2010;5(12):1–10.
180. Barber MN, Risis S, Yang C, Meikle PJ, Staples M, Febbraio MA, et al. Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. PLoS One. 2012;7(7):1–12.
181. Lu Y, Wang Y, Ong CN, Subramaniam T, Choi HW, Yuan JM, et al. Metabolic signatures and risk of type 2 diabetes in a Chinese population: an untargeted metabolomics study using both LC-MS and GC-MS. Diabetologia. 2016;59(11):2349–59.
182. Guasch-Ferré M, Hruby A, Toledo E, Clish CB, Martínez-González MA, Salas-Salvadó J,

et al. Metabolomics in prediabetes and diabetes: A systematic review and meta-analysis. Diabetes Care. 2016;39(5):833–46.

183. Lee CC, Watkins SM, Lorenzo C, Wagenknecht LE, Il'Yasova D, Chen YDI, et al. Branched-chain amino acids and insulin metabolism: The Insulin Resistance Atherosclerosis Study (IRAS). Diabetes Care. 2016;39(4):582–8.
184. Menni C, Fauman E, Erte I, Perry JRB, Kastenmüller G, Shin SY, et al. Biomarkers for type 2 diabetes and impaired fasting glucose using a nontargeted metabolomics approach. Diabetes. 2013;62(12):4270–6.
185. Lotta LA, Scott RA, Sharp SJ, Burgess S, Luan J, Tillin T, et al. Genetic Predisposition to an Impaired Metabolism of the Branched-Chain Amino Acids and Risk of Type 2 Diabetes: A Mendelian Randomisation Analysis. PLoS Med. 2016;13(11):1–22.
186. Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, He Y, et al. Novel biomarkers for pre-diabetes identified by metabolomics. Mol Syst Biol. 2012;8(615):1–11.
187. Hamley S, Kloosterman D, Duthie T, Man CD, Visentin R, Mason SA, et al. Mechanisms of hyperinsulinaemia in apparently healthy non-obese young adults : role of insulin secretion, clearance and action and associations with plasma amino acids. Diabetologia. 2019;62(12):2310–24.
188. She P, Reid TM, Bronson SK, Vary TC, Hajnal A, Lynch CJ, et al. Disruption of BCATm in Mice Leads to Increased Energy Expenditure Associated with the Activation of a Futile Protein Turnover Cycle. Cell Metab. 2007;6(3):181–94.
189. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol. 2014;10(12):723–36.
190. She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. Am J Physiol - Endocrinol Metab. 2007;293:1552–63.
191. White PJ, Lapworth AL, An J, Wang L, McGarrah RW, Stevens RD, et al. Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid oxidation and acyl-glycine export. Mol Metab. 2016;5(7):538–51.
192. Slawik M, Vidal-Puig AJ. Lipotoxicity, overnutrition and energy metabolism in aging. Ageing Res Rev. 2006;5(2):144–64.
193. Mollard RC, Seńéchal M, MacIntosh AC, Hay J, Wicklow BA, Wittmeier KDM, et al. Dietary determinants of hepatic steatosis and visceral adiposity in overweight and obese youth at risk of type 2 diabetes. Am J Clin Nutr. 2014;99(4):804–12.
194. Jornayvaz FR, Birkenfeld AL, Jurczak MJ, Kanda S, Guigni BA, Jiang DC, et al. Hepatic insulin resistance in mice with hepatic overexpression of diacylglycerol acyltransferase 2.

PNAS. 2011;108(14):5748–52.

195. Aguer C, McCoin CS, Knotts TA, Thrush AB, Ono-Moore K, McPherson R, et al. Acylcarnitines: Potential implications for skeletal muscle insulin resistance. FASEB J. 2015;29(1):336–45.
196. Serra MC, Ryan AS, Goldberg AP. Reduced LPL and subcutaneous lipid storage capacity are associated with metabolic syndrome in postmenopausal women with obesity. Obes Sci Pract. 2017;3(1):106–14.
197. Yang Q, Vijayakumar A, Kahn BB. Metabolites as regulators of insulin sensitivity and metabolism. Nat Rev Mol Cell Biol. 2018;19(10):654–72.
198. Ling J, Chaba T, Zhu LF, Jacobs RL, Vance DE. Hepatic ratio of phosphatidylcholine to phosphatidylethanolamine predicts survival after partial hepatectomy in mice. Hepatology. 2012;55(4):1094–102.
199. Li Z, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, et al. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. Cell Metab. 2006;3(5):321–31.
200. van der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. Biochim Biophys Acta. 2017;1859(9):1558–72.
201. Lehmann R, Franken H, Dammeier S, Rosenbaum L, Kantartzis K, Peter A, et al. Circulating lysophosphatidylcholines are markers of a metabolically benign nonalcoholic fatty liver. Diabetes Care. 2013;36(8):2331–8.
202. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial Overload and Incomplete Fatty Acid Oxidation Contribute to Skeletal Muscle Insulin Resistance. Cell Metab. 2008;7(1):45–56.
203. Haeusler RA, Astiarraga B, Camastra S, Accili D, Ferrannini E. Human Insulin Resistance Is Associated With Increased Plasma Levels of 12α -Hydroxylated Bile Acids. Diabetes. 2013;62:4184–91.
204. Haeusler RA, Camastra S, Nannipieri M, Astiarraga B, Castro-perez J, Xie D, et al. Increased Bile Acid Synthesis and Impaired Bile Acid Transport in Human Obesity. J Clin Endocrinol Metab. 2016;101:1935–44.
205. Vincent RP, Omar S, Ghozlan S, Taylor DR, Cross G, Sherwood RA, et al. Higher circulating bile acid concentrations in obese patients with type 2 diabetes. Ann Clin Biochem. 2013;50(4):360–4.
206. Anderson KE, Kok E, Javitt NB. Bile Acid Synthesis in Man: Metabolism of 7α-Hydroxycholesterol-’4C and 26-Hydroxycholesterol-3H. J Clin Endocrinol Metab. 1972;51(1):112–7.

207. Schwarz M, Lund EG, Setchell KDR, Kayden HJ, Zerwekh JE, Bjo I, et al. Disruption of Cholesterol 7 ⎵ -Hydroxylase Gene in Mice. J Biol Chem. 1996;271(30):18024–31.
208. Shapiro H, Kolodziejczyk AA, Halstuch D, Elinav E. Bile acids in glucose metabolism in health and disease. J Exp Med [Internet]. 2018;215(2):383–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29339445
209. Wahlstrom A, Sayin SI, Marschall H-U, Backhed F. Review Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. Cell Metab. 2016;24:41–50.
210. Devlin AS, Fischbach MA. A biosynthetic pathway for a prominent class of microbiota-derived bile acids. Nat Chem Biol [Internet]. 2015;11(9):685–90. Available from: http://dx.doi.org/10.1038/nchembio.1864
211. Chiang JY. Recent advances in understanding bile acid homeostasis. F1000Research. 2017;6(2029):1–8.
212. Li T, Chiang JYL. Bile acids as metabolic regulators. Curr Opin Gastroenterol. 2015;31(2):159–65.
213. Prawitt J, Caron S, Staels B. Bile acid metabolism and the pathogenesis of type 2 diabetes. Curr Diab Rep. 2011;11(3):160–6.
214. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of Bile Acids and Bile Acid Receptors in Metabolic Regulation. Physiol Rev. 2009;89:147–91.
215. Trabelsi MS, Lestavel S, Staels B, Collet X. Intestinal bile acid receptors are key regulators of glucose homeostasis. Proc Nutr Soc. 2017;76(3):192–202.
216. Cariou B, Harmelen K Van, Duran-sandoval D, Dijk TH Van, Grefhorst A, Abdelkarim M, et al. The Farnesoid X Receptor Modulates Adiposity and Peripheral Insulin Sensitivity in Mice. J Biol Chem. 2006;281(16):11039–49.
217. Katsuma S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem Biophys Res Commun. 2005;329(1):386–90.
218. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab. 2009;10(3):167–77.
219. Glicksman C, Pournaras DJ, Wright M, Roberts R, Mahon D, Welbourn R, et al. Postprandial plasma bile acid responses in normal weight and obese subjects. Ann Clin Biochem. 2010;47(5):482–4.
220. Sun W, Zhang D, Wang Z, Sun J, Xu B, Chen Y, et al. Insulin resistance is associated with total bile acid level in type 2 diabetic and nondiabetic population: A cross-sectional study. Medicine (Baltimore). 2016;95(10):1–6.
221. Schulze MB, Hoffmann K, Boeing H, Linseisen J, Rohrmann S, Möhlig M, et al. An

accurate risk score based on anthropometric, dietary, and lifestyle factors to predict the development of type 2 diabetes. Diabetes Care. 2007;30(3):510–5.

222. Bello O, Ladwa M, Hakim O, Marathe C, Shoajee-Moradie F, Charles-Edwards G, et al. Differences in the link between insulin sensitivity and ectopic fat in men of Black African and White European ethnicity. Eur J Endocrinol. 2019;(5).
223. Tishkoff SA, Reed FA, Friedlaender FR, Ranciaro A, Froment A, Hirbo JB, et al. The Genetic Structure and History of Africans and African Americans. Science (80-). 2009;324(5930):1035–44.
224. Murray T, Beaty T, Mathias R, Rafaels N, Grant A, Faruque M, et al. African and Non-African Admixture Components in African Americans and An African Caribbean Population. Genet Epidemiol. 2010;34(6):1–14.
225. Zakharia F, Basu A, Absher D, Assimes TL, Go AS, Hlatky MA, et al. Characterizing the admixed African ancestry of African Americans. Genome Biol. 2009;10(12).
226. World Health Organisation. Global status report on noncommunicable diseases 2014. WHO. 2014;1–302.
227. Tulloch-Reid MK, Hanson RL, Sebring NG, Reynolds JC, Premkumar A, Genovese DJ, et al. Both subcutaneous and visceral adipose tissue correlate highly with insulin resistance in african americans. Obes Res. 2004;12(8):1352–9.
228. Goedecke JH, Dave J a., Faulenbach M V., Utzschneider KM, Lambert E V., West S, et al. Insulin Response in Relation to Insulin Sensitivity. Diabetes Care. 2009;32(5):860–5.
229. Tatsukawa Y, Misumi M, Kim YM, Yamada M, Ohishi W, Fujiwara S, et al. Body composition and development of diabetes: A 15-year follow-up study in a Japanese population. Eur J Clin Nutr. 2018;72(3):374–80.
230. Franklin RM, Ploutz-Snyder L, Kanaley JA. Longitudinal changes in abdominal fat distribution with menopause. Metabolism. 2009;58(3):311–5.
231. George C, Goedecke JH, Crowther NJ, Jaff NG, Kengne AP, Norris SA, et al. The role of body fat and fat distribution in hypertension risk in urban Black South African women. PLoS One. 2016;11(5):32–40.
232. Jaff NG, Norris SA, Snyman T, Toman M, Crowther NJ. Body composition in the Study of Women Entering and in Endocrine Transition (SWEET): A perspective of African women who have a high prevalence of obesity and HIV infection. Metabolism. 2015;64(9):1031–41.
233. Crowther NJ, Norris SA. The Current Waist Circumference Cut Point Used for the Diagnosis of Metabolic Syndrome in Sub-Saharan African Women Is Not Appropriate. PLoS One. 2012;7(11):1–8.
234. Micklesfield LK, Reid S, Bewerunge L, Rush EC, Goedecke JH. A proposed method to

measure body composition in obese individuals using dual-energy X-ray absorptiometry. Int J Body Compos Res. 2007;5(4):147–51.

235. Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444(7121):881–7.
236. Cornier M-A, Despres J-P, Davis N, Grossniklaus DA, Klein S, Lamarche B, et al. Assessing Adiposity: A Scientific Statement From the American Heart Association. Circulation [Internet]. 2011;124(18):1996–2019. Available from: http://circ.ahajournals.org/cgi/doi/10.1161/CIR.0b013e318233bc6a
237. Gutch M, Kumar S, Razi S, Gupta K, Gupta A. Assessment of insulin sensitivity/resistance. Indian J Endocrinol Metab. 2015;19(1):160–4.
238. Pearson ER. Type 2 diabetes: a multifaceted disease. Diabetologia. 2019;62:1107–12.
239. Goedecke JH, Olsson T. Pathogenesis of type 2 diabetes risk in black Africans: a South African perspective title. J Intern Med. 2020;1–11.
240. Richter L, Norris S, Pettifor J, Yach D, Cameron N. Cohort Profile : Mandela ' s children : The 1990 birth to twenty study in South Africa. Int J Epidemiol. 2007;36(3):504–11.
241. Mtintsilana A, Micklesfield LK, Chorell E, Olsson T, Goedecke JH. Fat redistribution and accumulation of visceral adipose tissue predicts type 2 diabetes risk in middle-aged black South African women: a 13-year longitudinal study. Nutr Diabetes. 2019;9(1):12.
242. Zeng Y, Mtintsilana A, Goedecke JH, Micklesfield LK, Olsson T, Chorell E. Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women: a prospective cohort study. Metabolism. 2019;95:57–64.
243. World Health Organization. Obesity:preventing and managing the global epidemic. World Health Organization - Technical Report Series. Geneva, Switzerland; 2000.
244. Mtintsilana A, Micklesfield LK, Chorell E, Olsson T, Shivappa N, Hebert JR, et al. Adiposity Mediates the Association between the Dietary Inflammatory Index and Markers of Type 2 Diabetes Risk in Middle-Aged Black South African Women. Nutr intake Quant food Freq Quest. 2019;11(1246):1–19.
245. Utzschneider KM, Kahn SE, Polidori DC. Hepatic Insulin Extraction in NAFLD Is Related to Insulin Resistance Rather Than Liver Fat Content. J Clin Endocrinol Metab. 2019;104(5):1855–65.
246. International Diabetes Federation. Diabetes Atlas. 8th ed. Cho NH, Kirigia J, Claude J, Ogurstova MK, Guariguata L, Rathmann W, et al., editors. Brussels, Belgium: IDF; 2017. 1–147 p.
247. Grosso G, Marventano S, Yang J, Micek A, Pajak A, Scalfi L, et al. A comprehensive meta-analysis on evidence of Mediterranean diet and cardiovascular disease: Are

individual components equal? Crit Rev Food Sci Nutr. 2017;57(15):3218–32.
248. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW. Obesity is associated with macrophage accumulation in adipose tissue. Anaesth Intensive Care. 2003;112(12):1796–808.
249. Kim CS, Park HS, Kawada T, Kim JH, Lim D, Hubbard NE, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int J Obes. 2006;30(9):1347–55.
250. Alberti KGM, Zimmet P, Shaw J, Grundy SM. The IDF consensus worldwide definition of the metabolic syndrome. Diabet Med. 2006;23(5):1–25.
251. Swindale A, Bilinsky P. Development of a universally applicable household food insecurity measurement tool: process, current status, and outstanding issues. J Nutr. 2006;136(5):1449S-1452S.
252. Goedecke JH, Mendham AE, Clamp L, Nono Nankam PA, Fortuin-De Smidt MC, Phiri L, et al. An exercise intervention to unravel the mechanisms underlying insulin resistance in a cohort of black south african women: Protocol for a randomized controlled trial and baseline characteristics of participants. J Med Internet Res. 2018;20(4):1–19.
253. Nel, Johanna Helena, Steyn N. Report on South African Food Consumption Studies Undertaken Among Population Groups (1983-2000) :Average Intakes of Foods Most Commonly Consumed. Pretoria: Department of Health; 2002. 191 p.
254. Steyn NP, Senekal M, Norris SA, Whati L, Mackeown JM, Nel JH. How well do adolescents determine portion sizes of foods and beverages ? Asia Pac J Clin Nutr. 2006;15(1):35–42.
255. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods. 2008;40(3):879–91.
256. Snel M, Jonker JT, Schoones J, Lamb H, De Roos A, Pijl H, et al. Ectopic fat and insulin resistance: Pathophysiology and effect of diet and lifestyle interventions. Int J Endocrinol. 2012;1–18.
257. Pou KM, Massaro JM, Hoffmann U, Vasan RS, Maurovich-Horvat P, Larson MG, et al. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: The Framingham Heart Study. Circulation. 2007;116(11):1234–41.
258. Perry RJ, Camporez JPG, Kursawe R, Titchenell PM, Zhang D, Perry CJ, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell. 2015;160(4):745–58.
259. Gregor MF, Hotamisligil GS. Inflammatory Mechanisms in Obesity. Annu Rev Immunol.

2011;29(1):415–45.
260. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose Expression of Tumor Necrosis Factor-x: Direct Role in Obesity-Linked Insulin Resistance. Science. 1993;259(January):87–92.
261. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet. 2017;389(10085):2239–51.
262. Chung ST, Sumner AE. Diabetes: T2DM risk prediction in populations of African descent. Nat Rev Endocrinol. 2016;12(3):131–2.
263. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19–39.
264. Panunzi S, Carlsson L, De Gaetano A, Peltonen M, Rice T, Sjöström L, et al. Determinants of Diabetes Remission and Glycemic Control after Bariatric Surgery. Diabetes Care. 2016;39(1):166–74.
265. Taylor R, Al-Mrabeh A, Zhyzhneuskaya S, Peters C, Barnes AC, Aribisala BS, et al. Remission of Human Type 2 Diabetes Requires Decrease in Liver and Pancreas Fat Content but Is Dependent upon Capacity for β Cell Recovery. Cell Metab. 2018;28(4):547–56.
266. American Diabetes Association. Economic costs of diabetes in the U.S. in 2017. Diabetes Care. 2018;41:917–28.
267. Goedecke JH, Utzschneider K, Faulenbach M V., Rizzo M, Berneis K, Spinas G a., et al. Ethnic differences in serum lipoproteins and their determinants in South African women. Metabolism. 2010;59(9):1341–50.
268. Wang Q, Holmes M V., Smith GD, Ala-Korpela M. Genetic support for a causal role of insulin resistance on circulating branched-chain amino acids and inflammation. Diabetes Care. 2017;40(12):1779–86.
269. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva, Switzerland: World Health Organization; 2006.
270. Lundstedt T, Seifert E, Abramo L, Thelin B, Nyström Å, Pettersen J, et al. Experimental design and optimization. Chemom Intell Lab Syst. 1998;42(1–2):3–40.
271. Jonsson P, Wuolikainen A, Thysell E, Chorell E, Stattin P, Wikström P, et al. Constrained randomization and multivariate effect projections improve information extraction and biomarker pattern discovery in metabolomics studies involving dependent samples. Metabolomics. 2015;11(6):1667–78.
272. Jiye A, Trygg J, Gullberg J, Johansson AI, Jonsson P, Antti H, et al. Extraction and GC/MS analysis of the human blood plasma metabolome. Anal Chem. 2005;77(24):8086–94.
273. Eriksson L, Trygg J, Wold S. CV-ANOVA for significance testing of PLS and OPLS®

models. J Chemom. 2008;22(11–12):594–600.
274. Jonsson P, Björkblom B, Chorell E, Olsson T, Antti H. Statistical loadings and latent significance simplify and improve interpretation of multivariate projection models. bioRxiv. 2018;
275. Razquin C, Toledo E, Clish CB, Ruiz-Canela M, Dennis C, Corella D, et al. Plasma lipidomic profiling and risk of type 2 diabetes in the PREDIMED trial. Diabetes Care. 2018;41(12):2617–24.
276. Soga T, Ohishi T, Matsui T, Saito T, Matsumoto M, Takasaki J, et al. Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan G-protein-coupled receptor. Biochem Biophys Res Commun. 2005;326(4):744–51.
277. Dugas LR, Chorell E, Plange-Rhule J, Lambert E V., Cao G, Cooper RS, et al. Obesity-related metabolite profiles of black women spanning the epidemiologic transition. Metabolomics. 2016;12(3):1–10.
278. Cornall LM, Mathai ML, Hryciw DH, Simcocks AC, O'Brien PE, Wentworth JM, et al. GPR119 regulates genetic markers of fatty acid oxidation in cultured skeletal muscle myotubes. Mol Cell Endocrinol. 2013;365(1):108–18.
279. Brighton CA, Rievaj J, Kuhre RE, Glass LL, Schoonjans K, Holst JJ, et al. Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. Endocrinology. 2015;156(11):3961–70.
280. Martínez-Moya P, Romero-Calvo I, Requena P, Hernández-Chirlaque C, Aranda CJ, González R, et al. Dose-dependent antiinflammatory effect of ursodeoxycholic acid in experimental colitis. Int Immunopharmacol. 2013;15(2):372–80.
281. Sousa T, Castro RE, Pinto SN, Coutinho A, Lucas SD, Moreira R, et al. Deoxycholic acid modulates cell death signaling through changes in mitochondrial membrane properties. J Lipid Res. 2015;56:2158–71.
282. Im E, Martinez J. Ursodeoxycholic Acid (UDCA) Can Inhibit Deoxycholic Acid (DCA)-induced Apoptosis via Modulation of EGFR/ Raf-1/ERK Signaling in Human Colon Cancer Cells. J Nutr. 2004;134:483–6.
283. Tomkin GH, Owens D. Obesity diabetes and the role of bile acids in metabolism. J Transl Intern Med. 2016;4(2):73–9.
284. Walajtys-Rode E, Williamson J. Effects of branched-chain alpha-ketoacids on the metabolism of isolated rat-liver cells. III. Interactions with pyruvate-dehydrogenase. J Biol Chem. 1980;255(2):413–8.
285. Goodpaster BH, Sparks LM. Metabolic Flexibility in Health and Disease. Cell Metab. 2017;25(5):1027–36.
286. Mccoin CS, Knotts TA, Adams SH. Acylcarnitines—old actors auditioning for new roles in

metabolic physiology. Nat Rev Endocrinol. 2015;11(10):617–25.
287. Blüher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best Pract Res Clin Endocrinol Metab. 2013;27(2):163–77.
288. Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends Endocrinol Metab. 2000;11(9):351–6.
289. McLaughlin T, Lamendola C, Liu A, Abbasi F. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. J Clin Endocrinol Metab. 2011;96(11):1756–60.
290. May A, Hazelhurst S, Li Y, Norris SA, Govind N, Tikly M, et al. Genetic diversity in black South Africans from Soweto. BMC Genomics. 2013;134(644):1–12.
291. Mohandas C, Bonadonna R, Shojee-Moradie F, Jackson N, Boselli L, Alberti KGMM, et al. Ethnic differences in insulin secretory function between black African and white European men with early type 2 diabetes. Vol. 20, Diabetes, Obesity and Metabolism. 2018. 1678–1687 p.
292. Steven S, Hollingsworth KG, Al-Mrabeh A, Avery L, Aribisala B, Caslake M, et al. Very low-calorie diet and 6 months of weight stability in type 2 diabetes: Pathophysiological changes in responders and nonresponders. Diabetes Care. 2016;39(5):808–15.
293. Goedecke JH, Micklesfield LK, Levitt NS, Lambert E V, West S, Maartens G, et al. Effect of Different Antiretroviral Drug Regimens on Body Fat Distribution of HIV-Infected South African Women. AIDS Res Hum Retroviruses. 2013;29(3):557–63.

APPENDICES

Appendix A: Declaration by student and co-authors

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

I, **Asanda Mtintsilana**, student number (**607019**), declare that this Thesis/Dissertation/Research Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis/Dissertation/Research Report.

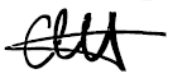
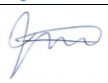
Signature of Student:  **Date**: 3 December 2020

Name of Primary Supervisor: Julia H. Goedecke

Signature of Primary Supervisor  **Date:** 23 November 2020

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

Article 1: Title: Fat redistribution and accumulation of visceral adipose tissue predicts type 2 diabetes risk in middle-aged black South African women: a 13-year longitudinal study
Journal name, year, volume and page numbers: *Nutrition and Diabetes* 2019; **9**: 1-12

Authors	Name	Signature	Date
1st author	Asanda Mtintsilana		3/12/2020
2nd author	Lisa K. Micklesfield		25/11/2020
3rd author	Elin Chorell		24/11/2020
4th author	Tommy Olsson		25/11/2020
5th author	Julia H. Goedecke		23/11/2020

Comments by primary supervisor:

……

……

Article 2: Title: Adiposity mediates the association between the dietary inflammatory index and markers of type 2 diabetes risk in middle-aged black South African women

Journal name, year, volume and page numbers: *Nutrients* 2019; **11**: 1-19

Authors	Name	Signature	Date
1st author	Asanda Mtintsilana		3/12/2020
2nd author	Lisa K. Micklesfield		25/11/2020
3rd author	Elin Chorell		24/11/2020
4th author	Tommy Olsson		25/11/2020
5th author	Nitin Shivappa		24/11/2020
6th author	James R. Hebert		24/11/2020
7th author	Andre P Kengne		24/11/2020
8th author	Julia H. Goedecke		23/11/2020

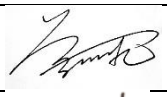
Comments by primary supervisor:

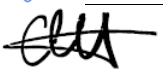
……

……

Article 3: Title: Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women

Journal name, year, volume and page numbers: *Metabolism, Clinical and Experimental* 2019; **95**: 57-64

Authors	Name	Signature	Date
1st author	Yingxu Zeng		25/11/2020
2nd author	Asanda Mtintsilana		3/12/2020

3rd author	Julia H. Goedecke		23/11/2020
4th author	Lisa K. Micklesfield		25/11/2020
5th author	Tommy Olsson		25/11/2020
6th author	Elin Chorell		24/11/2020

Comments by primary supervisor:

Although Miss Asanda Mtintsilana is not the first author on this publication, she made significant contributions. Briefly, as a PhD student and project coordinator, she was responsible for setting up procedures and overall project coordination, field work and recruitment, data collection, biochemical analyses, and data cleaning. In summary, Asanda was responsible for all the data used in this publication, excluding the metabolomics analysis. In addition to these tasks, she critically reviewed and edited the manuscript.

It also important to highlight that metabolomics is a complex technique that requires an expert or trained personnel as it involves multiple procedures. These include data acquisition using analytical instruments such as mass spectrometry, followed by data interpretation and the use of statistical analyses such as the multivariate statistical methods (i.e., principal component analysis and partial least squares (PLS) or its extension, orthogonal PLS). Collectively, these tasks require years of training, which Miss Mtintsilana was unable to attain during the course of her PhD degree. For these reasons, Dr Yingxu Zeng, as an expert in metabolomics procedures and statistical analyses was the deserving first author for this paper.

APPENDICES

Appendix B: Supporting information

Table 2S1: Characteristics of the participants according to the three glycaemic groups.

	NGT (n=91)		IFG/IGT (n=35)		T2D (n=16)		ANOVA		
	Baseline	**Follow up**	**Baseline**	**Follow-up**	**Baseline**	**Follow-up**	**Group**	**Time**	**Group x Time**
Age (years)	41 (36-47)	53 (48-59)*	46 (38-49)	57 (50-60)*	44 (40-46)	56 (52-58)*	0.125	<0.0001	0.258
Anthropometry									
Weight (kg)	75.6 (64.5-89.6)	83.0 (70.8-96.4)*	77.1 (67.7-84.9)	78.6 (72.6-100.2)*	78.3 (71.0-100.6)	81.4 (72.3-98.1)	0.460	<0.0001	0.109
BMI (kg/m^2)	30.8 (25.1-35.6)	33.4 (28.6-38.6)*	31.2 (26.9-33.7)	32.6 (29.0-37.4)*	32. 4 (29.0-37.0)	32.4 (28.5-38.9)	0.439	<0.0001	0.072
Waist circumference (cm)	86.1 ± 10.7	98.0 ± 12.5*	89.1 ± 10.0	100.8 ± 10.9*	98.4 ± 13.9^{aa}	106.6 ± 14.9*^{a}	0.003	<0.0001	0.160
Hip circumference (cm)	114.5 (105.6-123.0)	120.2 (110.5-129.0)*	112.0 (104.0-122.0)	116.2 (110-0-128.0)*	116.5 (108.0-123.8)	117.5 (106.0-131.0)	0.715	<0.0001	0.237
WHR	0.75 (0.70-0.80)	0.82 (0.77-0.85)*	0.80 (0.76-0.83)^{cc}	0.85 (0.80-0.89)*^{c}	0.86 (0.81-0.88)$^{aa\ b}$	0.88 (0.84-0.91)*$^{aa\ b}$	<0.0001	<0.0001	0.715
Body composition and body fat distribution									
Fat-free soft tissue mass (kg)	36.0 (31.7-39.2)	36.7 (32.08-39.8)	36.3 (33.4-39.2)	35.6 (33.6-39.8)	38.1 (33.6-43.0)	37.0 (33.1-42.7)	0.122	0.475	0.432
Fat mass (kg) $^{\#, \#\#}$	31.7 (24.4-40.2)	38.7 (29.5-48.3)*	32.9 (26.1-38.9)	35.8 (31.6-47.6)*	33.7 (29.4-46.6)	35.0 (29.0-48.2)	0.743	<0.0001	0.025
Body fat (%) $^{\#, \#\#}$	46.7 (40.5-51.1)	51.1 (45.3-54.5)*	46.2 (43.2-49.6)	50.2 (46.5-53.2)*	47.7 (43.6-49.4)	49.1 (44.7-54.0)	0.896	<0.0001	0.017
Trunk %FM	43.2 ± 4.3	44.6 ± 5.2*	46.3 ± 3.8^{cc}	48.6 ± 4.2*^{cc}	49.0 ± 4.6$^{aa\ b}$	51.3 ± 5.7*^{aa}	<0.0001	<0.0001	0.355
Leg %FM	45.6 (42.3-49.6)	42.9 (40.2-47.5)*	42.6 (38.8-44.2)^{cc}	39.6 (37.0-42.0)*^{cc}	39.9 (34.6-41.6)$^{aa\ b}$	36.6 (30.8-38.7)*$^{aa\ b}$	<0.0001	<0.0001	0.497
Arm %FM	10.9 (9.9-11.9)	11.5 (10.5-12.5)*	11.4 (10.7-13.0)^{c}	12.0 (10.2-14.1)	12.1 (11.2-13.2)^{aa}	12.6 (11.5-13.8)^{a}	0.006	0.0014	0.391
VAT (cm^2)	102 (71 -153)	151 (104 -193)*	134 (89 -163)^{c}	173 (147-230)*^{c}	156 (130-204)^{aa}	199 (160-249)*^{aa}	0.0005	<0.0001	0.705
SAT (cm^2) $^{\#, \#\#}$	410 (274 -539)^{a}	520 (376-614)*	445 (286-513)	478 (381-603)*	458 (378-600)	501 (378-620)	0.663	<0.0001	0.015

Data presented as means ± SD or median (25th-75th percentiles). The three categorical groups include participants that remained with NGT (n=91), transitioned to IFG/ IGT (, n=35) and those that developed T2D (n=16) at follow-up. BMI, body mass index; %FM (expressed as a percentage of sub-total fat mass); VAT, visceral adipose tissue area; SAT, subcutaneous adipose tissue area.

^{a}p<0.05, ^{aa}p<0.01 T2D group vs NGT group at baseline and/or follow-up;

^{b}p<0.05, ^{bb}p<0.01 T2D group vs IFG/IGT group at baseline and/or follow-up;

^{c}p<0.05, ^{cc}p<0.01 IFG/IGT group vs NGT group at baseline and/or follow-up.

*p<0.05 Baseline vs follow-up within a defined group.

$^{\#}$p<0.05 (group-by-time interaction) T2Dvs NGT; $^{\#\#}$p<0.05 (group-by-time interaction) T2D vs IFG/IGT

Table 2S2: Body fat and fat distribution measures as predictors of IFG/IGT and T2D risk at follow-up

Variables	Outcome Variables	RRR	95% CI	p-value	Model p-value
FM (kg)					
Baseline fat mass (kg)	NGT to IFG/IGT	1.00	0.96-1.03	0.806	0.059
Change fat mass (kg)		1.03	0.96-1.10	0.474	
Baseline fat mass (kg)	NGT to T2D	1.04	0.98-1.09	0.212	
Change fat mass (kg)		0.92	0.84-1.00	0.047	
Trunk FM (kg)					
Baseline trunk FM (kg)	NGT to IFG/IGT	1.72	1.23-2.41	**0.002**	<0.0001
Change trunk FM (kg)		1.12	0.99-1.28	0.078	
Baseline trunk FM (kg)	NGT to T2D	2.62	1.58-4.34	**<0.0001**	
Change trunk FM (kg)		1.02	0.86-1.21	0.797	
Arm FM (kg)					
Baseline arm FM (kg)	NGT to IFG/IGT	2.65	1.24-5.67	**0.012**	0.009
Change arm FM (kg)		1.16	0.71-1.90	0.541	
Baseline arm FM (kg)	NGT to T2D	2.74	1.11-6.72	**0.028**	
Change arm FM (kg)		0.71	0.38-1.32	0.281	
Leg FM (kg)					
Baseline leg FM (kg)	NGT to IFG/IGT	0.59	0.43-0.80	**0.001**	<0.0001
Change leg FM (kg)		1.09	0.91-1.32	0.346	
Baseline arm FM (kg)	NGT to T2D	0.47	0.31-0.73	**0.001**	
Change arm FM (kg)		0.85	0.65-1.12	0.254	
VAT (10 cm^2)					
Baseline VAT (10 cm^2)	NGT to IFG/IGT	1.20	1.05-1.38	**0.007**	0.001
Change VAT (10 cm^2)		1.11	1.01-1.24	**0.033**	
Baseline VAT (10 cm^2)	NGT to T2D	1.37	1.14-1.64	**0.001**	
Change VAT (10 cm^2)		1.16	1.03-1.31	**0.015**	
SAT (10 cm^2)					
Baseline SAT (10 cm^2)	NGT to IFG/IGT	1.02	0.95-1.08	0.582	0.073
Change SAT (10 cm^2)		1.01	0.96-1.07	0.615	
Baseline SAT (10 cm^2)	NGT to T2D	1.02	0.93-1.12	0.672	
Change SAT (10 cm^2)		0.91	0.85-0.99	0.019	
VAT (10 cm^2) and Leg FM (kg)					
Baseline VAT (10 cm^2)	NGT to IFG/IGT	1.10	0.94-1.29	0.242	<0.0001

Change VAT (10 cm^2)		1.09	0.96-1.24	0.173	
Baseline leg FM (kg)		0.66	0.46-0.94	**0.022**	
Change leg FM (kg)		1.01	0.81-1.27	0.897	
Baseline VAT (10 cm^2)	NGT to T2D	1.27	1.01-1.59	**0.041**	
Change VAT (10 cm^2)		1.25	1.05-1.49	**0.013**	
Baseline leg FM (kg)		0.62	0.38-1.00	**0.048**	
Change leg FM (kg)		0.69	0.49-0.96	**0.026**	

Data are presented as relative risk ratios, 95% confidence interval (CI) and p-values adjusted for age. Each model includes baseline body fat and fat distribution measures, the change in the body fat and fat distribution measures as predictor variables. Outcome variables include three groups; NGT (i.e. NGT participants, reference group categorised as “0”), IFG/IGT and T2D (exposure groups; categorised as “1” and “2”). All the body fat and fat distribution models were adjusted for the potential effects of age at baseline, and regional FM, VAT and SAT models were also adjusted for baseline body fat mass. FM, fat mass; VAT, visceral adipose tissue area; RRR, relative risk ratios; SAT, subcutaneous adipose tissue area.

Table 2S3: Body fat and fat distribution measures as predictors of IGM/T2D risk at follow-up, adjusted for menopausal status

Variables	Odds ratio	95% CI	p-value	Model p-value
FM (kg)				
Baseline fat mass (kg)	1.01	0.97-1.04	0.647	0.299
Change fat mass (kg)	0.99	0.93-1.04	0.613	
Menopausal status (follow-up)	0.91	0.49-1.69	0.767	
Trunk FM (kg)				
Baseline trunk FM (kg)	1.92	1.41-2.61	**0.000**	<0.001
Change trunk FM (kg)	1.08	0.97-1.21	0.164	
Menopausal status (follow-up)	0.96	0.50-1.86	0.915	
Arm FM (kg)				
Baseline arm FM (kg)	2.79	1.41-5.52	**0.003**	0.007
Change arm FM (kg)	0.97	0.63-1.49	0.889	
Menopausal status (follow-up)	0.95	0.51-1.79	0.875	
Leg FM (kg)				
Baseline leg FM (kg)	0.56	0.42-0.74	**0.000**	<0.0001
Change leg FM (kg)	1.01	0.86-1.20	0.881	
Menopausal status (follow-up)	1.06	0.55-2.07	0.855	
VAT (10 cm^2)				
Baseline VAT (10 cm^2)	1.26	1.11-1.43	**0.000**	<0.001
Change VAT (10 cm^2)	1.14	1.04-1.24	**0.006**	
Menopausal status (follow-up)	0.82	0.43-1.56	0.547	
SAT (10 cm^2)				
Baseline SAT (10 cm^2)	1.02	0.96-1.08	0.591	0.330
Change SAT (10 cm^2)	0.98	0.94-1.03	0.457	
Menopausal status (follow-up)	0.92	0.49-1.71	0.786	
VAT (10 cm^2) and Leg FM (kg)				
Baseline VAT (10 cm^2)	1.15	0.99-1.34	0.066	<0.0001
Change VAT (10 cm^2)	1.15	1.02-1.30	**0.021**	
Baseline leg FM (kg)	0.67	0.49-0.92	**0.013**	
Change leg FM (kg)	0.89	0.73-1.09	0.260	
Menopausal status (follow-up)	0.89	0.45-1.77	0.746	

Data are presented as odds ratios, 95% confidence interval (CI) and p-values adjusted for age. Each model includes baseline body fat and fat distribution measures, the change in the body fat and fat distribution measures as predictor variables. Outcome variables include two groups; NGT (i.e. NGT participants, reference group) and the IGM/T2D groups (IFG/IGT and T2D participants). All the body fat and fat distribution models were adjusted for the potential effects of age at baseline, and regional FM, VAT and SAT models were also adjusted for baseline body fat mass. FM, fat mass; VAT, visceral adipose tissue area; SAT, subcutaneous adipose tissue area.

Table 2S4: Regression coefficients from robust multiple linear models for the prediction of HbA1c, fasting insulin resistance (HOMA2-IR) and OGTT-derived insulin sensitivity (Matsuda Index) at follow-up, adjusted for menopausal status

	HbA1c				Insulin resistance (HOMA2-IR)				Insulin sensitivity (Matsuda Index)			
	β	**95% CI**	**p-value**	**R^2**	**β**	**95% CI**	**p-value**	**R^2**	**β**	**95% CI**	**p-value**	**R^2**
Body fat mass (kg)												
Baseline fat mass (kg)	0.01	0.00-0.01	**0.008**	0.08*	0.03	0.01-0.05	**0.001**	0.09*	-0.07	-0.11—0.02	**0.008**	0.06*
Change fat mass (kg)	0.00	-0.01-0.01	0.898		0.02	-0.02-0.05	0.344		-0.06	-0.14-0.03	0.196	
Menopausal status (follow-up)	0.07	-0.04-0.18	0.188		0.29	-0.04-0.61	0.084		-0.09	-1.73-002	**0.225**	
Trunk FM (kg)												
Baseline trunk FM (kg)	0.06	0.02-0.11	**0.004**	0.12*	0.26	0.12-0.39	**<0.0001**	0.17*	-0.63	-0.96—0.30	**<0.0001**	0.12*
Change trunk FM (kg)	0.01	-0.01-0.03	0.336		0.04	-0.01-0.10	0.105		-0.13	-0.26-0.01	0.063	
Menopausal status (follow-up)	0.09	-0.02-0.19	0.116		0.32	0.01-0.63	**0.042**		-0.79	-1.55—0.02	**0.046**	
Arm FM (kg)												
Baseline arm FM (kg)	-0.02	-0.13-0.09	0.706		0.11	-0.23-0.45	0.539		-0.37	-1.34-0.60	0.456	
Change arm FM (kg)	0.05	-0.03-0.12	0.199	0.09*	0.15	-0.08-0.37	0.200	0.09*	-0.72	-1.36—0.08	**0.027**	0.09*
Menopausal status (follow-up)	0.07	-0.04-0.18	0.192		0.30	-0.03-0.63	**0.071**		-0.96	-1.86—0.05	**0.039**	
Leg FM (kg)												
Baseline leg FM (kg)	-0.04	-0.08—0.00	**0.045**	0.10*	-0.20	-0.32-0.09	**0.001**	0.14*	0.47	0.16-0.78	**0.003**	0.09*
Change leg FM (kg)	-0.00	-0.03-0.03	0.967		0.04	-0.04-0.12	0.324		-0.10	-0.30-0.09	0.306	
Menopausal status (follow-up)	0.08	-0.02-0.19	0.121		0.33	0.02-0.64	**0.040**		-0.84	-1.64—0.04	**0.040**	
VAT area (10 **cm^2)**												
Baseline VAT (10 cm^2)	0.01	-0.00-0.03	**0.105**	0.09*	0.10	0.05-0.16	**<0.0001**	0.19*	-0.23	-0.37—0.08	**0.002**	0.13*
Change VAT (10 cm^2)	0.01	-0.01-0.02	0.492		0.07	0.03-0.12	**0.001**		-0.19	-0.31—0.08	**0.001**	
Menopausal status (follow-up)	0.07	-0.04-0.17	0.224		0.26	-0.05-0.56	0.095		-0.72	-1.54-0.10	0.086	
SAT area (10 cm^2)												
Baseline SAT (10 cm^2)	0.00	-0.01-0.01	0.425	0.08*	0.01	-0.02-0.04	0.415	0.10	-0.07	-0.16-0.03	0.164	0.07*
Change SAT (10 cm^2)	0.00	-0.00-0.01	0.423		0.01	-0.01-0.04	0.294		-0.06	-0.13-0.01	0.069	
Menopausal status (follow-up)	0.07	-0.04-0.18	**0.223**		0.27	-0.06-0.60	0.108		-0.78	-1.68-0.13	0.093	
VAT (10 cm^2) and Leg FM (kg)												
Baseline VAT (10 cm^2)	0.01	-0.01-0.03	0.403		0.09	0.03-0.15	**0.004**		-0.17	-0.35—0.00	**0.047**	
Change VAT (10 cm^2)	0.00	-0.01-0.02	0.651		0.07	0.03-0.12	**0.003**		-0.16	-0.29—0.03	**0.014**	
Baseline leg FM (kg)	-0.03	-0.08-00.02	0.209	0.10	-0.09	-0.22-0.05	0.215	0.20*	0.20	-0.19-0.59	0.304	
Change leg FM (kg)	-0.00	-0.03-0.03	0.789		-0.02	-0.11-0.06	0.604		-0.00	-0.23-0.23	0.998	
Menopausal status (follow-up)	0.08	-0.03-0.19	0.165		0.28	-0.02-0.59	**0.070**		-0.75	-1.57-0.08	0.075	

Data are presented as β-coefficients, 95% confidence intervals, R^2 for each model as well as p-values adjusted for age. Each model includes baseline body fat and fat distribution measures, the change in the body fat and fat distribution measures as predictor variables and HbA1c, fasting insulin resistance (HOMA2-IR) and OGTT-derived insulin sensitivity (Matsuda Index) measures at

follow-up as the outcome variables. All the body fat and fat distribution models were adjusted for the potential effects of age at baseline, and regional FM, VAT and SAT models were also adjusted for baseline body fat mass FM, fat mass. VAT, visceral adipose tissue area; SAT, subcutaneous adipose tissue area. *P value for the model <0.05.

Table 3S1: Robust linear regression analyses for measures of insulin secretion and clearance, with insulin sensitivity (Matsuda Index), adiposity and FLI as determinants

	Beta-cell glucose sensitivity, β-CGS (pmol/(min m^2 mM))			
	β	**95% CI**	**p-value**	**R^2**
Insulin sensitivity	-7.58	-12.04—3.12	**0.001**	0.10*
Body fat mass (kg)	0.06	-1.08-1.19	0.923	0.07*
FLI	0.10	-0.48-0.68	0.737	0.07*
VAT area (10 cm^2)	1.56	-0.91-4.03	0.214	0.07*
Leg FM (%FM)	-0.89	-3.6-1.67	0.493	0.07*

Data are presented as β-coefficients, 95% confidence intervals, R^2 for each model and p-values. Each model includes measures of insulin secretion and clearance (basal state and total) as the outcome variables, with insulin sensitivity (Matsuda index) and adiposity as independent variables. The models were adjusted for the potential effects of age and glycaemic groups (i.e., NGT, IGM and T2D). FLI, fatty liver index; FM, fat mass; VAT, visceral adipose tissue. *P value for the model <0.05. #P value for the model not significant (P>0.05).

Table 4S1. Demographic, behavioral and lifestyle factors characteristics of the participants.

	Overall	Negative E-DII group (n=107)	Positive E-DII group (n=83)	p-value
Sociodemographic factors				
Housing density (persons/room)	1 (0.7-1.3)	0.9 (0.6-1.2)	1.0 (0.8-1.3)	0.12
Asset index (out of 12 items)	9 (7-11)	9 (7-11)	9 (7-11)	0.87
Household Food insecurity score	4 (0-9)	3 (0-8)	4 (0-10)	0.21
Marital status				
Married	104 (54.7)	56 (52.3)	48 (57.8)	0.45
Unmarried	86 (45.3)	51 (47.7)	35 (42.2)	
Highest level of education, n (%)				
Primary education	29 (15.3)	16 (15.0)	13 (15.7)	0.22
Some secondary education	89 (46.8)	44 (41.1)	45 (54.2)	
Secondary education completed (grade 12)	41 (21.6)	28 (26.2)	13 (15.7)	
Tertiary education	31 (16.3)	19 (17.8)	12 (14.5)	
Employment, n (%)				
Employment	100 (52.6)	58 (54.2)	42 (50.6)	0.622
Unemployed	90 (47.4)	49 (45.8)	41 (49.4)	
Monthly income, n (%)				
1=No income	1 (0.5)		1 (1.2)	0.51
2=R1-4999	101 (54.0)	59 (56.2)	42 (51.2)	
3=R5000-9999	39 (20.9)	20 (19.0)	19 (23.2)	
4=R10000-19999	34 (18.2)	21 (20.0)	13 (15.9)	
5= (>R20000)	12 (6.4)	5 (4.8)	7 (8.5)	
**3 participants did not disclose their monthly income*				
Behavioral and lifestyle factors				
Smoking status, n (%)				
Never smoked	204 (96.3)	104 (97.2)	79 (95.2)	0.46

Current smoker	8 (3.7)	3 (2.8)	4 (4.8)	
Alcohol intake, n (%)				
Yes	49 (25.8)	24 (22.4)	25 (30.1)	0.23
No/Never	141 (74.2)	83 (77.6)	58 (69.9)	
Physical activity data				
Light (hr/d)	6.4 (5.4-7.4)	6.6 (5.5-5.2)	6.1 (5.2-7,2)	0.14
Moderate (mins/d)	37.4 (21.4-51.9)	41.5 (21.5-50.8)	33.7 (18.7-52.8)	0.31
Vigorous (mins/d)	0.1 (0.0-0.5)	0.2 (0.0-0.6)	0.0 (0.0-0.3)	0.19
Total MVPA (mins/d)	37.9 (21.6-53.2)	43.8 (21.7-53..0)	33.7 (18.7-53.5)	0.23
MVPA (% per day)	4.3 (2.3-5.9)	4.9 (2.4-6.0)	3.7 (2.2-5.7)	0.25
Steps per day	6590.4 (4871.7-8583.7)	6945.7 (5063.9-8736.1)	6203.0 (4792.8-8249.0)	0.12
Sedentary behavior				
Sedentary (hr/d)	8.5 ± 2.2	8.5 ± 2.3	8.4 ± 2.0	0.69
Sedentary (% per day)	50.8 ± 12.9	50.8 ± 13.7	50.9 ± 11.8	0.99
Standing (% per day)	36.9 ± 10.6	36.6 ± 11.4	37.2 ± 9.4	0.70
Stepping (% per day)	12.3 ± 4.2	12.6 ± 4.3	11.9 ± 4.0	0.31

Data presented as means ± SD, median (25th-75th percentiles) or frequency (%). E-DII, energy-adjusted dietary inflammatory index; MVPA, moderate-vigorous physical activity.

Table 4S2. Nutrient intake between the positive and negative E-DII score groups.

		Negative E-DII group (n=107)	Positive E-DII group (n=83)	p-value
Number (%)	**Overall**	**107 (56.3%)**	**83(43.7%)**	
Energy (kcal/d)	1464.8 (1115.0-1959.1)	1461.9 (1115.0-1962.1)	1490.3 (1111.1-1905.9)	0.66
Protein (g/d)	36.6 (28.7-52.5)	38.8 (29.4-57.7)	34.9 (26.8-46.8)	0.03
Protein (%E)	10.2 (8.5-12.6)	11.1 (9.4-13.0)	9.2 (7.8-10.8)	<0.0001
Total fat (g/d)	60.5 (45.4-85.9)	58.2 (41.5-79.6)	71.8 (52.5-99.1)	0.004
Total fat (%E)	38.1 (32.2-43.9)	35.3 (29.9-39.9)	42.4 (36.5-48.4)	<0.0001
Saturated fat (g/d)	14.2 (11.0-20.8)	13.2 (9.9-19.2)	17.1 (11.7-22.5)	0.007
Saturated fat (%E)	8.9 (7.6-10.4)	8.2 (7.3-9.5)	10.0 (8.6-11.3)	<0.0001
Monounsaturated fatty acid (g/d)	20.4 (14.7-29.8)	19.6 (13.6-27.2)	21.8 (15.7-33.9)	0.03
Monounsaturated fatty acid (%E)	12.6 (10.3-14.9)	11.9 (9.9-13.9)	13.6 (11.3-15.8)	0.001
Polyunsaturated fatty acid (g/d)	22.6 (14.7-30.7)	18.8 (12.0-28.4)	24.4 (17.7-34.2)	0.002
Polyunsaturated fatty acid (%E)	12.6 (8.8-16.9)	11.0 (8.4-14.4)	15.8 (10.3-19.0)	0.0001
Carbohydrates (g/d)	181.4 (135.6-247.3)	194.0 (142.5-256.9)	168.6 (125.6-239.4)	0.18
Carbohydrates (%E)	50.8 (45.5-56.7)	52.3 (48.1-58.3)	47.0 (41.4-54.0)	<0.0001
Alcohol	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.14
Cholesterol (mg)	132.3 (83.8-217.0)	132.6 (85.7-208.1)	132.1 (80.5-240.9)	0.89
Fibre (g/d)	12.0 (8.4-17.3)	14.5 (10.4-20.1)	9.2 (7.0-13.1)	<0.0001
Iron (mg/d)	8.5 (6.3-12.1)	9.7 (7.0-13.7)	7.8 (5.7-10.2)	0.005
Magnesium (mg/d)	159.4 (118.2-210.0)	175.7 (129.2-250.8)	136.3 (105.2-200.4)	0.002
Zinc (mg/d)	6.5 (5.0-9.0)	7.43 (5.7-10.3)	6.0 (4.3-7.5)	<0.0001)
Vitamin A (Retinol equivalent) (µg/d)	617.9 (354.7-1007.8)	664.0 (393.8-1039.9)	590.5 (300.4-1007.8)	0.21
Thiamine (mg/d)	1.0 (0.8-1.5)	1.1 (0.8-1.6)	1.0 (0.7-1.3)	0.03
Ribloflavin (mg/d)	0.8 (0.6-1.1)	0.7 (0.6-1.2)	0.8 (0.5-1.1)	0.57
Niacin (mg/d)	13.0 (9.8-18.2)	15.0 (10.9-21.0)	10.8 (8.5-15.5)	<0.0001
Vitamin B6 (mg/d)	1.8 (1.3-2.8)	2.0 (1.5-3.4)	1.4 (1.0-2.2)	<0.0001
Vitamin B12	1.9 (1.2-3.5)	2.0 (1.2-3.3)	1.9 (1.2-3.7)	0.78
Vitamin C (mg/d)	29.9 (15.7-65.3)	38.6 (17.2-69.6)	23.5 (12.9-53.0)	0.009
Vitamin D (µg/d)	3.9 (2.5-5.4)	3.8 (2.2-5.3)	4.1 (3.0-5.6)	0.22
Vitamin E (mg/d)	13.4 (8.0-20.5)	11.8 (6.9-18.5)	14.7 (10.8-24.3)	0.008

Values expressed as median (25th-75th percentiles).

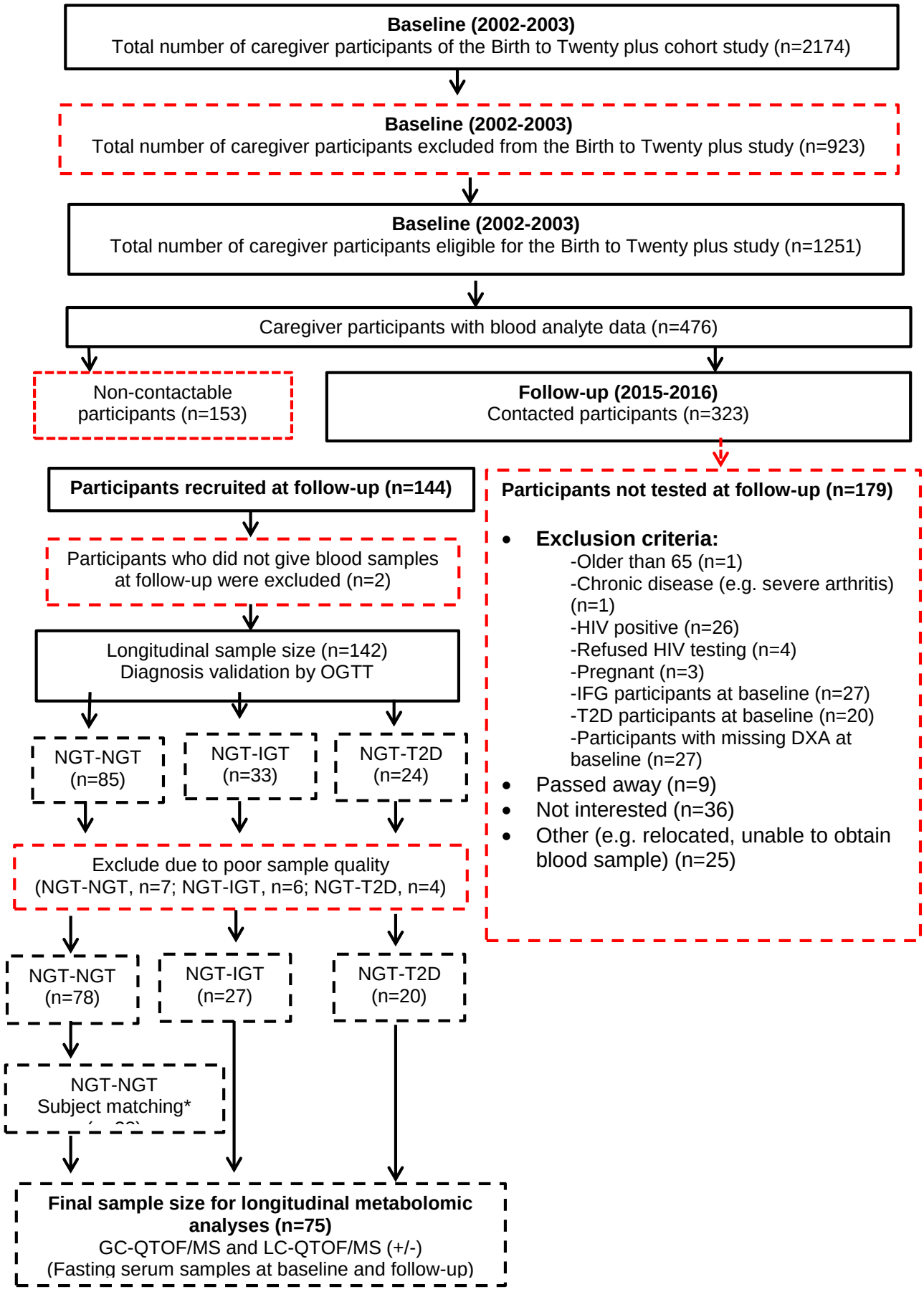


Figure 5S1. Study design and sample selection flow chart. Comprehensive metabolomics analyses were performed on serum samples at baseline (prior to disease development) and at 13-year follow-up.
*NGT-NGT subjects are matched with the other two groups with regards to anthropometric data. See Method

5S1: Research design and methods

Study population

This prospective study consists of two parts of data collection. The first part of data collection took place between 2002 and 2003 (baseline) and at the 13-year follow-up period between 2015 and 2016. Briefly, at baseline, 2174 caregivers of the Birth to Twenty Plus (BT20+) cohort were invited to visit a data collection facility at either Chris Hani Baragwanath Hospital in Soweto or the University of the Witwatersrand Medical School in Johannesburg. Of those invited, only 1251 were eligible for inclusion in the study as they fulfilled the criteria of being black SA female caregivers of the BT20+ cohort and residents in the urban township of Soweto (Crowther and Norris, 2012) (Figure S1). These participants underwent extensive testing that included interviews and underwent blood sampling. However, only 476 participants tested had complete blood analyte data at baseline. Between 2015 and 2016, contactable participants were asked to participate in the follow-up study if they fulfilled the following inclusion criteria: (1) having normal fasting glucose data at baseline; (2) female caregivers of the BT20+ cohort; (3) HIV negative on testing; (4) less than 65 years of age. Participants were excluded from the study when they had chronic or infectious diseases or were pregnant or lactating. At follow-up, all recruited women (n=144) underwent clinical measurements. Those without known type 2 diabetes underwent an oral glucose tolerance test (OGTT). WHO diabetes diagnostic criteria were used to diagnose IGT and type 2 diabetes. The final sample size for this study was n=75 (Figure S1). The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M010556 and M150530). The procedures and risks associated with the study were explained to the participants and those who signed the consent form were included. All testing procedures were performed at the South African Medical Research Council (MRC)/University of the Witwatersrand (WITS) Developmental Pathways for Health Research Unit (DPHRU) offices at the Chris Hani Baragwanath Hospital in Soweto.

Sample preparation and mass spectrometry metabolomics analyses

In short, 100 µL of plasma samples were extracted with 900 µL of extraction mixture

(methanol/water (90/10, v/v) spiked with isotope labelled internal standards), stored at –20 ˚C for 2 h in the freezer to precipitate the protein and subsequently centrifuged at 14000 RPM for 10 min at 4 ˚C. Aliquots of supernatant (200 µL) were collected and dried for GC-TOF/MS and LC-TOF/MS metabolomics analyses, respectively. For LC-TOF/MS analysis, isotope labelled internal standards including $13C_9$-phenylalanine, [$^{13}C_3$]-caffeine, [D_4]-cholic acid, [D_8]-arachidonic acid and [$^{13}C_9$]-caffeic acid were used (Sigma, St. Louis, MO, USA). For GC-TOF/MS analysis, isotope labelled internal standards consisted of: [$^{13}C_5$]-proline, [D_4]-succinic acid, [$^{13}C_5$, ^{15}N]-glutamic acid, [1,2,3-$^{13}C_3$]-myristic acid, [D_7]-cholesterol and [$^{13}C_4$]-disodium-ketoglutarate purchased from Cambridge Isotope Laboratories (Andover, MA); [$^{13}C_{12}$]-sucrose and [$^{13}C_4$]-palmitic acid obtained from Campro (Veenendaal, The Netherlands); [$^{13}C_6$]-glucose and [D_4]-putrescine purchased from Sigma Aldrich (Steinheim, Germany); [D_4]-salicylic acid purchased from Icon (Summit, NJ). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), 1% trimethylchlorosilane (TMCS) and pyridine (silylation grade) were purchased from Pierce Chemical Co.

Prior to GC-TOF/MS metabolomics analysis, a further two-step derivatization procedure was carried out to increase metabolites volatility (silylated) and reduce number of tautomeric form of each carbohydrate (methoxymated) (A et al. 2005). The derivatized samples (1 µL) were analysed on an Agilent 6890 gas chromatograph equipped with a 10 m × 0.18 mm i.d. fused silica capillary column with a chemically bonded 0.18-µm DB 5-MS stationary phase (J&W Scientific, Folsom, CA). The injector temperature was 270 °C. The column temperature was held at 70 °C for 2 min, increased by 40 °C min^{-1} to 320 °C, and held there for 1 min. The column effluent was introduced into the ion source of a Pegasus III time-of-flight mass spectrometer, GC-TOF/MS (Leco, St. Joseph, MI, USA). The transfer line and the ion source temperatures were 250 and 200 °C, respectively. Ions were generated by a 70-eV electron beam at an ionization current of 2.0mA. The spectra were recorded in the mass range 50–800 m/z at a rate of 30 spectra s^{-1}.

Prior to LC-TOF/MS metabolomics analysis, the samples were re-suspended in 10 + 10 µL of methanol and water. In each batch, an internal serum sample and pooled serum (quality control, QC) samples were injected for instrument quality control and instrument stability monitoring. In addition, dilution series of QC samples were analysed. Aliquots of plasma extracts (2 µL) were injected onto a Waters Acquity

UPLC HSS T3 C_{18} column (2.1 × 50 mm, 1.8 µm, Waters, Milford, MA, USA) in combination with a 2.1 mm x 5 mm, 1.8 µm VanGuard precolumn (Waters Corporation, Milford, MA, USA) held at 40 °C. The chromatographic separations of metabolites were carried out using a gradient solvent system consisted of water with 0.1 % formic acid (A) and acetonitrile/isopropanol (75/25, v/v) with 0.1 % formic acid (B) at a flow rate of 0.5 mL min^{-1}. The gradient started at 0.1 % B increasing linearly to 10 % B in 2 min and increased to 99 % B over 5 min which held for 2 min; the proportion of B was decreased to 0.1 % for 0.3 min and was further decreased to 0 % with the flow-rate increased to 0.8 mL min^{-1} for 0.5 min which held for 0.9 min; the initial conditions were restored in 0.1 min before the next injection. The detection of separated metabolites was performed using the Agilent 6550 Q-TOF mass spectrometer equipped with a jet stream electrospray ionization (ESI) source, operating in both positive and negative ion modes. A reference interface was connected for accurate mass measurements and reproducibility. Full scan MS spectra were collected in a centroid mode over the mass range 70-1700 m/z with an acquisition rate of 4 spectra s^{-1}. The capillary voltage was set at +4 kV and –4 kV with nozzle voltages of +300 V and –300V for positive and negative ion modes, respectively. Other MS parameters were applied as follows: gas temperature was set at 150 °C, drying gas flow was 16 L min^{-1}, and the pressure of nebulizer gas was 35 psig. The sheath gas flow was kept at 11 L $min^{-1}$with a temperature of 350 °C. The voltages of the fragmentor, skimmer, and octopole RF peak voltage were 380 V, 45 V and 750 V, respectively. The LC-TOF/MS data acquisition was performed in global profiling mode including the initial MS/MS scanning for the further targeted and untargeted data analysis. Additional targeted and untargeted MS/MS analyses of quality control samples were performed for targeting metabolites included in the in-house database and for elucidating the structures of untargeted metabolites, respectively. Data were acquired with MassHunter Acquisition Software B.05.01.

Data processing for GC-TOF/MS and LC-TOF/MS data

The GC-TOF/MS data were processed with an in-house MATLAB script (R2016a, The MathWorks, Natick, MA, USA). First, the chromatograms were aligned, based on internal standards, and the dataset was filtered to remove double peaks and noisy spectra. Only unique spectral profiles with a relative standard deviation (RSD) <40%, calculated from QC samples, were included in the subsequent sample comparison

modeling. Mass spectra of all putative metabolites were compared to data in our in-house spectral library, at the Swedish Metabolomics Centre (www.swedishmetabolomicscentre.se), and the publicly available Max Planck Institute library (http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html). We normalized the data with a cross-contribution compensating multiple standard normalization method, and multiple internal standards were included.

The LC-TOF/MS data were processed in both targeted and untargeted approaches by MassHunter Profinder version B.08.00 assisted by MassHunter Qualitative Analysis software version B.07.00, MassHunter PCDL manager version B.07.00 and Mass Profiler Professional™ 13.0 (all from Agilent Technologies Inc., Santa Clara, CA, USA). We performed a dual-feature extraction procedure, which included (i) a targeted feature extraction, where the retention time and mass spectra were compared to data in our in-house compound library and (ii) an untargeted feature extraction, designed to find putative novel metabolites that were not included in available databases.

Firstly, we performed the targeted data analysis to find the targeted molecular features (compounds) across all the samples in the batch by batch targeted feature extraction algorithm in Profinder. The extracted features were identified by matching the retention time and mass spectral data of the peaks against our in-house metabolite library. The match tolerance of masses deviation was set to ± 20 ppm and retention times deviation was adjusted to ± 0.15 min, respectively. Features were filtered with a peak height above 1000 counts and a match score above 50.

Next, we perform the untargeted data analysis to find the putative novel metabolites across the samples not included in available databases by batch molecular feature extraction algorithm in Profinder. The match tolerance of masses deviation was set to ± 10 ppm and retention times deviation was adjusted to ± 0.15 min, respectively, where up to 1500 features with a peak height above 1000 counts and a match score above 60 can be extracted.

Furthermore, we performed a filtering step on both targeted and untargeted features. Features were firstly filtered by manual inspection of the data chromatographic and mass spectral profiles and removal of non-Gaussian peaks as well as peaks with deviating % RSD-values > 10 ppm (Mass) and % RSD > 20 (target score of

molecular feature extraction). Next, to remove noisy features and background, we calculated an OPLS model for the extracted features using the concentration of the dilution series of QC samples as response. Features with VIP < 1 and with a negative correlation to the dilution series of QC samples were removed.

To extend the identification beyond the existing in-house databases, we compared the measured MS/MS spectra with data in publicly available spectral databases in combination with a manual inspection of the fragmentation pattern. Identification of untargeted compounds was carried out by extracting the MS/MS spectra using MassHunter Qualitative Analysis software and searching against the online metabolite databases such as METLIN (http://metlin.scripps.edu/), MassBank (http://www.massbank.jp), Human Metabolome Database (HMDB) (http://www.hmdb.ca/), *etc* in combination with manual inspection of the fragmentation pattern.

Metabolomics data pre-processing by PCA

Initial inspection by PCA of the 2003 dataset yielded no signs of groupings. However, we found nine corrupt samples with low quality chromatographic spectra that were excluded from further sample comparison modelling. The analysis of 2016 dataset revealed a clear grouping trend due to the presence of multiple masses from a polymer interferant (polyvinylpyrrolidone ($[C_6H_9NO]_n$) eluting from 3.3-4.3 min in LC-MS) in 40 % of the samples (7 NGT, 17 IGT, 12 with type 2 diabetes). We suspect that this polymer was introduced by the serum collection tubes used for these samples. Nevertheless, our metabolomics approach allowed the chromatographic separation of this polymer from most of metabolites prior to mass spectrometric analysis, thus greatly reducing the potential ion suppression effects. We found four metabolites (C8:1-carnitine, C8-carnitine, salicylic acid, cortisol), within the time-range of the potential pollutant, with slightly lower intensity in the samples with polymer interferant. The confounding effect from the interferant was minimized by performing a targeted metabolomics approach via the use of isotope-labelled internal standards for data normalization that eluted over the whole chromatographic range. For further sample comparison modelling, we excluded the variables (masses) derived from the interferant in the specific retention time range. No grouping trend

related to interferant was observed by PCA after the removal of these interfering masses (data not shown).

Annotation of unidentified features

We screened the unidentified putative metabolites, to find those that were significantly correlated with clinical measures using both multivariate and univariate statistics. From this, we found features P94, P103 and P104 displayed consistent positive correlations with all the clinical measures. Via MS/MS spectra, we were able to identify these three metabolites as fructosyl-BCAAs, *i.e.*, fructosyl-valine, fructosyl-isoleucine and fructosyl-leucine. However, it should be noted that no clear differentiation could be made between the isomer fructosyl-leucine and fructosyl-isoleucine due to their highly similar MS/MS fragmentation patterns. In addition, we highlighted features N3, N6, N9 and N10 that were positively correlated with clinical markers describing impaired insulin secretion and lipid metabolism after diagnosis. These features were metabolites of valine and isoleucine degradation, i.e. ketovaline, ketoisoleucine, α-hydroxyisobutyric acid and 2-hydroxyisovaleric acid.

Table 5S1. A list of the identified and classified metabolites

ID	Metabolite class	Metabolite name	Abbr.	Platform	HMDB ID	CAS No.	Direction in NGT-T2D at baseline	Significance at baseline	Direction in NGT-T2D at follow-up	Significance at follow-up
G39	Amino acid and derivative	Leucine	Leu	GC-MS	HMDB00687	61-90-5	↑	TRUE	↑	TRUE
P103	Amino acid and derivative	Fructosyl isoleucine	Fru-Ile	LC-MS	HMDB39780	87304-79-8	↑	TRUE	↑	TRUE
P104	Amino acid and derivative	Fructosyl leucine	Fru-Leu	LC-MS	HMDB37840	34393-18-5	↑	TRUE	↑	TRUE
L40	Amino acid and derivative	Leucine	Leu	LC-MS	HMDB00687	61-90-5	↑	TRUE	↑	TRUE
G197	Amino acid and derivative	2-Aminobutyric acid	Abu	GC-MS	HMDB00452	1492-24-6	↓	TRUE	NA	FALSE
G53	Amino acid and derivative	Creatinine	Cre	GC-MS	HMDB00562	60-27-5	↓	TRUE	NA	FALSE
G203	Amino acid and derivative	Beta-Alanine	β-Ala	GC-MS	HMDB00056	107-95-9	↓	TRUE	NA	FALSE
G57	Amino acid and derivative	4-hydroxyproline	4-Hyp	GC-MS	HMDB00725	51-35-4	NA	FALSE	↑	TRUE
G26	Amino acid and derivative	Alanine	Ala	GC-MS	HMDB00161	56-41-7	NA	FALSE	↑	TRUE
G36	Amino acid and derivative	Glutamic acid	Glu	GC-MS	HMDB03339	6893-26-1	NA	FALSE	↑	TRUE
G38	Amino acid and derivative	Isoleucine	Ile	GC-MS	HMDB00172	73-32-5	NA	FALSE	↑	TRUE
G199	Amino acid and derivative	Sarcosine	Sar	GC-MS	HMDB00271	107-97-1	NA	FALSE	↑	TRUE
G43	Amino acid and derivative	Valine	Val	GC-MS	HMDB00883	72-18-4	NA	FALSE	↑	TRUE
P94	Amino acid and derivative	Fructosyl valine	Fru-Val	LC-MS	HMDB37844	10003-64-2	NA	FALSE	↑	TRUE
G28	Amino acid and derivative	Allothreonine	allo-Thr	GC-MS	HMDB04041	24830-94-2	NA	FALSE	↓	TRUE
G29	Amino acid and derivative	Asparagine	Asp	GC-MS	HMDB00168	70-47-3	NA	FALSE	↓	TRUE
G35	Amino acid and derivative	Glycine	Gly	GC-MS	HMDB00123	56-40-6	NA	FALSE	↓	TRUE
G214	Amino acid and derivative	Histidine	His	GC-MS	HMDB00177	71-00-1	NA	FALSE	↓	TRUE
G48	Amino acid and derivative	Kynurenine	Kyn	GC-MS	HMDB00684	343-65-7	NA	FALSE	↓	TRUE
G40	Amino acid and derivative	Ornithine	Orn	GC-MS	HMDB00214	70-26-8	NA	FALSE	↓	TRUE
G61	Amino acid and derivative	Pyroglutamic acid	Pyro-glu	GC-MS	HMDB00267	98-79-3	NA	FALSE	↓	TRUE
G45	Amino acid and derivative	Threonine	Thr	GC-MS	HMDB00167	72-19-5	NA	FALSE	↓	TRUE
G204	Amino acid and derivative	Beta-Homoglutamine	β-HGln	GC-MS		7433-32-1	NA	FALSE	↓	TRUE
L45	Amino acid and derivative	L-Kynurenine	Kyn	LC-MS	HMDB00684	343-65-7	NA	FALSE	↓	TRUE
L2	Amino acid and derivative	Pyroglutamic acid	Pyro-glu	LC-MS	HMDB00267	98-79-3	NA	FALSE	↓	TRUE
L59	Acylcarnitine	Hexanoyl-carnitine (C6)	C6-ct	LC-MS	HMDB00705	6418-78-6	NA	FALSE	NA	FALSE
G37	Amino acid and derivative	Methyl histidine	1-MH	GC-MS	HMDB00479	368-16-1	NA	FALSE	NA	FALSE

G55	Amino acid and derivative	Asymetrical-n,n-dimethylarginine	ADMA	GC-MS	HMDB01539	30315-93-6	NA	FALSE	NA	FALSE
G46	Amino acid and derivative	Arginine	Arg	GC-MS	HMDB00517	74-79-3	NA	FALSE	NA	FALSE
G33	Amino acid and derivative	Citrulline (Arginine)	Cit-Arg	GC-MS	HMDB00904	372-75-8	NA	FALSE	NA	FALSE
G32	Amino acid and derivative	Citrulline (Ornthine)	Cit-Orn	GC-MS	HMDB00904	372-75-8	NA	FALSE	NA	FALSE
G27	Amino acid and derivative	Cysteine	Cys	GC-MS	HMDB00574	52-90-4	NA	FALSE	NA	FALSE
G42	Amino acid and derivative	Glutamine	Gln	GC-MS	HMDB00641	56-85-9	NA	FALSE	NA	FALSE
G63	Amino acid and derivative	Glycylvaline	Gly-val	GC-MS	HMDB28854	1963-21-9	NA	FALSE	NA	FALSE
G31	Amino acid and derivative	Lysine	Lys	GC-MS	HMDB00182	56-87-1	NA	FALSE	NA	FALSE
G34	Amino acid and derivative	Cystine	oxCys	GC-MS	HMDB00192	56-89-3	NA	FALSE	NA	FALSE
G52	Amino acid and derivative	Phenylalanine	Phe	GC-MS	HMDB00159	63-91-2	NA	FALSE	NA	FALSE
G47	Amino acid and derivative	Proline	Pro	GC-MS	HMDB00162	147-85-3	NA	FALSE	NA	FALSE
G44	Amino acid and derivative	Taurine	Tau	GC-MS	HMDB00251	107-35-7	NA	FALSE	NA	FALSE
G49	Amino acid and derivative	Tryptophan	Trp	GC-MS	HMDB00929	73-22-3	NA	FALSE	NA	FALSE
G50	Amino acid and derivative	Tyrosine	Tyr	GC-MS	HMDB00158	60-18-4	NA	FALSE	NA	FALSE
L33	Amino acid and derivative	Creatinine	Cre	LC-MS	HMDB00562	60-27-5	NA	FALSE	NA	FALSE
L57	Amino acid and derivative	Hippuric acid	HA	LC-MS	HMDB00714	495-69-2	NA	FALSE	NA	FALSE
L35	Amino acid and derivative	L-Methionine	Met	LC-MS	HMDB00696	63-68-3	NA	FALSE	NA	FALSE
L46	Amino acid and derivative	Phenylalanine	Phe	LC-MS	HMDB00159	63-91-2	NA	FALSE	NA	FALSE
L34	Amino acid and derivative	L-Proline	Pro	LC-MS	HMDB00162	147-85-3	NA	FALSE	NA	FALSE
L51	Amino acid and derivative	L-Tryptophan	Trp	LC-MS	HMDB00929	73-22-3	NA	FALSE	NA	FALSE
L54	Amino acid and derivative	Gamma-glutamyl-leucine	γ-Glu-Leu	LC-MS	HMDB11171	2566-39-4	NA	FALSE	NA	FALSE
L42	Acylcarnitine	Glutaroyl-carnitine (C5-DC)	C5-DC-ct	LC-MS	HMDB62587	102636-82-8	↑	TRUE	↑	TRUE
L65	Acylcarnitine	Dodecenoyl-carnitine (C12:1)	C12:1-ct	LC-MS	HMDB13326		NA	FALSE	↓	TRUE
L69	Acylcarnitine	Tetradecenoyl-carnitine (C14:1)	C14:1-ct	LC-MS		835598-21-5	NA	FALSE	↓	TRUE
L72	Acylcarnitine	Hexadecenoyl-carnitine (C16:1)	C16:1-ct	LC-MS			NA	FALSE	↓	TRUE
L73	Acylcarnitine	Linoleoyl-carnitine (C18:2)	C18:2-ct	LC-MS		36816-10-1	NA	FALSE	↓	TRUE
L71	Acylcarnitine	Linoleneoyl-carnitine (C18:3)	C18:3-ct	LC-MS			NA	FALSE	↓	TRUE
L36	Acylcarnitine	Acetyl-carnitine (C2)	C2-ct	LC-MS	HMDB00201	3040-38-8	NA	FALSE	↓	TRUE
L41	Acylcarnitine	Propionyl-carnitine (C3)	C3-ct	LC-MS	HMDB00824	17298-37-2	NA	FALSE	↓	TRUE
L63	Acylcarnitine	Decenoyl-carnitine (C10:1)	C10:1-ct	LC-MS			NA	FALSE	NA	FALSE
L64	Acylcarnitine	Decanoyl-carnitine (C10)	C10-ct	LC-MS	HMDB62631		NA	FALSE	NA	FALSE

L66	Acylcarnitine	Dodecanoyl-carnitine (C12)	C12-ct	LC-MS	HMDB02250	25518-54-1	NA	FALSE	NA	FALSE
L68	Acylcarnitine	Hydroxymyristoyl-carnitine (C14:0-OH)	C14:0-OH-ct	LC-MS			NA	FALSE	NA	FALSE
L67	Acylcarnitine	Tetradecdienoyl-carnitine (C14:2)	C14:2-ct	LC-MS			NA	FALSE	NA	FALSE
L70	Acylcarnitine	Myristoyl-carnitine (C14)	C14-ct	LC-MS	HMDB05066	25597-07-3	NA	FALSE	NA	FALSE
L75	Acylcarnitine	Palmitoyl-carnitine (C16)	C16-ct	LC-MS		2364-67-2	NA	FALSE	NA	FALSE
L78	Acylcarnitine	Oleoyl-carnitine (C18:1)	C18:1-ct	LC-MS		38677-66-6	NA	FALSE	NA	FALSE
L84	Acylcarnitine	Stearoyl-carnitine (C18)	C18-ct	LC-MS		1976-27-8	NA	FALSE	NA	FALSE
L80	Acylcarnitine	Eicosadieneoyl-carnitine (C20:2)	C20:2-ct	LC-MS			NA	FALSE	NA	FALSE
L47	Acylcarnitine	Butyryl-carnitine (C4)	C4-ct	LC-MS	HMDB02013	25576-40-3	NA	FALSE	NA	FALSE
L52	Acylcarnitine	Tiglyl-carnitine (C5:1iso)	C5:1-iso-ct	LC-MS	HMDB02366	64191-86-2	NA	FALSE	NA	FALSE
L55	Acylcarnitine	Valeryl-carnitine (C5)	C5-ct	LC-MS	HMDB13128		NA	FALSE	NA	FALSE
L56	Acylcarnitine	Isovaleryl-carnitine (C5iso)	C5-iso-ct	LC-MS	HMDB00688	31023-24-2	NA	FALSE	NA	FALSE
L44	Acylcarnitine	Hydroxyisovaleryl-carnitine (C5iso-OH)	C5iso-OH-ct	LC-MS	HMDB61189		NA	FALSE	NA	FALSE
G90	Carbohydrate	Turanose	Tur	GC-MS	HMDB11740	547-25-1	↑	TRUE	NA	FALSE
G120	Carbohydrate	cis-Inositol	c-Ino-ol	GC-MS		488-59-5	NA	FALSE	↑	TRUE
G78	Carbohydrate	Galactose	Gal	GC-MS	HMDB00143	59-23-4	NA	FALSE	↑	TRUE
G209	Carbohydrate	Galactitol	Gal-ol	GC-MS	HMDB00107	608-66-2	NA	FALSE	↑	TRUE
G83	Carbohydrate	Glycero-gulo-heptose	G-Hep	GC-MS		3146-50-7	NA	FALSE	↑	TRUE
G128	Carbohydrate	Laminaribiose	Lam	GC-MS		34980-39-7	NA	FALSE	↑	TRUE
G88	Carbohydrate	Maltose	Mal	GC-MS	HMDB00163	69-79-4	NA	FALSE	↑	TRUE
G81	Carbohydrate	Mannose	Man	GC-MS	HMDB00169	3458-28-4	NA	FALSE	↑	TRUE
G98	Carbohydrate	Mannitol	Man-ol	GC-MS	HMDB00765	69-65-8	NA	FALSE	↑	TRUE
G219	Carbohydrate	Sorbitol-6-phosphate	S-6-P	GC-MS	HMDB05831	20479-58-7	NA	FALSE	↑	TRUE
G73	Carbohydrate	Sorbose	Sor	GC-MS	HMDB01266	87-79-6	NA	FALSE	↑	TRUE
G222	Carbohydrate	Xylose	Xyl	GC-MS	HMDB00098	58-86-6	NA	FALSE	↑	TRUE
G108	Carbohydrate	Xylitol	Xyl-ol	GC-MS	HMDB02917	87-99-0	NA	FALSE	↑	TRUE
G75	Carbohydrate	1,5-anhydro-D-Glucitol	1,5-AG	GC-MS	HMDB02712	154-58-5	NA	FALSE	↓	TRUE
G85	Carbohydrate	Sorbitol	Sor-ol	GC-MS	HMDB05831	20479-58-7	NA	FALSE	↓	TRUE
G198	Carbohydrate	2-Deoxy-galactose	2-D-gal	GC-MS	HMDB12327	25494-04-6	NA	FALSE	NA	FALSE
G92	Carbohydrate	Fructose	Fru	GC-MS	HMDB00660	53188-23-1	NA	FALSE	NA	FALSE
G94	Carbohydrate	Fucose	Fuc	GC-MS	HMDB29196	2438-80-4	NA	FALSE	NA	FALSE

G95	Carbohydrate	Glucose	Glc	GC-MS	HMDB00122	50-99-7	NA	FALSE	NA	FALSE
G102	Carbohydrate	N-Acetyl glucosamine	GluNAc	GC-MS	HMDB00215	7512-17-6	NA	FALSE	NA	FALSE
G76	Carbohydrate	Lactose	Lac	GC-MS	HMDB41627	5965-66-2	NA	FALSE	NA	FALSE
G97	Carbohydrate	Lyxose	Lyx	GC-MS		1114-34-7	NA	FALSE	NA	FALSE
G103	Carbohydrate	N-Acetyl mannosamine	ManNAc	GC-MS	HMDB01129	3615-17-6	NA	FALSE	NA	FALSE
G101	Carbohydrate	myo-Inositol	m-Ino-ol	GC-MS	HMDB00211	87-89-8	NA	FALSE	NA	FALSE
G72	Carbohydrate	Rhamnose	Rha	GC-MS	HMDB00849	3615-41-6	NA	FALSE	NA	FALSE
G106	Carbohydrate	Ribitol	Rib-ol	GC-MS	HMDB00508	488-81-3	NA	FALSE	NA	FALSE
G105	Carbohydrate	Sakebiose	Sak	GC-MS	HMDB29882	497-48-3	NA	FALSE	NA	FALSE
G121	Carbohydrate	scyllo-Inositol	s-Ino-ol	GC-MS	HMDB06088	488-59-5	NA	FALSE	NA	FALSE
G107	Carbohydrate	Sucrose	Suc	GC-MS	HMDB00258	57-50-1	NA	FALSE	NA	FALSE
G129	Carbohydrate	Trehalose	Tre	GC-MS	HMDB00975	99-20-7	NA	FALSE	NA	FALSE
G221	Carbohydrate	Xylobiose	Xob	GC-MS	HMDB29894	6860-47-5	NA	FALSE	NA	FALSE
G223	Carbohydrate	Xylulose	Xul	GC-MS	HMDB00751	527-50-4	NA	FALSE	NA	FALSE
G1	Carboxylic acid	L-Lactic acid	LacA	GC-MS	HMDB00190	79-33-4	↑	TRUE	↑	TRUE
L3	Carboxylic acid	Ketoleucine	K-leu	LC-MS	HMDB00695	816-66-0	↑	TRUE	↑	TRUE
G10	Carboxylic acid	Citric acid	CitA	GC-MS	HMDB00094	77-92-9	↓	TRUE	NA	FALSE
G22	Carboxylic acid	Isocitric acid	Iso-CitA	GC-MS	HMDB00193	320-77-4	↓	TRUE	NA	FALSE
G132	Carboxylic acid	3-Hydroxybutyrate	3-HB	GC-MS	HMDB00357	300-85-6	NA	FALSE	↑	TRUE
G122	Carboxylic acid	3-Hydroxyisobutyrate	3-HIB	GC-MS	HMDB00023	2068-83-9	NA	FALSE	↑	TRUE
G217	Carboxylic acid	Oxalic acid	OA	GC-MS	HMDB02329	144-62-7	NA	FALSE	↑	TRUE
N3	Carboxylic acid	α-hydroxybutyrate	2-HB	LC-MS	HMDB00008	600-15-7	NA	FALSE	↑	TRUE
N10	Carboxylic acid	Ketoisoleucine	K-Ile	LC-MS	HMDB00491	1460-34-0	NA	FALSE	↑	TRUE
N6	Carboxylic acid	Ketovaline	K-Val	LC-MS	HMDB00019	759-05-7	NA	FALSE	↑	TRUE
L1	Carboxylic acid	L-Lactic acid	LacA	LC-MS	HMDB00190	79-33-4	NA	FALSE	↑	TRUE
G25	Carboxylic acid	4-hydroxyphenylacetic acid	4-HPA	GC-MS	HMDB00020	156-38-7	NA	FALSE	NA	FALSE
G9	Carboxylic acid	Adipic acid	AdiA	GC-MS	HMDB00448	124-04-9	NA	FALSE	NA	FALSE
G202	Carboxylic acid	Azelaic acid	AzeA	GC-MS	HMDB00784	123-99-9	NA	FALSE	NA	FALSE
G24	Carboxylic acid	Benzoic acid	BenA	GC-MS	HMDB01870	65-85-0	NA	FALSE	NA	FALSE
G123	Carboxylic acid	cis-Aconitic acid	cis-AA	GC-MS	HMDB00072	585-84-2	NA	FALSE	NA	FALSE
G2	Carboxylic acid	Fumaric acid	FA	GC-MS	HMDB00134	110-17-8	NA	FALSE	NA	FALSE

G8	Carboxylic acid	Glyceric acid	GlyA	GC-MS	HMDB00139	473-81-4	NA	FALSE	NA	FALSE
G212	Carboxylic acid	Glycolic acid	GlyA	GC-MS	HMDB00115	79-14-1	NA	FALSE	NA	FALSE
G213	Carboxylic acid	Hippuric acid	HA	GC-MS	HMDB00714	495-69-2	NA	FALSE	NA	FALSE
G4	Carboxylic acid	Malic acid	MA	GC-MS	HMDB00744	6915-15-7	NA	FALSE	NA	FALSE
G193	Carboxylic acid	Picolinic acid	PA	GC-MS	HMDB02243	98-98-6	NA	FALSE	NA	FALSE
G119	Carboxylic acid	Pyruvic acid	PyruA	GC-MS	HMDB00243	127-17-3	NA	FALSE	NA	FALSE
G3	Carboxylic acid	Succinic acid	SucA	GC-MS	HMDB00254	110-15-6	NA	FALSE	NA	FALSE
G23	Carboxylic acid	trans-Aconitic acid	trans-AA	GC-MS	HMDB00958	4023-65-8	NA	FALSE	NA	FALSE
G6	Carboxylic acid	α-ketoglutaric acid	α-KG	GC-MS	HMDB00208	328-50-7	NA	FALSE	NA	FALSE
L7	Carboxylic acid	Citric acid	CitA	LC-MS	HMDB00094	77-92-9	NA	FALSE	NA	FALSE
L4	Carboxylic acid	Malic acid	MA	LC-MS	HMDB00744	6915-15-7	NA	FALSE	NA	FALSE
G124	Fatty acid	Palmitic acid methyl ester	C16:0-ME	GC-MS		112-39-0	↓	TRUE	NA	FALSE
G216	Fatty acid	Octadecanoic acid methyl ester	C18:0-ME	GC-MS	HMDB34154	112-61-8	↓	TRUE	NA	FALSE
G13	Fatty acid	Eicosadienoic acid (20:2 n-6)	C20:2	GC-MS	HMDB05060	2091-39-6	↓	TRUE	NA	FALSE
L15	Fatty acid	2-Hydroxypalmitic acid (16:0-OH)	C16:0(2-OH)	LC-MS	HMDB62548	764-67-0	↓	TRUE	NA	FALSE
G17	Fatty acid	Palmitic acid (16:0)	C16:0	GC-MS	HMDB00220	57-10-3	NA	FALSE	↑	TRUE
G20	Fatty acid	Margaric acid (17:0)	C17:0	GC-MS	HMDB03229	373-49-9	NA	FALSE	↑	TRUE
G21	Fatty acid	Stearic acid (18:0)	C18:0	GC-MS	HMDB00827	57-11-4	NA	FALSE	↑	TRUE
G125	Fatty acid	trans-Oleic acid (trans 18:1 n-9)	trans C18:1	GC-MS	HMDB00573	112-79-8	NA	FALSE	↑	TRUE
L12	Fatty acid	Palmitic acid (16:0)	C16:0	LC-MS	HMDB00220	57-10-3	NA	FALSE	↑	TRUE
L14	Fatty acid	β-Hydroxypalmitic acid (16:0-OH)	C16:0(3-OH)	LC-MS	HMDB61658	928-17-6	NA	FALSE	↑	TRUE
L19	Fatty acid	Stearic acid (18:0)	C18:0	LC-MS	HMDB00827	57-11-4	NA	FALSE	↑	TRUE
G14	Fatty acid	Arachidonic acid (20:4 n-6)	ARA	GC-MS	HMDB01043	506-32-1	NA	FALSE	NA	FALSE
G11	Fatty acid	Lauric acid (12:0)	C12:0	GC-MS	HMDB00638	143-07-7	NA	FALSE	NA	FALSE
G16	Fatty acid	Myristoleic acid (14:1 n-5)	C14:1	GC-MS	HMDB02000	544-64-9	NA	FALSE	NA	FALSE
G19	Fatty acid	Palmitoleic acid (16:1 n-7)	C16:1	GC-MS	HMDB03229	373-49-9	NA	FALSE	NA	FALSE
G12	Fatty acid	Linoleic acid (18:2 n-6)	C18:2	GC-MS	HMDB00673	60-33-3	NA	FALSE	NA	FALSE
G208	Fatty acid	cis-11-Eiocosenoic acid	C20:1	GC-MS	HMDB02231	26764-41-0	NA	FALSE	NA	FALSE
G194	Fatty acid	8,11,14-Eicosatrienoic acid (C20:3 n-6)	DGLA	GC-MS		55682-88-7	NA	FALSE	NA	FALSE
G15	Fatty acid	DHA (22:6 n-3)	DHA	GC-MS	HMDB62579	6217-54-5	NA	FALSE	NA	FALSE
G127	Fatty acid	DHA methyl ester(22:6 n-3)	DHA-ME	GC-MS		301-01-9	NA	FALSE	NA	FALSE

G126	Fatty acid	EPA (20:5 n-3)	EPA	GC-MS	HMDB01999	10417-94-4	NA	FALSE	NA	FALSE
L20	Fatty acid	Arachidonic Acid (20:4 n-6)	ARA	LC-MS	HMDB01043	506-32-1	NA	FALSE	NA	FALSE
L9	Fatty acid	Myristic acid (14:0)	C14:0	LC-MS	HMDB00806	544-63-8	NA	FALSE	NA	FALSE
L11	Fatty acid	Palmitoleic acid (16:1 n-7)	C16:1	LC-MS	HMDB03229	373-49-9	NA	FALSE	NA	FALSE
L18	Fatty acid	Oleic acid (18:1 n-9)	C18:1	LC-MS	HMDB00207	112-79-8	NA	FALSE	NA	FALSE
L17	Fatty acid	Linoleic acid (18:2 n-6)	C18:2	LC-MS	HMDB00673	60-33-3	NA	FALSE	NA	FALSE
L16	Fatty acid	α-Linolenic Acid (18:3 n-3)	C18:3	LC-MS	HMDB01388	463-40-1	NA	FALSE	NA	FALSE
L21	Fatty acid	DHA (22:6 n-3)	DHA	LC-MS	HMDB62579	6217-54-5	NA	FALSE	NA	FALSE
G99	Lipid	Glycerol-3-phosphate	G-3-P	GC-MS	HMDB00126		↓	TRUE	↓	TRUE
L100	Lipid	PAF(16:2)	PAF(16:2)	LC-MS			↓	TRUE	↓	TRUE
G100	Lipid	Glycerol-2-phosphate	G-2-P	GC-MS	HMDB02520	17181-54-3	↓	TRUE	NA	FALSE
L28	Lipid	PE(18:2/0:0)	LPE(18:2)	LC-MS	HMDB11507		↓	TRUE	NA	FALSE
L74	Lipid	PC(14:0/0:0)	LPC(14:0)	LC-MS	HMDB10379		NA	FALSE	↓	TRUE
L79	Lipid	PC(16:1/0:0)	LPC(16:1)	LC-MS	HMDB10383		NA	FALSE	↓	TRUE
L91	Lipid	PC(17:0/0:0)	LPC(17:0)	LC-MS	HMDB12108		NA	FALSE	↓	TRUE
L88	Lipid	PC(18:1/0:0)	LPC(18:1)	LC-MS	HMDB10385		NA	FALSE	↓	TRUE
L83	Lipid	PC(18:2/0:0)	LPC(18:2)	LC-MS	HMDB10386		NA	FALSE	↓	TRUE
L76	Lipid	PC(20:5/0:0)	LPC(20:5)	LC-MS	HMDB10397		NA	FALSE	↓	TRUE
L103	Lipid	PAF(14:0)	PAF(14:0)	LC-MS			NA	FALSE	↓	TRUE
L97	Lipid	PAF(14:1)	PAF(14:1)	LC-MS			NA	FALSE	↓	TRUE
L106	Lipid	PAF(16:0)	PAF(16:0)	LC-MS			NA	FALSE	↓	TRUE
L96	Lipid	PAF(16:3)	PAF(16:3)	LC-MS			NA	FALSE	↓	TRUE
L95	Lipid	PAF(18:5)	PAF(18:5)	LC-MS			NA	FALSE	↓	TRUE
G117	Lipid	1-Monopalmitoylglycerol	1-MPG	GC-MS	HMDB31074	542-44-9	NA	FALSE	NA	FALSE
G67	Lipid	Campesterol	Camp	GC-MS	HMDB02869	474-62-4	NA	FALSE	NA	FALSE
G68	Lipid	Cholesterol	Chol	GC-MS	HMDB00067	57-88-5	NA	FALSE	NA	FALSE
G114	Lipid	PC(16:0/0:0)	LPC(16:0)	GC-MS	HMDB10382		NA	FALSE	NA	FALSE
G115	Lipid	PC(18:0/0:0)	LPC(18:0)	GC-MS	HMDB10384		NA	FALSE	NA	FALSE
L10	Lipid	CMPF	CMPF	LC-MS		86879-39-2	NA	FALSE	NA	FALSE
L62	Lipid	Cortisol	Cor	LC-MS		50-23-7	NA	FALSE	NA	FALSE
L87	Lipid	PC(16:0/0:0)	LPC(16:0)	LC-MS	HMDB10382		NA	FALSE	NA	FALSE

L92	Lipid	PC(18:0/0:0)	LPC(18:0)	LC-MS	HMDB10384		NA	FALSE	NA	FALSE
L77	Lipid	PC(18:3/0:0)	LPC(18:3)	LC-MS	HMDB10388		NA	FALSE	NA	FALSE
L86	Lipid	PC(20:3/0:0)	LPC(20:3)	LC-MS	HMDB10393		NA	FALSE	NA	FALSE
L82	Lipid	PC(20:4/0:0)	LPC(20:4)	LC-MS	HMDB10395		NA	FALSE	NA	FALSE
L85	Lipid	PC(22:5/0:0)	LPC(22:5)	LC-MS	HMDB10403		NA	FALSE	NA	FALSE
L81	Lipid	PC(22:6/0:0)	LPC(22:6)	LC-MS	HMDB10404		NA	FALSE	NA	FALSE
L90	Lipid	PC(O-16:0/0:0)	LPC(O-16:0)	LC-MS		52691-62-0	NA	FALSE	NA	FALSE
L89	Lipid	PC(P-16:0/0:0) or PC(O-16:1/0:0)	LPC(P-16:0)	LC-MS			NA	FALSE	NA	FALSE
L26	Lipid	PE(16:0/0:0)	LPE(16:0)	LC-MS	HMDB11503		NA	FALSE	NA	FALSE
L30	Lipid	PE(18:0/0:0)	LPE(18:0)	LC-MS	HMDB11130		NA	FALSE	NA	FALSE
L29	Lipid	PE(18:1(9Z)/0:0)	LPE(18:1(9Z))	LC-MS	HMDB11506		NA	FALSE	NA	FALSE
L94	Lipid	PAF (12:0)	PAF (12:0)	LC-MS			NA	FALSE	NA	FALSE
L105	Lipid	PAF(15:0)	PAF(15:0)	LC-MS			NA	FALSE	NA	FALSE
L104	Lipid	PAF(16:1)	PAF(16:1)	LC-MS			NA	FALSE	NA	FALSE
L102	Lipid	PAF(18:3)	PAF(18:3)	LC-MS			NA	FALSE	NA	FALSE
L99	Lipid	PAF(18:4)	PAF(18:4)	LC-MS			NA	FALSE	NA	FALSE
L101	Lipid	PAF(20:5)	PAF(20:5)	LC-MS			NA	FALSE	NA	FALSE
L98	Lipid	PAF(20:6)	PAF(20:6)	LC-MS			NA	FALSE	NA	FALSE
L23	Lipid	Sphingosine-1-phosphate	S1P	LC-MS		26993-30-6	NA	FALSE	NA	FALSE
R1	No class	LPC18:2/LPE18:2	LPC/LPE (18:2)	LC-MS			↑	TRUE	NA	FALSE
L38	No class	Uric acid	UA	LC-MS	HMDB00289	69-93-2	↓	TRUE	↑	TRUE
G220	No class	Ursodeoxycholic acid	UDCA	GC-MS	HMDB00946	128-13-2	↓	TRUE	NA	FALSE
G69	No class	α-Tocopherol	α-VE	GC-MS	HMDB01893		↓	TRUE	NA	FALSE
G131	No class	γ-Tocopherol	γ-VE	GC-MS	HMDB01492	1406-66-2	↓	TRUE	NA	FALSE
L58	No class	Caffeine	Caf	LC-MS	HMDB01847		↓	TRUE	NA	FALSE
L25	No class	Chenodeoxycholic acid glycine conjugate	G-CDCA	LC-MS	HMDB00637	640-79-9	↓	TRUE	NA	FALSE
L13	No class	Inosine	Ino	LC-MS	HMDB00195	58-63-9	↓	TRUE	NA	FALSE
G207	No class	Deoxycholic acid	DCA	GC-MS	HMDB00626	83-44-3	NA	FALSE	↑	TRUE
G215	No class	Indole-3-acetic acid	IAA	GC-MS	HMDB00197	87-51-4	NA	FALSE	↑	TRUE
G60	No class	Putrescine	Put	GC-MS	HMDB01414	110-60-1	NA	FALSE	↑	TRUE
G65	No class	Uric acid	UA	GC-MS	HMDB00289	69-93-2	NA	FALSE	↑	TRUE

L24	No class	Glycodeoxycholate	G-DCA	LC-MS	HMDB00631	360-65-6	NA	FALSE	↑	TRUE
G62	No class	Acetaminophen	AAP	GC-MS	HMDB01859	103-90-2	NA	FALSE	NA	FALSE
G200	No class	Adenosine	Ado	GC-MS	HMDB00050	58-61-7	NA	FALSE	NA	FALSE
G112	No class	Adenosine-5-monophosphate	AMP	GC-MS	HMDB00045	61-19-8	NA	FALSE	NA	FALSE
G195	No class	Cholic acid	CA	GC-MS	HMDB00619	81-25-4	NA	FALSE	NA	FALSE
G206	No class	Cholic acid	CA	GC-MS	HMDB00619	81-25-4	NA	FALSE	NA	FALSE
G205	No class	Chenodeoxycholic acid	CDCA	GC-MS	HMDB00518	474-25-9	NA	FALSE	NA	FALSE
G66	No class	Hydeoxycholic acid	HDCA	GC-MS	HMDB00733	83-49-8	NA	FALSE	NA	FALSE
G54	No class	Hypoxanthine	Hyp	GC-MS	HMDB00157	68-94-0	NA	FALSE	NA	FALSE
G113	No class	Inosine	Ino	GC-MS	HMDB00195	58-63-9	NA	FALSE	NA	FALSE
G58	No class	Urea	Urea	GC-MS	HMDB00294	57-13-6	NA	FALSE	NA	FALSE
G110	No class	Uridine	Uri	GC-MS	HMDB00296	58-96-8	NA	FALSE	NA	FALSE
G111	No class	Xanthine	Xan	GC-MS	HMDB00292	69-89-6	NA	FALSE	NA	FALSE
R2	No class	LPC16:0/LPE16:0	LPC/LPE (16:0)	LC-MS			NA	FALSE	NA	FALSE
R4	No class	LPC18:0/LPE18:0	LPC/LPE (18:0)	LC-MS			NA	FALSE	NA	FALSE
R3	No class	LPC18:1/LPE18:1	LPC/LPE (18:1)	LC-MS			NA	FALSE	NA	FALSE
L49	No class	p-Acetamidophenol	AAP	LC-MS	HMDB01859	103-90-2	NA	FALSE	NA	FALSE
L43	No class	Adenosine	Ado	LC-MS	HMDB00050	58-61-7	NA	FALSE	NA	FALSE
L22	No class	3-β-hydroxyandrost-5-en-17-one sulfate	DHEA-S	LC-MS	HMDB01032	651-48-9	NA	FALSE	NA	FALSE
L27	No class	Glycocholic acid	G-CA	LC-MS	HMDB00138	475-31-0	NA	FALSE	NA	FALSE
L39	No class	Hypoxanthine	Hyp	LC-MS	HMDB00157	68-94-0	NA	FALSE	NA	FALSE
L8	No class	Indoxylsulfuric acid	IS	LC-MS		487-94-5	NA	FALSE	NA	FALSE
L37	No class	Niacinamide	Niacin	LC-MS	HMDB01406	98-92-0	NA	FALSE	NA	FALSE
L31	No class	Taurochenodeoxycholic acid	T-CDCA	LC-MS	HMDB00951	516-35-8	NA	FALSE	NA	FALSE
L32	No class	Taurodeoxycholate	T-DCA	LC-MS	HMDB00896	516-50-7	NA	FALSE	NA	FALSE
L53	No class	Theophylline	Theo	LC-MS	HMDB01889	58-55-9	NA	FALSE	NA	FALSE
L50	No class	Theobromine	TheoB	LC-MS	HMDB02825	83-67-0	NA	FALSE	NA	FALSE
L48	No class	Pantothenic Acid	VB5	LC-MS	HMDB00210	79-83-4	NA	FALSE	NA	FALSE
L6	No class	Xanthine	Xan	LC-MS	HMDB00292	69-89-6	NA	FALSE	NA	FALSE

Direction: ↓ lower level in NGT-T2D and ↑ higher level in NGT-T2D. Significance: metabolites that fulfilled the statistical significance criteria calculated from the OPLS-DA model loadings.

Table 5S2. List of unannotated metabolites in the fasting serum of black South African women

Baseline						Follow-up					
Name	**Analytical method**	**Mass**	**R.T.**	**Direction in NGT-T2D at baseline**	**Significance at baseline**	**Name**	**Analytical method**	**Mass**	**R.T.**	**Direction in NGT-T2D at follow-up**	**Significance at follow-up**
N106	LCMS untarget NEG	312.0621	0.8825	↓	TRUE	P15	LCMS untarget POS	132.0251	0.6325	↓	TRUE
N266	LCMS untarget NEG	594.0290	0.7625	↓	TRUE	N38	LCMS untarget NEG	198.0896	3.615	↓	TRUE
N137	LCMS untarget NEG	358.0397	0.77	↓	TRUE	N238	LCMS untarget NEG	553.2867	2.38	↓	TRUE
N175	LCMS untarget NEG	448.3559	7.2425	↓	TRUE	P386	LCMS untarget POS	1000.6443	6.11	↓	TRUE
N156	LCMS untarget NEG	404.0195	0.7575	↓	TRUE	P300	LCMS untarget POS	631.7972	3.18	↓	TRUE
N84	LCMS untarget NEG	272.0777	2.115	↓	TRUE	P227	LCMS untarget POS	513.0195	3.205	↓	TRUE
N255	LCMS untarget NEG	572.0476	0.765	↓	TRUE	P207	LCMS untarget POS	493.3169	5.975	↓	TRUE
N235	LCMS untarget NEG	548.0496	0.77	↓	TRUE	N55	LCMS untarget NEG	230.0774	1.1975	↑	TRUE
N161	LCMS untarget NEG	426.3712	7.38	↓	TRUE	N160	LCMS untarget NEG	426.2070	5.325	↑	TRUE
N209	LCMS untarget NEG	502.4016	7.3925	↓	TRUE	P53	LCMS untarget POS	184.1218	1.96	↑	TRUE
P89	LCMS untarget POS	268.2202	6.88	↓	TRUE	P8	LCMS untarget POS	125.0843	1.96	↑	TRUE
P35	LCMS untarget POS	159.0693	1.4775	↓	TRUE	N135	LCMS untarget NEG	356.1674	7.205	↑	TRUE
N257	LCMS untarget NEG	574.4601	7.2175	↓	TRUE	N129	LCMS untarget NEG	345.1424	1.5975	↑	TRUE
N165	LCMS untarget NEG	430.1620	2.1125	↓	TRUE	N9	LCMS untarget NEG	118.0628	1.975	↑	TRUE
N148	LCMS untarget NEG	382.0377	0.7625	↓	TRUE	N174	LCMS untarget NEG	447.2976	5.085	↑	TRUE
N86	LCMS untarget NEG	272.2358	6.83	↓	TRUE	N48	LCMS untarget NEG	216.1724	5.615	↑	TRUE
N182	LCMS untarget NEG	454.3277	6.205	↓	TRUE	N93	LCMS untarget NEG	293.1477	1.3175	↑	TRUE
P333	LCMS untarget POS	702.0275	3.49	↓	TRUE	N7	LCMS untarget NEG	116.0492	1.7875	↑	TRUE
P379	LCMS untarget POS	885.4202	3.625	↓	TRUE	N261	LCMS untarget NEG	584.2664	4.475	↑	TRUE
N261	LCMS untarget NEG	584.2664	4.475	↓	TRUE	N228	LCMS untarget NEG	528.2165	4.3225	↑	TRUE
N191	LCMS untarget NEG	468.3824	7.1975	↓	TRUE	N125	LCMS untarget NEG	332.2705	7.195	↑	TRUE

P174	LCMS untarget POS	440.2019	1.2425	↓	TRUE	N61	LCMS untarget NEG	232.0530	1.1975	↑	TRUE
P316	LCMS untarget POS	662.2501	3.64	↓	TRUE	P119	LCMS untarget POS	327.1323	2.105	↑	TRUE
N238	LCMS untarget NEG	553.2867	2.38	↓	TRUE	P37	LCMS untarget POS	161.0432	0.5225	↑	TRUE
N325	LCMS untarget NEG	869.2702	2.115	↓	TRUE	P41	LCMS untarget POS	166.0161	0.93	↓	TRUE
N38	LCMS untarget NEG	198.0896	3.615	↓	TRUE	P166	LCMS untarget POS	423.3352	5.76	↓	TRUE
N207	LCMS untarget NEG	499.9378	5.7775	↓	TRUE	P152	LCMS untarget POS	397.3190	5.655	↓	TRUE
N173	LCMS untarget NEG	446.3393	7.095	↓	TRUE	P222	LCMS untarget POS	507.3296	6.22	↓	TRUE
N108	LCMS untarget NEG	312.2304	6.085	↓	TRUE	P201	LCMS untarget POS	481.3090	6.0925	↓	TRUE
N269	LCMS untarget NEG	596.5002	7.5575	↓	TRUE	P231	LCMS untarget POS	519.3351	6.13	↓	TRUE
P198	LCMS untarget POS	477.2880	6.16	↓	TRUE	P230	LCMS untarget POS	519.3329	6.045	↓	TRUE
N131	LCMS untarget NEG	348.1173	1.175	↓	TRUE	N272	LCMS untarget NEG	602.7720	3.1575	↓	TRUE
N103	LCMS untarget NEG	310.0501	0.9125	↓	TRUE	N94	LCMS untarget NEG	294.1218	2.5925	↓	TRUE
N169	LCMS untarget NEG	439.3042	6.5475	↓	TRUE	P137	LCMS untarget POS	367.2701	5.075	↓	TRUE
N160	LCMS untarget NEG	426.2070	5.325	↑	TRUE	P198	LCMS untarget POS	477.2880	6.16	↓	TRUE
P72	LCMS untarget POS	246.1201	1.8225	↑	TRUE	P63	LCMS untarget POS	217.1319	1.32	↓	TRUE
N28	LCMS untarget NEG	181.3198	1.3525	↑	TRUE	N165	LCMS untarget NEG	430.1620	2.1125	↓	TRUE
P67	LCMS untarget POS	232.1457	1.7525	↑	TRUE	P384	LCMS untarget POS	996.6051	6.155	↓	TRUE
N98	LCMS untarget NEG	299.1489	2.9775	↑	TRUE	N274	LCMS untarget NEG	607.3089	5.975	↓	TRUE
P181	LCMS untarget POS	456.2671	3.15	↑	TRUE	N37	LCMS untarget NEG	198.0755	1.5875	↓	TRUE
N31	LCMS untarget NEG	188.0727	1.67	↑	TRUE	P138	LCMS untarget POS	369.2889	5.315	↓	TRUE
P96	LCMS untarget POS	280.1414	1.7925	↑	TRUE	P286	LCMS untarget POS	602.7754	3.16	↓	TRUE
N88	LCMS untarget NEG	276.1131	3.255	↑	TRUE	P247	LCMS untarget POS	541.3112	5.85	↓	TRUE
P128	LCMS untarget POS	356.2269	1.865	↑	TRUE	P256	LCMS untarget POS	549.3767	6.8675	↓	TRUE
N136	LCMS untarget NEG	356.2065	1.8675	↑	TRUE	N20	LCMS untarget NEG	154.0268	2.9525	↓	TRUE
P186	LCMS untarget POS	465.3197	6.98	↓	TRUE	P233	LCMS untarget POS	521.3502	6.4475	↓	TRUE
N155	LCMS untarget NEG	399.9439	5.2275	↓	TRUE	P187	LCMS untarget POS	467.3025	5.84	↓	TRUE
P88	LCMS untarget POS	268.0817	1.4575	↓	TRUE	P56	LCMS untarget POS	191.0605	1.5575	↓	TRUE
N167	LCMS untarget NEG	437.2895	6.5375	↓	TRUE	P145	LCMS untarget POS	379.2494	5.925	↓	TRUE
N71	LCMS untarget NEG	246.1216	1.5775	↓	TRUE	N187	LCMS untarget NEG	464.3124	5.905	↓	TRUE
N51	LCMS untarget NEG	224.1419	5.37	↓	TRUE	P150	LCMS untarget POS	388.2158	2.5575	↓	TRUE
P231	LCMS untarget POS	519.3351	6.13	↓	TRUE	P2	LCMS untarget POS	90.0470	2.125	↓	TRUE
N186	LCMS untarget NEG	463.3075	6.625	↓	TRUE	P14	LCMS untarget POS	131.0944	1.085	↑	TRUE

P109	LCMS untarget POS	306.0360	1.455	↓	TRUE	N14	LCMS untarget NEG	132.0788	3.035	↑	TRUE
P99	LCMS untarget POS	283.0912	1.4575	↓	TRUE	N2	LCMS untarget NEG	90.0321	0.5875	↑	TRUE
P258	LCMS untarget POS	553.2868	2.3825	↓	TRUE	N184	LCMS untarget NEG	456.2542	5.655	↑	TRUE
P18	LCMS untarget POS	136.0390	1.46	↓	TRUE	N32	LCMS untarget NEG	188.1405	5.015	↑	TRUE
P28	LCMS untarget POS	151.0498	1.4575	↓	TRUE	P89	LCMS untarget POS	268.2202	6.88	↑	TRUE
N138	LCMS untarget NEG	366.2774	6.6025	↓	TRUE	N311	LCMS untarget NEG	768.3436	3.1975	↑	TRUE
P230	LCMS untarget POS	519.3329	6.045	↓	TRUE	N310	LCMS untarget NEG	767.8409	3.195	↑	TRUE
P178	LCMS untarget POS	451.3057	3.1	↓	TRUE	N67	LCMS untarget NEG	242.1262	2.915	↑	TRUE
N190	LCMS untarget NEG	465.3232	6.7475	↓	TRUE	P22	LCMS untarget POS	143.0946	0.4	↑	TRUE
N211	LCMS untarget NEG	504.4175	7.505	↓	TRUE	N149	LCMS untarget NEG	382.1818	7.2475	↑	TRUE
N195	LCMS untarget NEG	477.2863	6.155	↓	TRUE	P123	LCMS untarget POS	343.1274	1.51	↑	TRUE
P165	LCMS untarget POS	422.1926	2.3825	↓	TRUE	N113	LCMS untarget NEG	314.2460	6.4775	↑	TRUE
N105	LCMS untarget NEG	311.8460	4.8375	↓	TRUE	N178	LCMS untarget NEG	449.3145	5.495	↑	TRUE
P49	LCMS untarget POS	180.0656	2.17	↓	TRUE						
N234	LCMS untarget NEG	545.2085	3.2575	↓	TRUE						
N111	LCMS untarget NEG	314.1279	4.6175	↓	TRUE						
N264	LCMS untarget NEG	592.4710	7.28	↓	TRUE						
P176	LCMS untarget POS	444.1765	1.47	↓	TRUE						
P87	LCMS untarget POS	267.0979	1.4	↓	TRUE						
N212	LCMS untarget NEG	505.3175	6.1275	↓	TRUE						
N231	LCMS untarget NEG	538.4245	7.1975	↓	TRUE						
N168	LCMS untarget NEG	438.3343	6.845	↓	TRUE						
N267	LCMS untarget NEG	594.4871	7.475	↓	TRUE						
N99	LCMS untarget NEG	300.2667	7.195	↓	TRUE						
N72	LCMS untarget NEG	247.9130	4.66	↓	TRUE						
N69	LCMS untarget NEG	245.9158	4.6625	↓	TRUE						
N55	LCMS untarget NEG	230.0774	1.1975	↓	TRUE						
P121	LCMS untarget POS	340.2298	2.85	↑	TRUE						

Direction: ↓ lower level in NGT-T2D and ↑ higher level in NGT-T2D compared to NGT-NGT group by OPLS-DA model. Significance: metabolites that fulfilled the statistical significance criteria calculated from the OPLS-DA model loadings.

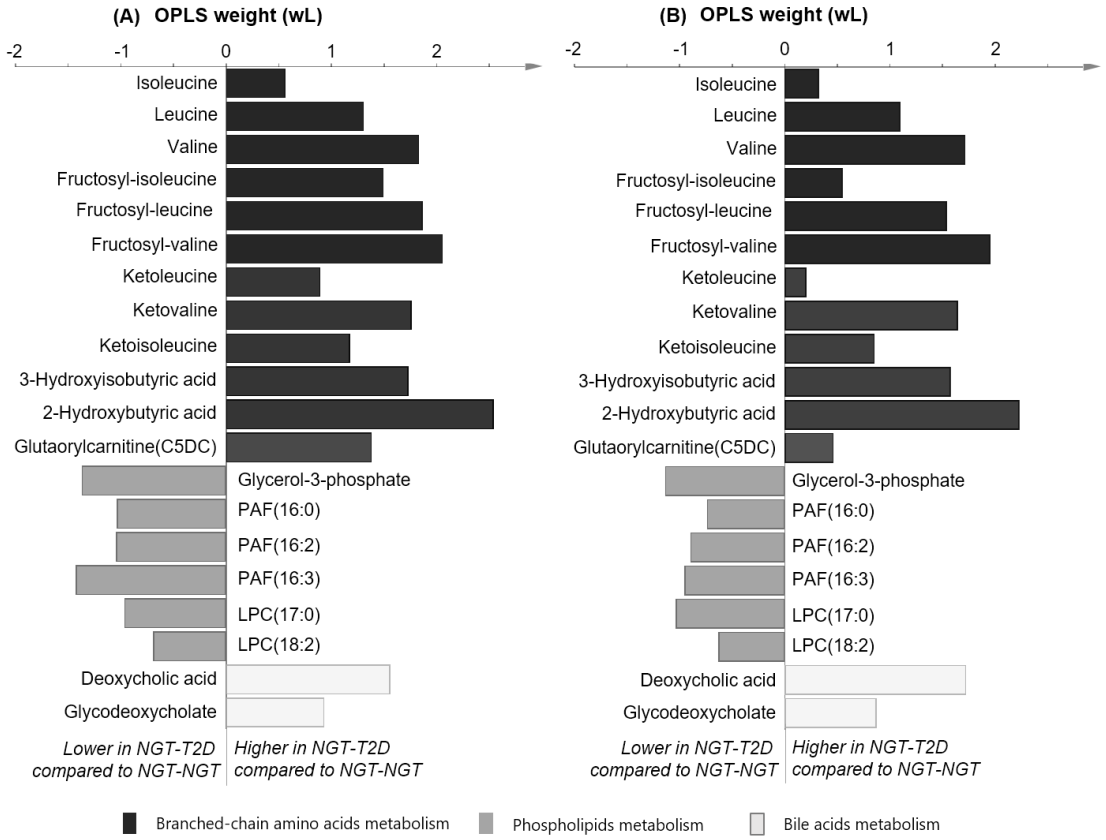


Figure 5S2. Comparison of metabolite patterns of type 2 diabetes at follow-up in black South African women by OPLS-DA models, (A) including all NGT-T2D subjects and (B) excluding NGT-T2D subjects with diabetes medications. The overall metabolite pattern remains the same but less pronounced outspoken due to the reduced number of individuals explore.

APPENDIX C

Ethics certificates

R14/49 Asanda Mtintsilana

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150530

NAME: (Principal Investigator)	Asanda Mtintsilana
DEPARTMENT:	MRC/Wits Developmental Pathways for Health Research Unit
PROJECT TITLE:	Identification of Metabolites and Metabolic Pathways that Predict Impaired Glucose Metabolism in Black South African Women
DATE CONSIDERED:	29 May 2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Associate Prof Julia Goedecke
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2015/06/29

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

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

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

06 February 2020
Person No: 607019
TAA

Dear Miss Asanda Mtintsilana

Doctor of Philosophy: Change of title of research

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

From:	**The identification of metabolites and metabolic pathways that predict impaired glucose tolerance in black South African Women**
To:	**Delineating the pathophysiology of type 2 diabetes in middle-aged black South African women**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG

R14/49 Ms A Mtintsilana

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200999

NAME: (Principal Investigator)	Ms A Mtintsilana
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Developmental Pathways to Health Research Unit Medical School University
PROJECT TITLE:	Delineating the pathophysiology of type 2 diabetes in middle-aged Black South African women
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Renewal of M150530
SUPERVISOR:	Profs JH Goedecke & L Micklesfield; Dr E Chorell
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/10/15

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary 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 **September** and will therefore reports and re-certification will be due early in the month of **September** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

APPENDIX D

Participant questionnaire

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

METABOLOMICS STUDY: Delineating the pathophysiology of type 2 diabetes in middle-aged black South African women

PARTICIPANT OUESTIONNAIRE

DATE: Day | | | **Month** | | | **Year** | | | | |

MTB ID NUMBER: | | | | | | | |

BONE ID NUMBER: | | |

CHECK LIST TABLE

Components	Tick
Contact details and ID Copy	
Study information sheet	
Informed consent form	
Section A: HIV rapid test	
HIV consent form	
Section B: Demographics and Socio-Economic Status (SES)	
Section C: General Health	
Photograph of medication (s)	
Section D: Lifestyle	
Section E: Weight Management Practices	
Section F: General Health Questionnaire (GHQ-28)	
Section G: Anthropometry	

Height	
Weight	
Waist circumference	
Hip circumference	
DXA	
Section H: Blood pressure	
Section I: Blood sampling	
Glucose	
Insulin, C-peptide	
HbA1c	
Erythrocytes analysis	
Storage of plasma	
Serum (metabolomics, fasting samples)	
Inflammatory markers, adipokines & extra serum storage	
Section J: Oral Glucose Tolerance Test (OGTT)	
Section K: Activity monitoring	
Accelerometer (Actigraph)	
ActivPALs	
Section L: Dietary Intake	
Food Frequency Questionnaire (FFQ)	
Feedback (comment)	
Payment (R 100)	
Date and signature: _____	

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

PARTICIPANT Information sheet

Title of research

Delineating the pathophysiology of type 2 diabetes in middle-aged black South African women

Invitation

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

I would like to invite you to consider participating in the research study entitled; **Delineating the pathophysiology of type 2 diabetes in middle-aged black South African Women.** Before, agreeing to participate, it is important that you understand the purpose of the study, the study procedures, benefits and risks as well as your right to withdraw from the study at any time. If you have any questions, do not hesitate to ask me. You should not agree to take part unless you are satisfied with all the procedures involved. If you decide to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will also be given a copy to keep.

Purpose

I am doing a research study to determine which factors affect blood glucose (sugar) levels and risk for diabetes in black South African (SA) women. Briefly, when you eat, the body breaks food down into glucose and transports it into the blood. At the same time, the pancreas releases insulin (a hormone) so that the body can use glucose for energy and store it for future use. This process usually keeps your blood glucose levels from getting too high or too low. However, if your body doesn't respond to insulin (insulin resistance), the glucose in your blood will increase and so your pancreas has to produce more and more insulin. When your body can no longer produce enough insulin to maintain your blood glucose levels in the normal range, you may develop a disease called type 2 diabetes. The exact causes of insulin resistance and type 2 diabetes in black SA women are poorly understood. Therefore, I am conducting this study to understand how factors such as socio-economic status (e.g. level of education, your income) and lifestyle factors (diet, alcohol intake, smoking and physical activity) alter the metabolic pathways involved in glucose metabolism thereby increasing the risk for type 2 diabetes in black SA women. The results obtained from this study might help us to have a better understanding of the biological processes that are involved in insulin resistance and type 2 diabetes in black SA women and might also assist us in identifying those who might be at risk for developing T2D.

What do we expect from the women who participate in this study?

If you agree to participate in the study, you will be required to visit the DPHRU offices at the Chris Hani Barangwanath Hospital on a morning that is convenient to you, but after an overnight fast (nothing to eat or drink, except water, from 10pm the night before). During your visit you will be asked to complete a series of questionnaires by interview, which will include questions on measures of SES (i.e. housing index, employment and income), personal and family history of disease (e.g. diabetes, obesity, high blood pressure, and cardiovascular disease), reproductive history, food security, and stress. In addition questions on lifestyle factors, including smoking and alcohol intake, medication and supplement use will be included. Furthermore, habitual dietary food intake will be measured using a Food Frequency Questionnaire (FFQ). You will also be asked to wear small devices, one on your hip and one on your thigh, for 7 days to measure your physical activity and sedentary time.

You will be asked to donate a sample of blood and then undergo an oral glucose tolerance test (OGTT) to determine whether you are at risk for developing T2D. Your weight and height will be measured, as well as your waist and hip circumferences. In addition, you be required to undergo some tests to measure your body fat, muscle mass, and bone density, using a special X-ray scan. This is safe, non-invasive and painless. Your blood pressure will also be measured.

Procedures and potential risks

Demographic, socio-economic status and lifestyle questionnaire

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

Food frequency questionnaire (FFQ)

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

Blood sampling and oral glucose tolerance test (OGTT)

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

Blood sampling will be performed by inserting a small plastic tube into a vein in your arm and a small plastic tap or valve will be attached onto it so that blood samples can be drawn (1 teaspoon = 5 ml) before the 2 hour OGTT test. The first blood sample (40 ml) will be drawn for the measurement of blood lipids (fats), glucose (sugar) and various hormones (e.g. insulin) and metabolites in the fasted state (i.e. before you eat anything). You will then be asked to drink a cup of water containing 75 g of glucose (sugar). Thereafter, blood samples (5 mls or 1 teaspoon each) will be drawn from your arm at 10, 20, 30, 60, 90 and 120 min after the glucose ingestion for the later measurement of changes in glucose and insulin levels.

After your test you we will give you something to eat and drink. Sometimes when blood is taken you may feel a prick at the place where the needle enters your body. Afterwards there may be a little bruise, pain and local infection (which are associated with normal blood sampling). All procedures will be supervised and carried out by appropriately trained medical personnel using sterile techniques to minimise any risks of infection. This test is used routinely for both research and medical purposes. A total of 70 ml of blood will be drawn, which is substantially less than that of typical drawn when donating blood (±500ml).

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

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

Physical activity

You will be asked to wear two motion sensors (accelerometer and activPAL) for 7 days, to measure your activity and sedentary patterns, respectively. They are the size of a small matchbox and one is worn on the waist with a lightweight belt and the other is attached to the thigh using a waterproof

plaster. You will be instructed on how to use the monitors. There are no side-effects associated with wearing the monitors.

HIV test

We would like to test your HIV status. This is very important for the metabolomics study as HIV has been shown to alter your metabolite composition. The HIV test is voluntary, however , if you refuse to test, you will not be allowed to participate in the study. It is also important to note that if you decide to test, you will be given pre-test counseling by a fully trained registered counsellor. If you test positive, you will be excluded from the study. This test is always strictly confidential and can only happen if you agree. There is no way in which anyone can link your HIV status to your name as all results in this study are coded with a number. You will have your finger pricked with a sterile needle and the drop of blood will be tested on specific HIV testing kits to check for HIV antibodies ("body soldiers" that protect you from infections). If the report says negative it means that you are HIV negative. The window period will be explained. If the report states positive, it means that you are HIV positive. 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. If it happens that we cannot tell whether you are negative or positive, refer you to a special clinic for further testing.

Possible risks

There are no appreciable risks associated with the blood sampling and OGTT, other than those associated with routine blood sampling, including discomfort, bruising, swelling and local infection. All procedures will be supervised and carried out by a nursing sister and appropriately trained medical personnel using sterile techniques to minimise any risks of infection. A maximum of 80 ml of blood will be drawn during the entire study, which is 1/6th of that drawn during standard blood donation.
The only risk associated with the DXA scan is exposure to radiation. However, the radiation exposure with a DXA scan is less than half that of a chest x-ray (11.3 microSieverts).

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

Possible benefits

To you:

You will receive your own results, including body composition (weight, height, waist circumference, muscle and fat mass and bone density), serum lipid profile, blood pressure, risk for diabetes, physical fitness and dietary analysis, with some recommendations made by a dietician on how to adapt your dietary intake to improve your health. If you have any abnormal results, you will be given a referral letter and directed to the appropriate health practitioner or local clinic.

To others:

The results obtained from this study might help us to have a better understanding of the biological processes that are involved the risk for type 2 diabetes in black SA women. Also, the results of the study might assist us in the early identification of those who might be at risk of developing type 2 diabetes. Early detection of type diabetes might be beneficial for the prevention of long term health consequences and reduce economic costs associated with type 2 diabetes, especially in developing countries such as SA.

Costs to you

These tests will not cost you anything. You will be given something to eat and fruit juice once your blood tests have been completed. Once you have completed all the tests, you will be given transport money (R 100).

Voluntary participation in research:

You can refuse to take part in this study and you are free to withdraw from the study without any reason at any time. Your refusal to participate will not result in any penalty or loss of benefits to which you are otherwise entitled.

Records of your participation in this research:

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

Ethical approval:

This study protocol has been submitted to the University of the Witwatersrand, Human Research Ethics Committee (HREC), and written approval has been granted by the committee.

Publication of the results of the research:
The results of this research may appear in scientific publications without identifying you in any way.

Your questions

You may contact me (or my supervisors) at any time to ask questions, and if you have any questions concerning the tests, we will explain them to you. Our contact numbers are on this information sheet (see below), which you will be given to take home.

You will have a copy of this information sheet to keep

When you sign the consent forms, it means that:

- You have heard and understood the information
- We have answered your questions.
- You have decided to be part of this study, but you have the right to withdraw at any time without any prejudice. This study is completely voluntary.
- You have agreed to the following items:
    - Complete questionnaires by interview
    - HIV test
    - Routine body measurements
    - Body fat , muscle mass and bone density measurement using an X-ray scan
    - Blood sampling (70 ml, which is about 14 tablespoons of blood) after fasting and during the oral glucose tolerance test.
    - Wearing an accelerometer (ActiGraph) and activPALs for 7 days to physical activity and sedentary time, respectively
- The procedures to be carried out have been explained to you. The possible discomfort, risks and benefits have also been described to you.

Contact details of researchers

If you have any questions about this study, please feel free to contact us at:

Name: Miss Asanda Mtintsilana (PhD student and Project coordinator)
Cell phone: 072 217 3051
Email: mtintsilana.asanda@gmail.com

Name: Honorary Associate Professor Julia Goedecke (supervisor)
Work telephone: 021 938 0267
Cell phone: 082 825 5616

Email: julia.goedecke@mrc.ac.za

Name: Dr Lisa Micklesfield (co-supervisor)
Work telephone: 021 650 4570
Cell phone: 083 943 3172
Email: lisa.micklesfield@wits.ac.za

INFORMED CONSENT

I, _____ (*insert full name*), agree to participate in the research study entitled **identification of metabolites and metabolic pathways that predict impaired glucose metabolism in black South African women.** The goal and methods of the study are clear to me.

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, 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 the information sheet and have been given an opportunity to ask any questions and have my questions answered. I understand that I have the right to refuse to participate in the study.

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

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

2. I have the right to not answer any or all questions posed in the interviews and to not participate in any or all of the procedures / assessments.

3. The University of the Witwatersrand Human 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 metabolomics 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 a health service if any result is out of the normal range or a problem is detected in the course of the study.

PARTICIPANT (Caregiver)

Printed Name	Signature	Date and Time

RESEARCH ASSISTANT:

Printed Name	Signature	Date and Time

SECTION A: HIV RAPID TESTING

CONSENT FOR HIV TESTING

Additional consent to: Delineating the pathophysiology of type 2 diabetes in middle-aged black South African women

I _____ (*insert full name*), agree to provide a blood sample for the purposes of confidential HIV testing. I understand that it is necessary for me to have this test to participate in the research study. If I test positive, I cannot participate in the study entitled **identification of metabolites and metabolic pathways that predict impaired glucose metabolism in black South African women,** conducted by the Developmental Pathways for Health Research Unit (DPHRU), within the Department of Paediatrics at the University of the Witwatersrand. I am aware that I will receive pre and post-test counselling from a qualified HIV counsellor, and will be referred to an appropriate health care clinic if necessary. I understand the implications of performing the test.

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.

PARTICIPANT

Printed Name	Signature	Date and Time

HIV RAPID TEST

Name _____

ID number: _____

Date of Birth _____ Age: _____

Physical Address:

_____

_____

SUBJECT CODE:

You can only take the HIV test if you have <u>signed the HIV consent form.</u>

Have you signed the HIV consent?

YES	NO

HIV test results (click appropriate box)

Negative	
Positive	

TO BE KEPT SEPARATE FROM QUESTIONNAIRE DATA

SECTION B: DEMOGRAPHIC AND SOCIEO-ECONOMIC DETAILS

1. CURRENT MARITUS STATUS (tick option that applies)

Single	
Married/cohabiting	
Widowed	
Separated/Divorced	

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

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

3. CAREGIVER'S CONFIRMATION DETAILS

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

4. GENERAL HOUSEHOLD INFORMATION

Questions	**Answers**

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

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

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

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

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

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

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

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

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

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

Earth/sand/mud		Stone/Brick		Cement	

Wood		Vinyl/linoleum		Other	
Carpet		Ceramic tiles			

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

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

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

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

12. HOUSEHOLD FOOD INSECURITY ACCESS SCALE

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

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

13. EMPLOYMENT AND INCOME

13. 1 Are you currently employed? (tick)	YES NO
13.2 If YES TO 13.1, what type of employment?	FORMAL INFORMAL

13.3 If YES to 13.1, which best describes the work that you are employed to do? (tick relevant option)

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

15.4 Other household incomes (tick appropriate box):

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

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

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

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

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

SECTION C: GENERAL HEALTH

1. Are you pregnant?

Y	N

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

Y	N

2.1 If YES, what date was it?_____

3. Do you have regular periods?

Y	N

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

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

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

3.3 How would you classify your menopausal stage?

Pre-menopausal	
Peri-menopausal "menopausal transition" e.g. menstrual irregularity, hot, flashes, mood changes	
Post-menopausal (e.g. 12 month period without menstruation)	

4. How many children do you have?_____

5. How many pregnancies have you had? _____

6. When was your last pregnancy?_____

7. Contraception and fertility control:

Contraceptive method	**Tick the appropriate box (give name where appropriate)**	**Duration**
7.1 None		
7.2 Oral contraceptive pill		
7.3 Injection (state frequency of injection)		
7.4 Female sterilisation		

7.5 IUD “Intra-Uterine Device” loop		
7.6 Patch		
7.7 Other and specify		

8. MEDICATION AND SUPPLEMENT USE

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

	8.9.1 Did you take medicine? (yes/no) What medication(s) did the nurse or doctor prescribe you? State the dosage, frequency and duration of use.	NO.....

9. QUALITY OF LIFE AND CLINICAL CONDITIONS

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
9.1	Would you say your health is poor, average, good, or very good/excellent?	POOR..... AVERAGE ..... GOOD..... VERY GOOD/EXCELLENT.....
9.2	Do you personally think that you are underweight, normal weight or overweight?	UNDERWEIGHT..... NORMAL WEIGHT ..... OVERWEIGHT. ..... OBESE..... ..... DON'T KNOW.....
	Has a **doctor** or **nurse** or **health worker** at a **clinic** or at **hospital told you** that you had or have any of the following conditions:	
9.3	High Blood Pressure?	YES ..... NO ..... DON'T KNOW.....
9.4	Heart attack or angina (chest pains)?	YES ..... NO ..... DON'T KNOW.....
9.5	Stroke?	YES ..... NO ..... DON'T KNOW.....
9.6	High blood cholesterol or fats in the blood?	YES ..... NO ..... DON'T KNOW.....
9.7	Diabetes or Blood Sugar?	YES ..... NO ..... DON'T KNOW.....
9.8	Emphysema/Bronchitis?	YES ..... NO ..... DON'T KNOW.....
9.9	Asthma?	YES ..... NO ..... DON'T KNOW.....
9.10	Sore joints, e.g. Arthritis, gout?	YES ..... NO ..... DON'T KNOW.....
9.11	Osteoporosis?	YES ..... NO ..... DON'T KNOW.....
9.12	Epilepsy / fits?	YES ..... NO ..... DON'T KNOW.....

9.13	TB?	YES ..... NO ..... DON'T KNOW.....
9.14	How many episodes of TB have you ever been treated for?	NUMBER OF TB EPISODES……..... Are you currently on TB medications? ……..... ..... When was your last TB episode: ……..... ...
9.15	Cancer?	YES ..... NO ..... DON'T KNOW..... If yes, what? …… …..

10. FAMILY AND MEDICAL HISTORY

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
	Now I would like to ask you about your family. Do you have a **close blood relative** (father, mother, brother, sister or child) who has ever had any of the following conditions:	
8. 1	High Blood Pressure?	YES ..... NO ..... DON'T KNOW ..... If yes, who? …… …… …… …… …… …… …….....
8.2	Heart attack or angina or chest pain when exerting himself/herself?	YES ..... NO ..... DON'T KNOW ..... If yes, who…… ….. ……..…… …… …… ……....
8.3	Was this relative younger or older than 50 years old	YOUNGER THAN 50 YEARS.....

NO.	QUESTIONS AND FILTERS	CODING CATEGORIES
	when they first had a heart attack, angina or chest pain?	…… …… OLDER THAN 50 YEARS ..... …… …… DON'T KNOW ..... …… …… .....
8.4	Stroke?	YES ..... NO ..... DON'T KNOW ..... If yes, who? …… ……... …… …… …… …… …….....
8.5	Diabetes?	YES ..... NO ..... DON'T KNOW ..... If yes, who? …… …… …… …… …… …… Adult/ child onset? …… …. …… …… ……
8.6	Obesity? (Were they abnormally large? Or have difficulty moving?)	YES ..... NO ..... DON'T KNOW ..... If yes, who? …… …… …… …… …… …… …… …… ……

SECTION C: LIFESTYE

	1. TOBACCO USE (WHO STEPwise Questionnaire)	
1.1	Do you **currently smoke** any tobacco products, such as cigarettes, cigars, or pipes?	YES..... NO .....
1.2	Do you currently smoke tobacco products **daily**?	YES..... NO .....
1.3	**How old** were you when you first **started** smoking daily?	YEARS OLD _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ IF "YOU DON'T REMEMBER",.....
1.4	If you do not remember how old you were, do you remember how long ago it was?	WEEKS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ _
		MONTHS AGO _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
		YEARS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ _
1.5	On average, how many of the following items do you smoke each day?	MANUFACTURED CIGARETTES _ _ _ _
	(CHECK EACH ITEM, IF NOT SMOKING AN ITEM, CODE 00)	HAND-ROLLED CIGARETTES _ _ _ _ _
		PIPES FULL OF TOBACCO _ _ _ _ _ _ _
		CIGARS/CHEROOTS/CIGARILLOS _ _ _ _
		OTHER_ _
1.6	**In the past,** did you ever smoke daily?	YES..... NO .....
1.7	**How long ago did you stop** smoking daily?	WEEKS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ MONTHS AGO _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ YEARS AGO_ _ _ _ _ _ _ _ _ _ _ _ _ _ _

2. Do you use snuff?

2.1 IF YES, how often do you use snuff?

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

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

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

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

4. ALCOHOL INTAKE

1 standard drink is equal to 10 g of pure alcohol:

- 200 ml of beer
- 1 glass of wine
- 1 tot (25 ml) spirits
- 1 small glass (50ml) of sherry/port

4.1 How often do you have a drink containing alcohol?

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

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

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

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

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

SECTION E: WEIGHT MANAGEMENT PRACTICES

1. Have you tried to lose weight?	YES / NO
2. How many times have you tried to lose weight?	1 time / 2 times / 3 or more

3. Which of the following methods did you use to lose weight?

3 healthy, smaller meals a day		
Increased exercise		
Leaving out one or more meals		
Eating less or nothing between meals		
Diet formula/milkshakes/powders		
Weigh-less or any other slimming club		
Low carbohydrate, high protein		
Pills to lose water		
Laxatives		
Herb mixtures		
Stretch clothes to make you perspire		
Machines which “break” down fat		
Injections that help to break down fat		
Fasting (one or more days)		
Other (specify)		

4. Which ONE of the following best describes you at the MOMENT?

I am completely satisfied with my present weight	
I would like to lose 1-3 kg	
I would like to lose 4 or more kg	
I would like to put some weight	

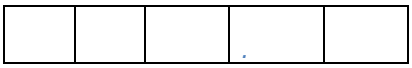
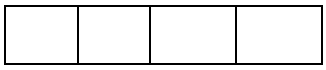
SECTION F: General health Questionnaire (GHQ-28)

The following questions examine your changes in mood, feelings, and behaviours in the **past four weeks**. Please answer the questions by choosing the appropriate box.

SOMATIC SYMPTOMS			
1. Been feeling perfectly well and in good health?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
2. Been feeling in need of a good tonic?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
3. Been feeling run down and out of sorts?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
4. Felt that you are ill?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
5. Been getting any pains in your head?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
6. Been getting a feeling of tightness or pressure in your head?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
7. Been having hot or cold spells?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
ANXIETY AND INSOMNIA			
1. Lost much sleep over worry?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
2. Had difficulty in staying asleep once you are fall off "to sleep"?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
3. Felt constantly under strain?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
4. Been getting edgy and bad tempered?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
5. Been getting scared or panicky for no good reason?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
6. Found everything getting on top of you?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
7. Been feeling nervous and strung-up all the time?			
1. Less than normal	2. No more than usual	3.Rather more than usual	4. Much more than usual

SOCIAL DYSFUNCTION			
1. Been managing to keep yourself busy and occupied?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
2. Been taking longer to do the things you do?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
3. Felt on the whole you were doing things well?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
4. Been satisfied with the way you've carried out your task?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
5. Felt that you are playing a useful part in things?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
6. Felt capable of making decisions about things?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
7. Been able to enjoy your normal day-to-day activities?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
DEPRESSION			
1. Been thinking of yourself as a worthless person?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
2. Felt that life is entirely hopeless?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
3. Felt that life isn't worth living?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
4. Thought of the possibility that you might "make away" with yourself/			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
5. Found at times you couldn't do anything because your nerves were too bad?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
6. Found yourself wishing you were dead and away from it all?			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual
7**. Found that the idea of taking your own life kept coming into your mind?**			
1. Less than normal	2. No more than usual	3. Rather more than usual	4. Much more than usual

SECTION G: ANTHROPOMETRIC MEASUREMENT

- STANDING HEIGHT: (mm) 
- WEIGHT: (kg) 
- WAIST CIRCUMFERENCE: (mm) 
- HIP CIRCUMFERENCE (mm) 

DEXA: □ Full Body □ Half Body

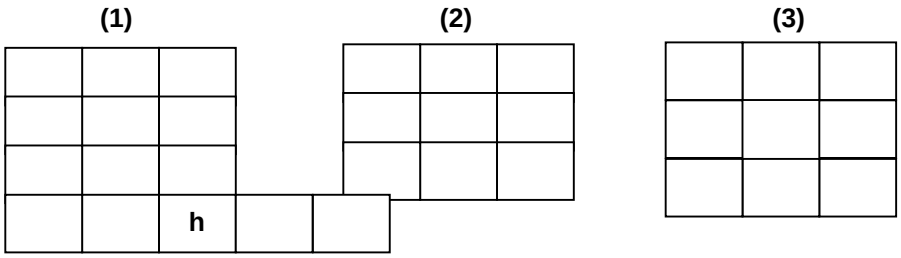
Comments:

_____

SECTION H: BLOOD PRESSURE

Left arm

- SYSTOLIC BP
- DIASTOLIC BP
- PULSE
- TIME OF BP



Comments:

_____

SECTION I: BLOOD SAMPLING

Date of testing: _____ Time of testing: _____

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

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

Good ☐ Fair ☐ Poor ☐

If POOR, explain why:
_____

Date of last menstrual cycle: _____

Fasting blood samples: Tubes: purple □ , grey □, red/yellow □

Tube	**Analyses**
1 X Grey tube	Glucose
1 X Yellow tube	Insulin, C-peptide
1 X Purple tube	HbA1c
1 X Purple tube	Erythrocytes (fatty acids)
1 X Purple tube	Storage of plasma
1 X Yellow tube	Metabolomics
2 X Yellow tubes:	
Inflammatory markers	
Adipokines	
Extra serum storage	

SECTION J: ORAL GLUOSE TOLERANCE TEST

Tubes: , grey □, red/yellow □

Drink: □ Time: _____ Comments: _____

10 min: □ Time: _____ Comments: _____

20 min □ Time: _____ Comments: _____

30 min: □ Time: _____ Comments: _____

60 min □ Time: _____ Comments: _____

90 min: □ Time: _____ Comments: _____

120 min: □ Time: _____ Comments: _____

SECTION K: ACTIVITY MONITORING

1. Accelerometer (Actigraph)

1.1 Serial number of the monitor:_____

1.2 Date collected:_____

1.3 Date returned:_____

RECORDING DAY	**Sedentary (min/d)**	**Light (min/d)**	**Moderate (min/d)**	**Hard (min/d)**	**Very hard (min/d)**	**Total MVPA (min/d)**	**%MVPA**	**Step counts (n/d)**
DAY 1								
DAY 2								
DAY 3								
DAY 4								
DAY 5								
DAY 6								
DAY 7								

2. ActivPAL

1.1 Serial number of the monitor:_____

1.2 Date collected:_____

1.3 Date returned:_____

RECORDING DAY	Sedentary (min/d)	Sedentary (%/d)	Standing (%/d)	Stepping (%/d)	Steps (n/d)
DAY 1					
DAY 2					
DAY 3					
DAY 4					
DAY 5					
DAY 6					
DAY 7					

SECTION L: DIETARY INTAKE

1. Which of the following do you usually eat most of he time? **(Mark only one per column)**						**MILK / MILK PRODUCTS**	
				SPREAD		Full cream / Maas	
CHICKEN/POULTRY		**RED MEAT**		Butter		2% or low fat	
With skin		Fatty meat		Hard margarine (brick)		Skim	
Without skin		Lean meat		Soft margarine (tub)		Blends	
None		None		None		None	

2. How often do you usually eat the following? **(Mark each line)**	**Never Occasionally**	**Weekly**	**Daily**
Deep fried food e.g. chips			
Shallow fried foods e.g. eggs			
Crisps e.g. packet of chips			
Processed meats e.g. polony, viennas			

3. How would you describe your alcohol intake?	
None	
Less than 1 drink per day	
1 – 3 drinks per day	
4 + drinks per day	

4. How often during the past week did you eat the following? **(Mark every item)**

Food item	Never	1-3 times per week	4-6 times per week	1 time a day	2 times a day	3+ times a day		Never	1-3 times per week	4-6 times per week	1 time a day	2 times a day	3+ times per day
Red meat (any type)							Spinach (marog)						
Chicken (any type)							Carrots						
Tinned fish							Tomato (raw/cooked)						
Organ meat e.g. liver, offal							Green peas						
Eggs (any type)							Green beans						
Milk /yoghurt / maas to drink / on cereals							Mixed vegetables						
Milk in tea / coffee							Pumpkin/ butternut						
Cheese (except cottage)							Sweet potato						
Legumes eg baked beans, lentils							Potato (any preparation)						
Peanuts and nuts							Citrus fruit e.g. orange						
Brown / whole wheat bread / rolls							Pure orange/guava juice						
Breakfast cereal (instant)							Bananas						
Oats porridge							Mangoes						
Soft margarine (tub)							Apples/pears						
Broccoli, cauliflower, Brussels sprouts							Avocado						

7

PARTICIPANT (Caregiver)

Printed Name	Signature	Date and Time

RESEARCH ASSISTANT:

Printed Name	Signature	Date and Time

APPENDIX E

Turnitin report

607019:PhD_Thesis_Report_submission.docx

ORIGINALITY REPORT

19%	10%	18%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.mdpi.com Internet Source	3%
2	www.nature.com Internet Source	1%
3	Asanda Mtintsilana, Lisa K. Micklesfield, Elin Chorell, Tommy Olsson, Julia H. Goedecke. "Fat redistribution and accumulation of visceral adipose tissue predicts type 2 diabetes risk in middle-aged black South African women: a 13-year longitudinal study", Nutrition & Diabetes, 2019 Publication	1%
4	Julia H. Goedecke, Tommy Olsson. "Pathogenesis of type 2 diabetes risk in black africans: a south african perspective", Journal of Internal Medicine, 2020 Publication	1%
5	Stephanie T Chung, Mirella Galvan-De La Cruz, Paola C Aldana, Lilian S Mabundo et al. "Postprandial insulin response and clearance among black and white women: The Federal Women's Study", The Journal of Clinical Endocrinology & Metabolism, 2018 Publication	<1%

among black and white women: The Federal Women's Study", The Journal of Clinical Endocrinology & Metabolism, 2018
Publication

6	www.endocrine.org Internet Source	<1%
7	Asanda Mtintsilana, Lisa K. Micklesfield, Elin Chorell, Tommy Olsson et al. "Adiposity Mediates the Association between the Dietary Inflammatory Index and Markers of Type 2 Diabetes Risk in Middle-Aged Black South African Women", Nutrients, 2019 Publication	<1%
8	Erdmann, J.. "Weight-dependent differential contribution of insulin secretion and clearance to hyperinsulinemia of obesity", Regulatory Peptides, 20090108 Publication	<1%
9	Louise M. Goff, Meera Ladwa, Olah Hakim, Oluwatoyosi Bello. "Ethnic distinctions in the pathophysiology of type 2 diabetes: a focus on black African-Caribbean populations", Proceedings of the Nutrition Society, 2019 Publication	<1%
10	worldwidescience.org Internet Source	<1%

11	Goedecke, Lisa K. Micklesfield, Tommy Olsson, Elin Chorell. "Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women: a prospective cohort study", Metabolism, 2019 Publication	<1%
12	Goedecke, Julia H., Asanda Mtintsilana, Siphiwe N. Dlamini, and Andre Pascal Kengne. "Type 2 diabetes mellitus in African women", Diabetes Research and Clinical Practice, 2017. Publication	<1%
13	journals.plos.org Internet Source	<1%
14	"Minutes of The 43rd General Assembly of The European Association for The Study of Diabetes", Diabetologia, 2008 Publication	<1%
15	"Abstracts 2007", Diabetologia, 2007 Publication	<1%
16	Meera Ladwa, Oluwatoyosi Bello, Olah Hakim, Fariba Shojaee-Moradie et al. "Insulin clearance as the major player in the hyperinsulinaemia of black African men without diabetes", Diabetes, Obesity and Metabolism, 2020 Publication	<1%

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

TURNITIN REPORT COVERLETTER

Candidate name	Asanda Mtintsilana
Student number	607019
E-mail	mtintsilana.asanda@gmail.com
Degree for which report is submitted for	PhD
Department	MRC/Wits Developmental Pathways for Health Research Unit (DPHRU), Department of Paediatrics, School of Clinical Medicine
Title of proposed research (current title)	Delineating the pathophysiology of type 2 diabetes in middle-aged black South African women.
Primary supervisor	Professor Julia H. Goedecke (SA MRC)
Email	julia.goedecke@mrc.ac.za
Co-supervisor	Associate Professor Lisa Micklesfield (DPHRU, Wits)
Email	lisa.micklesfield@wits.ac.za
Co-supervisor	Dr Elin Chorell (Umeå University, Sweden)
Email	elin.chorell@umu.se

This letter to inform you that Miss Asanda Mtintsilana submitted her thesis report on Turnitin, 24 November 2020 (Submission ID: 1456315012) to detect the level of plagiarism in her thesis report. She received a Turnitin similarity score of 19% **(see the Turnitin originality report with primary sources in Appendix E of her thesis)**, which was mostly made of < 1% similarity scores from the sources. We have reviewed her Turnitin report and found that the majority of the similarities come from short phrases or words such as "between black African and white European", "are described in detail", "although there are", "risk of developing", and "as well as adipose tissue", rather than long sentences or paragraphs. Based on the matches detected by the software, we believe that the Turnitin similarity score of 19% is acceptable and does not reflect any overt plagiarism.

Please contact us immediately if you need further clarification.

Signature of primary supervisor	
Date	26 November 2020
Signature of co-supervisor	
Date	26 November 2020
Signature of co-supervisor	
Date	26 November 2020