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THE IMPACT OF DIABETES MELLITUS IN PREGNANCY ON MATERNAL HEALTH OUTCOMES

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

Supervisors

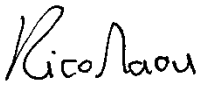
Professor Naomi Levitt
Professor Shane Norris

Johannesburg, South Africa
2021

Declaration

This dissertation is submitted for the degree of Doctor of Philosophy at the University of Witwatersrand. The research described herein was conducted under the supervision of Professor Shane Norris in the Department of Paediatrics, University of Witwatersrand and Professor Naomi Levitt in the Department of Endocrinology, University of Cape Town, between period January 2018 and December 2020.

This work to the best of my knowledge is original, except where acknowledgements and references are made to previous work. Neither this, nor any substantially similar dissertation has been or is being submitted for any other degree or diploma or other qualification at any other University.



Signature

12th day of November 2021

Dedication

"Every great dream begins with a dreamer. Always remember, you have within you the strength, the patience and the passion to reach for the stars to change the world".
Harriet Tubman

Professor Huddle, without you I may have never realized this dream!

To my dearest husband Stefano and two beautiful girls, Michela and Sofia who were behind me all the way. It is with thanks to you all that I achieved this dream.

Acknowledgements

I am deeply indebted to Professor Shane Norris for a priceless opportunity to pursue my PhD under his supervision. I appreciate his vast knowledge and skills, guidance, and input in building a critical intellectual argument, hypothesis generation and writing the reports from research proposal, ethics application, publications, and the whole thesis. He provided not only the platform, but also brought on board international expertise in my area of interest.

A special thanks go to Professor Naomi Levitt for co-supervising my work all the way from Cape Town. She was always ready to hop on a plane to meet up and discuss some critical issues surrounding the project and was always readily available to assist and advise were necessary. I thank you for your expertise and time devoted in preparing my academic arguments and execution of hypotheses.

Furthermore, I need to thank various other experts and colleagues, for contributing in various ways. Professor Huddle, from the outset of my career in Internal Medicine and Endocrinology, you have been a phenomenal mentor and colleague. Your intimate knowledge you have always shared with me during my years of training as a clinician and more recently the support you have given me during my PhD path has been invaluable. To a newly acquired colleague and friend who set out on her PhD journey alongside me, Larske Soepnel, thank you for being a pillar of strength as we tackled the many challenges together with the blissful moments in achieving our goals throughout this process. I wish you all the best with your PhD. Professor Kerstin Klipstein-Grobusch, the mentoring and guidance you provided throughout this journey was a welcome addition to my research. Your keen knowledge and ongoing support were deeply appreciated.

To the research team at the DPHRU, a special word of thanks to the research nurses and assistants, especially Sr Gladys who selflessly supported this project despite the numerous challenges. Without you this would not have been possible. Thank you for your hard work and dedication. I am eternally grateful. To the laboratory staff, Elisa Masiakwala and Yusuf Guman- thank you for all your hard work in the laboratory and for seeing this project through from start to end. Your meticulous work ethic and dedication to the study is so appreciated. To Wihan Taljaard, your intimate knowledge and kind manner was a tremendous help with setting up our REDCap data capturing platform. Thank you! To Glory Chidumwa, your generous contribution in assisting me with understanding statistics and applying it correctly to the thesis is commendable and I am grateful to you for having provided me with this valuable tool.

The women and their babies in my study- thank you for your contribution to Science and for helping me better understand the consequences of gestational diabetes in our country. I hope that through this work we can assist in improving health care platforms to encourage better screening and awareness around screening for non-communicable diseases following a diabetic pregnancy together with interrupting the vicious cycle of intergenerational disease.

Lastly, I am so grateful to my husband Stefano and two daughters, Michela and Sofia who were a constant inspiration on my journey.

Thesis Material and Contributions

Together with my supervisors, I contributed to the conceptualisation of the new data collection for this thesis. I contributed to the development of the study protocol, which included designing the data collection templates, formulating the questionnaire, the standard operating procedures and training staff where necessary. I was actively involved in performing all data collection which included interviewing participants, performing anthropometric measurements, blood venesection, performance of an oral glucose tolerance test and ultrasonography. In addition, I contributed to the project management, quality control and data management, as well as statistical analysis of the results presented.

Funding and Awards

This study was made possible through the support of the SAMRC/Wits Developmental Pathways for Health Research Unit. The support of the SAMRC/Wits Developmental Pathways for Health Research Unit, towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the author and not necessarily attributed to the Centre of Excellence in Human Development.

Presentations and publications

Presentations

Scientific meetings

1. **Characteristics of women with diabetes in pregnancy in Soweto and the associated maternal and neonatal outcomes**

   Conference: Research day University of Witwatersrand

   Date: August 2018

   Place: University of the Witwatersrand, South Africa.

2. **The cardiometabolic outcomes in a cohort of women exposed to hyperglycaemia first detected in pregnancy at 3-6 years post-partum in an urban South African setting.**

   Conference: DOHaD around the world seminar series

   Date:7th April 2021

Conference proceedings

1. **Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV.**

   Conference: International Congress of Endocrinology (ICE)

   Date: December 2018

   Place: Cape Town, South Africa.

2. **The cardiometabolic outcomes in a cohort of women exposed to hyperglycaemia first detected in pregnancy at 3-6 years post-partum in an urban South African setting.**

   Conference: ENDO 2021(virtual)

   Date: March 20-23rd 2021

3. **The metabolic outcomes following hyperglycaemia first detected in pregnancy in Soweto, South Africa**

   Conference: SEMDSA (South Africa)

   Date: 25-28th March 2021

   Awarded prize for best oral presentation in the “Diabetes” category.

Publications

The research findings from this PhD were written up as three separate manuscripts all submitted to peer-reviewed journals. Two manuscripts were accepted for publication and published by the respective journals and the last manuscript has been submitted and is currently under review. The signed co-author agreement for the use of each publication within this thesis can be found in Appendix A.

1. **Nicolaou V**, Soepnel L, Huddle KRH, Klipstein-Grobusch, Levitt N & Norris, S.A. (2019) Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV. *International Journal of Obstetrics and Gynecology*, 147: 1-9. DOI: 10.1002/ijgo.12956. (Appendix I)

2. **Nicolaou V,** Soepnel L**,** Huddle KRH, Levitt N, Klipstein-Grobusch & Norris, S.A. (2020) Maternal and neonatal outcomes following the introduction of oral hypoglycaemic agents for gestational diabetes were comparable to insulin monotherapy within two historic cohorts. *South African Medical Journal*, 110: 154-158. doi:10.7196/SAMJ. 2020.v110i2.14024.(Appendix J)

3. **Nicolaou V,** Soepnel L**,** Huddle KRH, Klipstein-Grobusch, Levitt N & Norris, S.A The cardiometabolic outcomes in a cohort of women exposed to hyperglycaemia first detected in pregnancy at 3-6 years post-partum in an urban South African setting, submitted to *PLOS ONE Journal* on 31st July 2021-provisionally accepted subject to minor revisions. (Appendix K)

PhD student's contribution to each publication:

The student was responsible for the overall study design and the conceptualisation of each sub-study, as well as project coordination that involved overseeing data collection, designing of standard operating procedures, and training of staff. Data cleaning, statistical analyses and interpretation of results were performed by the student as was the writing and revision of each manuscript. Co-authors provided methodological advice, some statistical assistance, and critically reviewed the manuscripts.

Abstract

Background

Hyperglycaemia in pregnancy, encompassing all types of diabetes, is on the rise with an alarming global prevalence of 17%, of which 84% of these being accounted for by hyperglycaemia first detected in pregnancy (HFDP). Alongside the well-established adverse short-term outcomes of poorly managed or untreated disease, HFDP has a significant impact on the future health of both mother and offspring, playing a crucial role in the global diabetes epidemic. Until recently, HFDP, for which the highest prevalence occurs in low to middle income countries (LMIC), played a minor role in the shadow of more obvious determinants of maternal and fetal morbidity in these low-resource settings. More recently it has been recognised as an important public health issue given its immediate burden on maternal health services and the transgenerational and population level impact of the far-reaching ramifications for mother and child alike. Though our knowledge surrounding the burden, determinants, and immediate consequences of HFDP in South Africa (SA) is growing, the appreciation of its long-term consequences is poorly explored. Understanding and appreciating the adverse health consequences of HFDP creates an informed platform for developing health interventions for a condition which is aggressively fuelling the non-communicable diseases (NCD) burden.

Objective:

Our study seeks to investigate the cardiometabolic outcomes in an urban cohort of black SA women with a history of HFDP, 3-6 years following their delivery, compared to a group of women non-exposed to HFDP on the backdrop of a high human immunodeficiency virus (HIV) burden. Additionally, we explore the safety and efficacy of exposure to oral hypoglycaemic agents (OHAs) on maternal and neonatal outcomes.

Design and Methods:

An initial retrospective review of all women identified with diabetes in pregnancy, including type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and HFDP at the Gestational Endocrine Clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) for the period 2012 through to 2018 was performed in order to investigate the immediate maternal and neonatal outcomes as well as to explore the impact of HIV infection and exposure to OHAs on these outcomes. Twin pregnancies and more than one pregnancy for the same individual

during the time frame were excluded. Secondly, a prospective follow up study of women identified with prior HFDP between 2014 and 2017 were recruited for a cross-sectional analysis between March and November 2019 (3-6 years following their index pregnancy) and compared with women time-matched post-partum, who tested negative for HFDP (non-HFDP group) using the same diagnostic test and criteria. Two hundred and four participants were enrolled of which 103 were exposed and 101 non-exposed to HFDP. All participants were subject to a detailed questionnaire as well as various measurements including anthropometric parameters, blood pressure, the performance of an oral glucose tolerance test (OGTT), fasting serum creatinine, insulin, lipids, and glycated haemoglobin (HbA1C), urine analysis, body composition analysis via dual-energy x-ray (DXA) and ultrasonography of the carotid arteries to determine intima media thickness (cIMT) and the presence of plaque. For the retrospective review, descriptive statistics were used for comparing various maternal and neonatal outcomes by diabetes groups and for statistically significant outcomes, logistic regression analysis was performed using backwards selection following univariate analysis. For the prospective evaluation of HFDP exposed vs. non-exposed HFDP participants, unadjusted and multivariate adjusted odds ratio were estimated for the outcomes T2DM, metabolic syndrome (MetS) and 10-year cardiovascular risk calculated using Framingham risk score (FRS) from logistic regression models. Further multivariate models were designed to explore the potential association of historical maternal factors with the outcomes including fat mass index (FMI), T2DM, and cIMT using logistic or linear regressions.

Results:

Of the initial 1071 diabetic pregnancies retrospectively identified, majority of the women had pregestational diabetes (57%), with the remainder having HFDP (43%). Within the HFDP group, 51% had “overt” GDM (DIP) vs. 49% “true” gestational diabetes (GDM). Despite good glycaemic control across all the groups (as measured by HbA1C at term), adverse maternal and neonatal outcomes were higher than in the background population with initial maternal HbA1C and body mass index (BMI) being predictors of poorer neonatal outcomes. Overall HIV prevalence within the group was 24%, lowest in those with HFDP (17.4%) and accounted for significantly higher perinatal mortality (PNM) in HIV-infected 9.4% than non-HIV-infected pregnancies 1.8%, $p<0.001$. The exposure to either oral hypoglycaemic agents (metformin and glibenclamide) versus insulin on immediate maternal and neonatal outcomes in two cohorts of women diagnosed with HFDP, was found to be safe and effective and was only significant for

an association between glibenclamide and macrosomia. In the prospective, cross-sectional analysis of 103 women exposed to HFDP (identified using 75g OGTT, adopting IADPSG criteria) versus 101 women not exposed to HFDP, higher rates of progression to all three outcomes were noted with 44.6% of those exposed progressing to T2DM (adjusted risk of 10.5(95%CI 3.7-29.5)), 40.8% having MetS (adjusted risk 6.3(2.2-18.1)) and higher cardiovascular risk as identified by FRS (adjusted risk 4.3(1.6-11.5)), however cIMT did not remain significant after adjusting for confounders. Though HFDP exposure was a significant predictor for T2DM, MetS and cardiovascular risk, certain maternal factors present either pre-pregnancy, during the index pregnancy and/or post-partum were significantly associated with the outcomes. HIV, however, was not found to play a role in progression to any of these outcomes. Body composition was significantly altered in women exposed to HFDP versus those non-exposed with significantly higher FMI (13.9 vs. 12.3, p=0.008) and visceral fat indices between the groups and this remained significant after adjusting for confounders.

Conclusion:

The growing epidemic of obesity, T2DM, and cardiovascular diseases (CVD), particularly amongst LMIC, poses a significant public health burden in addition to the persistent infectious disease scourge. Our study confirmed the significant immediate adverse maternal and neonatal outcomes in women with diabetes in pregnancy and provided reassuring data on the safety and efficacy of OHAs in pregnancy when compared with the gold standard of insulin. Additionally, we identified a group of young black women at high risk of cardiometabolic diseases in the short-term following HFDP exposure. Appreciating these risks on the background of the growing prevalence of HFDP assists in providing evidence to incorporate universal screening programmes for detecting HFDP, expansion of specialized antenatal services for these women in addition to the post-partum interventions including targeted screening, counselling, and lifestyle and/or therapeutic interventions in order to preserve future maternal health and interrupt the intergenerational cycle of chronic diseases. Future prospective studies are needed to explore the best timing and impact of these interventions in curtailing these adverse outcomes and improving cardiometabolic health in this vulnerable group of women.

Preface

"*The future belongs to those who believe in their dreams*".

Eleanor Roosevelt

Pregnancy offers the clinician a unique opportunity to meaningfully affect the outcome of two individuals. Additionally, the post-partum period offers a further opportunity to influence future health for both mother and child alike. With this knowledge, my passion and interest in the field of diabetes in pregnancy, was first sparked when Professor Huddle, Head of Clinical Medicine at the Chris Hani Baragwanath Academic Hospital at the time, offered for me to take over the head position at the Gestational Endocrine Clinic in 2015. My background as a specialist endocrinologist devoted to full-time service in the state hospital was the perfect platform from which to grow and continue a much-needed service like this. The Gestational Endocrine Clinic was first opened at the hospital in May 1983 by Professor Huddle with the intent of improving maternal and fetal outcomes for women with a diabetic pregnancy. The clinic served an impoverished, predominantly Black African community of Soweto. The impact such a service had on maternal health was profound and became clinically evident when two papers were published in the early 1990s and again in 2000, highlighting the dramatic improvement in both maternal and child health outcomes following a diabetic pregnancy when compared to women who remain undetected and untreated. As you can imagine, I was certainly honoured and delighted to take on this opportunity and further continue to improve maternal and fetal health. Moreover, it is extremely rewarding managing such motivated women who had themselves and their offspring's best interest at heart. Their enthusiasm is evident in their regular attendance and excellent compliance at the clinic, which ensures their improved immediate outcomes for mother and child when compared to the dire consequences for those left untreated. Sadly, on the contrary, their post-partum follow-up was inexistent, apparent in their poor attendance at their prescribed six-week post-partum oral glucose tolerance test to rule out diabetes. More so, I noted that many of these women returned to our adult diabetic clinic some years later, with full-blown diabetes, unfortunately, some with diabetic-related complications. Data mostly from high to middle income countries has demonstrated that these women are highly vulnerable and are at risk of various cardiometabolic consequences. This highlighted the urgent need to implement platforms for ongoing surveillance among these women. I became pre-occupied with the notion that we as clinicians were afforded an excellent opportunity to change the eventuality of these women. Their diabetic pregnancies can be

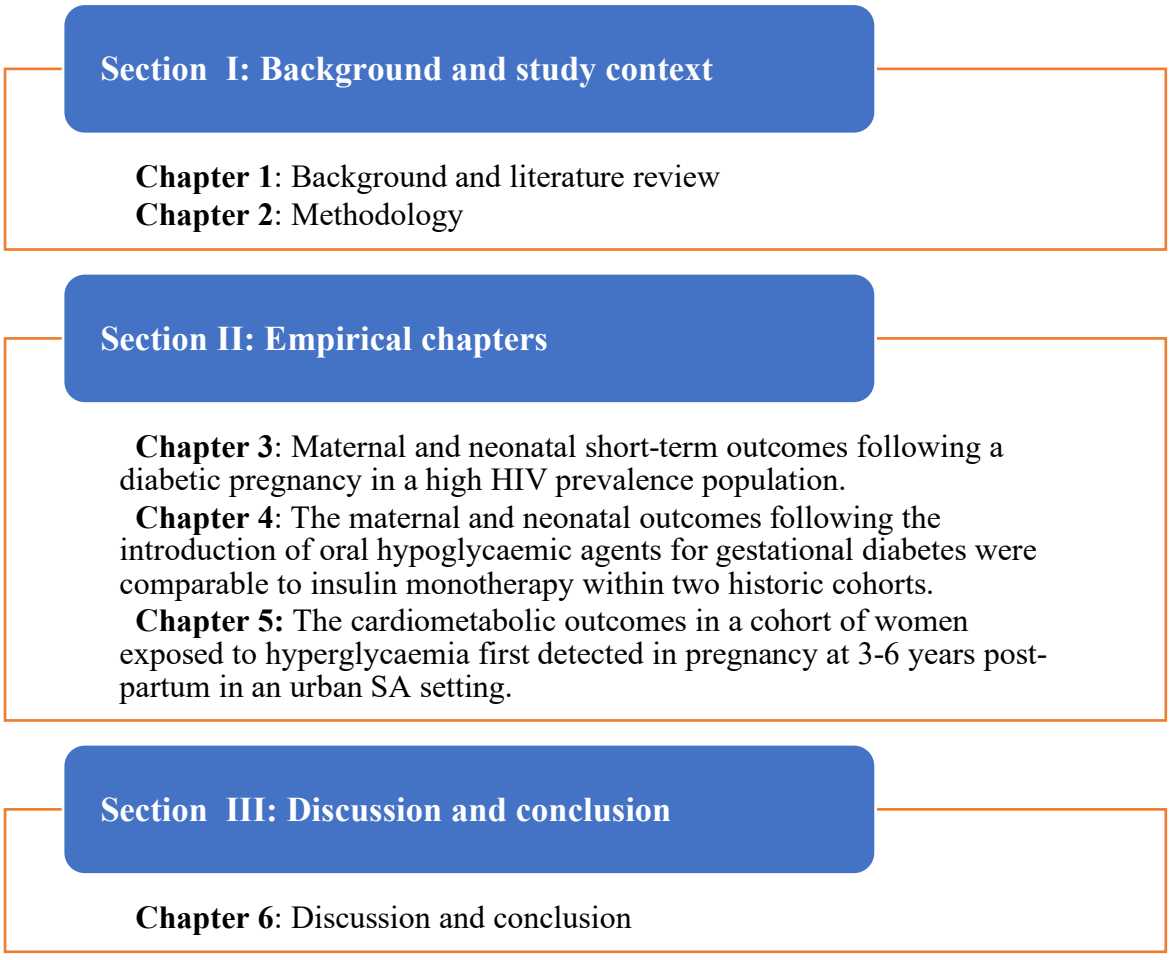
viewed as a window of opportunity to identify and potentially prevent the development of a burdensome and dreadful chronic illness if simple preventative strategies are employed during the peripartum period, including simple achievable measures which embrace lifestyle interventions like dietary measures and regular exercise. To achieve this goal, I realized that I needed to create a better awareness amongst these at-risk individuals, as well as health care professionals and public health services alike. In order to accomplish this, I needed to conduct research. The opportunity came knocking, when I received a call from Professor Shane Norris in August 2017, affording me the opportunity of joining his team at the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU), to embark on a PhD in the field of diabetes in pregnancy and its intermediate to long-term consequences. Of course, I was elated as this was a topic remarkably close to my heart. Furthermore, Professor Norris is renowned for his knowledge and interest in the field of maternal health. The time had finally arrived to make a difference in the lives of these women and their offspring by exploring their post-partum consequences with the goal of providing evidence to change public health policies to prevent various non-communicable diseases.

Now, I have reached the end of a very challenging but certainly rewarding journey and I have learnt that "*Life to me is a journey-you never know what may be your next destination*"

David Russel

Structure of the thesis

This thesis, which was pursued through three academic papers, can be divided into three main sections as indicated below. The introduction section provides context for the research aims through a thorough discussion of the background literature, and presentation of the conceptual framework and problem statement, as well as an overview of the methodology used. This section is followed by empirical findings presented as papers in chapters 3 through 5. The final section encompasses a general discussion that addresses the implications of the results, the study limitations, and proposes areas for future research.



Structure of this PhD thesis



Table of Contents

Declaration *ii*

Dedication *iii*

Acknowledgements *iv*

Thesis Material and Contributions *vi*

Funding and Awards *vi*

Presentations and publications *vii*

Abstract *ix*

Preface *xii*

Structure of the thesis *xiv*

List of Tables *xx*

List of Figures *xxi*

Nomenclature *xxii*

SECTION 1 BACKGROUND AND STUDY CONTEXT *1*

Chapter 1 *2*

1.1 Problem statement *2*

1.2 Conceptual framework *4*

1.3 Background and definitions *7*

1.4 Diagnostic criteria *8*

1.5 Prevalence *10*

1.6 Study context within South Africa *12*

1.7 Pathophysiology of gestational diabetes *14*

1.8 Health systems: Screening *16*

1.8.1 Antepartum screening 16

1.8.2 Post-partum screening 17

1.9 Health systems: Clinical management *17*

1.9.1 Antepartum 17

1.9.2 Post-partum 19

1.10 Significance of diabetes in pregnancy *20*

1.10.1 Short-term consequences 20

1.10.2 Long-term consequences 21

1.11 HIV and the risk of dysglycaemia and cardiovascular diseases 34
1.12 Gaps in the literature 35
1.13 Justification for the study 36
1.14 Overall aim 36
1.15 Hypotheses 37
Chapter 2- Methodology 38
2.1 Study setting 38
2.2 Study design and population 41
2.3 Data collection and management 42
2.3.1 Data collection instruments and measurements 47
2.3.2. Sample size 51
2.4 Ethical considerations 52
SECTION 2 53
Chapter 3- Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV. 54
3. 1 Introduction 55
3.2 Materials and Methods 56
3. 3 Results 57
3. 4 Discussion 60
Chapter 4 – The maternal and neonatal outcomes following the introduction of oral hypoglycaemic agents for hyperglycaemia first detected in pregnancy were comparable to insulin monotherapy within two historic cohorts. 71
4. 1. Introduction 72
4.2. Materials and methods 73
4.2.1 Study population 73
4.2.2 Definitions 73
4.2.3 Clinical Practice 75
4.3 Statistical methods 76
4.4. Results 76
4.4.1 Comparison of the two cohorts of HFDP patients 77
4.4.2 Effect of oral hypoglycaemic agents on maternal and neonatal outcomes within 2010-2014 cohort (Table 4.3) 77
4.5. Discussion 78
Chapter 5- Cardiometabolic outcomes of women exposed to hyperglycaemia first detected in pregnancy at 3-6 years post-partum in an urban South African setting 85

5.1 Introduction *86*

5.3 Sample size calculation *88*

5.4 Data collection *88*

5.4.1 Questionnaire 88

5.4.2 Anthropometrics 89

5.4.3 Biological samples and OGTT 90

5.4.4 Carotid and femoral imaging 90

5.4.5 Biochemistry and laboratory analyses 91

5.5 Outcomes *91*

5.6 Ethics *92*

5.6 Statistical analysis *92*

5.7 Results *93*

5.7.1 Baseline and follow up demographics, maternal factors, anthropometrics, and biochemical parameters between the groups. 93

5.7.2 Stratified analysis of maternal outcomes including diabetes, metabolic syndrome and cardiovascular risk based on exposure to HFDP (Table 5.2) 94

5.7.3 Body composition measures between the groups (Table 5.3) 95

5.7.4 Strength of association of HFDP exposure and progression to diabetes, MetS and high CVD risk (Table 5.4) 95

5.7.5 Predictors of FMI and progression to type 2 diabetes and cardiovascular risk using multiple variable logistic and linear regression. 96

5.7.6 HIV influence on outcomes 96

5.8 Discussion *97*

SECTION 3 *119*

Chapter 6- Discussion and Conclusion *120*

6.1 Consolidated findings *120*

6.2 Hypotheses revisited *124*

6.3 Emerging research themes *124*

6.3.1 Underestimation of cardiovascular risk in young women 124

6.3.2. Strengthening health care systems in South Africa 131

6.4 Theoretical relevance *136*

6.5 Implications *138*

6.6 Strengths and limitations *139*

6.7 Future studies *140*

6.8 Conclusion *142*

7.0 References *143*

8.0 Appendices *177*

APPENDIX A: AUTHORS/CO-AUTHORS DECLARATION *177*

APPENDIX B: MATERNAL INFORMATION AND CONSENT SHEETS *180*

APPENDIX C: QUESTIONNAIRE *186*

APPENDIX D: PROTOCOLS FOR BIOSPECIMEN COLLECTION *197*

APPENDIX E: PROTOCOL FOR PERFORMING OGTT AND PROCESSING OF BLOODS *214*

APPENDIX F: PROTOCOL FOR DXA MEASUREMENT *218*

APPENDIX G: PROTOCOL FOR CAROTID AND FEMORAL INTIMA MEDIA THICKNESS ASSESSMENT *221*

APPENDIX H: ETHICS CLEARANCE CERTIFICATES *226*

APPENDIX I: PUBLISHED PAPER I *229*

APPENDIX J: PUBLISHED PAPER II *238*

APPENDIX K: SUBMITTED PAPER III *243*

APPENDIX L: PLAGIARISM DECLARATION *291*

List of Tables

Table 1. 1 Varying diagnostic criteria for HFDP 9
Table 1. 2 IADPSG criteria utilized for the diagnosis of HFDP. 10
Table 1. 3 Common risk factors for gestational diabetes mellitus (79) 17
Table 1. 4 Adverse outcomes associated with uncontrolled hyperglycaemia in pregnancy. 21
Table 1. 5 HAPO study primary and secondary outcomes 22
Table 1. 6 Long-term consequences following HFDP. 22
Table 1. 7 Summary of systematic reviews for progression to T2DM, CVD and MetS following HFDP. 33

Table 2. 1 Statistical analysis overview 43
Table 2. 2 Overview of various cardiovascular risk prediction models 49
Table 2. 3 The sample size required to determine the following outcomes among black SA women with a history of HFDP 52

Table 3. 1 Demographic, obstetric, comorbidity, and diabetes characteristics of participants with T1DM, T2DM, and HFDP. 64
Table 3. 2 Neonatal and maternal outcomes of participants with T1DM, T2DM, and HFDP. 65
Table 3. 3 Comparison of baseline characteristics in metformin monotherapy and glibenclamide-exposed groups versus insulin monotherapy. 66
Table 3. 4 a. Definition and criteria for diagnosis of diabetes in pregnancy, including T1DM, T2DM, and HFDP. b. Definition of maternal and neonatal outcomes. 67

Table 4. 1 Comparison of maternal characteristics, glycaemic control, and outcomes between 1992-2002 and 2010-2014 HFDP pregnancy cohorts as diagnosed by 100g oral glucose tolerance test and random plasma glucose 81
Table 4. 2 Maternal and neonatal outcomes for the 1992-2002 and 2010-2014 HFDP pregnancy cohorts 82
Table 4. 3 Comparison of oral agents vs. insulin on maternal and neonatal outcomes 83

Table 5. 1 Maternal demographics, characteristics and outcomes at index pregnancy and follow-up. 100
Table 5. 2 Stratified analysis of maternal outcomes by exposure to HFDP. 104
Table 5. 3 Maternal body composition outcomes by exposure to HFDP. 107
Table 5. 4 Frequency of the outcome's diabetes, metabolic syndrome and CVD event as predicted by FRS and the adjusted odds ratio for the association with a history of HFDP 109
Table 5. 5 Linear regression model for log-transformed FMI. Values are standardized regression coefficients (β) with 95% confidence interval, and p-values. 110
Table 5. 6 Multivariate analysis of factors associated with risk of progression to diabetes. Values are standardized odds ratios (OR) with 95% confidence interval, and p-value..... 112
Table 5. 7 Linear regression model for log-transformed carotid intima media thickness (cIMT). Values are standardized regression coefficients (β) with 95% confidence interval, and p-value. 114

Table 5. 8 Definitions of diagnostic criteria for HFDP, maternal pregnancy factors and outcomes..... 116

Table 6. 1 Summary table of objectives and findings..... 121

List of Figures

Figure 1. 1 Conceptual model adapted (21)..... 6
Figure 1. 2 Timeline of evolution of criteria to diagnose HFDP 1964-present (36) 8
Figure 1. 3 Median (interquartile range) prevalence (%) of HFDP by WHO region, 2005–2015 (48) 11
Figure 1. 4 Country-specific prevalence of HFDP according to different diagnostic criteria (47)..... 12
Figure 1. 5 Leading causes of mortality with percentages (%), diabetes mellitus being the second commonest (55). 13
Figure 1. 6 Changes in glucose homeostasis during pregnancy (66). 15
Figure 1. 7 Potential major determinants for the progression from HFDP to T2DM and other co-morbidities based on a life course perspective (22) 23
Figure 1. 8 Modifiable and non-modifiable risk factors shared by CVD and T2DM (137) ... 26
Figure 1. 9 Natural history of cardiovascular diseases on the background of various risk factors (144) 27
Figure 1. 10 Associations between pre-pregnancy risk factors, adverse pregnancy outcomes, and post pregnancy cardiometabolic risk factors and outcomes (84) 30
Figure 1. 11 Intergenerational cycle of diseases following HFDP. 34

Figure 2. 1 Map displaying Soweto, Johannesburg in the Gauteng province..... 40
Figure 2. 2 Flow diagram of the study design 46

Figure 3. 1 Neonatal outcomes in metformin monotherapy and glibenclamide-exposed groups versus insulin monotherapy in participants with T2DM and HFDP. 68
Figure 3. 2 Neonatal outcomes in HIV-infected and uninfected participants with T1DM, T2DM, and HFDP. 69
Figure 3. 3 Flow chart of clinical practice for patients attending the gestational endocrine clinic. 70

Figure 5. 1 Flow diagram of the study and eligible participants 118

Figure 6. 1 Prevention options in women with gestational diabetes mellitus (136)..... 130
Figure 6. 2 Conceptual framework revisited 137

Nomenclature

ACOG	American College of Obstetricians and Gynaecologists
ADA	American Diabetes Association
ANOVA	Analysis Of Variance
aOR	Adjusted odds ratio
BMI	Body mass index
CAD	Coronary artery disease
CCA	Common carotid artery
CHBAH	Chris Hani Baragwanath Academic Hospital
cIMT	Carotid intima media thickness
CS	Caesarean section
CT	Computerised tomography
CRP	C-reactive protein
CVD	Cardiovascular disease
DIP	Diabetes in pregnancy
DPHRU	Developmental Pathways for Health Research Unit
DXA	Dual energy X-ray absorptiometry
FIGO	International Federation of Gynaecology and Obstetrics
fIMT	Femoral intima media thickness
FMI	Fat mass index
FRS	Framingham risk score
GDM	Gestational diabetes
GDP	Gross domestic product
HAART	Highly active antiretroviral therapy
HAPO	Hyperglycaemia and Pregnancy Outcome
HbA1c	Glycated haemoglobin
HC	Hip circumference
HDL	High-density lipoprotein
HDP	Hypertensive disorders of pregnancy
HFDP	Hyperglycaemia first detected in pregnancy
HIV	Human Immunodeficiency Virus
HMIC	High to middle income countries
HTN	Hypertension

hsCRP	High sensitivity C-reactive protein
IADPSG	International Association of Diabetes and Pregnancy Study Group
ICAM-1	Intercellular Adhesion Molecule 1
IDF	International Diabetes Federation
IL-6	Interleukin 6
LBW	Low birth weight
LDL	Low-density lipoprotein
LGA	Large for gestational age
LMIC	Low to middle income countries
MRC	Medical research council
MRI	Magnetic resonance imaging
MetS	Metabolic syndrome
NCD	Non-communicable diseases
NDDG	National Diabetes Data Group
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health Care Excellence
OGTT	Oral glucose tolerance test
OHA	Oral hypoglycaemic agents
PAI-1	Plasminogen activator inhibitor-1
PIH	Pregnancy-induced hypertension
PNM	Perinatal mortality
PWV	Pulse wave velocity
RCT	Randomised controlled trial
RDS	Respiratory distress syndrome
REDCap	Research Electronic Data Capture
SA	South Africa
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SES	Socioeconomic status
SGA	Small for gestational age
SSA	Sub-Saharan Africa
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TG	Triglycerides

TNF-α	Tumour necrosis factor alpha
USA	United States of America
VAT	Visceral adipose tissue
VCAM-1	Vascular cell adhesion protein 1
WC	Waist circumference
WHR	Waist: hip ratio
WHO	World Health Organisation

Units and Symbols

The International System of Units (SI) has been used throughout this thesis. Of note, glucose is reported in mmol/l throughout this thesis.

%	percentage
>	greater than
<	smaller than
≥	greater than or equal to
±	plus/minus
g	gram
hr	hour
kg	kilogram
kg/m^2	kilogram per square metre
km^2	square kilometre
mmol/L	millimoles per litre
mmHg	millimetre of mercury

SECTION 1 BACKGROUND AND STUDY CONTEXT

"Wherever the art of medicine is loved, there is also a love of humanity".
Hippocrates

Chapter 1

1.1 Problem statement

Worldwide, type 2 diabetes mellitus (T2DM) remains an important area of public health concern since it is one of the major causes of early illness and death. The increasing global prevalence remains concerning as it contributes to an increasing health and financial burden around the world. Estimates from the International Diabetes Federation (IDF) suggests that diabetes affects about 463 million people globally which is projected to increase to 700 million people by 2040 (1), with the prediction that Africa will be subject to the greatest increase in numbers. In 2014, 14.2 million adults aged 20–79 years had diabetes, with over two-thirds of them being unaware of their diabetic status (2). Women are not immune from this with 199 million of them currently living with diabetes, with predictions that this will increase to 313 million by 2040 (1). The prevalence of T2DM in African women doubled, from 4.1% in 1980 to 8.9% in 2014 (3). A recent systematic review exploring the prevalence of dysglycaemia in African women of childbearing age, found the prevalence of T2DM was 7% (4). This growing burden of diabetes, is mostly driven by epidemiologic, demographic, and nutritional changes (5,6).

Whilst type 1 diabetes mellitus (T1DM) and T2DM is a state of glucose intolerance occurring outside of pregnancy, gestational diabetes (GDM), recently renamed by the World Health Organization as hyperglycaemia first detected in pregnancy (HFDP), collectively defines it as "any degree of glucose intolerance with onset or first recognition during pregnancy" (7,8).This includes glucose readings that fall within the impaired glucose tolerance (IGT) diagnostic range termed "true" GDM, as well as those within the diagnostic range for diabetes termed "overt" GDM or diabetes in pregnancy(DIP)(9).The prevalence of HFDP, the most commonly encountered hyperglycaemic disorder encountered in pregnancy has doubled in the past 10-15 years owing to the obesity epidemic (10,11) . Sadly, little is known about prevalence rates of HFDP in Africa, with figures varying according to different regions partly due to differences in study populations, survey methodology, and diagnostic methods used (12). A major barrier to appreciating the burden of HFDP in both developed and developing countries is the lack of consensus on the definition of HFDP as well as the absence of universally accepted standards for diagnosis and screening (13). Additionally, HFDP plays a minor role in the shadow of more obvious determinants of maternal and fetal morbidity in low-resource settings. It has only more recently been acknowledged in developing countries as a public health issue given its far-

reaching ramifications for mother and child alike. Alongside the well-established adverse short-term outcomes of poorly managed or untreated disease, HFDP, more specifically GDM, can have a marked impact on the future health of both mothers and their offspring, with HFDP playing a significant role in the global diabetes epidemic together with increasing cardiovascular risk and disease (CVD) and development of the metabolic syndrome (MetS) in these young women (14–16). Epidemiological evidence has consistently shown that women with a prior history of HFDP have a 30-84% chance of having a recurrence of HFDP in a subsequent pregnancy (17,18), 20-40% chance of developing MetS within 2-20 years (16)and 17-63% developing T2DM within 5-15years (14). Furthermore, if a woman with diabetes becomes pregnant, her offspring is at an increased risk of developing T2DM, obesity and CVD in adulthood, (19) thereby accelerating the intergenerational risk of T2DM, obesity and CVD. This has implications for the health of the population. Elucidating the causes of these disorders, the prediction and possible prevention or delay of the development of these chronic disease states is of paramount importance. Raising public awareness of this condition and the risks associated with it, remain an important area of public health and this will certainly pave the way for improved maternal and fetal health.

A further novel area of interest is the growing problem of diabetes amongst those with human immunodeficiency virus (HIV). Following the successful implementation of combination antiretroviral therapy (ART), and the resultant prolongation of life expectancy, several chronic conditions including diabetes mellitus and CVD has emerged (19). The mechanisms of these are multifactorial and includes shared environmental risk factors for diabetes such as sedentary lifestyle as well as HIV-specific effects encompassing chronic inflammation and ART related factors (20). The risk of developing diabetes together with other metabolic perturbations, such as dyslipidaemia and insulin resistance, places these individuals at risk for CVD. Taken together, HFDP and HIV co-existing in pregnancy potentially identifies a population of young women at risk of T2DM and CVD, which is of particular relevance in low to middle income countries (LMIC) where the heaviest burden of the HIV epidemic is felt. Furthermore, the contribution of HIV on the background of HFDP and the risk of developing diabetes and CVD is not studied or known.

In conclusion, the diabetes and obesity epidemics together with increasing CVD among women of reproductive age, poses an enormous public health challenge globally. Both the immediate burden on maternity health services, as well as the transgenerational and population level

impact of these metabolic diseases, highlights the need to focus on better antenatal detection and management together with screening and interventional measures during the post-partum period of this condition if we are to curtail this public health impact.

1.2 Conceptual framework

The framework for this study depicted in Figure 1.1, which was adapted from Zhang, C. *et al.* (21), has been designed and structured around the existing body of knowledge in the field of HFDP, mostly extracted from high to middle income countries (HMIC). Our setting which derives from a LMIC, is unique in that our population is predominantly Black African with a high burden of HIV disease. Furthermore, socioeconomic transitions have exposed women to deleterious living conditions, poor dietary habits, and sedentary behaviours. The indirect consequences of both the unique environmental and societal factors among these black women and how they shape the maternal trajectories of the measured outcomes are unknown.

On this background, the conceptual framework was designed to incorporate all these distinctive aspects together with acknowledging the significant role of HFDP exposure and how this influences the development of cardiometabolic diseases. The framework adopts a life-course approach and recognises the potential independent influence of non-modifiable and modifiable pre-conception, antenatal and post-partum maternal factors on these outcomes. Non-modifiable factors include ethnicity, age and genetics whilst modifiable pre-conception factors which may interact and influence both immediate and short-term maternal outcomes include maternal body mass index (BMI), co-morbidities including hypertension (HTN), as well as various socioeconomic factors (22,23). Additionally the HIV epidemic which disproportionately affects women in South Africa (SA) 26% (24), is another factor that cannot be ignored; its influence within this context has yet to be explored. Antenatal factors, including disease-related factors including severity of hyperglycaemia and management together with other pregnancy co-morbidities like maternal BMI contribute to these outcomes. Post-partum factors like weight gain and breastfeeding have also been shown to influence the outcomes measured (26–29). For the purposes of this study each component of the life-course model will be explored within the context of exposure to HFDP in terms of its role on the outcomes in an attempt to disentangle various mediators from HFDP exposure. Whilst this study aims to explore and define the post-partum cardiometabolic consequences following HFDP, including progression to T2DM and MetS as well as assessing cardiovascular risk/disease by utilising a life-course approach, the additional interplay of HIV and ART on this relationship will be

evaluated, given the known metabolic perturbations including insulin resistance, diabetes and dyslipidaemia in HIV-infected adults since the introduction of effective ART.

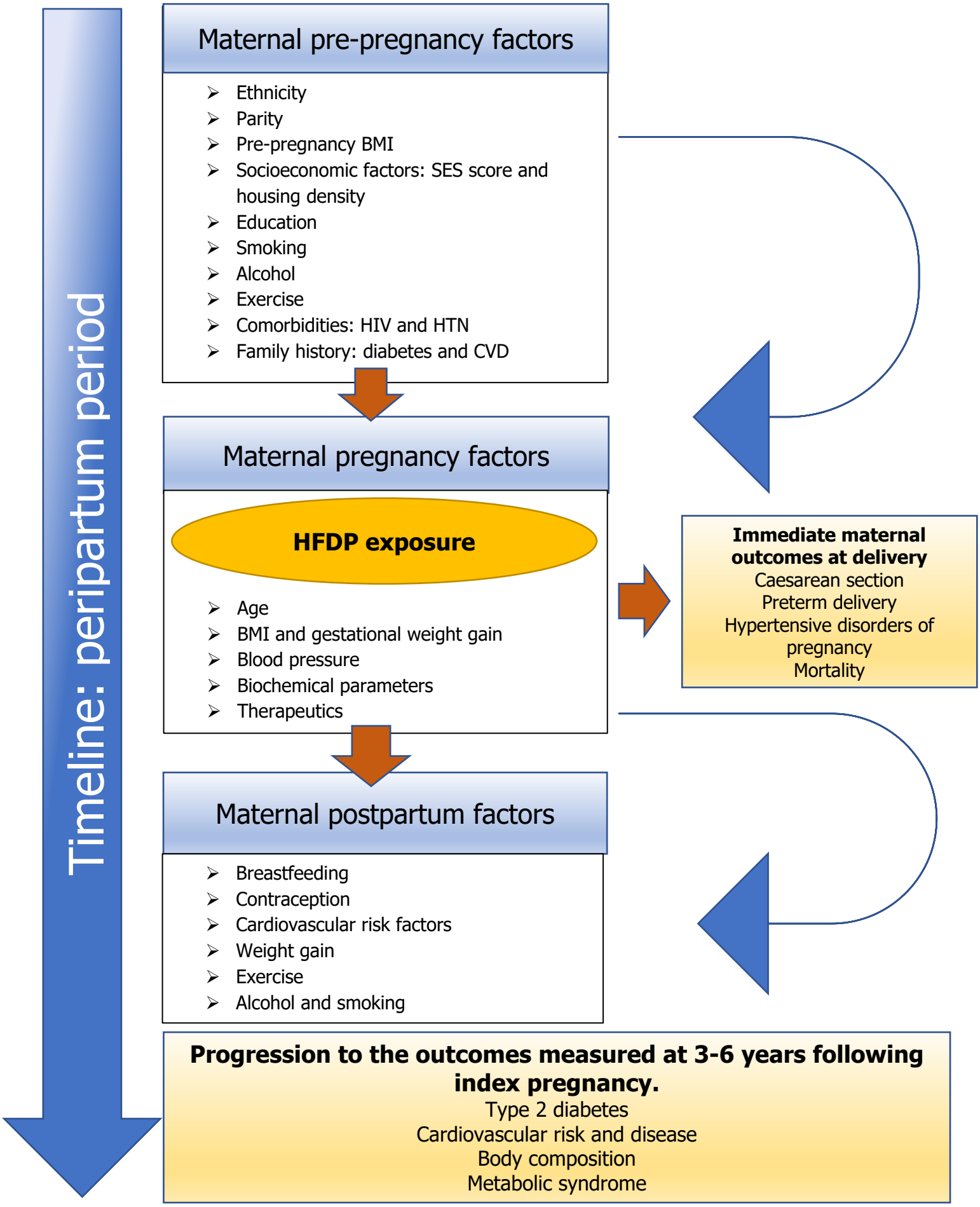


Figure 1. 1 Conceptual model adapted (21)

1.3 Background and definitions

Since the first description of diabetes in pregnancy in 1824 by Bennewitz in Germany (29), the landscape of HFDP has remained a dynamic entity with the prevalence, definitions, screening protocols and diagnostic criteria continuously changing from as early as the 1960's. The lack of a global approach to HFDP amongst both international and local organisations has created havoc in this field with the ultimate result being variations in prevalence and practice within countries. Further compounding this problem is the fact that LMIC, including sub-Saharan Africa (SSA) often must adopt different strategies given their resource poor infrastructure and unavailability of diabetes care services. There remains a lot of controversy surrounding HFDP, from classification to screening to diagnosis and treatment.

The prior definition of GDM as a glucose-intolerant state with onset or first recognition during pregnancy (30) was recently challenged by the International Association of Diabetes in Pregnancy Study Group (IADPSG) (8). They appreciated that a significant proportion of women with undiagnosed pre-existing diabetes (DIP) were being referred to as "GDM" when it was clearly evident that these women have diabetes which incidentally was first detected upon screening during pregnancy. The evidence for this being their degree of hyperglycaemia or their early manifestation (before 24 weeks). The varying terminology adopted for describing HFDP varies among national organisations. More recently, the World Health Organisation (WHO) (7), American Diabetes Association (ADA) (31) and the International Federation of Gynaecology and Obstetrics (FIGO) (32), have adopted the IADPSG recommendations to differentiate between pregnant females with probable pre-existing diabetes first manifesting in pregnancy, referred to as "overt" diabetes or DIP, versus, a transient manifestation of pregnancy-related insulin resistance, referred to as "true" GDM, which mostly develops during the second half of pregnancy. This new definition broadly grouped together as HFDP intends to differentiate between the varied severities of HFDP, with GDM being distinguished as the milder degree of HFDP and DIP the more serious form.

For the purpose of this thesis, the terms pre-gestational diabetes in pregnancy (pre-conceptional type 1 or type 2 diabetes) and HFDP (HFDP) which includes "overt" GDM/ (DIP), and "true" GDM will be used to distinguish the different types of diabetes. HFDP was diagnosed according to the internationally recommended IADPSG criteria following risk-factor based screening, which is in line with the local clinical practice at the Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, Johannesburg. It is important to note, that prior to

the introduction of the term HFDP, most studies used the term GDM to describe any hyperglycaemia detected in pregnancy, hence most likely including the DIP subgroup. Current standing is that most regional bodies have moved towards adopting the latest WHO guidelines (7).

1.4 Diagnostic criteria

The ongoing debate as to the optimal approach for the diagnosis of HFDP has resulted in a variety of diagnostic criteria being endorsed by different organisations (Table 1.1). Historically, diagnostic testing and thresholds for HFDP were in existence from the early 1960's. The original diagnostic criteria for HFDP by O'Sullivan and Mahan (33) were not based on the perinatal risk but instead were based on the maternal risk of developing diabetes later in life. Since then, there has been a shift to changing the criteria to focus on infant outcomes with two general oral glucose tolerance test (OGTT) tests predominating, one based on a 2-hour, 75g OGTT with two plasma glucose values and diagnostic criteria like those used outside of pregnancy(34) , and another one based on a 100g 3-hour OGTT, with four pregnancy-specific plasma glucose cut-offs (35). Figure 1.2 outlines a historical timeline regarding the introduction of the different diagnostic tests advocated by varying international authorities.

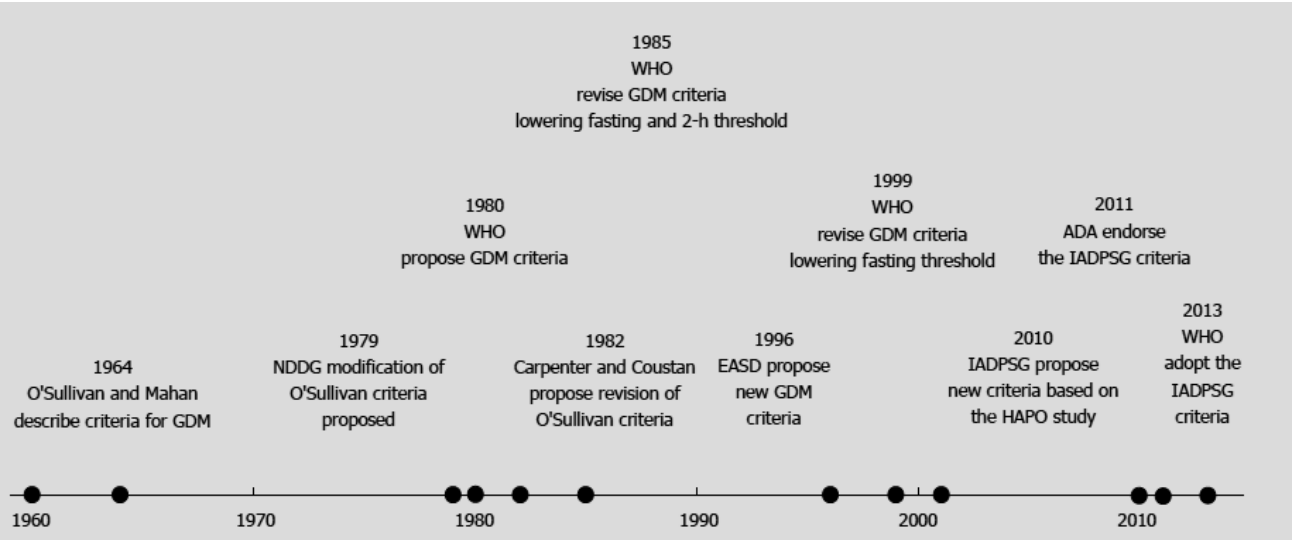


Figure 1 Timeline of evolution of criteria used to diagnose gestational diabetes from 1964-present. NDDG: National Diabetes Data Group; WHO: World Health Organisation; EASD: European Association for the Study of Diabetes; ADA: American Diabetes Association; IADPSG: International Association of Diabetes and Pregnancy Study Groups; GDM: Gestational diabetes; HAPO: Hyperglycemia and pregnancy outcomes study.

Figure 1. 2 Timeline of evolution of criteria to diagnose HFDP 1964-present (36)

To date, an absence of a scientific consensus remains regarding an optimal cut-off point to diagnose women with HFDP. Different authorities utilise different glucose challenge tests with varying glucose thresholds, which has led to much confusion and inconsistencies amongst countries resulting in differences in health system practices. After several international workshops and many decades of research, there is still no unified global approach to HFDP.

Table 1. 1 Varying diagnostic criteria for HFDP

	Diagnostic test	Blood glucose values (mmol/L) FPG	1hr	2hr	3hr	Diagnostic criteria
O'Sullivan and Mahan criteria 1964	100g OGTT (3 hour)	5.0	9.2	8.1	6.9	2 or more values above limit
O'Sullivan and Mahan criteria 1973	50g OGTT (1 hour)		7.2			If 1hr diagnostic threshold exceeded, to perform 100gm OGTT
National Diabetes Data Group (NDDG), 1979	100g OGTT (3 hour)	5.8	10.6	9.2	8.1	2 or more values above limit
Carpenter and Coustan,1982	100g OGTT (3 hour)	5.3	10.0	8.6	7.8	2 or more values above limit
World Health Organization (WHO),1999	75g OGTT (2 hour)	7.0		7.8		1 value above limit
American Diabetes Association (ADA), 2009	100g OGTT/ 75g OGTT (2 hour)	5.3	10.0	8.6		2 or more values above limit
IADPSG 2010	75g OGTT (2 hour)	5.1	10.0	8.5		2 or more values above limit
WHO 2013	75g OGTT (2 hour)	5.1	10.0	8.5		2 or more values above limit

SEMDSA (SA) 2012	75g OGTT (2 hour)	7.0		7.8		As per WHO 1999
SEMDSA (SA) 2017	75g OGTT (2 hour)	5.1	10.0	8.5		As per IADPSG 2010

Abbreviations: IADPSG International Association of Diabetes and Pregnancy Study Group, WHO World Health Organisation, SEMDSA Society for Endocrinology, Metabolism and Diabetes of South Africa, FPG fasting plasma glucose

Following the outcomes of the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study, in which a linear relationship between blood glucose and adverse pregnancy outcomes was demonstrated (37), the IADPSG proposed lower diagnostic cut-offs thresholds to indicate a positive diagnosis which has subsequently been adopted by the WHO in 2013 (7) and other organisations. This landmark study investigated the adverse maternal and neonatal outcomes associated with various degrees of hyperglycaemia following a 75g OGTT, in order to work towards an international consensus for outcome-based criteria for HFDP. The resulting IADPSG criteria, shown in Table 1.2 distinguish between GDM and DIP diagnosed in pregnancy. The diagnostic threshold for GDM is lower than previously used criteria such as the Carpenter and Coustan (35)or National Diabetes Data Group (NDDG) criteria for a 100g OGTT(38). Eighteen percent of women would have met criteria for HFDP if we applied the IADPSG criteria retrospectively to the HAPO cohort (39).

Table 1. 2 IADPSG criteria utilized for the diagnosis of HFDP.

	GDM	DIP
Fasting plasma glucose	≥5.1 mmol/L	≥7.0 mmol/L
One-hour plasma glucose	≥10.0 mmol/L	
Two-hour plasma glucose	≥8.5 mmol/L	≥11.1 mmol/L
Random plasma glucose / HbA1c		≥11.1 mmol/L/ ≥6.5%

Abbreviations: GDM gestational diabetes, DIP diabetes in pregnancy

1.5 Prevalence

Over the years an increase in the worldwide prevalence of HFDP has been observed. This increase appears to parallel the increasing prevalence of T2DM and obesity. Whilst HFDP prevalence is known to be influenced by ethnicity and genetic susceptibility, the nutrition

transition is a significant contributing factor towards this increased prevalence (40). According to the International Diabetes Federation (IDF), in 2017 there were approximately 131.4 million live births globally to women aged 20-49 years. Of those births, 16.2% (21.3 million) were affected by hyperglycaemia during pregnancy; 86.4% involved GDM, 7.4% involved overt diabetes first detected in pregnancy (DIP) and 6.2% involved pre-existing diabetes diagnosed before pregnancy (42).

Although variabilities exist across countries for HFDP screening (43) and diagnostic cut-off points for the OGTT (44), reported prevalence's are 2%–6% for Europe (43), 7% for North America (32), and 1%–9% and 4%–24% for white British and South Asian women, respectively, in England and Southern Ireland (45). Of concern is that almost 90% of cases of HFDP occur in LMIC, where access to maternal healthcare is limited (46). This was highlighted in a recent review (47) which included 77 studies representing both HMIC and LMIC in an effort to appreciate the global prevalence of HFDP. Overall, North Africa and the Middle East were found to have the highest prevalence of HFDP with Europe having the lowest (Figure 1.3).

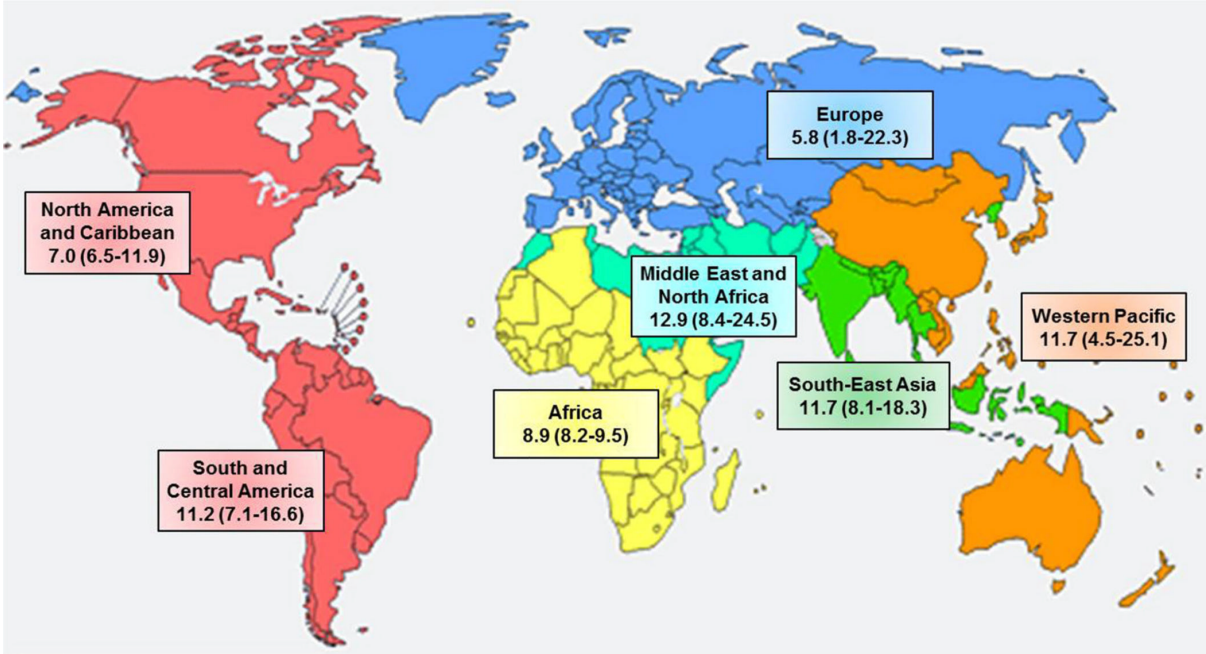


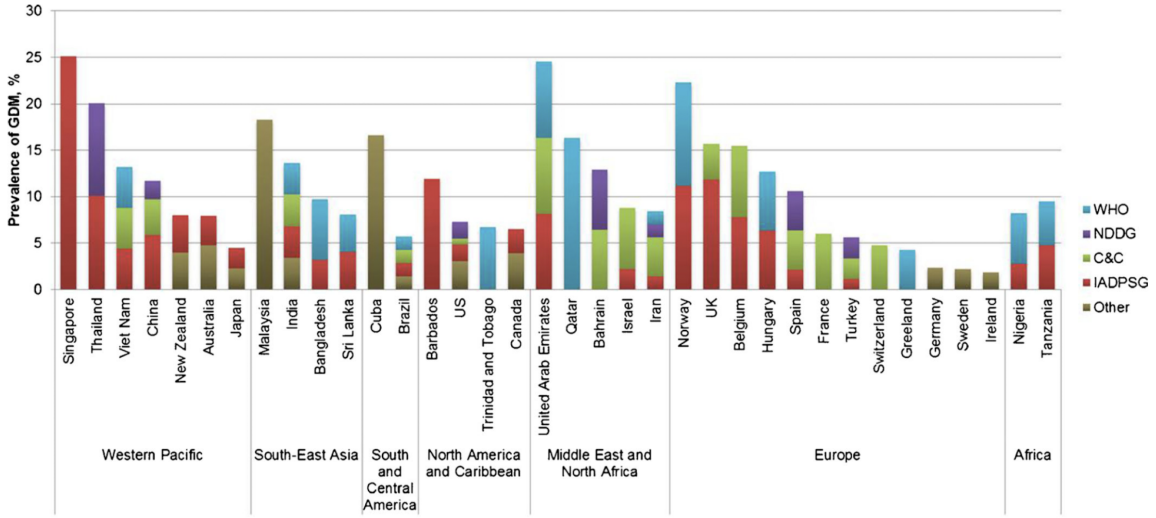
Figure 1. 3 Median (interquartile range) prevalence (%) of HFDP by WHO region, 2005–2015 (48)

Since prevalence estimates are subject to diagnostic criteria applied, the same study specified varying diagnostic criteria when giving country specific estimates with notable variations within and between countries (Figure 1.4). Since the introduction and widespread adoption of

the IADPSG criteria, in which a lower threshold of glucose is used to diagnose HFDP, the prevalence of HFDP has further increased by two to threefold and even up to sevenfold compared to the previous criteria (49–51).

In Africa, limited representation and varying diagnostic criteria limit the accuracy of determining the prevalence as shown by a metanalysis in 2014 which demonstrated that the prevalence is highly variable ranging from 0-14% (11) A more recent metanalysis (52), including 22 studies adopting the latest diagnostic criteria indicated that the pooled prevalence for Africa was 13.6% and 14.2% for sub-Saharan Africa (SSA).

Overall, prevalence estimates across populations are fraught with difficulties with numerous factors at play. This includes the employment of varying screening approaches and diagnostic criteria as well as differing inherent characteristics of the study population.



Abbreviations: C &C Carpenter and Coustan criteria, IADPSG International Association of Diabetes and Pregnancy Study Groups, NDDG National Diabetes data groups, WHO World Health Organisation, other included International Classification of Diseases codes and local guidelines or criteria

Figure 1. 4 Country-specific prevalence of HFDP according to different diagnostic criteria (47)

1.6 Study context within South Africa

Urbanisation and recent socio-economic changes in SA have fuelled a rapid nutritional transition and an epidemiological shift especially in the urban black population (53). The nutrition transition is characterised by a rapid shift towards high fat and sugar consumption,

refined foods, acculturation, and increased physical inactivity (54). This is accompanied by a dramatic shift in the burden of disease from communicable disease like HIV to non-communicable diseases (NCD) like diabetes and HFDP. In practice however, countries in transition are found to display a double burden of NCDs and persistent infectious diseases as well as introduction of new or re-emerging infectious diseases such as HIV and tuberculosis. Furthermore, this burden is amplified by a lack of access to diabetes prevention, diagnosis and appropriate management (55), with an estimated 70% of people with diabetes in Africa being unaware of their diagnosis (42). The overall impact of this is highlighted by the fact that diabetes is the second leading cause of death in SA and the top cause of death amongst SA females (56) (Figure 1.5).

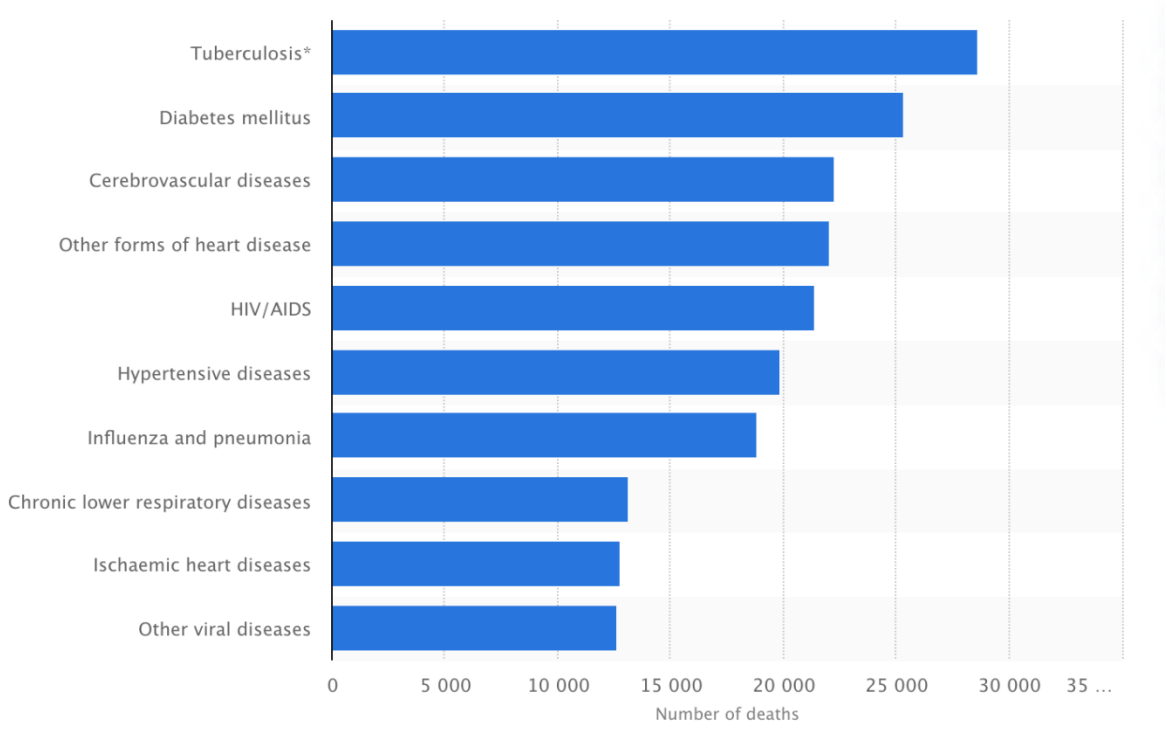


Figure 1. 5 Leading causes of mortality with percentages (%), diabetes mellitus being the second commonest (55)

The knowledge surrounding HFDP is slowly gaining momentum, however, detection of disease and access to specialist care facilities remain limited for the majority of pregnant women with diabetes and as a result these women and their offspring experience poor outcomes and high complication rates. Prevalence rates are limited to those areas with tertiary care facilities with currently only a few local prevalence studies having been conducted for HFDP in SA. Two small studies (56,57) found the prevalence of HFDP to be 8.8% in some regions,

though this figure is most likely an under-representation given the lack of universal screening (1)and poor referral of high-risk patients for screening from the local clinics to tertiary centres. Two more recent studies from Johannesburg, employing universal screening and the same diagnostic criteria , found varying prevalence's of GDM with 25% (1) found at a local clinic, and 9% at a tertiary hospital in the Soweto region(59). Laboratory inaccuracies may have accounted for these varying prevalence's.

Universal screening for HFDP is currently not adopted in SA (60), given our poor and often lacking resources. The detection of HFDP at best, is based on maternal risk-factor identification at the local clinics followed by referral to a specialist centre for the performance of an OGTT. Furthermore, disparities exist in screening methods and selection of risk factors which are used for screening methods throughout the various centres in the country. Needless to say, HFDP in SA remains under-reported (58) and for the most part poorly managed.

Though our knowledge surrounding the burden, determinants, and immediate consequences of HFDP in SA is growing, the appreciation of its long-term consequences is poorly explored in our setting. Understanding and appreciating the adverse health consequences of HFDP creates an informed platform for developing health interventions for a condition which is aggressively fuelling the NCD burden. Our study seeks to investigate the cardiometabolic profiles of women with a history of HFDP 3-6 years following their delivery compared to women nonexposed to HFDP on the backdrop of HIV.

1.7 Pathophysiology of gestational diabetes

Several metabolic and hormonal adaptations occur during pregnancy to ensure adequate maternal transfer of nutrients to the growing foetus. Physiologically, as the pregnancy progresses, an increase in insulin resistance is naturally observed, peaking during late pregnancy (61,62). This is due to the maternal tissues, particularly skeletal muscle and adipose tissue, becoming less sensitive to insulin and as a result maternal glucose disposal decreasing by approximately 50% (63). As glucose readily crosses the placenta (62,65), the insulin-resistant state prevents or reduces maternal somatic tissues from absorbing the blood glucose thus shunting it to the foetus. In response to the insulin resistant environment, the maternal pancreatic β-cells increase insulin secretion in order to maintain glucose homeostasis; insulin secretion is thought to increase by 200-250% in a normal pregnancy (62). Figure 1.6 illustrates

how maternal insulin sensitivity decreases as pregnancy progresses. This results in more insulin production which facilitates glucose being shunted to the foetus. The increased insulin resistance of pregnancy subsides very quickly following delivery suggesting that pregnancy-related hormones are key factors in achieving the insulin-resistant state.

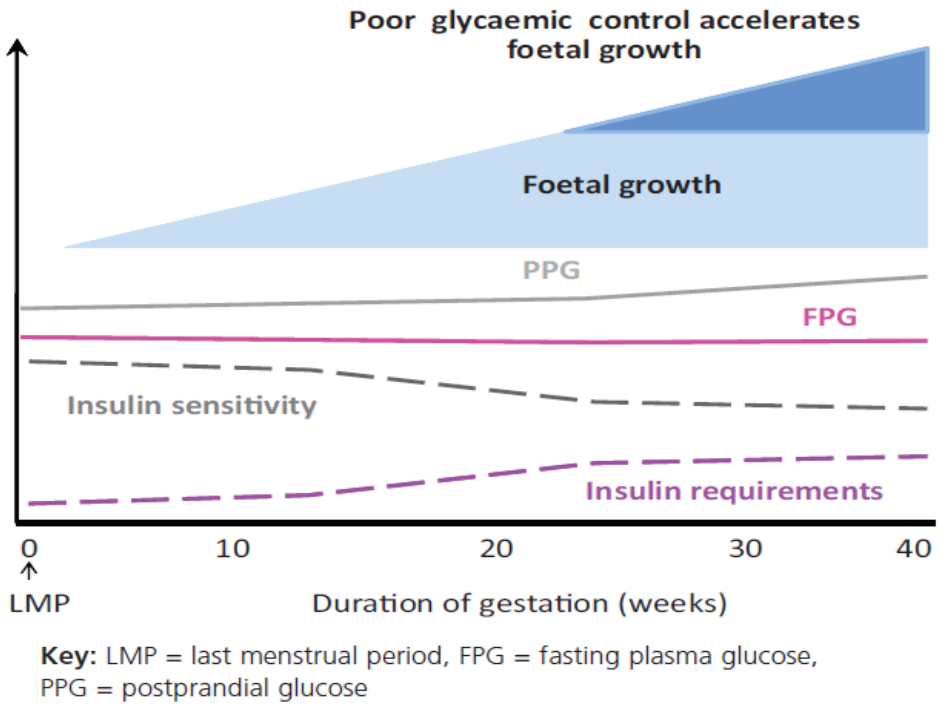


Figure 1. 6 Changes in glucose homeostasis during pregnancy (66)

Pregnancy can be viewed as a "stress test" for the development of glucose intolerance. The hormonal changes of pregnancy may incite an unmasking of the genetic predisposition for T2DM in women manifesting with GDM. Appearance of many hormones, including human placental growth hormone human placental lactogen, oestrogen, progesterone, prolactin, cortisol, and tumour necrosis factor-alpha (TNF-α), particularly during late pregnancy, triggers a state of insulin resistance by counteracting the effects of insulin thereby increasing insulin requirements. The promotion of a state of insulin resistance and hence a diabetogenic environment is incurred through these changes in the hormonal milieu along with the manifestation of subclinical inflammation (64,67). Exaggeration of this state in a pregnant woman ultimately manifests as GDM. Major determinants for GDM are the result of both pancreatic β-cell insufficiency and increased insulin resistance, with genetic factors and other socioeconomic and lifestyle processes contributing too (68). Gestational diabetes mellitus usually resolves after delivery with approximately 95% of affected women reverting to normal

glucose metabolism after delivery of their babies. The removal of the placenta and pregnancy hormones thought to facilitate the insulin-resistant state allows for normal glucose metabolism to be reinstated (69).

1.8 Health systems: Screening

1.8.1 Antepartum screening

The issue of universal versus risk-factor based screening remain an area of controversy and is mostly dictated by different organisations based on local prevalence rates and resource availability. Ideally, universal screening should be applied to high-risk pregnant women in order to identify all potential HFDP cases. However, cost-constraints often dictate a more conservative approach with selective screening predominating. A recent study performed in SA where risk-based screening is mostly employed, showed that adopting universal screening locally would significantly increase HFDP prevalence rates (59), which has significant implications in a country with limited resources.

The epidemiological studies of risk factors for HFDP are limited and are influenced by numerous confounders (70,71). The comparison of different findings across studies remains challenging given the inconsistencies in diagnostic criteria and measurements of risk factors. Despite this several risk factors emerge consistently and unsurprisingly, overlap with those for T2DM. These include, age, obesity, family history of T2DM and certain ethnic heritages (72) More specific to HFDP are, a prior history of HFDP, previous fetal macrosomia and previous unexplained perinatal death (Table 1.3)(52) . Interestingly one strong risk factor for HFDP includes varying ethnicity (73). These ethnic/racial disparities have best been appreciated in countries like USA, Canada and Australia in which multi-ethnic populations are present (70,71). Women found to have any one of these risk-factors should be offered a screening test for HFDP. Of importance, up to 20% of women diagnosed with HFDP have no risk factors(74) with a recent meta-analysis suggesting that a risk-factor based screening approach is a poor predictor of women who have an abnormal glucose tolerance test (75). The same conclusion was reached by a local study (59). Not all HFDP pregnancies are exposed to these risk factors, and selective screening based on risk factors has been found to miss from 24%-48% of cases that would be identified by universal screening (76,77). Moreover, to further complicate

matters, practises with respect to timing of screening also varies, with between 24-28 weeks most commonly being practiced (78).

Table 1. 3 Common risk factors for gestational diabetes mellitus (79)

1. Older age 2. High weight 3. Pre-pregnancy BMI of ≥27 kg/m2 4. High parity 5. Having previously given birth to a baby weighing 4 kg or more. 6. A family history of T2DM 7. A previous diagnosis of HFDP 8. Belonging to a particular ethnic group (Hispanic, African, African American, Asian)

Abbreviations: BMI body mass index, HFDP hyperglycaemia first detected in pregnancy.

1.8.2 Post-partum screening

The increasing prevalence of diabetes and the MetS among all demographics, including women of childbearing age, has challenged the prior notion that HFDP mostly resolves after delivery. A growing body of literature has shown that post-partum follow-up of women with HFDP remains critical since these women are at high risk of developing T2DM and other cardiometabolic diseases (80–87). Nonetheless, a substantial proportion of women fail to return for follow-up (88) owing to either patient or provider-related barriers with this area remaining grossly underprioritized. Studies have identified patient unawareness, lack of time, fear of receiving bad news and other social/economic reasons for patients not adhering to follow-up visits (89,90). Inconsistent guidelines regarding screening intervals, screening methods and lack of patient ownership are a few identified provider-related barriers. In SA, the overburdened health care system is a major rate limiting hurdle in providing post-partum screening with knowledge surrounding additional barriers in LMIC being very poorly explored (91).

1.9 Health systems: Clinical management

1.9.1 Antepartum

Since hyperglycaemia, is a major driver of maternal and fetal morbidity, treatment of HFDP is effective for preventing adverse pregnancy outcomes. The best care available for mothers with diabetes in pregnancy, should ideally be offered by a multidisciplinary team of specialists,

where three major factors need to be considered, severity of glucose intolerance, maternal response to therapy and effect on fetal growth.

Worldwide, the mainstay of therapy remains diet and exercise, which is adequate for the majority of HFDP patients (9,72,92). Whilst the ideal diet remains controversial, a general consensus of limiting excessive carbohydrate intake and distributing it evenly throughout the day is prescribed. A systematic review found that a low glycaemic index diet to be the most effective at managing maternal glucose levels and reducing birth weight in the offspring (93). Frequent self-monitoring of capillary blood glucose levels pre- and post-prandially remains imperative and directs therapy. For those not achieving normoglycaemia despite this (10-30%), pharmacotherapy in the form of oral hypoglycaemic agents or insulin is required.

Whilst insulin remains the standard of care since it does not cross the placenta, has not been found to be teratogenic and its long-term safety has been established (32,92), OHA's have gained a lot of interest over the past two decades as they are inexpensive, and easier to use than insulin making them particularly attractive for LMICs. Currently, no oral agents are licensed for use during pregnancy given the that both agents cross the placenta and their long-term safety data is limited which has translated into restrictions in their usage, particularly in United States of America (USA). Their use in pregnancy is endorsed by some major guidelines (60,71,93–95) with countries like Europe and SA frequently using them as first line agents (96).

Over the last while, the efficacy and safety of these agents in the treatment of HFDP have been evaluated through several meta-analyses, the results of which have been inconsistent due to lack of evidence from head-to-head randomised controlled trials (RCT). In one of the more recent meta-analysis (96), glyburide was ranked the worst with the highest incidence of macrosomia, pre-eclampsia, hyperbilirubinemia, neonatal hypoglycaemia, preterm birth, and low birth weight (LBW), despite the fact that it was as effective as insulin, whilst metformin (together with insulin when required), had the lowest risk of macrosomia, pregnancy HTN, large for gestational age (LGA), respiratory distress syndrome (RDS), preterm birth and LBW. Prior meta-analyses have been in accordance with these findings, however the most recent metanalysis which included 41 RCT found that though metformin is safe and effective for the treatment of HFDP, there was a higher incidence of preterm birth when compared to insulin and higher gestational weight when compared to glyburide (97) The frequency of treatment

failure has been shown to be similar for both OHA, with 16-17% of women requiring additional pharmacotherapy (32). One review (96) showed that glyburide was more effective at achieving glycaemic control in women with HFDP with lower treatment failure rates. Metformin, with its documented beneficial effects on both glycaemia and immediate pregnancy outcomes, make it a useful substitute during pregnancy, though its postnatal long-term effects are still to be better defined. The beneficial effects of metformin on glycaemia and pregnancy outcomes place it as a good alternative for its use during pregnancy, however the postnatal long-term effects require more evidence (80,81). Some human and animal studies have shown that metformin during pregnancy is associated with excessive postnatal weight gain and obesity in the offspring (98,99). There is no long-term safety data available for the offspring for glyburide exposure in pregnancy. Though the short-term safety of OHA versus insulin has been explored by numerous studies (96,100–107), there is a paucity of research evaluating their long-term effects and safety (104).

To date, insulin remains the golden standard of pharmaceutical treatment of hyperglycaemia in pregnancy, given that concerns for the long-term consequences of OHA, including metformin and glibenclamide, remain. Most professional societies (ADA, ACOG) advocate its use as first-line treatment of HFDP after failure of lifestyle modification, whilst others (NICE) promote initial use of OHA following 20 weeks' gestation prior to switching to insulin.

1.9.2 Post-partum

The importance in detecting impaired glucose tolerance other than diabetes, lies in its value in predicting future T2DM as is the case in screening for cardiovascular risk factors in determining cardiovascular risk and subsequent disease. Lifestyle interventions, including weight loss programs and dietary advise remain the cornerstone of therapy in the primary prevention of these consequences. Pharmacologic interventions have also been shown to prevent progression to T2DM (108), whilst the detection and management of each cardiovascular risk factor is advisable in decreasing cardiovascular morbidity and mortality. Encouraging exclusive breastfeeding offers further protection from developing diabetes (109). Contraceptive advice is important since recurrent HFDP pregnancies further increase the risk of progression to T2DM. The optimal contraceptive method for women with a history of HFDP remain unknown, though there are a few studies supporting the use of combined oral contraceptive use (110–112). Hormonal contraception has been shown in large cohort studies

to not increase the risk of developing T2DM in healthy women (113,114), though the metabolic changes appreciated with progestins may be magnified in a group of women with a higher risk of T2DM. For the most part, the effect of contraceptive hormones on the subsequent risk of developing T2DM following HFDP is unclear with the current consensus supporting the safety of all contraceptive methods (115). In the setting of obesity, HTN or dyslipidaemia, either mechanical methods or hormonal methods which are vascular neutral are prescribed (116).

1.10 Significance of diabetes in pregnancy

Uncontrolled hyperglycaemia during pregnancy, exposes both the mother and child to an increased risk of a range of adverse outcomes (117)**.** The diagnosis of HFDP has implications for the pregnancy and also for the future maternal health as well as their offspring. Furthermore, it contributes indirectly to maternal mortality and morbidity by augmenting the risk of the four main causes of maternal death globally, including haemorrhage, HTN, sepsis and obstruction of labour (118).

1.10.1 Short-term consequences

Poorly controlled diabetes in pregnancy, including both pre-existing and HFDP poses adverse perinatal and long-term outcomes for both the mother and foetus (Table 1.4). The risk increases as the maternal fasting plasma glucose increases. Immediate maternal risks include pre-eclampsia (20-30%), polyhydramnios (20%) and CS (30%) which can result in preterm delivery. In the foetus, spontaneous abortions and congenital anomalies may arise if dysglycaemia is present in the first trimester. Later in the pregnancy, elevated glucose levels may give rise to excessive fetal growth. This in the presence or absence of macrosomia and LGA infant can result in concomitant birth trauma. Immediate neonatal complications include respiratory problems and metabolic complications (119). A 20-year audit (1983-2002) performed at the CHBAH gestational diabetic clinic, acknowledged these well-established short-term risks in their “control” group, (those with diabetes in pregnancy who either presented late or only had a maximum of 2 weeks of intervention) (120,121). Needless to say, it is well established that achieving normoglycaemia early on in pregnancy can reduce these complications (122).

Table 1. 4 Adverse outcomes associated with uncontrolled hyperglycaemia in pregnancy.

Maternal	Fetal	Neonatal
Pregnancy induced hypertension	Miscarriage	Respiratory distress syndrome
Polyhydramnios	Stillbirth	Hypoglycaemia
Pre-term delivery	Macrosomia	Hyperbilirubinemia
	Congenital anomalies	Hypomagnesaemia/ hypocalcaemia
		Polycythaemia

1.10.2 Long-term consequences

Despite the fact that the majority of women who have HFDP, will experience normalized glucose tolerance in the immediate post-partum period, the development of either subtype of HFDP during pregnancy constitutes a maternal phenotype at increased subsequent risk for developing T2DM, MetS and, with time, overt CVD, including coronary artery disease and HTN (14–16,123).

A landmark study conducted, the HAPO study (38), was a large, multinational, ethnically diverse group of women with the main objective to assess four primary outcomes in relation to varying degrees of maternal hyperglycaemia. Short-term maternal and neonatal outcomes measured are listed in Table 1.5. These women after undergoing a 2-hour 75gm OGTT demonstrated that lower glucose levels than those diagnostic of diabetes were associated with adverse pregnancy outcomes along a continuum. A follow-up study (124,125) of the same cohort assessed the long-term risk of any disorders of glucose metabolism among mothers and their offspring, together with adiposity measures among their children 10 to 14 years postpartum. The major findings were that a significant proportion of the mothers with a history of HFDP developed a disorder of glucose metabolism (52.2%) vs. 20.1% of mothers without HFDP (OR 3.44 [95%CI 2.85-4.14]) and higher glucose levels and insulin resistance was noted in the offspring upon exposure to higher glucose levels in utero. This finding was independent

of maternal and childhood BMI and a family history of diabetes. Childhood measures of adiposity utilising BMI was not statistically significant between the groups.

Table 1. 5 HAPO study primary and secondary outcomes

	Maternal	**Neonatal**
Primary outcomes	Caesarean section delivery	Birth weight >90th centile
		Cord blood C-peptide >90th centile
		Neonatal hypoglycaemia
Secondary outcomes		Premature delivery
		Shoulder dystocia
		Birth injury
		ICU admissions
		Hyperbilirubinemia
		Pre-eclampsia

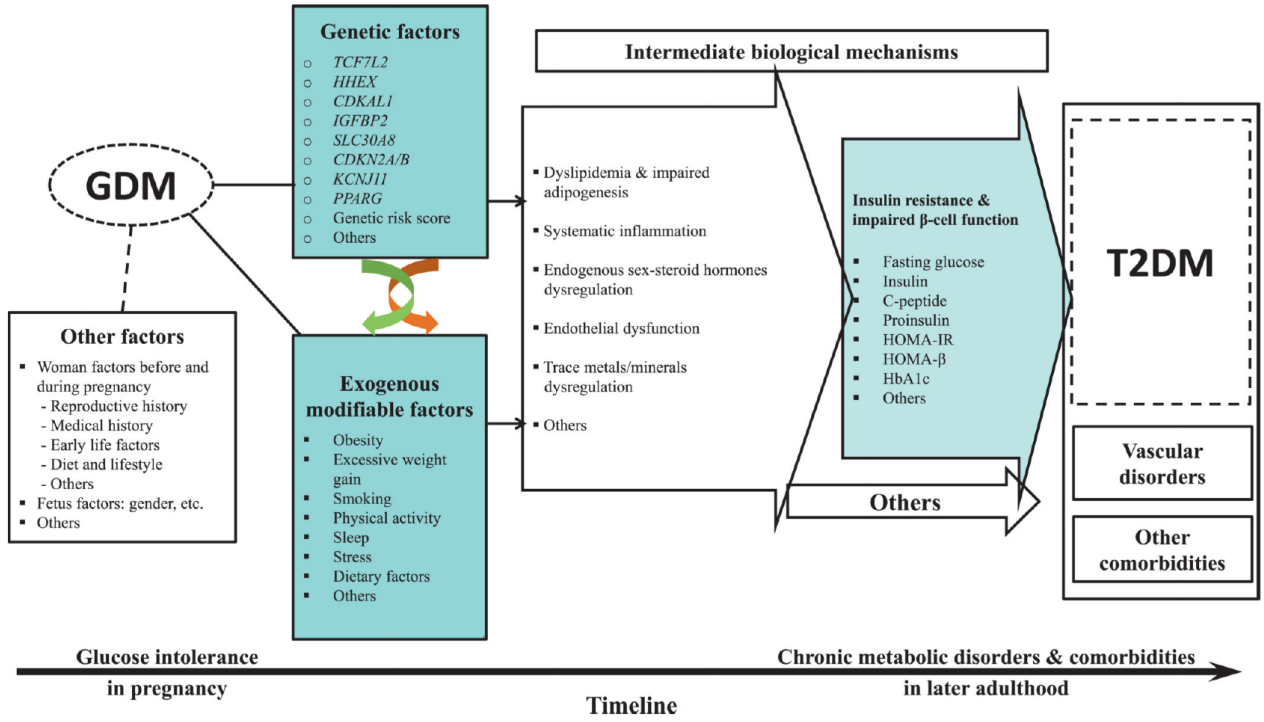
The long-term consequences for both mother and child following HFDP, which are mostly cardiometabolic, are outlined in Table 1.6.

Table 1. 6 Long-term consequences following HFDP.

Maternal	**Offspring/Children**
Impaired glucose tolerance	Impaired glucose tolerance
Diabetes (T2DM and T1DM)	T2DM
Cardiovascular disease	Obesity/MetS
Obesity/MetS	Cognitive impairment

The potential mechanisms by which this progression to the numerous co-morbidities occurs is best depicted in Figure 1.7, in which the interplay of modifiable and non-modifiable factors

present both at pre-conception and during conception together with intermediate biological mechanisms during the post-partum period augment the development of these outcomes on the background of glucose intolerance during pregnancy.



Abbreviations: GDM gestational diabetes, T2DM type 2 diabetes mellitus

Figure 1. 7 Potential major determinants for the progression from HFDP to T2DM and other co-morbidities based on a life course perspective (22)

1.10.2.1 Type 2 diabetes

HFDP is fuelling the T2DM epidemic with over a half of mothers with HFDP developing diabetes in ten years, making HFDP one of the strongest predictors of T2DM (14,123). Since HFDP includes undiagnosed pre-existing T2DM as well as GDM, this risk of progression may reflect the underlying T2DM epidemic within the population. An earlier metanalysis involving 20 studies (600, 000 women) spanning 50 years, showed that women with HFDP have an increased risk of developing T2DM compared with those who had a normoglycaemic pregnancy (RR7.43, 95% CI 4.79-11.51) (14). A more recent metanalysis which included 30 cohort studies, incorporating 2,626,905 pregnant women, supported these earlier findings of a substantially increased risk of diabetes with an adjusted risk of 17.92, however it also added that the greatest risk was between 3-6 years following HFDP and amongst the young age group less than 40 years, which is particularly alarming (123). The risk of progression has been shown

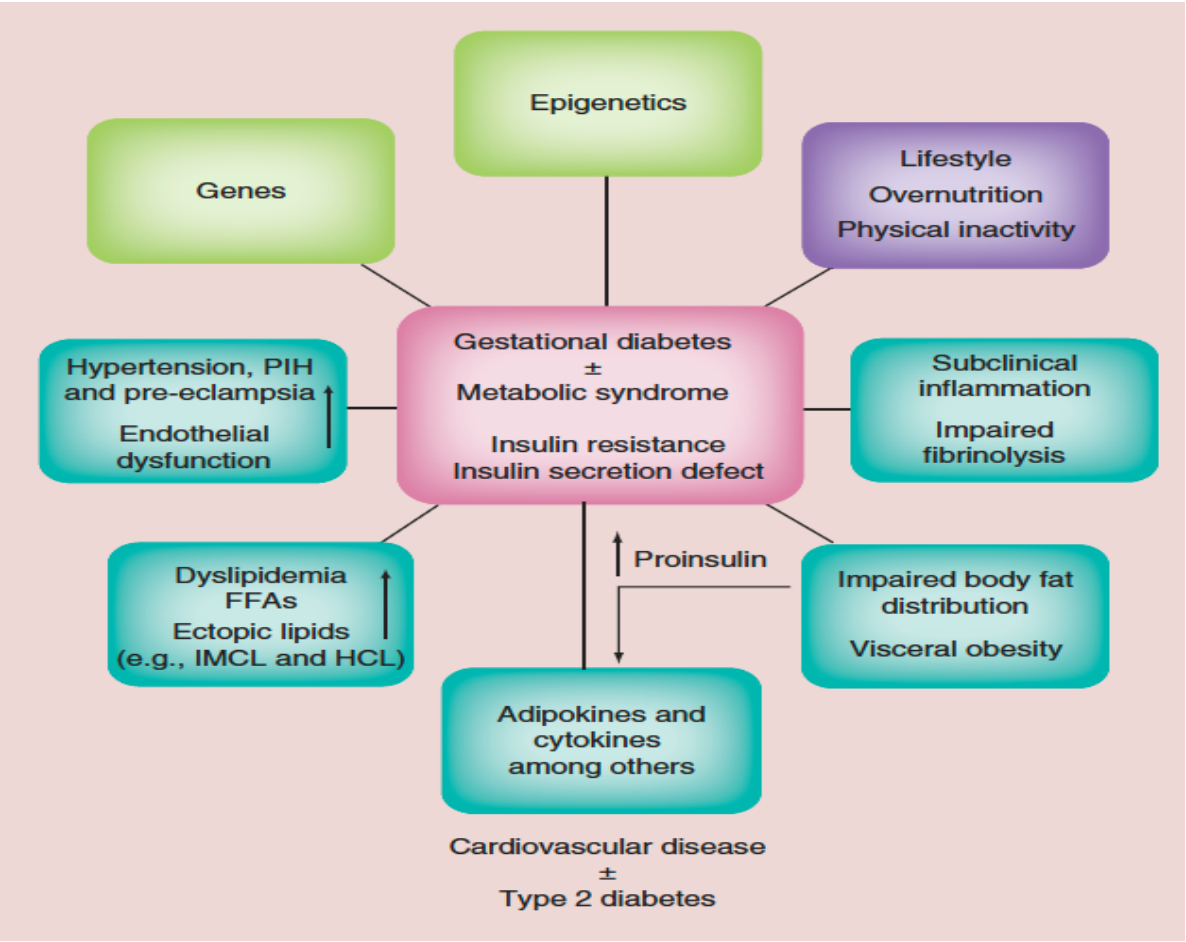
to vary with ethnic background, obesity and post-partum weight gain, family history of diabetes and the need for insulin during pregnancy amongst others (126). These risk factors vary by study with the most recent metanalysis showing that advanced maternal age and obesity have no role to play (123).

Among developing and LMIC countries which are suffering the burden of escalating prevalence's of HFDP and T2DM, data on the risk of progression to T2DM remains limited and little is known about modifiable factors that may lower this risk. Though a higher risk has been found in black women elsewhere in the world (127), most studies have not included women from Africa, with the applicability of previously recognised racial disparities in progression to T2DM, being uncertain. The variation in either maternal insulin sensitivity or secretion may account for these ethnic differences. This was appreciated in a study which focused on ethnic differences at three months postpartum where 31% of women of non-European ethnicity and 18% of women of European ethnicity were glucose tolerant (128). In SA, two studies in women of mixed ethnic background were found to have high rates of progression to dysglycaemia and T2DM as early as 6-12 weeks post-partum (129) as well as 5-6 years post-partum following a HFDP pregnancy(130). These studies had limited black ethnic representation.

The overall need for long-term follow-up and screening for diabetes is essential following all HFDP pregnancies and is generally prescribed at six weeks post-partum followed yearly thereafter (32,61,72). In resource limited settings, since women with HFDP represent a substantial proportion of the population which may be difficult to target as a whole, identifying women at highest risk of progressing to T2DM includes identifying additional factors that may further assist in targeting prevention, with factors like insulin use in pregnancy, higher BMI, recurrent HFDP being important considerations (37) . Advocating simple lifestyle intervention programmes to promote weight loss following HFDP has been shown to reduce the risk of progression (130), though the effectiveness of these interventions remains to be fully established. Furthermore, thiazolididiones and metformin, have been demonstrated to decrease the risk of developing T2DM in HFDP women who are found to have various forms of dysglycaemia postpartum (131,132).

1.10.2.2 Cardiovascular disease

Cardiovascular diseases are the most common cause of death globally, representing 30% of all deaths (133). The increase in deaths attributable to CVD is largely owing to an increase in risk factors including poor diet, sedentary lifestyle, obesity, hyperlipidaemia and importantly the increasing prevalence of T2DM. Diabetic women in particular have a four-to six-fold increased risk of developing coronary artery disease (CAD) (134). HFDP can be considered a "pre-diabetic state" with studies demonstrating similar cardio-metabolic alterations including obesity, insulin resistance, increased atherogenic lipid profile, increased inflammatory markers like highly sensitive CRP (hsCRP), HTN, and endothelial dysfunction with an increased carotid intima–media thickness (cIMT) as early as a few months postpartum (135). These "common soil" modifiable and non-modifiable risk factors shared by CVD and T2DM are depicted in Figure 1.8. It should be appreciated that there may be unrecognised factors both during and after pregnancy that contribute to the observed association of HFDP and subsequent CVD. These may include associated conditions in pregnancy or latent unrecognised cardiovascular risk determinants.



Abbreviations: FFA Free fatty acid; HCL Hepatocellular lipid content; IMCL Intramyocellular lipid content; PIH Pregnancy-induced hypertension.

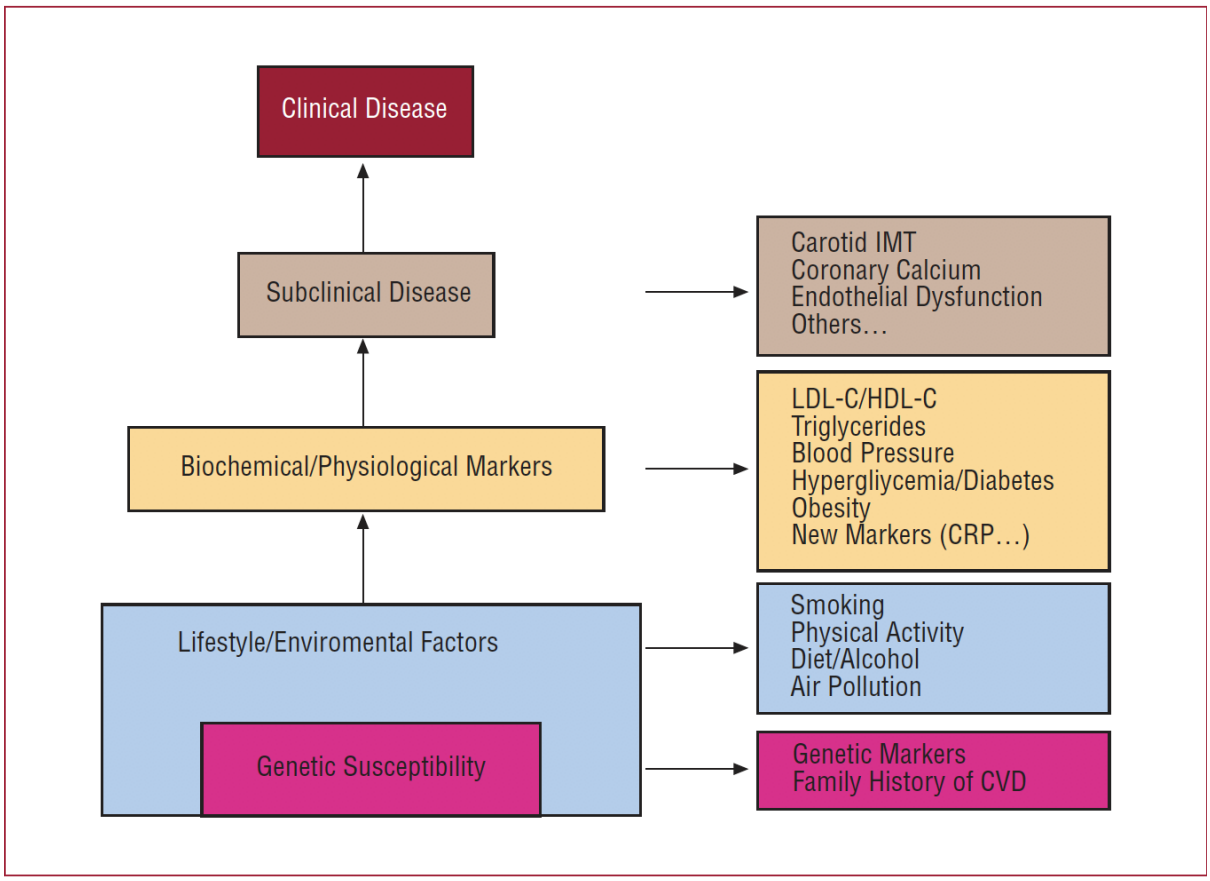
Figure 1. 8 Modifiable and non-modifiable risk factors shared by CVD and T2DM (137)

Whereas some studies have shown that women with HFDP, develop CVD if they progress to T2DM, (83,136,137)others have argued that this increased cardiovascular risk can manifest independently of diabetes (138–140).

A recent meta-analysis involving more than five million women demonstrated that women with a history of HFDP have a two-fold higher risk of major cardiovascular events in the years following the index pregnancy. In addition, the risk presents as early as the first decade following HFDP and importantly is independent of the development of T2DM (14).

The exact pathophysiological mechanisms linking pregnancies complicated by diabetes mellitus with future cardiovascular health are presently unknown. Endothelial dysfunction known to be an important initiating factor in the development of atherosclerosis is an independent predictor of CVD in the general population (141). Studies of women with HFDP have shown that there is functional and biochemical evidence of abnormal vascular endothelial function during pregnancy (142) and up to several years postpartum. Endothelial dysfunction

can be measured accurately by several non-invasive techniques which can evaluate large artery function through measures such as flow mediated dilatation or pulse wave velocity, or structural changes, measured by cIMT. More recently various novel biomarkers, including inflammatory markers and oxidative stress related biomarkers, have been found to valuable in predicting CVD risk (143). The natural history of CVD exists along a continuum with a progression from a subclinical state to clinical disease in the presence of various genetic, lifestyle and biochemical markers which act as risk factors (Figure 1.9). The identification of subclinical disease through various surrogate markers is imperative in order to prevent disease.



Abbreviations: IMT intima media thickness, LDL low density lipoprotein, HDL high density lipoprotein, CRP C-reactive protein, CVD cardiovascular disease.

Figure 1. 9 Natural history of cardiovascular diseases on the background of various risk factors (144)

Surrogate markers for cardiovascular disease

Carotid intima media thickness (cIMT) detects early atherosclerosis and strongly predicts heart disease and stroke, particularly in women (145). The relationship between HFDP and thickened cIMT is controversial (146). More recently, several studies published on cIMT in

women who have had HFDP, found that a history of HFDP can be considered a risk factor for atherosclerosis even before the onset of diabetes or MetS (147–149). A meta-analysis of fifteen studies (2247 subjects) found that HFDP is related to larger cIMT and the relation is stronger in obese HFDP patients with the association already existing at the time of pregnancy and remaining significant years after pregnancy (137).

Femoral intima media thickness (fIMT) has more recently been explored as a new marker in evaluating the global cardiovascular risk in patients with ischemic heart disease. The Bogalusa Heart Study found that fIMT, like cIMT is also correlated with the presence and number of cardiovascular risk factors. The usefulness in combining ultrasonography of the femoral artery in conjunction with multiple risk factors profiling for risk stratification in patients with atherosclerotic disease can be helpful (150).

2. **Cardiovascular biomarkers**

Subclinical inflammation is known major risk factor for future CVD in the general population. Inflammatory mechanisms have been linked to a higher risk of CVD later in life for women with a history of HFDP(151), though the mechanisms behind vascular injury and CVD are not well understood (152). More recently, a variety of novel biomarkers reflecting a broad range of pathological events involved in the progression of atherosclerosis, have been reported in association with cardiovascular risk. Certain biomarkers of endothelial dysfunction have been found to be elevated in the early postpartum period in women with a background of HFDP, of which these include intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin (147,153). A further case control study (154)found significantly higher C-reactive protein (CRP), interleukin-6 (IL-6), and plasminogen activator 1(PAI-1) and lower adiponectin concentrations than in control subjects. Though, after adjustment for confounders, only a high CRP and low adiponectin levels were associated with HFDP. Another marker, microalbuminuria, known to be a signal of impaired endothelial function in T2DM (155) has also been reported in women whose pregnancies were complicated by HFDP (156). Although this marker potentially outlines a possible pathophysiological mechanism for future cardiometabolic disease, to date small studies with numerous confounders make the interpretation of these results limited.

3**. Pulse wave velocity**

Arterial stiffness is best assessed utilising carotid to femoral pulse wave velocity (PWV) (157). Pulse wave velocity increases the less distensible a vessel is (158) and this is proportional to the number of cardiovascular risk factors present, such as diabetes or MetS (159,160). A raised PWV has been found in epidemiological studies to be predictive of cardiovascular events (157). During the post-partum period, studies have found that endothelial and vascular smooth muscle reactivity, measured by flow-mediated dilation and arterial pulse wave velocity, were found to be reduced in women with HFDP (161,162).

4. **Risk stratification-cardiovascular scores**

No single cardiovascular surrogate marker provides a highly sensitive and specific recognition of CVD and hence an attractive strategy has been to combine various evaluations to better define the presence of early disease. Scoring systems, much like the Framingham risk score (FRS)(163) has evolved to estimate the statistical risk of disease. This score is now recommended by numerous international guideline committees for CVD prevention since it has been validated across different populations. However, in young women with unique risk factors beyond the traditional risk factors (sex-specific, hsCRP) there is currently a lack of evidence and protocols for implementation of risk stratification strategies. Furthermore, the lack of prospective data in the African setting as well as the absence of locally validated total CVD risk assessment tools, makes risk assessment challenging. The FRS, although validated in white and black populations, has well established limitations in non-white, female and young individuals (164).

One study from SA (165) looking the applicability of Framingham laboratory- and non-laboratory-based and National Health and Nutrition Examination Survey (NHANES) I non-laboratory-based equations in black men and women confirmed that the use of non-laboratory scores in clinical practice may be a feasible alternative in predicting cardiovascular risk in our resource-constrained settings. Cardiovascular disease risk prediction tools that include important pregnancy-related factors alongside the traditional CVD risk factors will improve screening for these high-risk individuals, given that an obstetric history of one or more prior pregnancies complicated GDM implies an increased risk for later CVD for the affected woman and should therefore be part of a complete CVD risk factor assessment in women.

In order to better assess global risk, integration of multiple biomarkers and hence all risk factors which can assist in incremental risk prediction must be included in diagnosis. This will assist with tailoring therapy and to monitor the effects of therapy in a cost-effective manner.

There is compelling evidence to suggest that adverse pregnancy outcomes including HFDP serve as a warning for future CVD events (Figure 1.10) with numerous studies supporting the association of conditions such as preeclampsia, preterm delivery amongst others with CVD (84,166,167). Given that cardiovascular mortality is thus encountered more frequently, these women represent an important and potentially cost-effective subset of the population for diabetes and CVD prevention.

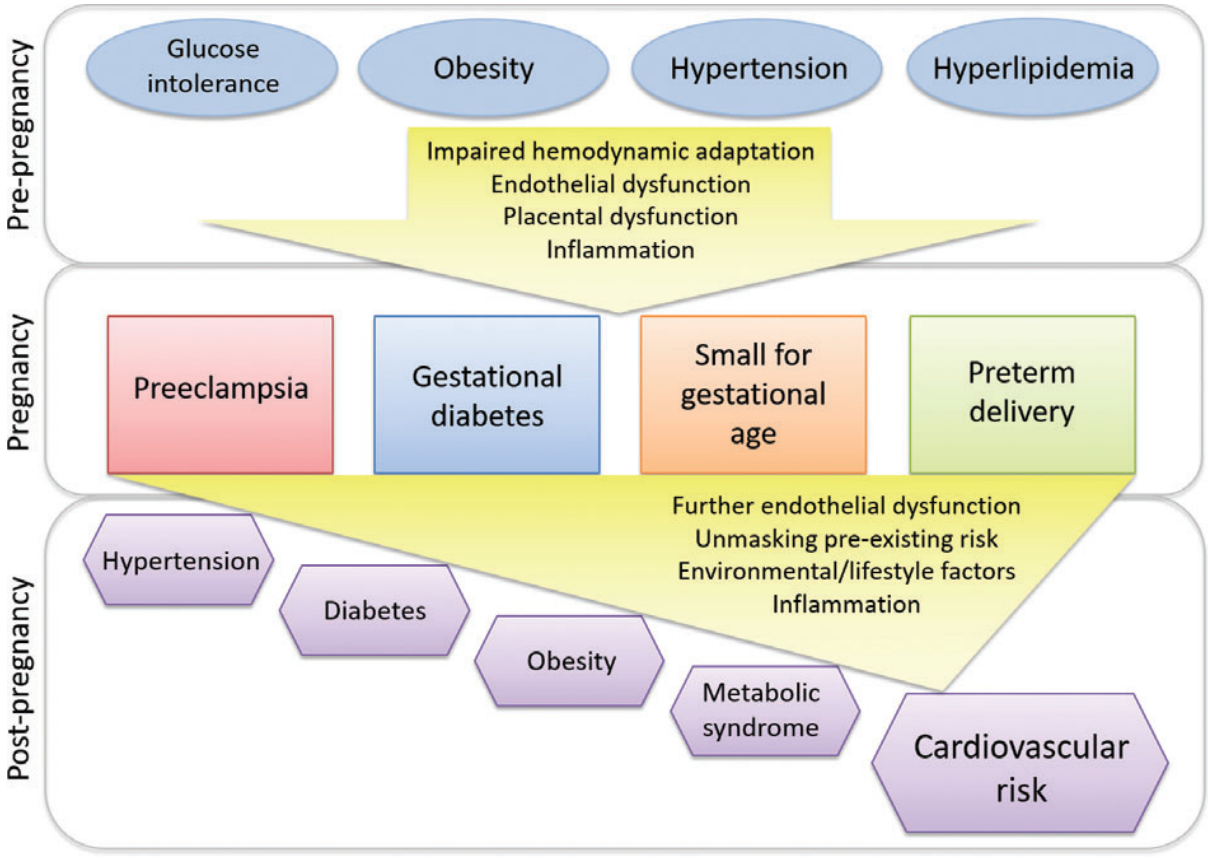


Figure 1. 10 Associations between pre-pregnancy risk factors, adverse pregnancy outcomes, and post pregnancy cardiometabolic risk factors and outcomes (84)

1.8.2.3 Body composition and obesity

The alarmingly high prevalence of overweight and obesity among women of childbearing age (168) in both developed and developing countries (169,170) is concerning given the immediate and long-term implications for mother and child. Immediate pregnancy complications include HFDP (171), pre-eclampsia, stillbirth, congenital abnormalities, and delivery of LGA infants

with the long-term consequences for the mother including T2DM, heart disease and HTN (172) whilst observational studies provide evidence for effects of maternal obesity on the offspring's risks which include obesity, coronary heart disease, stroke, T2DM and asthma (173,174). Pregnancies complicated by both obesity and HFDP influence these outcomes independently. The long-term effects of HFDP on maternal body composition and obesity is less well explored.

Data from a metanalysis of observational studies have shown that various proxies of adiposity such as BMI, weight gain, waist circumference (WC) and hip circumference (HC) and their association with T2DM and CVD risk, is causal (175), independent of overall body fatness. More specifically, body fat distribution, with upper body fat (android and visceral fat) being proportionately correlated with this risk compared to lower body fat depots (gynoid and leg fat) (176). Conventional anthropometric measurements such as waist-hip ratio (WHR) or imaging methods like commuted tomography (CT), magnetic resonance imaging (MRI) and dual-energy-absorptiometry (DXA) have demonstrated this (177). More recently quantifying body fat distribution has been shown to be causally related to CVD risk (178). Observational studies have demonstrated that although conventional anthropometry offers information regarding both fat distribution and total adiposity, it however provides weaker predictions of T2DM and CVD risk factors whereas other imaging platforms such as CT, MRI or DXA are more informative. The greatest limitations in performing CT and MRI remain the cost, radiation exposure and variability in image analysis. DXA has overcome these issues and provides accurate volumetric assessments of both total and regional adiposity (179) with more recent advances in DXA technology enabling accurate quantification of visceral adipose tissue (VAT).

1.10.2.4 Metabolic syndrome

The MetS is considered as the concomitant clustering of central obesity, hypertriglyceridemia, low high-density lipoprotein cholesterol, (HDL) hypertension (HTN), and dysglycaemia. Currently there are many MetS definitions in existence with varying criteria being employed by different organizations to define this, which has led to numerous controversies in the field including varying prevalence estimates with some researchers questioning the utility of these criteria and even the existence of the syndrome. In 2009, representatives from various organizations attempted to resolve the differences between the definitions and proposed

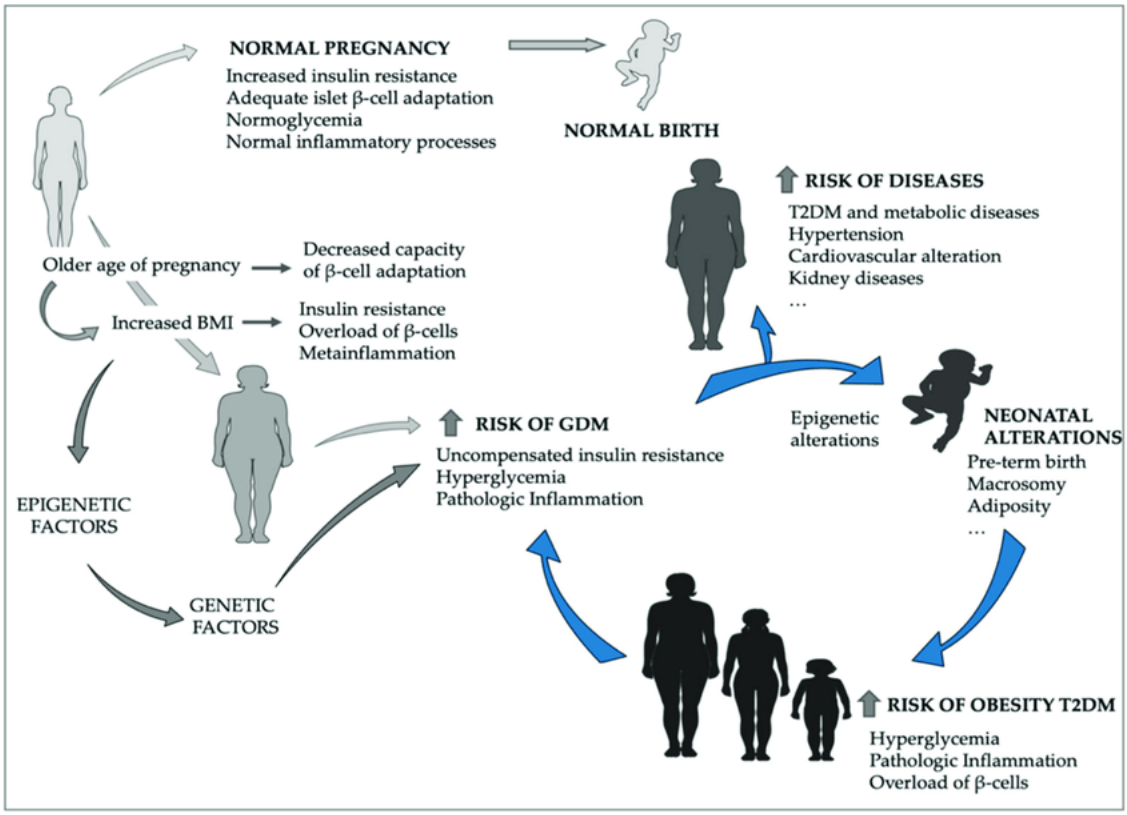
"harmonizing the MetS" with five components (180) in which there is no obligatory component and three abnormal findings out of five would qualify a person. Though the cut-points were uniformly defined for all components, regional cut-points for waist circumference are advocated. Despite the ongoing debates this constellation of risk factors identifies a group of individuals at high-risk of T2DM and CVD. The rise in the obesity prevalence is considered to be the major determinant of the concomitant increased in obesity related comorbidities such that many women who are obese have T2DM or HTN, which together aggravates the existence of the MetS. HFDP shares several common risk factors (family history of T2DM, increased age, and raised BMI) with T2DM and similarly with MetS. More so, HFDP and MetS are intimately related to each other and develop in the "common soil" of insulin resistance, together with endothelial dysfunction and low-grade inflammation. A meta-analysis of several studies including 5,832 women demonstrated an increased risk of MetS following a HFDP pregnancy (15). Even in the absence of pre-pregnancy obesity, there is a fivefold additional independent risk of developing MetS compared to controls without HFDP in women with a history of HFDP(181). In terms of predicting the development of MetS following a HFDP pregnancy, data is lacking for identifying antepartum characteristics of these women. Factors such as pre-pregnancy obesity, weight gain during follow-up and fasting glucose level (100 mg/dL (5.6mmol/L) at the oral glucose tolerance test (OGTT) during the index pregnancy were predictors of developing MetS as found by Akinci et al (13,182).

An overview of all relevant systematic reviews regarding the risk of progression to the aforementioned cardiometabolic disorders is summarised in Table 1.7.

Table 1. 7 Summary of systematic reviews for progression to T2DM, CVD and MetS following HFDP.

Author/year	Country	Study design	Sample size	Outcome measures	Results
Bellamy 2009 (13)	HMIC	Systematic review and analysis of cohort studies	675 455 participants (20 studies)	Risk of T2DM	RR of 7.43 for T2DM
Zhu 2016 (46)	HMIC LMIC	Literature review of studies 1960-2015	27 studies	Risk of progression T2DM	RR range: 1.3 Brazil 47.3 USA
Song 2018 (122)		Systematic review and analysis of cohort studies (1969-2014)	2626 905 participants (30 studies)	Risk of progression to T2DM by duration ff HFDP	Overall RR 7.76 <3 yrs. RR 5.37 3-6 yrs.- RR 16.5 6-10 yrs.- RR 8.2
Archambault 2014 (183)	HMIC	Scoping review 1991-2008	11 studies	Risk of CVD	OR Angina 2.9 CAD 1.6 Heart failure 0.7
Li 2018 (184)	HMIC	Systematic review and metanalysis of cohort studies (2006-2017)	3417 020 (7 studies)	Risk of CVD, CAD and stroke	Overall RR 1.74 CAD: RR2.09 Stroke: RR 1.25
Kramer 2019 (14)	HMIC	Systematic review and metanalysis (2013-2019)	5390 591 (9 studies)	Overall risk CVD Risk of CVD in women without T2DM Risk of CVD within 10 years ff GDM	Overall RR 1.98 RR 1.56 in women without T2DM RR within first decade 2.31
Xu 2014 (15)	HMIC LMIC	Systematic review (1979-2013)	5832 (17studies)	Risk of MetS following HFDP	OR 3.96 Outcome influenced by ethnicity and initial BMI

The importance in detecting and managing HFDP and providing ongoing follow-up lies in the long-lasting health consequences, with an increased risk for T2DM, CVD and obesity in both mother and child contributing to a vicious intergenerational cycle of NCD which impacts the population as a whole (Figure 1.11).



Abbreviations: GDM gestational diabetes, BMI body mass index, T2DM type 2 diabetes mellitus

Figure 1. 11 Intergenerational cycle of diseases following HFDP.

1.11 HIV and the risk of dysglycaemia and cardiovascular diseases

Though the introduction of ART for the treatment of HIV infection has conferred survival and quality of life benefits, the burden of metabolic perturbations such as insulin resistance, diabetes and dyslipidaemia have been of significant concern since the introduction of these agents. These associations have been explored by recent studies (185,186) with the pathogenesis being poorly defined and compounded by the differential prevalence of risk factors in HIV individuals including the HIV infection itself, ART therapy, patient factors as well as genetic susceptibility.

The overall prevalence of all disorders of glucose metabolism in all HIV-infected individuals on ART has been reported to be as high as 25% (187). The risk of diabetes in pregnancy, (including HFDP), in HIV infected pregnancies has been poorly studied and current data remain conflicting (188). A systematic review of the incidence and prevalence of T2DM in HIV infected individuals in Africa, though limited by a small number of studies and high heterogeneity, showed that there was no association (189). . As pregnancy is a known risk factor for impaired glucose tolerance, this together with the potential additional burden of protease

inhibitor-based ART in the co-infected HIV patient, may contribute to the development of impaired glucose tolerance and HFDP (190).

Furthermore, both T2DM and HIV infection are independently associated with an increased risk of atherosclerosis (192,193). The metabolic alterations as a result of both the HIV infection and its therapies have been implicated as a possible reason for the increased risk of CVD in HIV, with more recent data suggesting that immune activation and inflammation may also play a role (194). Taken together, HFDP and HIV co-existing in pregnancy potentially identifies a population of young women at risk of CVD.

An understanding of metabolic and cardiovascular risks in the HIV infected population remains critical, given the increasing burden of this condition. The same cardiometabolic consequences following HFDP are poorly studied, yet are of particular interest, as more compelling evidence accumulates regarding these long-term effects.

1.12 Gaps in the literature

The burden of HFDP is on the rise alongside the obesity epidemic (10). More alarming is that 90% of cases of hyperglycaemia in pregnancy occur in LMIC (195). The well-established immediate adverse outcomes of uncontrolled disease for both mother and foetus places unnecessary pressure on the already overwhelmed health care services in these countries. South Africa is not unique in this regard with an ailing public health infrastructure, often lacking in resources and very limited access to specialist centres where expertise is available to manage conditions like these. Although there is a growing body of evidence regarding HFDP in Africa, our knowledge surrounding maternal and child outcomes beyond the immediate post-partum period remains sparse. More so, the applicability of the available data for HFDP mostly pertains to HMIC with predominantly Caucasian ethnic representation, hence hampering the applicability to our mixed, predominantly black ethnic groups. The long-term outcomes following HFDP, including metabolic and cardiometabolic consequences have been explored elsewhere (79,86,150,196), with limited data available in SA. To my knowledge there are mere two prospective studies that investigated the rate of progression to T2DM in a mixed ethnic population at 6 weeks and 5-6 years post-partum (129,130). In addition, one of these studies explored the prevalence of individual cardiovascular risk factors, including the MetS in women with a history of HFDP (197) Furthermore, little is known about the impact of unique factors of particular importance to LMICs, including HIV-infection on these outcomes. Exploring

these long-term cardiometabolic outcomes on the background of our high obesity and HIV rates in a predominantly Black ethnic group would be novel and contribute to the paucity of data that exists for SSA.

1.13 Justification for the study

Over the past two decades, the global development agenda has placed important emphasis on improving maternal health and rightly so. Yet despite some gains, 830 women die daily from preventable causes (198). Diabetes in pregnancy is one such condition affecting 1 in 6 pregnancies around the world (1). HFDP to date, has remained an "invisible" maternal and child health issue given the unreliable profile of the global and local burden of this condition. Furthermore, HFDP is fuelling the T2DM epidemic. Over a half of mothers with HFDP will develop diabetes in 10 years, making HFDP one of the strongest predictors of T2DM. This study aims to gain better insight into the impact HFDP has on maternal health outcomes. Furthermore, it will contribute to the paucity of data regarding the influence of HIV during pregnancy on the post-partum cardiometabolic status. It aims to highlight the vulnerability of these women and their associated risk of developing NCD including T2DM, CVD and MetS following HFDP.

Gaining a better understanding of the consequences of HFDP on maternal health will provide evidence for the importance of universal screening, expanding the availability of antenatal services, and importantly postnatal screening and interventions to delay the onset and potentially prevent the development of these conditions. Health economies worldwide have been called to invest in primary preventative approaches as the costs of cardiometabolic diseases have gained momentum and remain an expensive cost burden once these diseases have fully progressed. HFDP is of particular public health importance in the context of urban SA, due to the combination of a lack of current evidence and an ongoing disproportionate increase in NCD's with HFDP playing a minor, though important role in the shadow of more obvious determinants of maternal and perinatal morbidity and mortality. Evidence-based data is needed in order to propose changes to current policies governing maternal health.

1.14 Overall aim

The overarching aim of this study was to establish the short-term cardiometabolic consequences of diabetes in pregnancy together with mediating factors by performing a cross-sectional analysis three to six-years post-delivery.

Three main objectives emerged as a way of achieving the aforementioned:

1. To describe maternal and neonatal outcomes in diabetic pregnancies including fetal miscarriage, preterm delivery, PIH, pre-eclampsia/eclampsia, CS rates and maternal death following diabetes in pregnancy considering how HIV and the exposure to OHA influenced these outcomes.
2. Examine the safety and efficacy of OHA on maternal and neonatal outcomes in the setting of HFDP exposure vs. insulin monotherapy amongst predominantly Black women, in an urban setting.
3. Establish the risk of dysglycaemia (impaired fasting glucose, impaired glucose tolerance and development of T2DM), CVD (pre-clinical and clinical disease states) and MetS in women with a history of HFDP for a period three-six years post-partum and compare it to women non-exposed to HFDP for the same period. Additionally, to investigate the potential role of HIV during pregnancy on these outcomes as well as to assess body composition and fat mass distribution as measured by DXA in women with a history of HFDP compared to women non-exposed to HFDP.

1.15 Hypotheses

With reference to the objectives above the hypothesis was that a history of HFDP leads to a higher likelihood of progressing to T2DM, MetS and higher CVD risk in the short-term following the index pregnancy when compared to a group of women non-exposed to HFDP among black SA women.

Chapter 2- Methodology

This chapter comprises the study setting and design, data management and analyses plan as well as ethical considerations. Data for this study was obtained from patient hospital records from the Gestational Endocrine Diabetic clinic at the CHBAH for the purposes of both retrospective and prospective studies. The study spanned two years. More detail on specific methodology and statistical analyses used is described in each of the empirical papers (Section 2).

2.1 Study setting

The study was conducted in Soweto situated in the Gauteng province of SA, only 15km from the centre of Johannesburg. Gauteng being the smallest province in SA in terms of land coverage, accounts for only 1.5% of the total land area of the country and is almost exclusively urban and has the highest population and population density (199). Gauteng is the economic and financial hub of SA producing 34% of SA gross domestic product (GDP) and approximately 10% of the GDP for the African continent (200,201), however, despite the economic advantages of the province, Gauteng has the 4th highest unemployment in SA (202). Gauteng is also home to the City of Johannesburg, the largest city in SA (201). Johannesburg emerged as an industrial and mining centre in the latter half of the 19th century after the discovery of gold. The discovery of gold and platinum led to the need for vast amounts of labour in order to extract the precious metals from the alluvial and reef deposits. Since 1904, Soweto (abbreviation for South West Township- So-We-To) was developed to accommodate black gold miners and black people who were displaced from their homes in Johannesburg, which were designated for whites only, following the passing of the Group Areas act of 1950 which imposed racial segregation on residential areas (203).

Currently, Soweto (Figure 2.1) is home to around 40% of Johannesburg's residents and includes thirty-four suburbs, covering 200km^2. The region remains mostly populated by black South Africans, most of which are female and originate from the isiZulu ethnic group. In terms of living conditions, 84.2% of dwellings are formal with 55 % of them having piped water inside and 90% of dwellings have electricity and flushing toilets connected to a sewerage system (204). Soweto now is a community in flux, neither first nor third world. The stresses of the broad social, economic and political changes in SA are reflected here.

In SA, private and public health systems exist in parallel. The public system serves the vast majority of the population but is chronically underfunded and understaffed.
The CHBAH, the third largest hospital in the world is a 3000-bed teaching hospital for the University of Witwatersrand, based in Soweto and serving mostly the surrounding population. It was first built in 1941 by the British military and served as a military hospital, then named the Imperial Military Hospital. After the second world war, the Transvaal Provincial Administration bought the hospital for £1 million. In 1997 it was renamed after the former general secretary of SA Communist Party, Chris Hani (205).

Approximately 60,000 patients are treated in the Maternity Hospital per year, with an average 1400-1600 deliveries per month (205). The hospital is only one of three in the country which provides access to a specialised combined maternity endocrine clinic, where diabetes in pregnancy is co-managed by endocrinologists and obstetricians. The Gestational Endocrine Clinic first opened in 1984, has seen major improvements in the care and outcomes of women with a diabetic pregnancy as well as their offspring children. On average 60 women are seen weekly and followed up frequently throughout their pregnancies until delivery. Screening for HFDP is not universal and is driven by risk factor profiles. Diagnostic testing and criteria for HFDP has changed since 2014 with the 75gm OGTT and IADPSG criteria being employed post 2014 and the 100gm OGTT and WHO 1999 criteria being adopted pre-2014 at the clinic. Women with diabetes or found to have HFDP are mostly referred by the surrounding Soweto clinics with a few women being referred by the neighbouring provinces where these services are lacking. There are 46 primary healthcare clinics and community centres scattered around Soweto, with only one district Hospital, Zola-Jabulani Hospital (206)

The Medical Research Council (MRC) and the Wits Developmental Pathways for Health Research Unit (DPHRU) is situated in the Department of Paediatrics in the School of Clinical Medicine and is based at the CHBAH. It is a well-established unit with a unique research platform which has substantial infrastructure and equipment. It has well-established links with the urban and rural SA communities. Numerous ongoing research projects are undertaken here which mostly address maternal and child health. One major project initiated in 1990 was the Birth to Twenty cohort (207), with a goal of examining the health and development of children born in Soweto.

DPHRU forms part of: (i) Wits Molecular Biosciences Research Thrust: Health for Africa, (ii) Wits Chronic Diseases of Lifestyle Research Thrust and the (iii) MRC Cardiovascular and Metabolic Diseases National Collaborative Research Programme.

All interviews, data collection and data capturing pertaining to studies are performed on site, as was the case for our study.

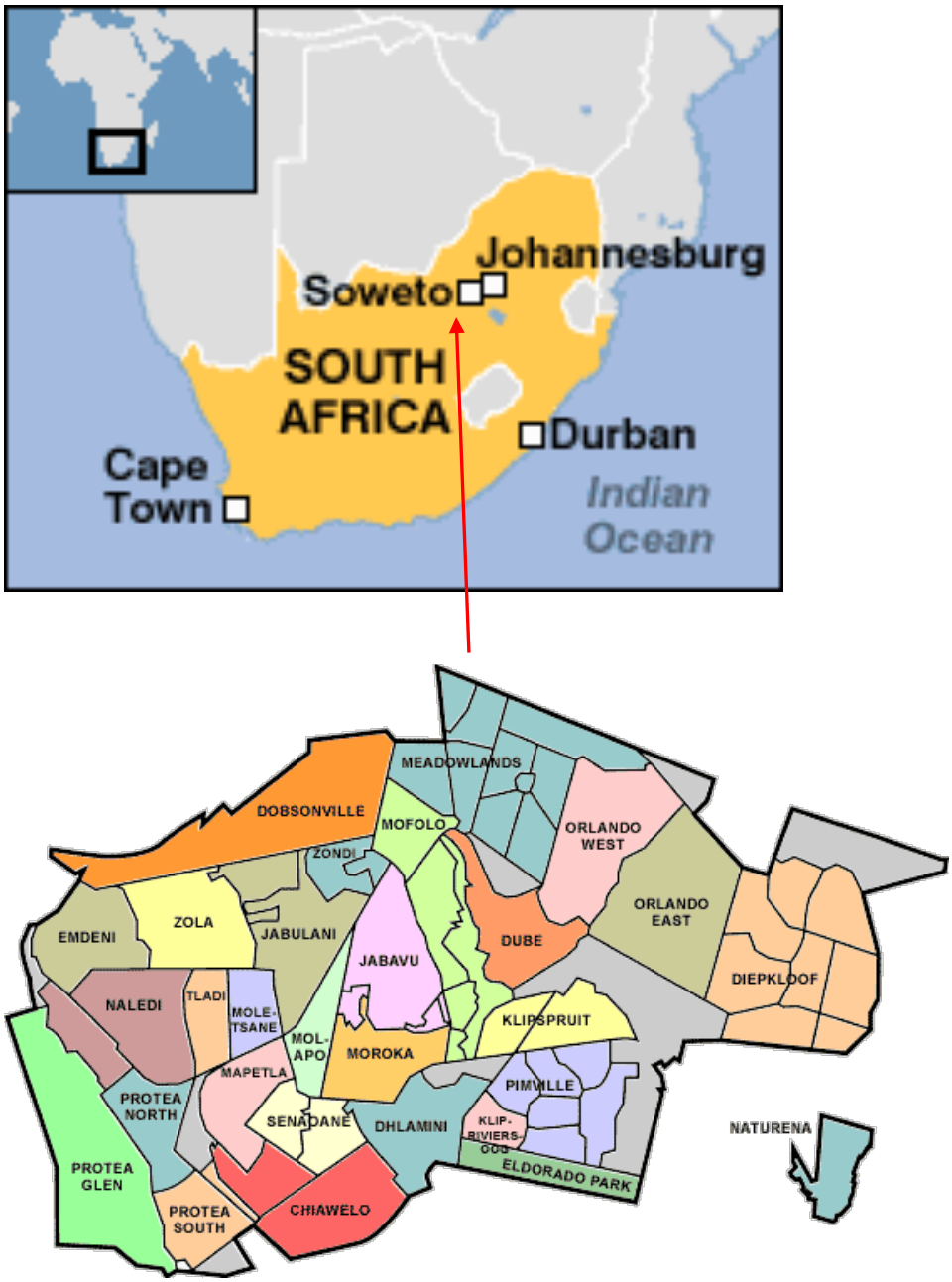


Figure 2. 1 Map displaying Soweto, Johannesburg in the Gauteng province.

2.2 Study design and population

The design of this study is two-fold with an initial retrospective component followed by a prospective, cross-sectional study.

An initial retrospective review of all medical records of women with a diabetic pregnancy identified between January 2010 and January 2017 (n=1263) from the gestational endocrine clinic was carried out. Of all the women identified with HFDP (n=653), it is notable that different diagnostic tests and criteria were employed pre-2014 (n=192) and post-2014 (n=461). These two groups were further separated for the purposes of analysis with the post-2014 HFDP group being incorporated with all pre-gestational diabetics(n=610) for the purpose of evaluating maternal and neonatal outcomes, Paper 1 (n=1071) and the pre-2014 group (n=192) separately reviewed in comparison to an earlier analysis of women with HFDP from the same clinic 1992-2002(n=348), utilizing the same test and criteria (Paper 2). Data for the retrospective arm was sourced and captured from an existing database at the Gestational Endocrine clinic at the CHBAH, when good record keeping was maintained, and the same diagnostic test was utilised for detecting HFDP. The database belongs to the Division of Endocrinology and is stored at the Gestational Endocrine clinic. Participants included all women with a diabetic pregnancy, including pre-gestational and HFDP, singleton pregnancies and women who were deemed to be 'controls' i.e., presented after 36 weeks or had less than two weeks of any medical intervention prior to delivery. Women were excluded if they were noted to have any other sub-type of diabetes, like steroid induced diabetes or secondary causes of pancreatic insufficiency and if a significant amount of data was missing. Any subsequent pregnancies identified for the same participant following the index pregnancy was excluded.

Women with HFDP identified from the retrospective study were invited to follow up in the prospective component of the study between March 2019 to November 2019. After exclusions, women post-2014 with HFDP (n=103) were recruited and randomly matched to controls (tested negative for HFDP) and evaluated cross-sectionally some 3 to 6 years post-partum (Figure 2.2). The controls were identified from an existing database at the DPHRU (S1000 and WDF, which was part of previous study (59) all of whom had a confirmed negative OGTT test using the same diagnostic test and criteria for the time period February 2014-January 2017. Eligible participants were recruited either telephonically or if unsuccessful, traced by visiting their

homes address. Women who did not consent, were not traceable or contactable, reported any cardiovascular event preceding their index pregnancy or were known or found to be pregnant were excluded.

2.3 Data collection and management

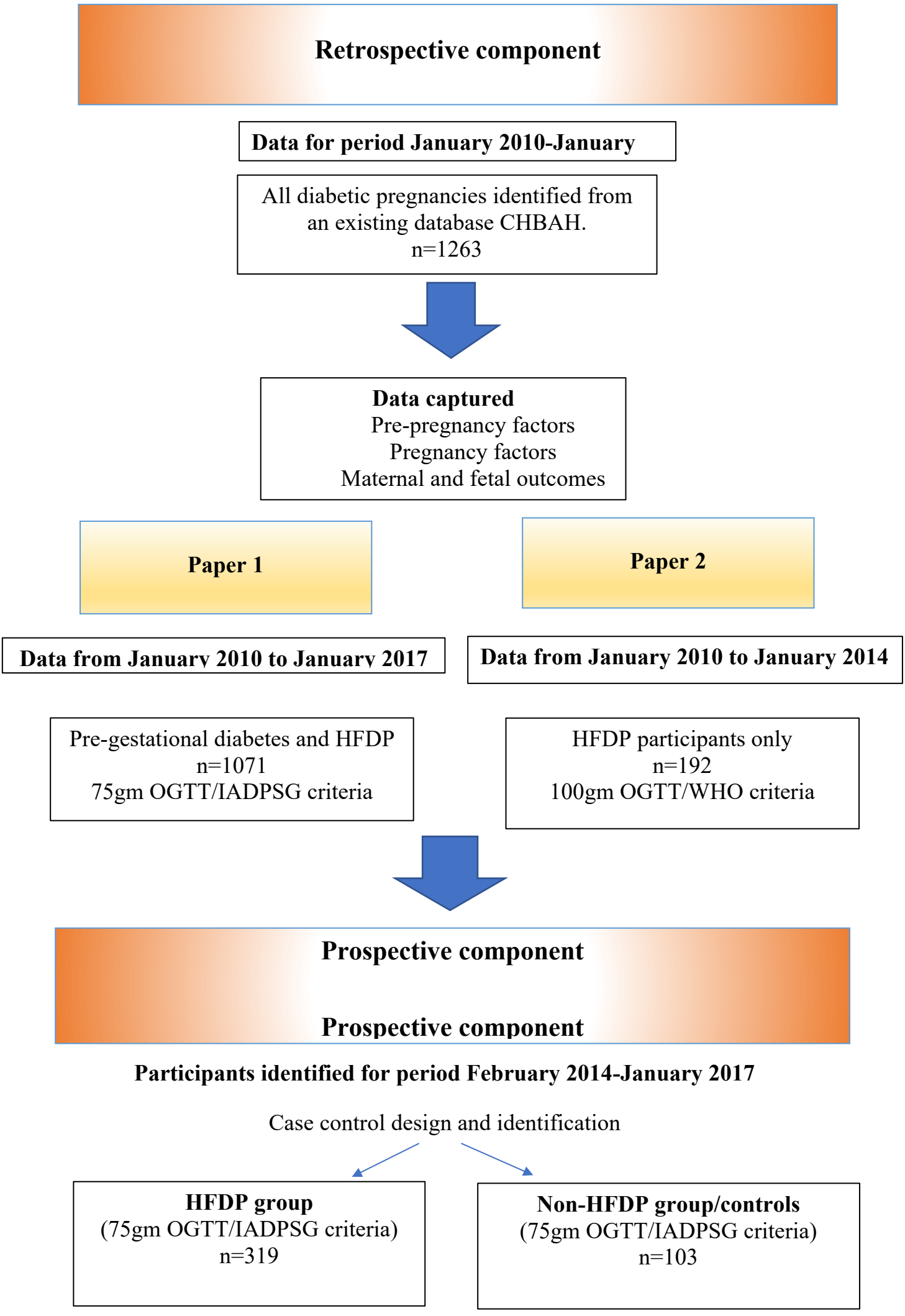
Details of the precise analytical approaches used for these analyses are described in each of the chapters 3 to 5 and summarised in Table 2.1 however additional information about specific analytical and data management components is provided in this section.

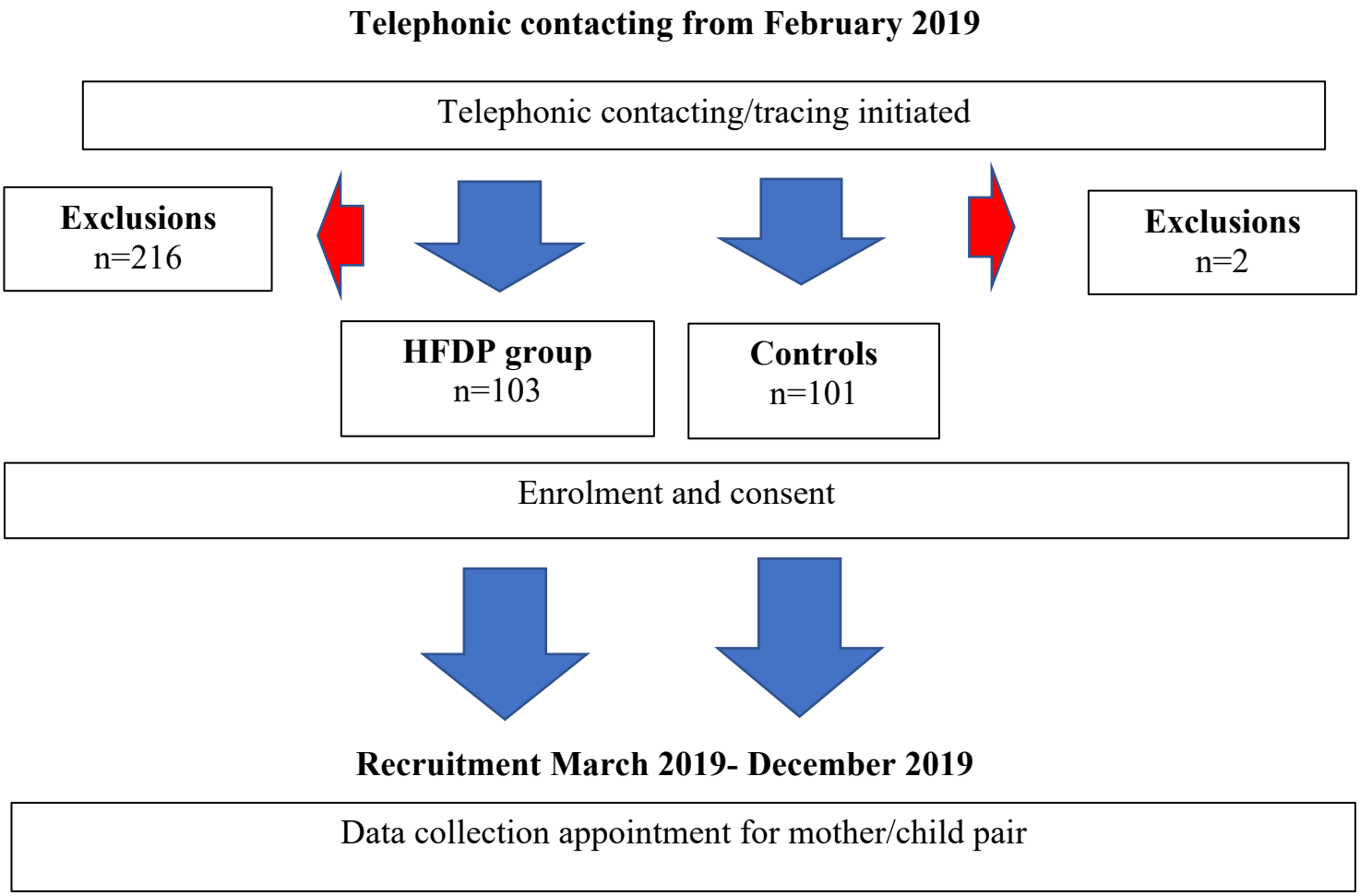
Data collected in this series of studies was captured onto the Research Electronic Data Capture (REDCap) data management platform (208), hosted by the University of the Witwatersrand, and is stored on a secure server at the DPHRU research facility with all information and files kept in locked premises. The data manager at the research facility is the gatekeeper and requests for access and usage of the data were submitted in accordance with research unit processes. Data was analysed by me and the findings from the analysis further discussed and reviewed with supervisors and co-authors. Variables pertaining to outcomes, exposures and covariates used in the analyses were extracted and merged using participants identification numbers. Checks were implemented to validate that the datasets were merged correctly. Data management by the author included conducting cleaning and quality checks on data pertaining to all key variables at relevant time points for this study. Data were evaluated for consistency, accuracy and outliers. Checks of all of the original response forms were conducted when missing or improperly defined data was detected. Recoding errors were corrected and where data was incorrectly captured from the original response forms, data were re-entered correctly. Data manipulation for the study included the creation of new variables (e.g., dummy variables and composite variables) and the recoding of existing variables (e.g., reverse coding), as well as converting or transforming variables (e.g., continuous variables to categorical variables). The cleaned datasets were then imported into the statistical software used for analysis; Stata version 13.0 (209).

Table 2. 1 Statistical analysis overview

Paper	Chapter	Statistical analysis
Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV	3	Descriptive statistics: categorical variables were reported as number and percentages and depending on normality, mean and standard deviation or median and interquartile range (IQR) for continuous variables. For statistical significance, a Student t-test or nonparametric Mann–Whitney U test was used for continuous outcomes, and chi-squared statistic or Fisher exact test was used for categorical outcomes. For analysis across more than two groups, analysis of variance (ANOVA) or the nonparametric Kruskal–Willis test was used. Statistical significance was set at a two-sided *P* value of <0.05. For statistically significant outcomes, logistic regression analysis was performed using backwards selection following univariate analysis at a significance level of *P*<0.20.
Maternal and neonatal outcomes following the introduction of oral hypoglycaemic agents for hyperglycaemia first detected in pregnancy were comparable to insulin monotherapy in two historical cohorts.	4	Comparison of the two cohorts was expressed as numbers and percentages for categorical variables and means and standard deviations for continuous variables. Statistical significance testing was done using student's t-test or the non-parametric Mann-Whitney U test for continuous outcomes, and chi-squared statistic or Fisher's exact test for categorical outcomes. For analysis across more than two groups, analysis of variance (ANOVA) or the non-parametric Kruskal Willis test were used. Statistical significance was set at a p-value of < 0.05.
Cardiometabolic outcomes of women exposed to hyperglycaemia first detected in pregnancy at 3-6 years post-partum in an urban South African setting	5	Mean and standard deviations were reported for normally distributed continuous variables and medians and interquartile ranges for non-normally distributed measured data. Number and percentages for categorical variables .

		Statistical differences between three groups (control, GDM and DIP), were tested using Analysis of Variance (ANOVA) or Kruskal-Wallis test. For categorical variables, Chi-squared test and Fischer's exact (small frequencies) was utilised. For continuous variables, a student's t-test or the non-parametric Mann-Whitney U was used. The statistical significance level was set at two-sided p-value <0.05. Multivariate logistic regressions were used to calculate the crude and adjusted odds ratios (OR) of progressing to T2DM, MetS and 10-year cardiovascular risk (FRS) considering confounders identified by bivariate analysis of factors ($p<0.05$). Multivariate models were designed to explore the relationship of maternal factors associated with the relevant outcomes adopting a timeline approach in which maternal factors present either at pre-pregnancy, index pregnancy and post-partum were assessed using logistic or linear regressions. The outcomes were adiposity in which fat mass index (FMI) was used as a surrogate, T2DM and cIMT as binary variables. The independent variables were identified using univariate analysis for each outcome. HFDP, an independent variable for all models was categorised according to degree of dysglycaemia as GDM and DIP. For logistic regression model diagnostics, results are expressed as OR and 95% confidence intervals (CI) and we assessed the following: linearity assumption using the Lowes graph, multicollinearity using variance inflation factors, model specification using the C-statistic, and confirmed the fit of the model using the Hosmer-Lemeshow goodness-of-fit test. We also checked for outliers as well as influential observations. For linear regressions cIMT were log-transformed to increase normality of residuals. Results are expressed as beta coefficient and 95% CI.


Retrospective component
Data for period January 2010-January
All diabetic pregnancies identified from an existing database CHBAH.
n=1263
Data captured
Pre-pregnancy factors
Pregnancy factors
Maternal and fetal outcomes
Paper 1
Paper 2
Data from January 2010 to January 2017
Data from January 2010 to January 2014
Pre-gestational diabetes and HFDP
n=1071
75gm OGTT/IADPSG criteria
HFDP participants only
n=192
100gm OGTT/WHO criteria
Prospective component
Prospective component
Participants identified for period February 2014-January 2017
Case control design and identification
HFDP group
(75gm OGTT/IADPSG criteria)
n=319
Non-HFDP group/controls
(75gm OGTT/IADPSG criteria)
n=103



Abbreviations: HFDP hyperglycaemia first detected in pregnancy, OGTT oral glucose tolerance test, IADPSG International Association of Diabetes and Pregnancy Study Group, CHBAH Chris Hani Baragwanath Academic Hospital, DPHRU Developmental pathways for Health Research Unit

Figure 2. 2 Flow diagram of the study design

2.3.1 Data collection instruments and measurements

Various methods and measurements were employed in the study; review of patient files, an administered questionnaire, maternal anthropometry, blood pressure measurements, body composition analysis, biochemical analysis including an OGTT, urine collection and ultrasonography imaging. The specific and detailed methodologies used to achieve each objective of this study are described in detail in the empirical papers.

Objective 1: To describe maternal and neonatal outcomes in diabetic pregnancies including fetal miscarriage, preterm delivery, pregnancy-induced hypertension, pre-eclampsia/eclampsia, CS rates and maternal death following diabetes in pregnancy considering how HIV and the exposure to OHAs influenced these outcomes.
Data from patient files at the CHBAH, Gestational Endocrine Clinic were retrospectively reviewed and captured into an electronic database (REDCap 9.3.1 Vanderbilt University Nashville, TN, USA) in order to achieve this objective. This study is described in Chapter 3.

Objective 2: Examine the safety and efficacy of oral hypoglycaemic agents on maternal and neonatal outcomes in the setting of HFDP exposure vs. insulin monotherapy amongst predominantly Black women, in an urban setting.
A retrospective audit of patient files was performed and compared to an earlier audit at the same clinic. Study described in Chapter 4.

Objective 3: Establish the risk of dysglycaemia (impaired fasting glucose, impaired glucose tolerance and development of T2DM), CVD (pre-clinical and clinical disease states) and MetS in women with a history of HFDP for a period three-six years post-partum and compare it to women non-exposed to HFDP for the same period. Additionally, to investigate the potential role of HIV during pregnancy on these outcomes as well as to assess body composition and fat mass distribution as measured by DXA in women with a history of HFDP compared to women non-exposed to HFDP.
A prospective case control evaluation of women with a history of HFDP (previously identified) vs. women non-exposed to HFDP at a 3-6-year interval following their index pregnancy. Data collection commenced from March 2019 through to November 2019 and took place at the DPHRU research site. Eligible participants attended a once-off visit where trained research assistants fluent in English and the most common African languages, explained the objectives

of the study and gained informed written consent. Participants also received information sheets (Appendix B) outlining what the purpose of the study was and what it involved. A questionnaire, various measurements including anthropometry, blood pressure, an OGTT, various biochemical measures, urine analysis, body composition analysis via DXA and ultrasonography of the carotid and femoral artery to measure IMT were performed.

DXA was selected to measure body composition and regional fat mass more accurately since conventional anthropometric measures such as waist and hip circumference are often limited by measurement variability and reproducibility as well as failing to discriminate between visceral and subcutaneous adipose tissue (VAT and SAT) (210). This is an important aspect to acknowledge since visceral obesity is considered to be more strongly associated with obesity related diseases such as T2DM and CVD more than other indices including BMI, waist circumference (WC) or waist: height ratio (WHR) (211). Though commuted tomography (CT) and magnetic resonance imaging (MRI) are considered "gold standard", for the measurement of VAT, these two imaging modalities are limited by their cost, together with radiation exposure for CT and the time required for MRI. DXA has been identified as an alternative method to estimate VAT in addition to accurately measuring whole body and regional distribution of fat and lean tissue (212,213). A prior study performed in SA confirmed the predictive ability and accuracy of DXA VAT measurement when looking at women (black and white) between the ages of 18-45 with no known co-morbidities. This study found that DXA VAT assessment performed as well as clinical read VAT from CT and better than anthropometric and demographic models (214).

Ten-year global cardiovascular risk prediction was determined by adopting the Framingham Risk Score (FRS) 2008. To date, numerous scoring systems are in existence and are outlined in Table 2.2 (215–220). The best known and probably the most widely used globally is the FRS. This score has been validated across different populations with several modified versions including QRISK2 and the Atherosclerotic Cardiovascular Disease (ASCVD) risk scores. These scores are used in clinical practice to identify and manage high-risk populations as well as to communicate risk effectively (221)

Table 2. 2 Overview of various cardiovascular risk prediction models

	Framingham risk score	SCORE	ASSIGN-SCORE	QRISK 1 and QRISK 2	PROCAM	WHO/ISH	Reynolds risk score
Population and sample type	General population, USA volunteers	Mostly random from general European population	Random sample from general population in Scotland	Health records of general practice attendees-not random	Healthy employees, volunteers, not random	N/A	Women: Health service employees Men: Physicians Not random, volunteers
Sample size	3639 men, 4522 women	117 098 men 88 080 women	6540 men 6757 women	1.28 million (1) 2.29 million (2)	18 460 men 8515 women	N/A	10 724 men 24558 women
Age range	30-75 years	40-65 years	30-74 years	35-74 years	20-75 years	40-79 years	45-80 years
Variables	Sex, age, total cholesterol, HDL cholesterol, SBP, smoking status, diabetes, HTN treatment	Sex, age, total cholesterol or total cholesterol/HDL ratio, SBP, smoking status,	Sex, age, total cholesterol, HDL cholesterol, SBP, smoking- no of cig, diabetes, area-based index of deprivation, family history of CHD	QRISK 1: sex, age, total cholesterol to HDL cholesterol ratio, SBP, smoking status, diabetes, area-based index of deprivation, family history, BMI and antihypertensive meds QRISK 2: also includes ethnicity and chronic diseases	Age, sex, LDL cholesterol, HDL cholesterol, diabetes, smoking, SBP	Sex, age, SBP, smoking status, diabetes +/- total cholesterol (different charts available for worldwide regions)	Sex, age, total cholesterol, HDL cholesterol, SBP, smoking status, family history of premature MI (parent age<60), HbA1c if diabetic, hsCRP

Calculates	10-year risk of CHD events originally Later version: 10-year risk of CVD events	10-year risk of CVD mortality	10-year risk of CVD events	10-year risk of CVD events	2 separate scores calculate 10-year risk of major coronary events and cerebral ischaemic events	10-year risk of CVD events	10-year risk of incident MI, stoke, coronary revascularisation or cardiovascular death

Abbreviations: BMI body mass index, CHD coronary heart disease, CRP C-reactive protein, CVD cardiovascular disease, Hb haemoglobin, HDL high-density lipoprotein, hsCRP high-sensitivity C-reactive protein, LDL low-density lipoprotein; MI myocardial infarction; SBP systolic blood pressure; WHO/ISH World Health Organization/International Society of Hypertension

The South African Heart Association and the Lipid and Atherosclerosis Society of Southern Africa (166), as well as the Southern African Hypertension Society in partnership with the National Department of Health, have endorsed a cardiovascular risk stratification approach for the management of CVD risk factors. Since the FRS has been validated in white and black populations and is transportable to other culturally diverse populations, this approach has been considered appropriate for local use (221–223). This approach has been considered relevant for local use since there is a lack of prospective data on the African continent together with the absence of locally validated total CVD risk assessment tools. This scoring system was best matched to our group of women since it has been validated for a younger age group (>30 years), stratifies risk by gender and includes a diagnosis of diabetes when calculating risk. Additionally, FRS has been shown to correlate well with cIMT(224) , which was used as surrogate marker of cardiovascular risk in this study. Overall, we felt the use of the FRS in this cohort of women in whom there was no history of any cardiovascular events, would best predict and communicate risk effectively the ten year all cardiovascular risk was stratified into three categories, low if the risk were less than 10%, intermediate if the risk were between 10% and less than 20% and high if the risk exceeded 20% This study is described in Chapter 5.

2.3.2. Sample size

Prior to commencement of the study, a sample size calculation was performed to determine the ideal sample size for 1) determining the risk of progression to T2DM, 2) determining progression to the MetS and 3) assessing cardiovascular risk and disease amongst a group of black African women exposed to HFDP vs those non-exposed in Soweto. All sample size calculation methods used a 5 % margin of error with a 95% confidence interval and a power of 80%.

Table 2. 3 The sample size required to determine the following outcomes among black SA women with a history of HFDP

Progression to T2DM
Given the reported estimated risk of developing T2DM following a HFDP pregnancy of 20-60% (13) and a 12% prevalence in the background population of SA women (225) a sample size of between 10-67 per group was calculated.
Progression to metabolic syndrome
Given the most recent metanalysis evaluating risk of progression to MS following HFDP, citing a 4-fold increase when compared to controls (16), a minimum sample size of n=64 per group was calculated utilising local data for background prevalence of MetS in black SA women of 42%
Prevalence of cardiovascular risk factors
Since no local data is available for this outcome, a metanalysis assessing cardiovascular events following HFDP vs. controls was used, where a 2-fold higher risk of CVD events post-partum compared with their peers was noted (14). A size of 98 per group was needed based on an estimated 7%[#] in the background population.

[*] All sample sizes were calculated using a population proportion estimate based on population parameters
[#] No data available for the prevalence of CVD events in young black women in our setting

Given the three sample size calculations, the sample size needed to establish CVD risk informed our decision to include a larger sample size.

2.4 Ethical considerations

The Human Research Ethics Committee at the University of the Witwatersrand (Medical) granted ethical clearance to perform this study (M180316 and M180204 see Appendix H).

SECTION 2
EMPIRICAL PAPERS

"To acquire knowledge, one must study; but to acquire wisdom one must observe."
Marilyn vos Savant

Chapter 3- Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV.

This chapter presents the research study that investigated the maternal and neonatal outcomes among urban black South African women following a diabetic pregnancy (including both pre-gestational diabetes and HFDP). These outcomes were reported in light of the background HIV burden together with the impact of OHA agents compared to the gold standard of insulin. This work has been published in the peer-reviewed journal, International Journal of Obstetrics and Gynecology. (Appendix I)

Nicolaou V, Soepnel L, *Huddle KRH, Klipstein-Grobusch, Levitt N & Norris, S.A. (2019) Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV International Journal of Obstetrics and Gynecology, 147: 1-9. DOI: 10.1002/ijgo.12956.*

3. 1 Introduction

The International Federation of Gynecology and Obstetrics (FIGO) has called for hyperglycaemia in pregnancy to be a global health priority (32) due to its rising prevalence, including in low- and middle-income countries (LMICs), and the serious associated consequences for mother and child (53). These include PIH, macrosomia, and stillbirth (37). Moreover, mothers with hyperglycaemia first detected in pregnancy (HFDP) have a higher chance of developing T2DM and cardiovascular complications postpartum, and metabolic consequences for the child contribute to an intergenerational cycle of disease (226,227).

Data about diabetes in pregnancy in SSA is limited. A recent review showed that merely 11% of countries had published prevalence data of HFDP (11). Reported rates of HFDP prevalence for Africa are in the range of 8.8%–9.3% (11,59,228). Within SA, varying rates of prevalence could be due to differences in population, screening practices, and diagnostic methods (57,59). Recent international recommendations for universal screening and the lower International Association of Diabetes and Pregnancy Study Groups (IADPSG) diagnostic criteria, distinguishing between overt GDM (DIP) and true GDM(GDM), are not uniformly implemented in SA, partly due to resource constraints (8,229). These discrepancies contribute to the underreporting and undermanaging of diabetes in pregnancy. A 20-year audit at a large urban hospital in SA between 1983 and 2002 showed the positive impact of management on maternal and neonatal outcomes for pre-gestational and HFDP (121). Since this publication, the same hospital has introduced OHA's, universal HIV monitoring and treatment, and lower diagnostic criteria for HFDP.

OHAs are not currently licensed for use during pregnancy due to lack of long-term safety data but use of metformin and glibenclamide is endorsed by major SA guidelines and is preferable to insulin in LMICs due to ease of use and affordability (60). Data from SA have shown a higher rate of macrosomia, neonatal hypoglycaemia, and perinatal mortality (PMR) in patients treated solely with glibenclamide (97,230). However, it is not clear whether these findings are a direct drug effect, and generalizability across SA is problematic due to disparities in ethnicity, diagnostic tests and criteria, and definitions of glycaemic control.

Additionally, understanding the metabolic risk of HIV-infected patients remains critical, since the overall prevalence of glucose metabolism disorders in HIV-infected individuals on

antiretroviral therapy (ART) is as high as 25% (188); pregnancy is an additional provoking factor (231). Treatment with ART has dramatically improved outcomes in HIV-infected pregnancies. However, the combined effect of hyperglycaemia, HIV, and ART exposure on maternal and neonatal outcomes remains unexplored.

Using data from one of the few specialized gestational endocrine clinics in SA, the aim of the present study was to investigate maternal and neonatal outcomes of T1DM, T2DM, and HFDP, and to explore the impact of HIV infection and exposure to OHAs on these outcomes, in order to create an informed platform to improve care for diabetes in pregnancy in LMICs.

3.2 Materials and Methods

We retrospectively analysed medical records of women attending the Gestational Endocrine Clinic at CHBAH for diabetes in pregnancy between 2012 and 2018. CHBAH is a 3000-bed teaching hospital with 1400–1600 deliveries per month in the large urban township of Soweto, with an antenatal HIV prevalence of 30%. The population attending the clinic is predominantly black African. Figure 3.3 shows a timeline of clinical practice after referral to the clinic. Participants with DIP were initiated on insulin based on a clinical decision where the degree of hyperglycaemia and presence of complications were taken into account. Excluding this, the first choice of pharmacological treatment for HFDP participants was metformin, which was escalated, if necessary, by either adding glibenclamide or transitioning to insulin, a decision based on levels of hyperglycaemia and the presence of diabetic complications. Those with T2DM on existing regimes were switched, where necessary, to either metformin and/or glibenclamide; otherwise, treatment was escalated as above. Only women with singleton pregnancies treated for T1DM, T2DM, and HFDP diagnosed according to the definitions in Table 3.4 were included in the present study. See Table 3.4 for definitions of maternal and neonatal outcomes. The Human Research Ethics Committee, University of the Witwatersrand (M180204) (Appendix H) approved this study.

Data were captured directly into an electronic database (REDCap, CO, USA), and subsequently imported into STATA 13 (StataCorp, TX, USA) (209). Baseline characteristics were described using number and percentage for categorical variables and, depending on normality, mean and standard deviation or median and interquartile range (IQR) for continuous variables. To test for normality, a skewness-kurtosis test was performed if the mean and median were

substantially different. When analysing the role of OHAs, participants that presented after a gestational age of 36 weeks or received less than 2 weeks of medical intervention were excluded.

For statistical significance, a Student t-test or nonparametric Mann–Whitney U test was used for continuous outcomes, and chi-squared statistic or Fisher exact test was used for categorical outcomes. For analysis across more than two groups, analysis of variance (ANOVA) or the nonparametric Kruskal–Willis test was used. Statistical significance was set at a two-sided *p* value of <0.05. For statistically significant outcomes, logistic regression analysis was performed using backwards selection following univariate analysis at a significance level of $p<0.20$, considering the following variables: maternal age, initial body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters), weight gain over pregnancy, gestational age at initial presentation, initial glycated haemoglobin (HbA1c), presence of chronic HTN, HIV, or anaemia, multiparity, glycaemic control by the third trimester, and exposure to OHAs. We reported those that reach statistical significance by $p<0.05$ in the final model. A positive outcome per variable ratio of 10 was adhered to for regression models.

3. 3 Results

There were 1071 diabetic pregnancies during the study period: 461 (43.0%) HFDP; 408 (38.1%) T2DM; and 202 (18.9%) T1DM (Table 3.1). DIP constituted 235 (51.0%) of HFDP cases and GDM 226 (49%). The median gestational age at initial presentation was 15.6–16.1 weeks for those with pre-gestational diabetes and 28.0–29.5 weeks for those with HFDP. The T1DM group was younger (mean 26.5 ± 5.6), had a higher initial HbA1c (median 8.3, IQR 7–10.2), higher frequency of retinopathy and nephropathy 17 (8.4%) and 4 (4.7%), respectively, and a lower BMI (29.3 ± 6.6) compared to the HFDP and T2DM groups. For T2DM, the median initial HbA1c was 7.6 (IQR 6.4–9.1), for DIP it was 7.2 (IQR 6.5–8.8) and for GDM it was 5.8 (IQR 5.4–6.3). All four groups were overweight by mean BMI at presentation. Overall, 234 (21.8%) women in the cohort had HTN, with the T2DM group accounting for the highest prevalence at 136 (33.3%).

All patients with T1DM received insulin. A total of 261 (56.6%) participants with HFDP and 347 (85.1%) participants with T2DM were exposed to OHAs. Of these, 31 (11.9%) in the

HFDP group and 217 (62.5%) in the T2DM group were switched to insulin for insufficient glycaemic control. Of all groups, 854 (94.9%) participants were controlled by the third trimester, with the lowest control in the DIP group at 187 (90.3%).

In terms of maternal outcomes, loss to follow-up was highest in the T1DM (n=37, 18.3%) and GDM (n=47, 20.8%) groups (Table 3.2). The number of Caesarean deliveries was 641 (72.2%) and the rate was highest in the T2DM group at 275 (78.4%). The rate of PIH was 9.2% (n=98) across all groups; the combined rate of pre-eclampsia and eclampsia was 2.7% (n=29). The rates of miscarriage and maternal mortality were highest in the T1DM group at 14 (6.9%) and 3 (1.5%), respectively. Initial HbA1c was the significant predictor for miscarriage when performing regression analyses (odds ratio [OR] 1.2, 95% confidence interval [CI] 1.13–1.55, *P*=0.001). When investigating "any obstetric complication" (the combined rate of miscarriage, hypertensive disorders of pregnancy, urinary tract infection, polyhydramnios, oligohydramnios, maternal death), no significant differences were found between the groups. Of offspring, 747 (92.6%) had an APGAR score ≥7 at 1-minute postpartum (Table 2). The rate of prematurity (n=237, 30.9%) was significantly lower in the GDM group (n=41, 22.8%) than the other groups, but not when correcting for initial HbA1c (OR 1.17, 95% CI 1.08–1.26, $p<0.001$) and lack of glycaemic control in the third trimester (OR 2.36, 95% CI 1.24–4.47, $p=0.009$). Macrosomia, with a prevalence of 71 (8.4%) in the whole cohort, was highest in the DIP group (n=24, 12.3%) and significantly lower in the GDM group (n=7, 4.0%). For both LBW, which was highest in the T1DM group, and macrosomia, the difference between the groups was no longer significant when adjusting for maternal BMI (OR 0.95, 95% CI 0.91–0.98, *P*=0.002; OR 1.05, 95% CI 1.00–1.09, $p=0.027$, respectively, for LBW and macrosomia), with multiparity (OR 0.61, 95% CI 0.37–0.99, $p=0.047$) and HTN (OR 1.85, 95% CI 1.04–3.29, $p=0.036$) being the other two significant predictors for LBW. Perinatal mortality was significantly higher in the T1DM group (n=10, 6.7%) when compared to T2DM and the HFDP group in its entirety ($p=0.019$), although HIV was the main statistical contributor in our model (OR 5.00, 95% CI 2.06–12.15, $p<0.001$). The T1DM group also had the most major congenital anomalies at 1.7% (n=2). Additional neonatal outcomes in the cohort included hypoglycaemia (n=10, 1.2%), respiratory distress syndrome (n=8, 1.0%), and jaundice (n=4, 0.5%), with no significant differences between groups.

When considering predictive factors for poor neonatal outcome (prematurity, LBW, macrosomia, PNM) per type of diabetes**,** initial HbA1C was found to significantly predict poor

neonatal outcome for T1DM. In the HFDP group, initial HbA1C (OR 1.17, 95% CI 1.00–1.37, *p*=0.044), gestational age at presentation (OR 0.95, 95% CI 0.91–0.98, *p*=0.005), and lack of glycaemic control by the third trimester (OR 3.12, 95% CI 1.21–9.08, *p*=0.019) were significant predictors for poor neonatal outcome. Within the T2DM group, no significant predictors were found.

When comparing outcomes for the group exposed to OHAs (metformin monotherapy and glibenclamide exposure) to insulin monotherapy (Table 3.3), the characteristics with significant differences between groups were weight gain over pregnancy and initial HbA1c (both higher in the insulin group), and the number achieving glycaemic control by the third trimester (lower in the insulin group). In addition, BMI at first presentation in those with T2DM was significantly higher in the glibenclamide group versus the insulin monotherapy group (34.6 vs 31.6 (IQR 31.6–41.8, 27.6–36.2). No significant differences were found in maternal outcomes between treatment groups.

T2DM: For the participants with T2DM, glibenclamide exposure was associated with a significantly higher prevalence of macrosomia in the T2DM group when compared to the insulin monotherapy group (n=10 [12.2%] vs 0, *p*=0.030) (Fig. 1). Participants with T2DM who were on glibenclamide but were switched to insulin (n=51, 12.5%) also had higher rates of macrosomia compared to insulin monotherapy (n=9 [19.6%] vs 0, *p*=0.003). PNM did not significantly differ between the groups.

HFDP: In the GDM metformin-exposed group, a higher number of offspring had LBW (17 [20.0%] vs 7 [8.4%], *p*=0.032) and a lower number presented with macrosomia than in the insulin monotherapy group (1 [1.2%] vs 9 [10.8%], *p*=0.009) (Fig. 3.1). There were too few cases of LBW and macrosomia in the HFDP group to pursue regression analysis. As with T2DM, PNM was not significantly different between groups.

HIV infection was found in 212 (24.0%) women in the cohort, the highest in the T1DM group with 45 (29.4%) participants and the lowest in the HFDP group with 72 (17.4%) participants. Maternal weight and BMI at first presentation, HTN, gestational age at presentation, and glycaemic control in the third trimester were similar among HIV-infected and uninfected participants. However, the HIV-infected group had a significantly higher rate of anaemia (61 [29.2%] vs 65 [9.7%], *p*≤0.001) and a lower initial HbA1C (median 6.7, IQR 5.6–8.5 vs median

7.1, IQR 6.1–8.7, *p*=0.013). A lower rate of macrosomia was observed in the HIV-infected HFDP group (0 vs 28 [10.0%], *p*=0.012) (Fig. 3.2). However, PNM was significantly higher in the HIV-infected T1DM (5 [14.7%] vs 0, *P*=0.002) and GDM groups (4 [7.0%] vs 3 [1.1%], *P*=0.017).

3. 4 Discussion

In this large retrospective study: (i) initial HbA1c, BMI, and glycaemic control were important predictors of maternal and neonatal outcomes; (ii) initial BMI was high in all diabetes groups, including T1DM; (iii) glibenclamide exposure was associated with higher macrosomia rates; and (iv) HIV infection was associated with higher PNM.

The rates of key adverse outcome including birth weight (macrosomia and LBW), prematurity, fetal anomalies, hypertensive disorders of pregnancy, maternal mortality, and PNM were similar to a previous audit from 1992 to 2002 at our clinic (121) and to another recent SA audit (232). However, macrosomia, PNM, and prematurity remain inferior to respective rates in SA for non-diabetic pregnancies (233–235). The rate of Caesarean delivery (n=641, 72.2%) was much higher in the present study than the estimated rate of Caesarean delivery at CHBAH of 39.8% in non-diabetic pregnancies (236), but only moderately higher than the 1992–2002 audit of diabetic pregnancies (62%), a difference that could be due to an overall increase in rates of Caesarean delivery over this period. When compared to two recent studies from the UK, rates of Caesarean delivery and PNM, which were in the range of 1.2–2.7%, were higher in our pre-gestational groups(237,238). However, comparisons to high-income countries (HIC) as a whole are complicated by differences in population, study design, diagnosis, and management (239,240).

Obesity, so common in our cohort and in South African women in general, not only augments the metabolic burden of pregnancy, it also increases the risk of adverse outcomes (241,242). In our cohort, BMI was found to be a significant predictor for macrosomia. Even the T1DM group had a high BMI at presentation, in contrast to the traditional set of characteristics attributed to T1DM. This indicates that the rising public health burden of MetS is impacting even unexpected population groups.

The T1DM group showed higher rates of fetal anomaly, maternal mortality, miscarriage, and

PNM than the other groups. This is most likely driven by the severity of hyperglycaemia, a trend that is consistent with the international literature (232,243). However, our high rates of PNM, compared to international studies (237,238) and South African background rates, reveals a critical area for improvement for women with pre-gestational diabetes, especially considering the unacceptably high initial HbA1c for both T1DM and T2DM and the lack of glycaemic control in 111 (49%) participants at initial presentation. This highlights the need for improved counselling and family planning for women of childbearing age with diabetes, for optimization of glycaemic control.

Notably, initial HbA1c and outcomes including prematurity and stillbirth were similar for the DIP and T2DM groups, but rates of macrosomia, PIH, and congenital anomalies were higher in the former, indicating that the DIP group most likely consisted of undetected T2DM. This highlights the importance of increased pre-conceptional detection of T2DM through strengthening of diabetic health systems in LMICs. The GDM group had fewer adverse outcomes than the DIP group, which consisted of more than 50% of HFDP. This low rate of adverse outcomes and the 97.5% glycaemic control attained indicates the successful treatment of this group, predominantly with metformin (n=171, 75.7%). The discrepancy with DIP reiterates that severity of initial hyperglycaemia is an important predictor for worse outcomes and lack of glycaemic control.

The HIV prevalence rate in our cohort of 212 (24.0%), though high in an international context, is similar to regional estimates of 28% (244). The lower HbA1C in the HIV-infected population was unexpected, as this group had higher rates of anaemia, which results in higher HbA1C if caused by iron deficiency anaemia (245,246). However, this relationship is complex since the cause of anaemia in our cohort was unknown and HbA1C has previously been found to underreport hyperglycaemia in HIV-positive patients (246). On the other hand, the observation that first visit mean blood glucose (MBG) was also lower in our HIV-infected group tends to affirm better control in this group. One possible explanation is that participants with known HIV infection may have increased contact with health services, improving lifestyle measures (247), although this was not reflected in earlier presentation for the HIV-infected group.

We found a higher PNM rate of 9.1% (16 cases) in HIV-infected participants. Although a background rate for PNM in HIV-infected patients on ART is not known, this was higher than the South African national PNM estimate for the general population (3.3%) (233). Previous

studies, in both HICs and LMICs, have found an increased PNM in HIV-infected pregnancies (248,249). When considering the consequences of HIV infection, the contribution of chronic generalized inflammation, indirect HIV-associated conditions, and side-effects of ART are difficult to unravel (187). The higher rates of anaemia in the HIV-infected group may have contributed to the high PNM rates, given that anaemia is associated with higher neonatal morbidity and mortality (250) .

All of the HIV-infected participants would have likely been placed on a lifelong ART regimen, in adherence with "test and treat" guidelines (251). However, a lack of documented information on both severity of HIV and exact use of ART prohibited us from further analyses in these areas. Regardless, the high risk of PNM and higher rates of anaemia in this combined diabetic and HIV-infected population marks them as a group of patients that could benefit from close monitoring and further research.

An unusually high number of HFDP patients required pharmaceutical treatment to achieve glycaemic control, which may be attributable to the high proportion of DIP women, and the use of risk-factor based screening. Importantly, the use of OHAs was adequate to achieve glycaemic targets in 261 (88.1%) GDM pregnancies, which is relevant in an LMIC setting due to their convenience and affordability. Evidence regarding safety is therefore highly important. For both T2DM and HFDP, maternal outcomes were not significantly different between patients exposed to glibenclamide and metformin compared to the gold standard of insulin. However, rates of macrosomia were higher among those exposed to glibenclamide (n=10 [12.2%] vs 0) despite a significantly lower initial HbA1c and better glycaemic control in the third trimester in this group. This was in line with results from a recent meta-analysis observing glibenclamide to be inferior to both metformin and insulin in terms of rates of macrosomia and neonatal hypoglycaemia (97). It has been suggested that the effect of glibenclamide may be due to a direct drug effect via placental transfer, but the relationship could also be due to the higher initial BMI in the glibenclamide group (235).

Reassuringly, we found no significant relationship between use of OHAs and prevalence of congenital anomalies, but a limited number of patients with T2DM (n=115, 25.8%) and HFDP (n=9,2.6%) were exposed to the drugs in the first trimester. Neither OHA negatively influenced PNM when compared to insulin. This contributes to the evidence that these agents may be a safe alternative to insulin in our setting (97,252).

Limitations of our study are related to the retrospective study design, resulting in missing data due to underreporting in files, including BMI, HIV status and treatment details, possible rates of pre-eclampsia/eclampsia, and some neonatal outcomes (hypoglycaemia, jaundice, and respiratory distress syndrome). Moreover, we did not have data on pre-pregnancy BMI and the use of BMI at initial presentation unadjusted for gestational age could have resulted in an overestimation of rates of obesity and overweight. It is important to note that the rate of miscarriage is likely an underestimation due to a lack of information on miscarriages in early gestation, since participants commonly presented during the second and third trimesters. Regression analyses could not be performed for all outcomes regarding HIV and treatment groups because of low positive outcomes. Loss to follow-up of 166 (15.4%) participants may have resulted in bias, although the only significant differences in baseline characteristics were younger age at presentation and higher primiparity rates in the group lost to follow-up.

The high rate of obesity and the significant impact of initial hyperglycaemia and glycaemic control on adverse outcomes emphasizes the need for improved pre-conception care, including increasing awareness of both obesity and diabetic health risks, earlier detection of T2DM, implementation of lifestyle interventions, and, in the case of pre-gestational diabetes, improved pre-conceptional glycaemic control. HIV-infected women with diabetes in pregnancy demonstrated a higher rate of PNM, which warrants further exploration. In addition, a greater understanding of the reasons for the increased rate of macrosomia associated with glibenclamide exposure is especially relevant in a LMIC setting, where the affordability and accessibility of OHAs make them an attractive alternative to insulin. These insights address gaps in diabetes health care in pregnancy that are meaningful for other LMICs where the double burden of infectious and non-communicable disease is impacting maternal and child health.

Table 3. 1 Demographic, obstetric, comorbidity, and diabetes characteristics of participants with T1DM, T2DM, and HFDP.

	n= 202	**T1DM**	**n= 408**	**T2DM**	**HFDP**			
					n= 235	**DIP**	**n= 226**	**GDM**
Maternal characteristics at initial presentation								
Maternal age (mean, SD)	202	26.9 (5.6)	403	34.0 (5.2)	235	32.4(5.3)	226	32.9(6.1)
GA, weeks (median, IQR)	200	15.6 (9.8–23.1)	407	16.1 (11.0-24.3)	235	28.0 (21.7-32)	224	29.5 (26.6-33.0)
BMI (mean, SD)	112	29.3 (6.6)	207	35.1 (8.1)	116	36.6 (7.9)	105	35.5 (8.5)
BP (mean, SD)	190	123/74.7 (16.0–11.5)	396	125.9/76.5 (15.0–11.6)	223	123.0/73.6 (14.0–10.9)	213	120.5/71.1 (14.2–9.6)
Obstetric history								
Primigravida (n, %)	200	69 (34.5)	407	21 (5.2)	235	20 (8.5)	226	22 (9.7)
Previous pregnancy losses (n, %)	200	49 (24.5)	407	147 (36.1)	235	94 (40.0)	226	99 (43.8)
Comorbidities at presentation								
Chronic hypertension (n, %)	202	27 (13.4)	408	136 (33.3)	234	35 (15.0)	225	36 (16.0)
HIV positive (n, %)	153	45 (29.4)	320	95 (29.7)	212	25 (11.8)	203	47 (23.1)
Anaemia (n, %)	199	46 (23.1)	403	56 (13.9)	235	25 (10.7)	226	34 (15.4)
Characteristics of diabetes								
Age at diagnosis, y (median, IQR)	196	21 (16–25)	383	31 (27–34)		NA		N/A
Initial Hba1c, % (median, IQR)	191	8.3 (7–10.2)	393	7.6 (6.4–9.1)	215	7.2 (6.5–8.8)	207	5.8 (5.4–6.3)
Control at initial presentation (n, %)	86	48 (55.8)	133	63 (47.3)	136	NA	78	NA
Control at third trimester [a] (n, %)	147	141 (95.9)	351	335 (95.4)	207	187 (90.3)	196	191 (97.5)
Target organ damage (n, %) Retinopathy Nephropathy	202 85	17 (8.4) 4 (4.7)	407 168	15 (3.7) 1 (0.6)	234 81	3 (1.3) 0	226 85	2 (0.9) 0
Therapy								
Exposure to metformin (n, %)	202	14 (6.9)	408	351 (86.0)	235	120 (52.8)	226	171 (75.7)
Exposure to glibenclamide (n, %)	202	1 (0.5)	408	151 (37.0)	235	80 (34.0)	226	76 (33.6)
Exposure to insulin (n, %)	202	202 (100.0)	408	270 (66.2)	235	134 (57.0)	226	25 (11.1)

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; HFDP, hyperglycaemia first detected in pregnancy; DIP, diabetes in pregnancy; GDM, gestational diabetes; SD, standard deviation; GA, gestational age; IQR, interquartile range; BMI, body mass index; BP, blood pressure; HbA1c, glycated haemoglobin; MBG, mean blood glucose.

[a] Control in third trimester = MBG <7.1 in third trimester.

Table 3. 2 Neonatal and maternal outcomes of participants with T1DM, T2DM, and HFDP.

	n	**T1DM**	**n**	**T2DM**	**HFDP**				***P* value**
					n	**DIP**	**n**	**GDM**	
Lost to follow-up	202	37 (18.3)	408	51 (12.5)	235	31 (13.2)	226	47 (20.8)	0.020 [b]
Maternal outcomes									
CD	155	104 (67.1)	351	275 (78.4)	199	142 (71.4)	177	120 (67.8)	0.015 [b]
Miscarriage	202	14 (6.9)	408	12 (2.9)	235	4 (1.7)	226	2 (0.9)	0.001 [b]
PIH	202	23 (11.4)	408	31 (7.6)	235	27 (11.5)	226	17 (7.5)	0.198
Pre-eclampsia/ Eclampsia	202	5 (2.5)	408	13 (3.2)	235	2 (0.9)	226	9 (4.0)	0.749
Maternal mortality	202	3 (1.5)	408	0 (0)	235	1 (0.4)	226	0 (0)	0.017 [b,c]
Neonatal outcomes									
Prematurity	165	57 (34.6)	358	117 (32.9)	212	65 (31.9)	195	41 (22.8)	0.065
Macrosomia	141	12 (8.5)	333	28 (8.4)	195	24 (12.3)	174	7 (4.0)	0.042 [b]
LBW	141	41 (29.1)	333	59 (17.7)	195	26 (13.3)	174	23 (13.2)	0.001 [b]
Major congenital anomaly	115	2 (1.7)	282	2 (0.7)	172	2 (1.2)	147	0 (0)	0.450 [c]
PNM	149	10 (6.7)	346	15 (4.3)	201	4 (2.0)	178	3 (1.7)	0.053 [c]
Stillbirth	149	8 (5.4)	346	11 (3.2)	201	4 (2.0)	178	3 (1.7)	0.225

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; HFDP, hyperglycaemia first detected in pregnancy; DIP, diabetes in pregnancy; GDM, gestational diabetes; CD, caesarean delivery; PIH, pregnancy-induced hypertension; LBW, low birth weight; PNM, perinatal mortality.

[a] Values are given as number (percentage).

[b] Statistical significance ($p<0.05$).

[c] Fisher exact test.

Table 3. 3 Comparison of baseline characteristics in metformin monotherapy and glibenclamide-exposed groups versus insulin monotherapy.

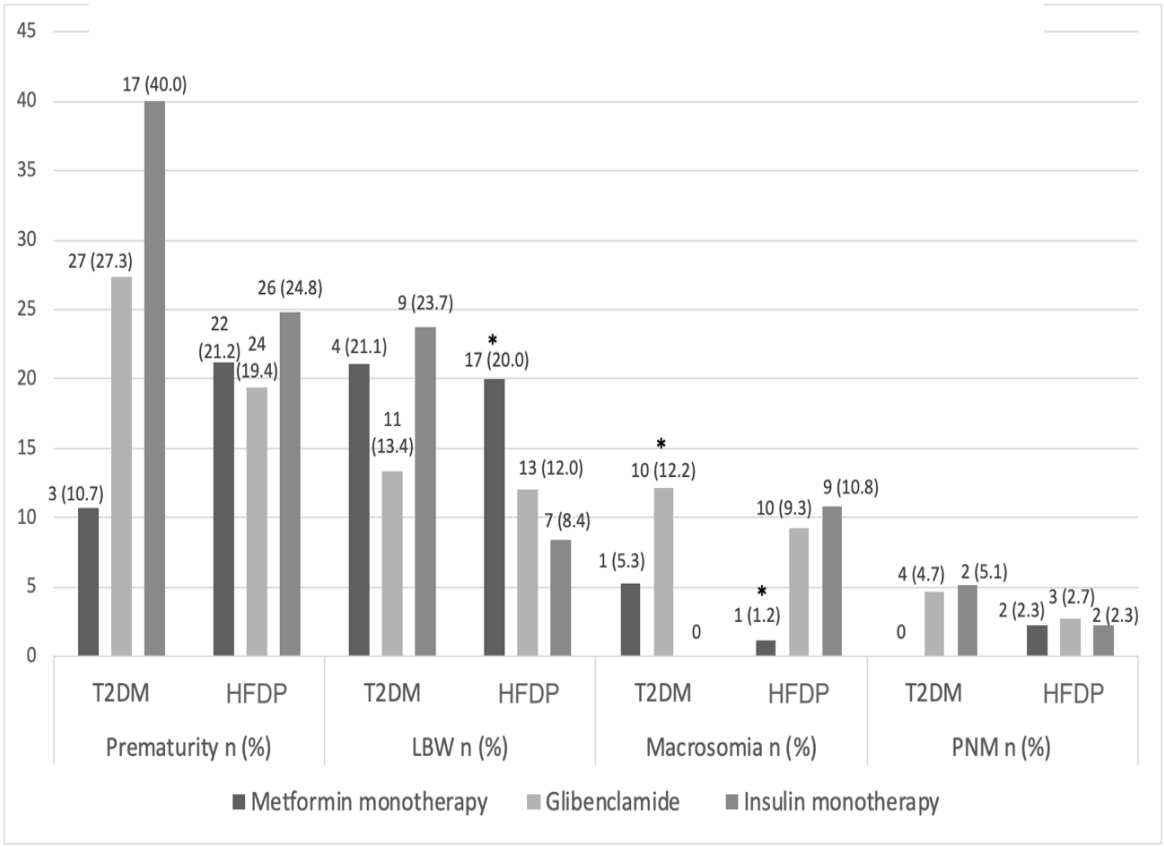
	n	**Exposed to metformin monotherapy [a] (n=106)**	n	**Exposed to glibenclamide [b] (n=296)**	n	**Exposed to insulin monotherapy (n=332)**	***P* value**
T2DM HFDP	408 461	29 (7.1) 106 (23.0)		100 (24.5) 124 (26.9)		46 (11.3) 106 (23.0)	<0.001 [a], <0.001 [b]
Age at presentation (median, IQR) T2DM HFDP	 26 106	 35 (28–38) 33 (39–37)	 99 124	 34 (30–37) 33 (29–37)	 46 106	 35 (31–38) 34 (29–38)	 0.518 [a], 0.391 [b] 0.660 [a], 0.593 [b]
BMI at first presentation (median, IQR) T2DM HFDP	 12 51	 32.95 (27.3–42.1) 33.8 (30.3–40)	 55 55	 34.6 (31.6–41.8) 36.7 (31.6–41.5)	 29 50	 31.6 (27.6–36.2) 35.35 (31.2–41.1)	 0.626 [a], 0.021 [b,c] 0.250 [a], 0.674
Weight gain over pregnancy (median, IQR) T2DM HFDP	 28 100	 3.5 (0.45–6.4) 0.95 (-0.2 to 2.5)	 97 117	 3.3 (1–7) 2.5 (0–6)	 43 101	 7.1 (2.5–11) 5 (1.9–9.8)	 0.008 [a,c], 0.003 [b,c] <0.001 [a,c], <0.001 [c]
Initial HbA1c (median, IQR) T2DM HFDP	 27 99	 6.2 (5.5–7.5) 5.8 (5.4–6.2)	 97 119	 6.7 (5.9–7.6) 6.2 (5.8–6.9)	 45 94	 7.9 (6.6–9.5) 8.55 (7–10.1)	 0.002 [a,c], <0.001 [b,c], <0.001 [a,c], <0.001 [b,c]
Number controlled at third trimester [d] (n, %) T2DM HFDP	 23 96	 22 (95.7) 96 (100)	 92 119	 92 (100) 117 (98.3)	 40 96	 36 (90.0) 86 (89.6)	 0.644 [a], 0.008 [b,c] 0.002 [a,c], 0.007 [b,c]

[a] *p* value for comparison between metformin monotherapy and insulin monotherapy groups.
[b] *p* value for comparison between glibenclamide and insulin monotherapy groups.
[c] Statistical significance (*P*<0.05).
[d] Control in third trimester = MBG <7.1 in third trimester.
Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; . HFDP, hyperglycaemia first detected in pregnancy IQR, interquartile range; HbA1c, glycated haemoglobin; MBG, mean blood glucose

Table 3. 4 a. Definition and criteria for diagnosis of diabetes in pregnancy, including T1DM, T2DM, and HFDP. b. Definition of maternal and neonatal outcomes.

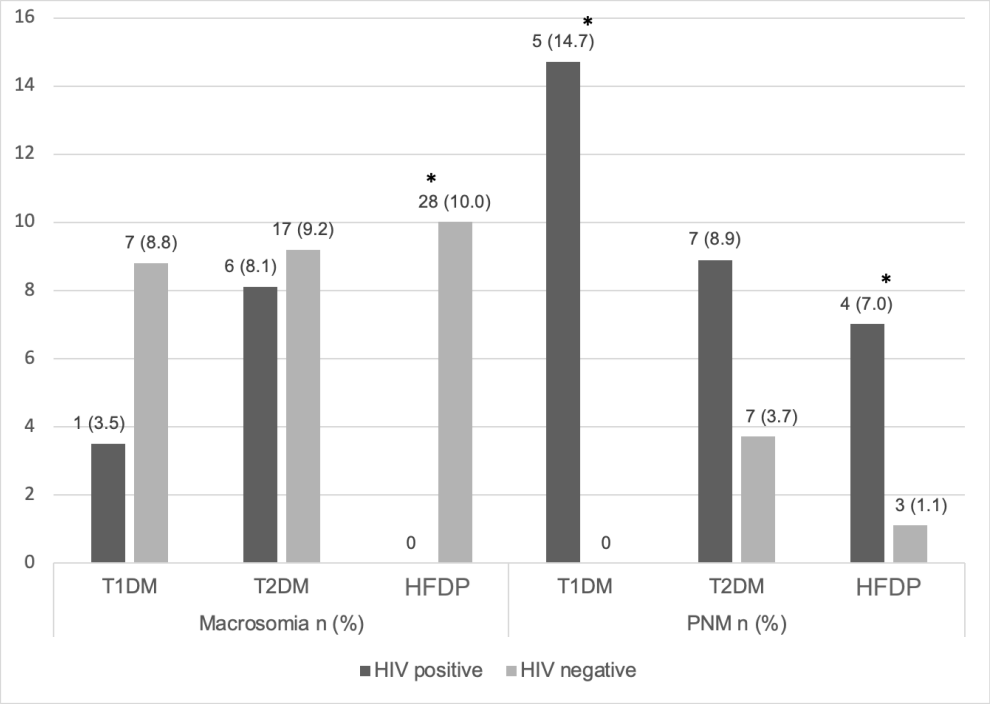
a.	
HFDP	Diabetes first discovered in pregnancy, diagnosed using a 2-h 75-g OGTT and the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, or a random plasma glucose ≥11.1 mmol/L. This method distinguished between true GDM(GDM) and overt GDM(DIP), diagnosed when fasting plasma glucose ≥7.0 mmol/L, or 2-h or random plasma glucose ≥11.1 mmol/L. For the purposes of this study, cases of GDM diagnosed pre-2014 by a 100-g 3-h oral glucose tolerance test were excluded. Diagnostic testing based on selective screening of risk factors for GDM, except for women universally screened in the context of a research study between 2014 and 2016.
Risk factors for HFDP	Persistent glycosuria, (first degree) family history of diabetes, previous unexplained perinatal losses, previous GDM, history of a macrosomic baby.
Pre-gestational diabetes	Diabetes diagnosed previous to conception, categorized as T1DM or T2DM according to the clinical diagnosis made on the hospital file.
b.	
Maternal outcomes	
Glycaemic control	Mean blood glucose <7.1 mmol/L for the 3rd trimester, measured by capillary blood glucose on a glucometer
Dietary Failure	Lack of glycaemic control after 2 weeks of dietary management
Maternal overweight	BMI ≥25
Maternal obesity	BMI ≥30
Nephropathy	Creatinine >71 ummol/L and MAC >30 mg/L
Anaemia	Haemoglobin levels < 11 g/dL
HIV-positive	Based on results from rapid or antibody ELISA tests before or at time of initial presentation
Hypertension	Blood pressure elevated > 140/90 mmHg for at least two measurements
Hypertensive disorders of pregnancy	Categorized as: Chronic hypertension: hypertension present before pregnancy PIH: hypertension presenting >20 completed weeks Pre-eclampsia: hypertension with proteinuria Eclampsia: hypertension with proteinuria and seizures
Neonatal outcomes	
Prematurity	Delivery <37 completed weeks gestational age
Miscarriage	loss occurring at <28 completed weeks gestational age
Stillbirth	Loss occurring at ≥29 weeks gestational age
END	Neonatal demise occurring in the first week of life
PNM	The number of stillbirths and early neonatal deaths out of the total number of deliveries
LBW	Birth weight <2500 g
Macrosomia	Birth weight ≥ 4000 g
Major congenital anomaly	Spinal, cardiac, central nervous system, renal, or digestive system anomaly.

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; HFDP, hyperglycaemia first detected in pregnancy; DIP, diabetes in pregnancy; GDM, gestational diabetes; OGTT, oral glucose tolerance test; PIH, pregnancy-induced hypertension; END, early neonatal death; PNM, perinatal mortality; LBW, low birth weight.



T2DM, type 2 diabetes; HFDP, hyperglycaemia first detected in pregnancy; LBW, low birth weight; PNM, perinatal mortality.

Figure 3. 1 Neonatal outcomes in metformin monotherapy and glibenclamide-exposed groups versus insulin monotherapy in participants with T2DM and HFDP.

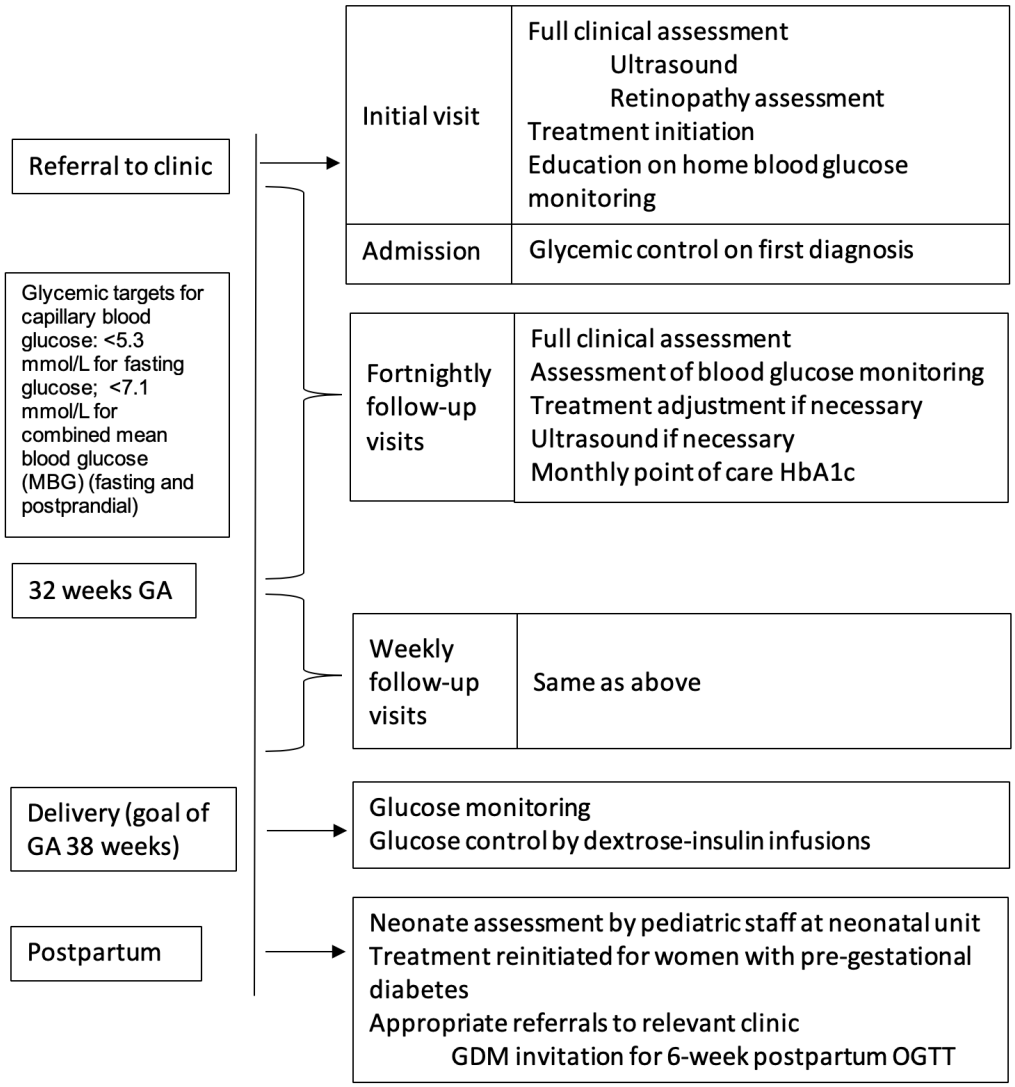


*= Significant difference between HIV positive and HIV negative groups (P<0.05)

T1DM, type 1 diabetes; T2DM, type 2 diabetes; HFDP, hyperglycaemia first detected in pregnancy

Figure 3. 2 Neonatal outcomes in HIV-infected and uninfected participants with T1DM, T2DM, and HFDP.

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GA, gestational age; GDM, gestational diabetes; OGTT, oral glucose tolerance test.

Figure 3. 3 Flow chart of clinical practice for patients attending the gestational endocrine clinic.

Chapter 4 – The maternal and neonatal outcomes following the introduction of oral hypoglycaemic agents for hyperglycaemia first detected in pregnancy were comparable to insulin monotherapy within two historic cohorts.

This chapter presents the research study that explored the effect following the introduction of OHA compared to insulin monotherapy on immediate maternal and neonatal outcomes between two cohorts of black African women with HFDP. This work has been published in the peer-reviewed journal, South African Medical Journal. (Appendix J)

Nicolaou V, *Soepnel L,* *Huddle KRH, Levitt N, Klipstein-Grobusch & Norris, S.A. (2020) Maternal and neonatal outcomes following the introduction of oral hypoglycaemic agents for gestational diabetes were comparable to insulin monotherapy within two historic cohorts South African Medical Journal, 110: 154-158. doi:10.7196/SAMJ. 2020.v110i2.14024.*

4. 1. Introduction

The landscape of hyperglycaemia first detected in pregnancy (HFDP) has remained dynamic, with the prevalence, definition, screening protocols, diagnostic criteria and therapeutic modalities continuously changing from as early as the 1960's. Most recently, HFDP has been defined as a disorder of glucose intolerance first encountered during pregnancy that is not clearly overt diabetes (8). HFDP has far-reaching well-known ramifications for both mother and infant (37). Global prevalence estimates are 16.9% (195,253) with recent studies in SA (SA) showing prevalence figures ranging from 9.1-25.8% (58,59).

Traditionally, HFDP is successfully managed with dietary and lifestyle interventions alone. However, insulin is introduced if glycaemic goals are not achieved (71,93,229) Oral hypoglycaemic agents (OHAs) are an attractive alternative if lifestyle measures fail, particularly in resource poor countries, due to low cost and ease of administration. However, their use for HFDP is surrounded by much debate regarding efficacy and safety, being recommended by some guidelines (60,71,91) and being omitted in others (31). One meta-analyses which included 13 studies, utilising different diagnostic tests and criteria for HFDP, with representation from both high and low-income countries investigated the safety of OHA's compared to insulin when treating HFDP. It demonstrated a higher preterm birth rate (relative risk RR1.51) in patients exposed to metformin monotherapy with a lower risk of both gestational HTN (RR 0.54) and post-prandial glucose levels (107). A further metanalysis of 15 studies showed a higher risk of macrosomia and neonatal hypoglycaemia for patients exposed to glibenclamide (97). Research from SA has generally shown reassuring safety outcomes in terms of fetal anomalies and maternal glycaemic control for both metformin and glibenclamide in patients with both T2DM in pregnancy and HFDP (230,254). However, a recent study showed a notably increased PNM in T2DM patients when exposed to glibenclamide, which could not be explained (230).

In the current study, we compared the fetal and maternal outcomes of two cohorts of patients with GDM treated at CHBAH a decade apart, where the first cohort (1992-2002) was exposed to insulin monotherapy and the more recent group (2010-2014) exposed to one or more treatment combinations including OHA monotherapy (metformin alone), OHA dual therapy (metformin plus glibenclamide), or insulin monotherapy. Furthermore, the outcomes within

the 2010-2014 cohort were compared between the insulin monotherapy group vs. those exposed to OHAs

4.2. Materials and methods

4.2.1 Study population

This study retrospectively compares two cohorts of women attending the Gestational Endocrine Clinic at CHBAH for GDM, the first between 1992 and 2002, and the second between 2010 and 2014. Characteristics and outcomes of the 1992-2002 cohort have been previously published and included for the purposes of this paper, (121) where patients were exclusively exposed to insulin monotherapy. A secondary analysis within the later cohort was performed with a medical record review of those exposed to OHA-use versus insulin monotherapy and their outcomes compared.

CHBAH, a 3000-bed teaching hospital with between 1400 and 1600 deliveries per month, serves the population of the sprawling township of Soweto, SA, ethnically predominantly black. All patients with singleton pregnancies treated at CHBAH for GDM between 1992-2002 and 2010-2014, diagnosed by either a random blood glucose ≥ 11.1mmol/L or fulfilling National Diabetes Data Group (NDDG) criteria following a 3-hour 100g OGTT were included in the study. Both cohorts adopted similar screening strategies for GDM. Participants with twin pregnancies, other subtypes of diabetes including pre-gestational diabetes, pancreatic diabetes, and steroid induced diabetes were excluded. A "control" group" was defined as patients who presented after 36 weeks gestational age (GA) or received <2 weeks of medical intervention, and these patients were excluded for the purposes of the study. The protocol for this study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M180316) (Appendix H).

4.2.2 Definitions

Diabetes was considered gestational if the diagnosis was first made during pregnancy in women referred because of the presence of risk factors for diabetes: persistent glycosuria, first-degree family history of diabetes, previous unexplained perinatal losses, previous gestational diabetes, history of macrosomic baby. Selective screening for HFDP was performed between 24-28 weeks gestational age. HFDP was diagnosed using a 100g 3-hour OGTT and NDDG criteria, which required two or more of the following plasma glucose levels for the diagnosis:

fasting plasma glucose (FPG) ≥ 5.8 mmol/L, one-hour post-glucose load of ≥ 10.6 mmol/L, two-hour post-glucose load of ≥ 9.2mmol/L, or a three-hour post glucose load of ≥ 9.2mmol/L. If IADPSG criteria were applied, HFDP was classified as DIP if baseline FPG was ≥≥ 7.0mmol/L or 2hr ≥ 11.0mmol/L.

Maternal characteristics and outcomes were defined as follows: gestational age was determined using an early ultrasound; glycaemic control: mean blood glucose (MBG) less <7.1 mmol/L for the 3rd trimester-calculated as a mean of daily self-monitored six-point profiles, including both fasting and 1 hour post-prandial; dietary failure was defined as failing to achieve glycaemic control following two weeks of dietary intervention. Diabetic nephropathy: microalbuminuria: creatinine level (MAC) >30mg/mmol on repeated testing, in absence of UTI and other renal disease; Body mass index (BMI): was considered obese if greater than 30 kg/m^2 at first visit, Anaemia: haemoglobin levels < 11 g/dL; HIV positive: based on results from rapid or antibody tests before or at time of initial presentation; Obstetric complications included: premature labour, Caesarean section (CS), miscarriage, hypertensive disorders of pregnancy, urinary tract infection, polyhydramnios, oligohydramnios, maternal death. Hypertension was diagnosed when blood pressure readings were elevated (> 140/90 mmHg) for at least two measurements. This was further categorised into hypertensive disorders of pregnancy (HDP) and chronic HTN. HDP was further classified into PIH where HTN developed after 20 weeks of pregnancy; preeclampsia where PIH was found in the presence of proteinuria on more than one occasion, and eclampsia where PIH was found in the presence of proteinuria and seizures. Chronic HTN was defined as HTN diagnosed prior to the pregnancy. Maternal hypoglycaemia was defined as one or more episodes of hypoglycaemia severe enough to necessitate the administration of intravenous dextrose.

Neonatal outcomes were defined as follows: Prematurity/preterm birth: delivery < 37 completed weeks gestational age; Miscarriage: loss in pregnancy occurring at < 29 weeks gestational age; Stillbirth: loss in pregnancy occurring at ≥ 29 weeks gestational age; Early neonatal death: neonatal demise occurring in the first week; PNM: the number of stillbirths and early neonatal deaths out of the total number of deliveries (successful deliveries and stillbirths); LBW: birth weight < 2500 grams; Macrosomia: birth weight ≥ 4000 grams; Major congenital anomalies; spinal, cardiac, central nervous system, renal, or digestive system anomaly.

*Definitions applied to both cohorts except for MBG and nephropathy, where the definitions for MBG in the earlier cohort was calculated as a mean of the fasting and 1 hour plasma glucose

levels, and nephropathy was defined by the presence of urine dipsticks positive for proteinuria (protein> 0.3g/L) on repeated testing.

4.2.3 Clinical Practice

The specialist clinic at CHBAH was established in 1983 and is comprised of a physician, obstetrician, paediatrician, and a diabetes nurse educator. The initial visit comprised of a full clinical assessment with ultrasound and treatment initiation. Initial ultrasound was used to assess for gestational age and to rule out any major fetal anomalies. All patients were screened for target organ damage, including retinopathy and nephropathy. Newly diagnosed women with HFDP (as per NDDG criteria)(38) were hospitalized for glycaemic stabilization. Patients were taught blood glucose monitoring by the nurse educator and fingerprick capillary blood samples were obtained using lancets and blood glucose levels were read using various different glucometers. In the earlier cohort, glucose levels were read visually using Haemo-glukotest 20-800R strips (Roche Diagnostics GmbH, Mannheim, Germany). Dietary advice was provided on an ongoing basis by a dietician and or nurse educator with a two-week trial of dietary monotherapy being prescribed where the fasting plasma glucose was < 7.0mm/L. For the period 1990 to 2010, insulin monotherapy was prescribed in the event of dietary failure, which was prescribed for a minimum period of 2 weeks. From 2010 onward, OHA (metformin and or glibenclamide) were introduced as follows: metformin monotherapy was prescribed in the event of dietary failure, then therapy was escalated with either the addition of glibenclamide or a switch to insulin monotherapy. Insulin was initiated at the outset in those where fasting glucose exceeded 7.0mmol/L or if maternal or fetal complications were present. Glycaemic targets were < 5.3 mmol/L for fasting glucose, and < 7.8 for a 1-hour post-prandial reading for the later cohort and levels between 4-7mmol/L were accepted for the earlier cohort. A combined mean blood glucose which included an average of the fasting and the 1-hour postprandial readings of < 7.1 mmol/L was included as glycaemic control as per institutional practice. Patient monitored daily 6-point profiles were also assessed based on SEMDSA criteria(60,255).

Following the initial assessment, patients were seen fortnightly or sooner if indicated. At each visit, patients were clinically assessed, the results of home blood glucose monitoring were noted, and treatment was adjusted if necessary. Ultrasounds were repeated at intervals to assess fetal growth and anomalies. Point of care glycated haemoglobin (HbA1c) was performed monthly using DCA Vantage analyser from Siemens which has been standardised and traceable

to the Diabetes Complications and Control Trial (DCCT). All patients had an HbA1c performed within a month prior to delivery. At 32 weeks, the visits were weekly until 37-38 completed weeks gestational age, when labour was induced. Time and mode of delivery were dictated by obstetric factors and glycaemic control with the goal of 38 completed weeks of pregnancy unless complications arose. During labour, glucose levels were monitored hourly and controlled using dextrose-insulin infusions. Oral and or insulin therapy was stopped postpartum, and only reinitiated based on results from in-patient self- glucose monitoring. All HFDP patients not requiring ongoing therapy were asked to return for a 6-week 75gm OGTT at the maternity clinic. All neonates were assessed by paediatric staff in the Neonatal Unit for clinical or biochemical abnormalities and managed accordingly.

The control group defined above was excluded for the purposes of this study

4.3 Statistical methods

Data was captured and entered directly into an electronic database (REDCap, Vanderbilt University, USA), cleaned and imported into STATA 13 (Texas, USA) (209) for statistical analysis. The comparison of the 1992-2002 vs 2010-2014 cohort was expressed as numbers and percentages for categorical variables and means and standard deviations for continuous variables. Comparisons between the two cohorts were made using an online statistical calculator MedCalc (256) (www.medcalc.org), where a chi-squared test was used for categorical outcomes and a t-test was employed for the purpose of continuous variables, to determine statistical significance

Baseline characteristics of the 2010-2014 cohort were described using number and percentage for categorical variables, and, depending on normality, mean and standard deviation or median and interquartile range for continuous variables. To test for normality, a skewness-kurtosis test was performed if mean and median were substantially different. Statistical significance testing within the 2010-2014 group was done using student's t-test or the non-parametric Mann-Whitney U test for continuous outcomes, and chi-squared statistic or Fisher's exact test for categorical outcomes. For analysis across more than two groups, analysis of variance (ANOVA) or the non-parametric Kruskal Willis test were used. Statistical significance was set at a p-value of < 0.05.

4.4. Results

4.4.1 Comparison of the two cohorts of HFDP patients

Maternal profiles

Table 4.1 presents maternal profiles of the 348 HFDP patients in the 1992-2002 and the 192 HFDP patients in the 2010-2014 cohort. The two cohorts were comparable in terms of maternal age at presentation, the rates of overall HTN (20.7% vs. 14.4%, p= 0.06) and the significant decrease in HbA1c at first booking vs. Hba1c pre-delivery (earlier cohort 7.1%. ± 1.7 to 6.6%± 1.2 p=0.0001 and later cohort 7.8% ± 1.8 vs. HbA1c at first booking 7.1% ± 1.4 p=0.000). In the later cohort, first presentation was earlier (27wks (7.5) vs. 28wks (6.4), p= 0.04), and more than 85% of all cases in this cohort achieved their glycaemic goals as measured by their mean blood glucose for the third trimester, with more than 95% of those on oral agents alone achieving good control by delivery. Failure rates for dietary monotherapy was high for both cohorts (98% vs. 95%). The need for insulin monotherapy was lower for the later cohort when compared to the earlier cohort 52.6% vs 95.1%.

Maternal and neonatal outcomes (Table 4.2)

Gestational age at delivery in the recent cohort was lower (36.4wks (3.65) vs. 37wks (1.6), p= 0.008), with a higher number of pre-term deliveries. Birth weight and macrosomia rates were statistically lower in the most recent cohort (3017gms (648) vs. 3204gms (657), p= 0.002 and 12.5% vs. 4.9% p= 0.01 respectively). The PNM was comparable between the cohorts (4.2% vs. 4.9%, p= 0.72) with fewer stillbirths and a non-significant increase in early neonatal deaths in the recent cohort.

4.4.2 Effect of oral hypoglycaemic agents on maternal and neonatal outcomes within 2010-2014 cohort (Table 4.3)

Within the 2010-2014 pregnancy cohort, DIP was diagnosed in 44% of patients based on a baseline FPG ≥7.0mmol/l. The mean FPG at first visit for the cohort was 7.3mmol/L (±2.14). Ninety-nine patients (53%) required insulin monotherapy at first diagnosis, with 45% requiring OHA's and 2% dietary monotherapy. Of those initiated on diet alone, 98% failed and required a switch to OHA's. Within the OHA group, 84 (43.7%) patients were exposed to metformin and 45 (23.4%) patients exposed to glibenclamide. Of the OHA group, 7.8% were switched to insulin. Within the cohort treatment groups, there were no significant differences observed in maternal age and BMI in the groups exposed to OHA compared to the insulin monotherapy.

For the insulin vs. glibenclamide exposed groups, initial HbA1C (8.9% IQR 7.2-10.1.vs 6.7% IQR 6.3-7.1, p=0.001) and weight gain (5kg IQR 2.2-9.6 vs 2kg IQR 0-4 p=0.00)) during pregnancy were significantly higher in the insulin group.

Of the 192 files recorded, outcomes were available for 162 patients, given a 16.7 % loss to follow-up rate. There were no differences in maternal and neonatal outcomes in terms of each oral agent vs. insulin. When comparing metformin to insulin, the incidence of prematurity, macrosomia, LBW and PNM were all non-significantly lower with metformin therapy. Outcomes for the glibenclamide vs. insulin exposed groups were comparable, including rates of macrosomia (11.4% vs 3.3%, p=0.19) and LBW (22.2% vs. 14.6%, p=0.35). CS rates, although not different amongst the groups, were high (79%, 78%, 65% respectively), with failed induction of labour being the commonest indication. No maternal mortality was reported for the cohort.

4.5. Discussion

In this comparative study: i) lower macrosomia rates and earlier GA at delivery were found in the later cohort exposed to both OHA and insulin therapy (2010-2014) vs. the earlier cohort (1992-2002) exposed to insulin monotherapy. ii) effectiveness of OHA was evident by the low number of participants that needed to be switched to insulin, and iii) maternal and neonatal outcomes were comparable in the group exposed to OHAs and those exposed to insulin alone for the 2010-2014 cohort.

When comparing the historical cohorts, the introduction of OHA's in the later cohort was effective in achieving good glycaemic control at delivery, with favourable and comparable maternal and neonatal outcomes, including maternal hypoglycaemia, CS-rates, macrosomia, major congenital anomalies, and PNM, in women treated with OHAs. This is of public health relevance in SA, where limited resources and access to specialised care for HFDP often translate into adverse outcomes, because it provides health care professionals with a cost-effective and less complex alternative with a similar safety profile to the gold standard of care, insulin.

Of interest was the lower birth weight and macrosomia rates in the cohort exposed to OHA's. One plausible explanation is the earlier gestational age at delivery which may have numerous

contributing factors. Additionally, maternal factors including HIV status, lower socioeconomic circumstances and lifestyle habits may also have played a role. Metformin has been found to be protective of macrosomia in some studies (257,258) though this was not appreciated in our study when metformin monotherapy was compared to insulin alone, but numbers were small for this comparison. Our study did not corroborate a finding from a robust meta-analysis, including seven randomized control trials of a significant relationship between glibenclamide exposure and higher birth weights, macrosomia rates, and neonatal hypoglycaemia compared with insulin therapy (97).

Lastly, PNM did not differ between the two cohorts 4.2% vs. 4.9% (p=0.72), and no higher PNM was found in the OHA-exposed group. This figure, while higher than the national rate of 3.1% (233) for all pregnancies compares favourably to a recent audit on diabetic pregnancies from Cape Town (232) and those published elsewhere in Africa which range from 5.2-25.4% (259)

In the 2010-2014 cohort, women were detected earlier, possibly owing to both the improved awareness amongst the public and the public health system through enhanced screening protocols at both primary and secondary level clinics. However, the high mean HbA1c at booking raises concern that many of the women had pre-gestational diabetes that had not been diagnosed before pregnancy highlighting a potential gap in the diabetes screening programmes. Furthermore, this burden of T2DM occurs alongside a rising prevalence of obesity (260), as is appreciated in the recent cohort with 61% of the women having a BMI >30. Despite this, most of our patients reached good glycaemic control by the third trimester (>86%) with similar outcomes when compared to studies, both internationally and locally(257,258) .

In the recent cohort, over half (53%) the patients required insulin from their first visit owing to the severity of their initial dysglycaemia, explained by the fact that close to 50% of the cohort had DIP. In the patients initiated on OHA's, the effectiveness of these agents in this group was evident in the small number of patients that needed to be switched from OHA to insulin (8%), and the good glycaemic control at delivery. Notably, both cohorts demonstrated high dietary failure rates when compared with figures elsewhere (95% and 98% vs 20-30%) (260)which is most likely due to the presence of more severe cases of dysglycaemia (as indicated by the high initial HbA1c) because of risk factor-based screening rather than universal screening. Furthermore, financial challenges encourage patients to consume a carbohydrate rich diet as opposed to fully embracing the proposed diet. Lastly, this discordance in the percentage of

patients failing dietary measures may be attributed to differences in definitions of glycaemic control, population characteristics and the population responses.

Limitations of the current study include the retrospective nature and small numbers within the cohorts. The diagnostic criteria employed, though relevant at the time, is no longer applicable and may limit reproducibility of this study. Furthermore, comparison for certain variables between the two cohorts was limited. We did not have access to the earlier cohort's database and hence could not merge the insulin monotherapy group with the later cohorts for analysis purposes. In addition, the definitions for nephropathy and the mean blood glucose calculations were different. Different assays for HbA1c were used at the two points in time, where an immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) was performed on whole blood by the NHLS in the earlier cohort and point of care testing was employed in the latter cohort making comparison of HbA1c between the cohorts problematic. As the prevalence rates of anaemia in the latest cohort were high (11%), decreased reliability of this test cannot be ruled out. Comparison of maternal and neonatal outcomes between cohorts was restricted to the variables obtained for the earlier cohort. This prohibited us from exploring whether the lower gestational age at delivery in the recent cohort may have translated into more cases of prematurity. Various confounders for outcomes were either poorly reported, like BMI, or not at all, like HIV status, in one or both of the cohorts. Strengths of the study included the comparison of patients from the same clinic 10 years apart, diagnosed utilising the same test and criteria, where the only difference was the introduction of oral hypoglycaemic agents.

Conclusions:

After the introduction of OHA's at the CHBAH gestational endocrine clinic, participants achieved similar levels of glycaemic control at delivery and showed similar outcomes when compared to insulin therapy. This is reassuring particularly for LMICs, where the OHAs are cheaper, more accessible, easier to use, and eliminate the availability and storage issues of insulin therapy. Further prospective studies with adequate sample sizes are needed to confirm our findings and to determine the impact of OHA on pregnancy outcomes both in the short and long-term.

Table 4. 1 Comparison of maternal characteristics, glycaemic control, and outcomes between 1992-2002 and 2010-2014 HFDP pregnancy cohorts as diagnosed by 100g oral glucose tolerance test and random plasma glucose

	1992-2002 cohort n=348 **Mean (SD) or n (%)**	**2010-2014 cohort n=192** **Mean (SD) or n (%)**	**p-value**
Maternal characteristics			
Maternal age at initial presentation (years)	33.9 (5.2)	33.0 (5.2)	0.06
GA[1] at first presentation (weeks)	28.3 (6.4)	27.0 (7.5)	0.04*
Hypertension[2]	50 (14.4)	39 (20.7)	0.06
Target organ damage Retinopathy Diabetic nephropathy	 5 (1.4) 1 (0.3)	 2(1.05) 0	 0.72 -
Glycaemic control and therapeutics			
HBA1c (%) at: ● first booking ● within a month of delivery	 7.1 (1.7) 6.6 (1.2)	 7.8 (1.8) 7.0(1.2)	 -# -#
No controlled by third trimester (by MBG)	-	139 (85)	-
Dietary therapy alone n (%)	17(4.9)	4 (2.1)	0.182
OHA[3] exposure n (%) Metformin Glibenclamide Switched from OHA[4] to insulin No. controlled by delivery on OHA alone	 N/A N/A N/A N/A	 84 (43.7) 46 (23.4) 15 (7.8) 59 (95)	 - - - - -
Insulin monotherapy n (%)	331 (95.1)	99 (52.6)	<0.0001*

All continuous variables are expressed as means ± standard deviations (SD) and categorical variables expressed as number and percentages. [1]GA: Gestational age; [2] Hypertension, includes all forms of HTN noted in pregnancy; [3] (MBG) mean blood glucose; [4](OHA) Oral hypoglycaemic agents.
[#] P-value omitted given different methodologies utilised for measuring HbA1c at different time points

Table 4. 2 Maternal and neonatal outcomes for the 1992-2002 and 2010-2014 HFDP pregnancy cohorts

	1992-2002 cohort n=348 Mean (SD) or n (%)	**2010-2014 cohort n=164 Mean (SD) or n (%)**	**p-value**
GA[1] at delivery (weeks)	37.0(1.6)	36.4(3.6)	0.008*
Live births	344(98.8)	161(98.3)	0.65
Caesarean section	215(61.8)	113(68.7)	0.10
Maternal hypoglycaemia	3(0.9)	1(1.0)	0.87
Birth weight (g)	3204 (657)	3017 (648)	0.002 *
Macrosomia	43 (12.5)	7(4.9)	0.011*
Stillbirths	10 (2.8)	3 (1.8)	0.49
Early neonatal deaths	5(1.4)	5(3.1)	0.193
Major congenital anomalies	8(2.3)	0	-
PNM[2]	15(4.2)	8(4.9)	0.72

All continuous variables are expressed as means ± standard deviations (SD)

All categorical variables expressed as number and percentages

[1] GA: Gestational Age; [2] PNM: Perinatal mortality

Table 4. 3 Comparison of oral agents vs. insulin on maternal and neonatal outcomes

	Metformin monotherapy[a] **n = 28**	**Exposed to glibenclamide[b]** **n = 45**	**Gold standard Insulin monotherapy** **n = 85**	**P-value**
Initial HbA1c (%)	6.95 (5.9-7.8)	6.7(6.3-7.1)	8.9(7.2-10.1)	[a]0.242 [b]0.000*
No. controlled prior to delivery by MBG (n, %)	27(96.4)	39(92.86)	64(86.49)	[a] 0.149 [b] 0.296
BMI Weight gain over pregnancy(kg)	32.8 (30.4-36.1) 1.7(0.3-3.4)	36.3(33.15-40.45) 2(0-4)	33.65(30.4-37.2) 5 (2.2-9.6)	[a] 0.837 [b] 0.0625 [a] 0.004* [b] 0.000*
Obstetric outcomes				
Total no complications	12(42.86)	22(52.38)	34(44.16)	[a] 0.906 [b] 0.390
Caesarean section (n, %)	22(78.6)	35(77.78)	55(64.7)	[a] 0.301 [b] 0.222
Maternal hypoglycaemia (n, %)	0	1(2.22)	1 (1.18)	[a] 1.000° [b] 1 .000°
Maternal mortality (n, %)	0	0	0	-
Miscarriage (n, %)	0	0	5(6.02)	[a] 0.328° [b] 0.161°
PIH/PE/E (n, %)	5(17.9)	7(15.56)	12(14.12)	[a] 0.761° [b] 0.800
Neonatal outcomes				
Prematurity (n, %)	5(17.86)	11(24.44)	20(24.10)	[a] 0.494 [b] 0.965
Macrosomia (n, %)	1(4)	4(11.43)	2(3.28)	[a] 1.000° [b] 0.186°
Low birth weight (n, %)	2(8.0)	8(22.22)	9(14.7)	[a] 0.498° [b] 0.350
Major congenital anomaly (n, %)	0	0	0	-
Stillbirth (n, %)	0	1(2.56)	2(2.86)	[a] 1.000° [b] 1.000°

Perinatal mortality (n, %)	1(3.70)	2(5.13)	4(5.71)	[a] 1.000° [b] 1.000°

[a] P value calculated between metformin and insulin monotherapy groups[b] P value calculated between glibenclamide exposed and insulin monotherapy groups

Note: of the total cohort n=192 the following numbers (%) were managed with diet alone 4(2.1%), metformin monotherapy 28(14.6%), combination of metformin and glibenclamide 45 (23.4%), switched from oral to insulin 30 (15.6%), insulin monotherapy 85(44.27). Control patients excluded.

Chapter 5- Cardiometabolic outcomes of women exposed to hyperglycaemia first detected in pregnancy at 3-6 years post-partum in an urban South African setting

This chapter addresses the main research question of this thesis which evaluates the maternal health and cardiometabolic consequences following HFDP compared with a group of women non-exposed to HFDP and further assesses these outcomes in light of the background burden of HIV. Alongside the growing epidemic of NCD's in SA, identifying a vulnerable group of women at significant risk of developing these comorbidities is a crucial step prior to exploring interventions, continuing research and implementation of potential policy change. This work has been submitted to the peer-reviewed journal, *PLOS ONE Journal* on 31st July 2021 and has been provisionally accepted subject to minor revisions. (Appendix K)

Nicolaou V, *Soepnel L,* *Huddle KRH, Levitt N, Klipstein-Grobusch & Norris, S.A. (2020) Cardiometabolic outcomes of women exposed to hyperglycaemia first detected in pregnancy at 3-6 years post-partum in an urban South African setting.*

5.1 Introduction

The non-communicable diseases (NCD) burden remains the leading cause of death worldwide, with diabetes and cardiovascular diseases (CVD) accounting for almost half the burden. In South Africa, diabetes and CVD are the second and third leading cause of death since 2014. In high-income countries, hyperglycaemia first detected during pregnancy (HFDP) has been associated with a sevenfold higher overall incidence of type 2 diabetes mellitus (T2DM) within the first decade following delivery (13)and an increased risk of CVD (14) and the metabolic syndrome (MetS) (15).

Prevalence figures for HFDP in Africa remain limited, with a few studies in South Africa demonstrating that 9-25% of women have HFDP (57–59). These figures may underrepresent the disease burden since risk-factor based screening and varying diagnostic tests and strategies are currently employed. Whilst numerous studies have explored the short term maternal and neonatal outcomes following HFDP, long-term outcomes for the African continent remain limited to a mere three studies. One study performed amongst a predominantly mixed ethnic ancestral group in Cape Town, South Africa demonstrated a 48% progression rate to T2DM at 5-6 years following the index pregnancy (130), although no control group was included. A high CVD risk and prevalence of MetS (60.9%) was demonstrated in the same cohort (197). A further smaller study amongst the same ethnic group from the same region established that the prevalence of T2DM at six-weeks post-partum was 27% (129).
Though racial and ethnic disparities for progression to T2DM following HFDP is well known with African American women being particularly vulnerable (127) owing to acculturation, lifestyle factors arising from social determinants in addition to genetic susceptibility and traditional risk factors (262), to our knowledge, the long-term CVD and metabolic impact of HFDP has not previously been explored in Black African women or compared to women in this setting without a history of HFDP.

Obesity, a well-known risk factor of HFDP, T2DM and CVD, is commonly encountered amongst black South African women (40.9%) and accounts for an estimated 87% of their diabetic risk (40,263). Obesity and weight-related characteristics including pre-pregnancy body mass index (BMI), post-pregnancy BMI and weight gain following the pregnancy, all have been shown to increase the risk of progression to T2DM following HFDP (264). The

pathophysiological mechanism behind this lies in the differential impact of regional fat deposition, with upper body(android and visceral fat) and lower body fat (gynoid and leg fat) showing directionally opposite associations with these risks (265) which too has been appreciated in black SA women (266,267). However, the impact of body composition characteristics on progression to T2DM, MetS and CVD following an HFDP pregnancy has yet to be explored in this setting.

Moreover, little is known about the impact of human immunodeficiency virus (HIV), which the healthcare sector in Africa is faced with, in addition to the growing burden of NCDs, on CVD and metabolic risk in the context of HFDP. The additional metabolic risk attributable to HIV is debatable, with an overall prevalence of glucose metabolism disorders in HIV-infected individuals on antiretroviral therapy (ART) in Africa ranging from 3-33.5% (190). A meta-analysis of 5 case-control studies in Africa did not demonstrate a significant relationship between HIV and exposure to ART and the prevalence of T2DM, as encountered in other studies in Europe and North America.

Given the growing prevalence of HFDP in South Africa and its well-known role in the intergenerational transmission of NCD, we sought to explore its impact on maternal CVD risk, development of T2DM and MetS in a group of Black African women with and without a prior history of HFDP. Secondary aims of our study were to explore how body composition differs between the two groups and if HIV-infection impacted the development of these outcomes following pregnancies with and without HFDP. To our knowledge this is the first study of its kind in Africa.

5.2 Study design and population

The study setting was the Chris Hani Baragwanath Academic Hospital(CHBAH), located in urban Soweto, South Africa. Between March and November 2019, we conducted a prospective cohort study in women previously diagnosed with HFDP (HFDP group) and women who tested negative for HFDP (non-HFDP group) using the same diagnostic test and criteria between February 2014 to January 2017. Both groups of women were derived from the same specialist clinic though were identified differently. The HFDP group were selected first and consisted mostly of women who were identified by risk-factor based screening and had attended a specialised gestational endocrine clinic at CHBAH for HFDP with their

pregnancy characteristics and outcomes have been previously published (268)A subgroup of the HFDP women were referred to the specialist clinic as a result of universal screening being performed by a research study. The “control group”, were women who tested negative for HFDP for the same time period and had undergone universal screening as part of a previous study (59) . Women were diagnosed using a 75-gram 2-hour oral glucose tolerance test(OGTT) with International Association of Diabetes in Pregnancy Study Group(IADPSG) criteria. HFDP comprised of GDM and DIP.

Participants were recruited telephonically and if unsuccessful traced by visiting their home address. Of the initial 319 HFDP cases identified, 206 were non-contactable/traceable, 4 declined to participate, 6 were pregnant. Difficulties tracing participants following delivery was mostly due to relocation or change of contact details. There were 845 women who screened negative for HFDP identified from the database of the previous study of which 103 women (13,195)were contacted in a random order until the number of mothers were the same as those in the HFDP group. Two hundred and four participants were enrolled at follow up, of which 103 had confirmed HFDP and 101 did not .

5.3 Sample size calculation

Sample size calculations were calculated for each of the three main outcomes(T2DM, MetS and CVD risk) using a two-sample proportion test based on population parameters using a 5% margin of error, a confidence interval of 95% and power of 80% to detect the effect. Given the reported estimated risk of developing T2DM following a HFDP pregnancy of 20-60% and 12% (13,195)in the background population a sample size of 15 per group was calculated. The minimum total sample size calculated for the outcome MetS was 64 given the risk of 40% following HFDP(15) and background prevalence of MetS of 10%. A size of 98 per group was needed based on a reported estimated risk of developing CVD of 17.6% (14) following HFDP vs. 7% in the background population. The sample size needed to establish CVD risk informed our final sample size.

5.4 Data collection

5.4.1 Questionnaire

A self-reported questionnaire was captured at the follow-up visit and incorporated maternal demographics, marital status, various socioeconomic parameters (SES), obstetrical history,

maternal complications and outcomes and postnatal factors (history of CVD risk factors, any vascular event/s, risk factors for the development of T2DM including recurrent HFDP pregnancies, breastfeeding following the index pregnancy, family history of diabetes or the presence of diabetes). Use of cholesterol-lowering or antihypertensive medication, smoking status and pack year history and ethanol consumption(ml/day) based on the quantity and frequency and physical activity was captured. Contraceptive use was self-reported and was categorized as none, oral contraceptives or injectable contraceptives and type. HIV status and therapy were noted where applicable. (See Table 5.8 for relevant definitions of maternal variables noted). The questionnaire was informed by the literature and adapted from several existing standard and recognised sources (269) in order to incorporate relevant factors.

5.4.2 Anthropometrics

Subjects underwent a physical examination for weight, height, waist and hip circumference and blood pressure measurements utilising standardised methods by qualified trained research assistants. Height (cm) was recorded to one decimal place using a wall mounted Holtain stadiometer (Crymych, UK) with subjects standing on a flat surface at a right angle to the vertical board of the stadiometer. Weight (kg) was measured on a SECA digital scale (Hamburg, Germany), to the nearest 0.1kg, which was calibrated and standardised using a weight of known mass. Participants wore light clothing and were asked to remove their shoes and socks. Blood pressure(mmHg) measurements were taken. Details pertaining to each measurement is outlined in Table 5. 1.

Dual–energy x–ray absorptiometry (DXA) (Hologic Discovery-A (S/N83145), Bedford, MA, USA) was used to determine whole-body composition since BMI alone is not an accurate indicator of body composition. This included subtotal (whole-body minus head) fat mass and fat-free soft tissue mass. Regional body fat, namely trunk, arm, leg, android, and gynoid fat mass(expressed in kg and as a percentage of subtotal fat mass, (% FM) were measured using DXA cut-off lines positioned at standard anatomical positions, as defined in the software (software version apex 4.2.0). FMI (kg/m^2) was calculated using height and body fat mass which offers superiority over BMI as a marker of obesity since the index is based on fat mass, not body weight, which is a combination of fat and lean components. In addition, abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue(SAT) were estimated using algorithms included in the DXA software, which have been shown to perform as well as clinical computed tomography (214). During data collection, a phantom

scan was performed each morning to) determine the coefficient of variance of the DXA machine and the coefficient of variance (CV) was less than 0,5% for all parameters. CVs for DXA parameters were <2 % for total fat mass, and 1% for fat–free soft tissue mass (FFSM).

5.4.3 Biological samples and OGTT

Point of care testing was performed for haemoglobin (Hemocue^{r} Hb 201) and HIV (Homemed HIV1/2 rapid test kit). An early morning midstream urine sample was collected from each participant to exclude pregnancy and perform bedside testing with a urine dipstick (Roche Combur) screening for any amount of glucose (glycosuria), albumin/protein (microalbuminuria and proteinuria), infection or renal disease.

A finger prick (OneTouch) fasting capillary glucose was performed at baseline and the OGTT was commenced irrespective of the result (Appendix E). At baseline, blood samples were drawn by a trained nurse after an 8-hour overnight fast for measurement of serum creatinine, lipogram, Hba1c, fasting insulin and glucose. Specimens were centrifuged and stored at -80^{0} C within 30 min of being drawn. An OGTT was performed for all participants, except those that self-reported an existing diabetes diagnosis, which was confirmed by either medical card record or drugs in-use. Participants ingested the 75g glucose load in 250ml water, administered by a research nurse. Subsequent blood samples were taken at 120 minutes post-glucose load. Categories of glucose intolerance were defined applying the 2006 WHO criteria (34). (Table 5.8)

5.4.4 Carotid and femoral imaging

Ultrasonographic assessment of the common carotid artery(CCA) and femoral artery(CFA) was performed (Linear-Array 12L-RS transducer with a B-mode Logic E Ultrasound machine, GE healthcare, CT, USA), to assess for intima media thickness (IMT) and for the presence of plaque. The IMT measurement was then performed on the posterior wall of the common carotid artery and common femoral artery in an area free of plaque, defined as the distance between two echogenic lines represented by the lumen-intima interface and media-adventitia interface of the arterial wall. The ultrasound machine software then detected the intima-lumen and the media-adventitia interfaces and calculated the minimum, maximum, and mean common carotid IMT(cIMT) and femoral IMT in millimetres and to 2 decimal places (270,271). All patients were positioned supine with the neck slightly hyperextended and rotated in the opposite

direction to the probe. A 45-degree angle wedge pillow was used to standardize lateral rotation. Measurements were performed by one observer with intra-observer variability 1.1%. (Appendix G)

5.4.5 Biochemistry and laboratory analyses

Plasma glucose was measured using Randox Rx Daytona chemistry analyser using enzymatic methods(Randox Laboratory Ltd, London, UK) glycosylated haemoglobin a1c (HbA1c) was measured using the Bio-Rad D-10™ Haemoglobin analyser using the HPLC method (catalogue number, 2200101) (Bio-Rad Laboratories, Inc. CA, USA). The precision and trueness of the Randox Rx Daytona chemistry analyser were verified using the clinical and laboratory standards institute document EP15. Coefficients of variation calculated from running 30 separate samples at 3 different times were 0.7% for glucose and 1.8% for HbA1c. Lipids including HDL, low-density lipoprotein(LDL), triglycerides(TG), total cholesterol concentrations were analysed on the Randox Rx Daytona chemistry analyser using enzymatic colorimetric (catalogue number, CH8311(HDL), CH8312 (LDL), TR8332 (trigs), CH8310 (Chol)) Randox Laboratories Ltd., London, UK). Enzymatic colorimetric assays were used to measure TG, total cholesterol and HDL cholesterol using the Roche modular auto analyser, while low-density lipoprotein cholesterol was calculated using direct methods/Friedewald formula. Fasting serum insulin concentrations were measured on the Immulite® 1000 Immunoassay system using the chemiluminescent method(catalogue number lkin1/ catalogue number, 10381429) (Siemens) chemiluminescent healthcare GMBH, Henkestr, Germany). Serum creatinine concentrations were analysed on the Randox Rx Daytona chemistry analyser using enzymatic methods (catalogue number, CR8317) (Randox Laboratories Ltd., London, UK). CVs calculated from running 40 separate samples in duplicate were, 0.8% for HDL and total cholesterol, 1.19% for TG and 3.9% for insulin and 0.7% creatinine.

5.5 Outcomes

The primary outcome was progression to and time to developing T2DM between women with HFDP (sub-categorised; GDM and DIP) and women without HFDP following their index pregnancy. Secondary outcomes included comparison of body composition measures, progression to MetS and CVD risk(utilising two surrogate measure, Framingham Risk Score(FRS) and cIMT between the groups. MetS was defined using the harmonised criteria

(181). Gender specific prediction for 10-year CVD risk was calculated using the modified Framingham risk score 2008 (FRS) (272). See Table 5.8 for definitions.

5.6 Ethics

This study was approved by the Human Research Ethics Committee at the University of the Witwatersrand (M180316). Informed consent, both verbal and written, was obtained from participants prior to enrolment in the study (Appendix H).

5.6 Statistical analysis

Data was captured using REDCap (208) (Vanderbilt University, Nashville, USA) and analysed using Stata software 13.0 (College station, USA) (209). Sensitivity analysis to address potential bias of missing data was performed. Mean and standard deviations were reported for normally distributed continuous variables (anthropometric parameters) and medians and interquartile ranges for non-normally distributed measured data (all other continuous variables). Number and percentages for categorical variables(chronic hypertension, family history of diabetes etc). Statistical differences between three groups(control, GDM and DIP), were tested using Analysis of Variance (ANOVA) or Kruskal-Wallis test. For categorical variables, Chi-squared test and Fischer's exact(small frequencies) was utilised. The statistical significance level was set at two-sided p-value <0.05.

Crude odds ratio (OR 95% CI) and multivariable adjusted odds ratio(aOR 95% CI) for T2DM, MetS, and 10-year cardiovascular risk calculated using FRS were estimated from logistic regression models. Covariates evaluated as potential confounders based on a priori hypotheses are included in Table 3. Covariates were excluded as confounders if they were not associated with both the dependent variables and independent variable (exposure to HFDP), $p<0.05$.

Further multivariable models were designed to explore the relationship of maternal factors associated with the relevant outcomes adopting a chronological approach in which maternal factors present either at pre-pregnancy (distal model 1), index pregnancy (intermediate model 2) and post-partum (proximal model 3) were assessed using logistic or linear regressions. The final model combined all variables from the three models. The outcomes explored in these multivariate models were fat mass index FMI (continuous, as surrogate for adiposity), T2DM (binary) and cIMT (continuous). The independent variables were identified using univariate analysis for each outcome. HFDP, an independent variable for all models, was categorised

according to degree of dysglycaemia as GDM and DIP. Both BMI and WHR were used as continuous variables for the purposes of the models. For logistic regression model diagnostics, results are expressed as OR and 95% confidence intervals(CI) and we assessed the following: linearity assumption using the Lowes graph, multicollinearity using variance inflation factors, model specification using the C-statistic, and confirmed the fit of the model using the Hosmer-Lemeshow goodness-of-fit test. We also checked for outliers. For linear regressions cIMT was log-transformed to increase normality of residuals. Results are expressed as beta coefficient and 95% CI. In order to assess the influence of HIV on the outcomes, it was included as binary variable into each of the multivariate models exploring maternal factors and outcomes.

5.7 Results

There were 103 women recruited in the HFDP group and 101 in the non-HFDP group after all exclusions were applied. There was <1% missing data and no biases detected through the sensitivity analyses. Details are shown in the study flow diagram (Figure 1)

5.7.1 Baseline and follow up demographics, maternal factors, anthropometrics, and biochemical parameters between the groups.

Most of the cohort was of Black African ancestry (n=198, 97%). Relevant baseline characteristics during the index pregnancy and at follow-up for the HFDP group and non-HFDP groups are shown in Table 1. Of the participants with prior HFDP, 45 (43.7%) had "overt" diabetes in pregnancy (DIP) with the remaining 58 (56.3%) classified as "true" GDM (GDM). When comparing the HFDP vs. non-HFDP groups at first booking during their index pregnancy, the median age was higher for the HFDP group, 32.5 (29-38 IQR) vs. 29.5 (25-34 IQR) respectively, with a median follow-up period of 3 years (IQR3-4) and their median BMI were 35.2 (IQR30.6-39.8) and 29.5 (IQR 25.0-32.9). Baseline prevalence of HIV during pregnancy was lower in the HFDP group 13 (12.7%) vs. 20(19.8%) amongst the non-HFDP group and this remained the case at follow-up. All women with HIV were on fixed dose combination of treatment whilst the majority(80%) were diagnosed at or before their index pregnancy. Forty-five (43.7%) of the women in the HFDP group experienced an obstetric complication vs.16 (15.8%) in the non-HFDP group. When comparing the DIP and GDM groups, the only variables significantly different at baseline were glucose values on

OGTT testing and exposure to therapeutic agents, with more in the DIP group being exposed to insulin 22 (48.9%) vs. 8 (14.8%).
At follow-up, the HFDP group remained obese (32.8 (29.1-39.2)) with elevated anthropometric measures, higher blood pressure measurements and glucose profiles when compared to the non-HFDP group. Their overall socioeconomic status was lower than those for the non-HFDP group.

5.7.2 Stratified analysis of maternal outcomes including diabetes, metabolic syndrome and cardiovascular risk based on exposure to HFDP (Table 5.2)

Of the HFDP group, 46 (44.6%) progressed to diabetes compared with 5 (4.9%) in the non HFDP group ($p<0.001$). Only 20 (42.5%) of the entire group were aware of their diabetes status and the average time to event was 30 months (SD +/-1.32). Both dysglycaemia (57 (55.9%) vs.14 (13.9%)) and insulin resistance (92 (90.2%) vs. 69 (68.3%), $p<0.001$) were significantly higher in the HFDP group. Within the HFDP group, all measures of dysglycaemia, insulin resistance and progression to T2DM were higher among the DIP group compared to the GDM group.
All CVD risk factors were higher in the HFDP group including diabetes 46(44.6%), hypertension 27 (26.1%), dyslipidemia 85 (82.5%), family history of CVD 20 (19.4%), history of ever smoking 10 (9.7%) and central obesity 66 (64.1%), though smoking and family history of CVD were not significantly different between the groups. Overall, the calculated FRS was significantly higher in the HFDP group 8.46 (4.9-14.4) vs. 3.48 (2.1-5.7) $p<0.001$, with an intermediate to score being present in over 40% of the HFDP individuals 42 (42%) vs. 8 (8%) $p<0.001$. Within the HFDP subtypes, DIP displayed a significantly higher intermediate-high risk category cardiovascular score of 26 (60.4%) vs. 16 (28%) in the GDM group. The overall median cIMT measurement, though within normal limits in all participants, was significantly higher in the HFDP group, with a cIMT of 0.48 (IQR 0.44-0.53) vs. 0.46 (IQR 0.42-0.5) $p=0.037$, though this was no longer significant after adjusting for age. No atherosclerotic plaque was noted at either carotid or femoral sites in any of the participants and there was only one reported event of CAD and one of cerebrovascular accident. Though these measured outcomes may be attenuated after correcting for differences in maternal age and BMI at index pregnancy, this has been explored in the subsequent regression models (Tables 5,6 &7).

The prevalence of the MetS was higher in the HFDP group, 42 (40.8%) vs. non-HFDP group 6 (5.9%) p<0. 001.Between the two subtypes of HFDP, more women in the DIP arm had MetS 25 (55.6%) vs 17 (29.3).

5.7.3 Body composition measures between the groups (Table 5.3)

Ninety-nine HFDP and 96 of the non HFDP women(n=195) had a DXA scan performed. All body composition variables, except total percent body fat, were significantly higher in the exposed vs. nonexposed groups, including the fat mass index (FMI) 13.9 (11.7-16.3) vs.12.3 (8.9-14.6) p=0.0008 and VAT:SAT ratio 0.20 vs.0.16. p<0.001. In particular, measures of visceral adipose tissue (VAT) were elevated in HFDP women. VAT volume was 517cm^3 (IQR372.6-610.2) vs. 322 cm^3 (IQR 219.2-469.2) p <0.001 and percentage trunk fat of 39.8% (IQR 35-43.6) vs 36.5% (IQR 29.4-42.5) p=0.0066 with android:gynoid ratio at 0.93(0.86-1.02) vs 0.86 (0.75-0.95) p <0.0001.After adjusting for BMI at baseline, only visceral adiposity indices remained significant between the groups.

5.7.4 Strength of association of HFDP exposure and progression to diabetes, MetS and high CVD risk (Table 5.4)

The crude OR (15.5 (CI 5.8-41.3, p<0.001)) for progressing to T2DM in the HFDP group remained significant(adjusted aOR 10.5(CI 3.7-29.5) p<0.001) after correcting for confounders. This significant risk persisted within the subtypes of HFDP, with a greater aOR 27.6 (8.7-87.4) in the DIP group. The crude OR for prior HFDP and its association with MetS was 10.9 (4.3-27.1 p<0.001) vs. aOR of 6.3 (CI 2.2-18.1), which remained significant (p=0.004) after adjusting for factors present at the index pregnancy including maternal age, parity, systolic blood pressure (SBP) and BMI. Additionally, the aOR within the HFDP subtypes and MetS risk remained significant . In the HFDP group, the association with an intermediate-high 10-year estimated CVD risk based on FRS was significant for crude OR 8.4 (3.6-19.1) p<0.001; and when adjusting for confounders including factors at index pregnancy including age, BMI, SBP (aOR of 4.3 (1.6-11.5, p=0.003) and this significance was retained in the DIP group.

5.7.5 Predictors of FMI and progression to type 2 diabetes and cardiovascular risk using multiple variable logistic and linear regression.

i) Factors associated with FMI

Linear regression models of relevant independent maternal factors present at pre-pregnancy (distal), pregnancy (intermediate) and post-partum (proximal) associated with log FMI is shown in Table 5. In the distal model only multiparty was significantly associated with FMI. In the intermediate model, BMI measured at first visit in pregnancy was significantly associated with the outcome, with BMI difference (post-partum BMI- pregnancy BMI) being the only significant factor in the proximal model. In the final model, only initial BMI in pregnancy and BMI difference was significantly associated with the outcome log FMI.

ii) Progression to T2DM (Table 5.6)

Multivariate logistic regression models examining the association between relevant maternal risk factors and progression to diabetes found that higher parity, family history of T2DM, and positive HIV status were significant in the distal model. Prior HFDP was significant in the intermediate model, with absence of exclusive breastfeeding being significant in the proximal model. However, in the combined final model only prior history of HFDP, a family history of T2DM and an elevated VAT:SAT ratio were independently associated with risk of progression to T2DM.

iii) Carotid intima media thickness (Table 5.7)

Linear regression models examining maternal factors associated with log cIMT in the various models are displayed in Table 7. These analyses show that of the variables included in the distal model, no variables were significant. In the intermediate model; maternal age, initial SBP remained significant with triglyceride levels and BMI difference being significant in the distal model. In the combined final model, maternal age and SBP at pregnancy and BMI difference were significantly associated with cIMT thickness.

5.7.6 HIV influence on outcomes

The prevalence of HIV within our cohort was 21.6% (n=44), of which 18.5% (n=19) were HIV reactive in the HFDP group. The independent influence of HIV on the measured

outcomes assessed in multivariate regression models was not found to be significant between women with a prior history of HFDP vs those without (Table 5,6 &7).

5.8 Discussion

We found that Black African women with compared to those without a history of HFDP have a (a) 10.5-fold increased risk for developing T2DM (4.6 and 27.6-fold for GDM and DIP groups respectively) (b) 6-fold increased risk of having MetS together with higher visceral adiposity; and (c) higher cardiovascular risk. HIV infection did not influence any of these outcomes.

The rate of progression to T2DM we found was similar to that reported from Cape Town (48% at 5-6 years post-partum) in predominantly mixed-ancestral women using the same diagnostic criteria (130). This is over 3-fold higher than the background prevalence rates of T2DM for black South African women (273) , and identifies a highly vulnerable population. This risk was significantly higher both in women with GDM, the less severe form of dysglycaemia in pregnancy, as well as women with DIP. Comparisons of our findings with international studies are challenging, as different study designs, lengths of follow-up, definitions and diagnostic criteria are employed. Nevertheless, a recent meta-analysis (123) identified the time period with the highest risk of progression as 3 to 6 years following the pregnancy, which aligns with our findings.

The modifiable risk factors associated with progression and risk of progression to T2DM have best been explored in high income countries (HIC) (79,81,196)with limited data for low to middle-income countries (LMIC) and very little African representation (80). Identification of these factors play a key role when implementing strategies to delay or prevent the onset of T2DM. The post-partum period offers a critical window of opportunity to implement such strategies and screen for diabetes in these high-risk individuals. In our study, though a history of HFDP and family history of T2DM and visceral adiposity were significantly associated with progression to T2DM , only adiposity is modifiable. Though these factors have been associated with progression in previous studies, two other studies in South Africa found that a range of measures of the extent of dysglycaemia to be significant predictors: fasting and 2-hr plasma glucose on OGTT in pregnancy diagnosis of HFDP before 24 weeks indicative of

undiagnosed/unrecognized pregestational diabetes, and exposure to insulin and OHA in pregnancy (129,130) , all of which are non-modifiable. The protective role of breastfeeding for T2DM(109), was not found to be significant in our study. The high number of women with HFDP who were unaware of their diabetes status indicates that postnatal assessment for diabetes is necessary even in an already overwhelmed LMIC health infrastructure.

Our study highlighted that, in addition to the high risk of progression to T2DM, women with a history of HFDP are more vulnerable to CVD as evidenced by their higher CVD risk scores and greater cIMT. Though their risk scores remained significantly elevated after correcting for obvious confounders, their cIMT, a surrogate marker for identifying pre-clinical atherosclerosis and hence often used as a surrogate CVD measure (274)was independent of prior HFDP and rather influenced by age, SBP and BMI difference. Though no cardiovascular events were reported in our study, their higher cardiovascular risk is likely to translate into CVD events with time as our cohort was young with a relatively short period of follow-up. Consequently, our study did not corroborate the findings of a recent metanalysis (14) demonstrating that women exposed to HFDP have a two-fold higher risk of cardiovascular events independent of the development of T2DM 10-25 years post-delivery.

The rates of obesity were high across both groups during the index pregnancy and follow-up, in keeping with high rates of obesity amongst Black women in SA (273). However, fat distribution differed in those with and without a history of HFDP; with the former having significantly greater upper body fat distribution, in particular, the visceral area. Visceral adiposity is known to be a strong predictor of diabetes and CVD independent of overall fatness (267,275). South African studies have demonstrated that visceral depots are generally lower in black African women compared to their white female counterparts, despite the higher insulin resistance and high rate of obesity in this group (273,276,277). Pregnancies complicated by both obesity and HFDP are known to independently influence immediate and long-term maternal outcomes. In our study, initial BMI at index pregnancy and weight gain post-pregnancy were not significantly associated with progression to diabetes or cardiovascular risk outcomes, but visceral adiposity was a significant predictor of progression to T2DM. A better appreciation of the role of obesity and body composition on our measured cardiometabolic outcomes would have been possible if it weren't for our lack of knowledge relating to fat distribution pre- and during pregnancy and BMI measurement at pre-pregnancy and early post-delivery, known to be independent risk factors for progression to

T2DM (273). It is therefore plausible that our study confirmed a high prevalence of MetS in women with HFDP with a 6-fold increased risk after adjusting for age, parity, BMI and SBP. Overall, altered body composition favouring visceral adiposity, together with the increasing burden of HFDP and its cardiometabolic consequences is the setting for the perfect storm in which the coexistence of these entities may perpetuate a vicious cycle fueling the NCD burden.

Strengths of our study were the inclusion of a control group of women with confirmed normoglycaemia on OGTT from the same period and setting as the HFDP group. The sample was adequately powered for establishing both the primary and secondary outcomes for our study. The use of DXA to assess maternal body composition at 3-5 years post-partum has not previously been reported from Africa. Lastly, exploring the impact HIV had on these outcomes which was insignificant contributes to the limited existing data.

A major limitation of our study was that this was a single centre study. Further limitations included missing pre-pregnancy BMI values for participants leading to difficulty in exploring this variable as a confounder. Difficulty tracing participants resulted in a small sample size and hence less power for sub-group comparisons in particular within the HFDP group (GDM vs. DIP). Self-reporting bias was a potential problem in the administered questionnaire. Cardiovascular risk and body composition outcomes were not measured in pregnancy for longitudinal comparison. However, these parameters are often influenced by the normal physiological adaptations of pregnancy, making interpretation difficult. The use of FRS to calculate cardiovascular risk in these women may have underestimated their risk since the formula doesn't consider other unique potential risk factors relating to HFDP such as recurrent HFDP and hypertensive disorders of pregnancy. The time of diabetes diagnosis is unclear as women did not have a 6-week post-partum OGTT, in some cases, HFDP may have never resolved post-partum. The small number of women who were HIV positive may have accounted for the lack of effect it had on the measured outcomes. The lack of data surrounding ART regimes was a further limitation as well as the fact that this was a single centre study.

Table 5. 1 Maternal demographics, characteristics and outcomes at index pregnancy and follow-up.

Index pregnancy		**HFDP group**			**Non-HFDP group**
		Total group n=103	GDM n=58	DIP n=45	n=101
Age(years)	(median/IQR†)	32.5 (29-38)	33(29-38)	32(30-37)	29.5 (25-34)
Primigravida	n (%)	8 (7.8)	5(8.8)	3(6.8)	54 (53.4)
GA[1] at presentation (weeks)	(median/IQR†)	30(26-33)	30(27-33)	30(24-32)	26(25-27)
BMI[2] at first visit	(median/IQR†)	35.2 (30.6-39.8)	34.3(30.5-38.2)	37.2(31.6-40.9)	29.5(25.0-32.9)
BP[3], (mmHg) Systolic BP DBP BP	(median/IQR†)	 121(112-129) 73(64-79)	 124(112-129) 71(62-77)	 119(113-129) 74(66-79)	 109(102.5-114.5) 69(64-73)
HIV[4] positive	n (%)	13(12.7)	11(18.9)	2(4.4)	20(19.8)
OGTT[5] results, mmol/L Fasting plasma glucose 60 mins 120 mins	 (median/IQR†)	n=95 6.1(5.3-7.4) 10.6(8.6-11.9) 10.0(8.0-11.9)	n=55 5.6(5.16-6.1) 9.25(8.1-10.65) 9.0(7.4-10)	n=40 7.6(6.5-9.3) 12.2 (11.4-14.3) 12.2 (11.35-15.05)	n=101 4.0(3.6-4.4) 5.8 (5.0-6.7) 5.2(4.5-6.3)
GA at delivery weeks Delivery <37 weeks	(median/IQR†) n (%)	n=100 38(37-38) 24(24)	n=55 38(37-38) 12(21.8)	n=45 37(36-38) 12(26.7)	n=90 39(37-40) 13(14.4)

Obstetric complications Caesarian section HDP[6]	n (%)	45(43.7) 67(66.3) 12(11.6)	21(36.2) 39(68.4) 6(10.3)	24(53.3) 28(63.6) 6(10.3)	16(15.8) 59(58.4) 11(10.9)
Exposure to OHA[7] Metformin Glibenclamide Exposure to insulin	n (%)	n=99 65(65.7) 31(31.3) 34(33.0)	n=54 38(70.4) 12(22.2) 8(14.8)	n=45 27(60) 19(42.2) 22(48.9)	 N/A N/A N/A
Neonatal outcomes Birth weight LBW[8] Macrosomia Anomalies	(median/IQR[†]) n (%)	n=99 3065 (2665-3430) 18(18.1) 5(5.0) 3(2.9)	n=56 3042.5(2650-3345) 11(19.6) 3(5.3) 2(3.5)	n=43 3140(2765-3685) 7(16.3) 2(4.6) 1(2.2)	n=100 3040(2792.5-3357.5) 17(17) 5(5) 2(2.1)
Demographic, anthropometric, and biochemical status at 3-6 years after the index pregnancy		**HFDP group**			**Non-HFDP group**
		Total group n=103	GDM n=58	DIP n=45	n=101
Age (years)	(median/IQR[†])	37.3 (33.1-42.7)	38.1(32.8-42.7)	37.1(33.9-41.7)	34.0(29.2-37.3)
Primigravida	n (%)	6(5.8)	3(5.2)	3(6.7)	13(12.8)
Time at follow-up (years)	(median/IQR[†])	3(3-4)	3(3-4)	3(3-4)	3(3-4)

SES[9]					
1) Marital status married	n(%)	38(36.9)	20 (34.4)	18(40)	15(14.8)
2) Education Secondary and higher		66(65.3)	40(71.4)	26(57.8)	73(23)
3) Household items	(mean/ SD ‡)	7.9(2.1)	7.8(2.0)	7.9(2.3)	8.3(1.9)
4) Housing density	(median/IQR†)	2.5(2-3)	2.5(2-4)	2.5(2-3)	2.0(1.5-3)
BP[3], (mmHg)					
Systolic BP	(median/IQR†)	115.1(108-126.6)	115(106.6-125)	117.5(112.-131.8)	113.7(104.6-120.3)
Diastolic BP		82.6(76.6-90.6)	81(73.3-89.6)	84.8(77.6-90.6)	79(73.3-86.6)
HIV positive	n(%)	19(18.6)	14(24.1)	5(11.1)	25(24.7)
Anthropometrics					
Waist circumference	(mean/SD ‡)	102(15.0)	100.2(14.4)	104.2(15.5)	90.9(16.6)
Hip circumference		115.1(12.2)	115.3(11.8)	114.9(12.8)	110.6(15.0)
BMI[2]	(median/IQR†)	32.8(29.1-39.2)	31.9(28.8-36.1)	33.8(29.5-39.7)	29.2(24.2-33.4)
BMI diff		-1.7(-3.7-0.3)	-1.5(-3.7-1.3)	-2.08(-3.2-(-0.56)	0.45(-2.7-3.3)
Weight diff		-3.6(-9.6-0.3)	-1.85(-9.6-3.3)	-5.2(-8.5-(-1.4)	-0.4(-8.7-10)
Glucose profiles					
Fasting glucose	(median /IQR†)	5.9(5.1-9.0)	4.9(4.6-5.2)	7.5(5.7-11.8)	4.9(4.6-5.3)
120 mins glucose		7.1(5.6-9.1)	5.5(4.8-6.1)	8.4(6.3-13.2)	5.5(4.8-6.1)

†IQR: interquartile range ‡SD: Standard deviation [1]GA: gestational age. [2]BMI: body mass index [3]BP: blood pressure. [4]HIV: human immunodeficiency virus.[5] OGTT: 75gm 2-hour oral glucose tolerance test [6]HDP: Hypertensive disorders in pregnancy including gestational hypertension, pre-eclampsia, eclampsia.

[7]OHA: oral hypoglycaemic agents [8]LBW: low birth weight. [9]SES: socioeconomic status. [10]IFG: impaired fasting glucose. [11]T2DM: type 2 diabetes [12] IGT: impaired glucose tolerance [13]FPG: fasting plasma glucose

Table 5. 2 Stratified analysis of maternal outcomes by exposure to HFDP.

Outcomes at follow-up		**HFDP group**			**Non-HFDP group**	**p-value**
		Total group n=103	GDM n=58	DIP n=45	n=101	
Diabetes status						
Type 2 diabetes mellitus	n(%)	46(44.6)	16(27.6)	30(66.7)	5(4.9)	*a0.0001 *b0.016
Status unknown		27(57.4)	13(81.2)	14(45.2)	4(100)	*a<0.001 *b<0.001
Dysglycaemia		57(55.9)	23(39.6)	34(77.3)	14(13.9)	*a<0.001 *b<0.001
Insulin resistance		92(90.2)	50(87.7)	42(93.3)	69(68.3)	*a<0.001 *b<0.001
Cardiovascular						
Cardiovascular risk factors						
Historical						
1.Age	median(IQR)	37.3(33.1-42.7)	37.1(33.9-41.7)	38.1(32.8-42.7)	34.0(29.2-37.3)	*a <0.001 *b0.001
2.Smoking						
Smoker-current/ever	n(%)	10(9.7)	6(10.3)	4(8.9)	8(7.9)	#a 0.65 #b0.901
3. Family history CVD[1]		20(19.4)	10(17.2)	10(22.2)	17(17.1)	a 0.65. b0.90
Blood pressure						
Chronic HTN[2] ≥ 140/90	n(%)	27(26.1)	10(17.2)	17(37.8)	12(11.9)	* 0.009 *b0.001
Known HTN		22(21)	8(13.8)	14(31.1)	11(10.9)	*a 0.03 *b0.005
Newly diagnosed		5(4.8)	2(3.5)	3(6.7)	1(0.9)	*#a0.02 *#b0.005

Biochemistry						
1. Proportion with dyslipidaemia		85(82.5)	46(79.3)	39(86.7)	73(72.2)	[a] 0.080 [*b]0.146
2. Triglycerides	n(%)	0.8(0.6-.1.14)	0.79(0.6-1.1)	0.9(0.6-1.2)	0.58 (0.44-0.8)	[*a] 0.000 [*b]<0.001
3. High density lipoprotein	median(IQR)	1.1(0.9-1.3)	1.1(0.9-1.3)	1.1(1-1.2)	1.1(0.91-1.36)	[a] 0.894 [*b]0.8770
4. Total cholesterol		4.2(3.6-4.8)	4.2(3.6-4.8)	4.2(3.8-4.8)	3.6(3.1-4.4)	[*a] 0.002 [*b]0.002
5. Low density lipoprotein		2.7(2.1-3.1)	2.7(1.9-3.2)	2.7(2.2-3.1)	2.2(1.7-2.7)	[a] 0.003 [*b]0.002
Cardiovascular risk		n=100	n=57	n=43	n=101	
a) Framingham risk score (FRS)	median(IQR)	8.46(4.9-14.4)	7.17(4.4-10.7)	11.7(5.8-16.7)	3.48(2.1-5.7)	[*a]<0.001 [*b]<0.001
Categories						
• High risk(>20%)		13(13)	5(8.7)	8(18.6)	0	[*a]<0.001 [*b]<0.001
• Intermediate(10-20%)	n(%)	29(29)	11(19.3)	18(41.8)	8(7.9)	
• Low(<10%)		58(58)	41(71.9)	17(239.5)	93(92.1)	
Non-lab-based FRS score	median(IQR)	43.9(25>30-62.8)	35.1(21.1-48.9)	53.2(35.5-70.2)	20.7(12.0-32.7)	[*a]<0.001 [*b]<0.001
b) Carotid intima media thickness(cIMT)						
• Thickened IMT (>0.8mm)	n(%)	0	0	0	0	--
• Overall mean CIMT	median(IQR)	0.48(0.44-0.53)	0.48(0.44-0.52)	0.49(0.43-0.55)	0.46(0.42-0.50)	[*a]0.0371 [b]0.113
• Right CCA [6]		0.47(0.43-0.51)	0.47(0.42-0.52)	0.46(0.42-0.55)	0.46 (0.42-0.51)	[a]0.3500 [b]0.590
• Left CCA		0.49(0.44-0.54)	0.5(0.45-0.54)	0.49(0.44-0.55)	0.46(0.43-0.51)	[*a]0.007 [*b]0.0274
3. Metabolic syndrome						
Metabolic Syndrome Harmonized criteria	n(%)	42(40.8)	17(29.3)	25(55.6)	6(5.9)	[a]<0.001 [*b]<0.001
BMI[3] ≥ 30	n(%)	71(68.9)	39(67.2)	32(71.1)	46(45.5)	[*a] <0.001 [*b]<0.003
Waist circumference ≥ 80cm		95(92.2)	54(93.1)	41(91.1)	77(77)	[*a]<0.001 [*b]<0.001
Waist: hip ratio (>0.85)		66(64)	33(56.9)	33(73.3)	26(26)	[*a]<0.001 [*b]<0.010

[a]p-value for control vs. hyperglycaemia detected in pregnancy; [b]p-value for control vs GDM and DIP. *indicates significance <0.05. [#] indicates chi exact test. [†]IQR: interquartile range [‡]SD: Standard deviation [1]CVD: cardiovascular disease. [2] HTN: hypertension. [3] BMI: body mass index. [4] CCA: common carotid artery.

Table 5. 3 Maternal body composition outcomes by exposure to HFDP.

Follow up		**HFDP group**			**Non-HFDP group**	**p-value**
		Total n=99	GDM n=55	DIP n=44	n=96	
Fat-free soft tissue mass (FFSM)/kg	median(IQR)	44.9(39.2-51.0)	44.1(38.7-49.2)	45.5(41.6-53.7)	41.9(36.2-46.1)	*[a]0.0006*[b]0.0007
Total body fat mass/kg	mean(SD)	36.1(9.6)	34.9(9.2)	37.5(10.1)	31.1(10.7)	*[a]0.0008 *[b]0.0011
Total lean body mass/kg	mean(SD)	48.1(7.2)	46.9(6.2)	49.7(8.3)	44.1(6.9)	*[a]0.0001 *[b]0.0005
Total body fat %		44.1(40-46.1)	44.3(40.2-47.1)	43.7(40.3-45.3)	43.5(38.9-46.9)	[a]0.42 [b]0.6146
Android fat/kg	median(IQR)	2.6(2.0-3.4)	2.4(1.9-3.4)	2.9(2.1-3.7)	1.9(1.3-2.7)	*[a]<0.001 *[b]0.0001
Visceral fat/kg	median(IQR)	1.5(1.2-1.9)	1.3(1.2-1.9)	1.6(1.3-2.0)	1.1(0.7-1.6)	*[a]<0.001 *[b]0.0001
Visceral fat %	median(IQR)	39.8(35-43.6)	38.4(35.4-43.6)	40.2(35.8-43.2)	36.5(29.4-42.5)	*[a] 0.0066 *[b]0.0237
Arm(right) fat mass/kg	median(IQR)	2.1(1.8-2.7)	2.1(1.8-2.6)	2.4(2.0-2.9)	1.8(1.3-2.2)	*[a] <0.001*[b]0.0001
Leg(right) fat mass/kg	median(IQR)	7.4(5.7-9.1)	7.4(5.6-9.0)	7.1(5.8-8.9)	6.9(5.2-8.5)	[a] 0.13 [b]0.3178

VAT[1] (volume) cm^3	median(IQR)	517.6(372.6-610.2)	510.6(361-594)	570.5(407.9-655.5)	322(219.2-469.2)	*[a]<0.001 *[b]0.0001
SAT[2](volume) cm^3	median(IQR)	2334.6(2005.1-3003.6)	2316.2(1995.4-2949.2)	2492.6(20240.8-3121.8)	1943.9(1430-2678.6)	*[a]0.0003 [b]0.0001

Ratios	**Total HFDP group**	GDM	DIP	**HFDP nonexposed**	**p-value**
VAT:SAT	0.20(0.16-0.24)	0.20(0.15-0.24)	0.21(0.17-0.24)	0.16(0.12-0.20)	*<0.001
Android: Gynoid	0.93(0.86-1.02)	0.93(0.85-1.02)	0.98(0.86-1.02)	0.86 (0.75-0.95)	[a*]<0.001
Trunk: limb fat mass	0.82(0.73-0.98)	0.81(0.70-0.94)	0.86(0.76-1.01)	0.72(0.60-0.83)	[a*]<0.001
FMI[3] (Fat mass/height[2])	13.9(11.7-16.3)	13.4(11.3-15.9)	14.6(12.2-18.0)	12.3(8.9-14.6)	[a*]0.0008
Lean mass//height[2]	18.7(17.1-20.8)	18.4(17.1-20.3)	19.6(17.0-21.8)	17.1(16.7-18.9)	[a*]<0.001

*indicates significance <0.05 [a]p-value for control vs. hyperglycaemia detected in pregnancy; [b]p-value for control vs GDM and DIP [1]VAT: visceral adipose tissue. [2]SAT: subcutaneous adipose tissue [3]FMI: fat mas

Table 5. 4 Frequency of the outcome's diabetes, metabolic syndrome and CVD event as predicted by FRS and the adjusted odds ratio for the association with a history of HFDP

	Diabetes			**Metabolic syndrome**			**Predicted cardiovascular risk (FRS)[b]**		
	Crude OR[a] (95% CI)	Adjusted OR[#] (95% CI)	p-value	Crude OR[a] (95% CI)	Adjusted OR[†] (95% CI)	p-value	Crude OR[a] (95% CI)	Adjusted OR[‡] (95% CI)	p-value
HFDP	15.5(5.8-41.3)	10.5(3.7-29.5)	[*]<0.001	10.9(4.4-27.9)	6.3(2.2-18.1)	<0.001	8.4(3.6-19.1)	4.3(1.6-11.5)	[*]0.003
GDM	7.3(2.5-21.3)	4.6(1.5-14.4)	0.008	6.5(2.4-17.8)	3.9(1.2-12.5)	[*]0.021	4.5(1.7-11.4)	1.7(0.52-5.55)	0.373
DIP	38.4(12.9-114.4)	27.6(8.7-87.4)	[*]<0.001	19.7(7.2-54.5)	10.5(3.3-32.8)	[*]<0.001	17.7(6.9-45.7)	1.5(0.23-10.0)	[*]<0.001

[a] p-values for all unadjusted OR were p <0.001

[b] FRS categorized as intermediate and high (10-year risk of >10% risk for CVD event)

[#]Confounders: Post-partum variables: Maternal age, BMI, Parity and family history diabetes

[†]Confounders: Index pregnancy variables: Maternal age, parity, SBP, BMI

[‡]Confounders: Index pregnancy variables: Maternal age, SBP, BMI

Table 5. 5 Linear regression model for log-transformed FMI. Values are standardized regression coefficients (β) with 95% confidence interval, and p-values.

Outcome: Fat mass index	**M1: Distal model**		**M2: Intermediate model**		**M3: Proximal model**		**M4:M1+M2+M3**	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Maternal pre-pregnancy factors								
Parity-multiparous	0.18(0.07-0.29)	**0.001**						
Education: secondary and higher	0.03(-0.46-0.53)	0.890						
SES[1] score	0.004 (-0.02-0.03)	0.718						
Smoking	-0.04(-0.21-0.13)	0.654						
Alcohol	0.08(-0.04-0.21)	0.182						
HIV[2] positive	-0.04(-0.16-0.07)	0.476						
Maternal pregnancy factors								
Age			-0.001(-0.008-0.005)	0.748				
Initial BMI[3]			0.031(0.02-0.03)	**<0.001**				
HFDP[4] exposure			0.019(-0.07-0.11)	0.683				
Maternal post-partum factors								
Exclusive breastfeeding					-0.086(-0.27-0.10)	0.374		
BMI difference[#]					0.009(0.003-0.016)	**0.005**		
Exercise					-0.086(-0.224-0.062)	0.266		
Combined models								
Parity							0.023(-0.065-0.112)	0.599
Education: secondary and up							0.167(-0.139-0.474)	0.283
SES score							0.002(-0.015-0.020)	0.818
Smoking							0.053(-0.066-0.172)	0.379
Alcohol							0.001(-0.892-0.091)	0.976
HIV positive							0.092(-0.012-0.198)	0.082
Age							-0.000(-0.006-0.006)	0.982
Initial BMI							0.045(0.039-0.051)	**<0.001**

HFDP exposure Exclusive breastfeeding BMI difference Exercise				0.029(-0.06-0.124) 0.033(-0.085-0.151) 0.043(0.034-0.051) -0.030(-0.126-0.065)	*0.539 0.581 **<0.001** *0.527
	Adj. R^2 =0.02 N=191	Adj. R^2 =0.36 N=187	Adj. R^2 =0.04 N=151		Adj. R^2 =0.66 N=144

[1]SES: socioeconomic asset score, [2]HIV: Human immunodeficiency virus, [3]BMI: body mass index, [4 5]HFDP: hyperglycaemia first detected in pregnancy

[#]BMI diff: the difference between BMI measured at post-partum visit minus BMI at pregnancy

Table 5. 6 Multivariate analysis of factors associated with risk of progression to diabetes. Values are standardized odds ratios (OR) with 95% confidence interval, and p-value.

Outcome Diabetes	**M1: Distal model**		**M2: Intermediate model**		**M3: Proximal model**		**M4: M1+M2 +M3**	
	OR (95%CI)	p value	OR (95% CI)	p value	OR (95%CI)	P value	OR (95%CI)	P value
Maternal pre-pregnancy factors								
Parity	5.14(1.82-14.50)	**0.002**						
Education: secondary and higher	0.20 (0.09-4.31)	0.309						
SES[1] score	0.88(0.74-1.05)	0.180						
Alcohol	0.60(0.211-1.71)	0.341						
Smoking	0.40(0.09-1.68)	0.213						
HIV[2]	0.29(0.108-0.82)	**0.019**						
Family history diabetes	4.67(2.15-10.13)	**<0.001**						
Maternal pregnancy factors								
Age			1.01(0.94-1.08)	0.704				
Initial BMI[3]			1.04(0.98-1.09)	0.151				
Initial SBP[4]			0.99(0.96-1.02)	0.886				
HFDP[5] exposure			12.9(4.33-38.45)	**<0.001**				
Maternal post-partum factors								
Exclusive breastfeeding					0.141(0.04-0.48)	**0.002**		
Exercise					2.28(0.86-6.05)	0.097		
Progesterone only contraception					1.11 (0.49-2.53)	0.794		
VAT:SAT[6] ratio					10.70(2.53-45.25)	**<0.001**		

Combined models					
Parity				0.94(0.19-4.55)	0.944
Education: secondary and up				0.32(0.01-8.50)	0.498
SES score				0.75(0.56-1.01)	0.062
Alcohol				0.79(0.18-3.51)	0.763
Smoking				0.31(0.04-2.07)	0.230
HIV				0.40(0.07-2.29)	0.307
Family history diabetes				4.37(1.80-15.80)	**0.010***
Age				0.96(0.88-1.06)	0.482
Initial BMI				1.03(0.94-1.13)	0.407
Initial SBP				0.99(0.95-1.04)	0.840
HFDP exposure				7.84(1.37-44.6)	**0.020***
Exclusive breastfeeding				0.23(0.05-0.96)	0.057
Exercise				1.00(0.25-4.01)	0.998
Progesterone only contraception				0.96(0.33-2.81)	0.955
VAT:SAT ratio				7.98(1.09-57.94)	**0.004***
	N=200 Adj. R^2 =0.18	N=193 Adj. R^2 =0.23	N=151 Adj R^2 =0.15	N=142 Adj. R^2 =0.37	

[1]SES: socioeconomic asset score, [2]HIV: Human immunodeficiency virus, [3]BMI: body mass index, [4]SBP: systolic blood pressure, [5]HFDP: hyperglycaemia first detected in pregnancy, [6]VAT:SAT ratio: visceral adipose tissue: subcutaneous adipose tissue ratio

Table 5. 7 Linear regression model for log-transformed carotid intima media thickness (cIMT). Values are standardized regression coefficients (β) with 95% confidence interval, and p-value.

Outcome CIMT	**M1: Distal variables**		**M2: Intermediate model**		**M3: Proximal model**		**M4: M1+M2 +M3**	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Maternal pre-pregnancy factors								
Parity	0.02(-0.011-0.070)	0.163						
Education: secondary and higher	0.02(-0.175-0.207)	0.868						
SES[1] score	0.003(-0.005-0.013)	0.432						
HIV[2] status	0.035(-0.011-0.081)	0.142						
Smoking	-0.019(-0.085-0.046	0.557						
Family history CVD[3]	0.009(-0.039-0.059)	0.695						
Maternal pregnancy factors								
Age			0.01(0.007-0.013)	**<0.001**				
Initial BMI[4]			0.002(-0.002-0.004)	0.075				
Initial SBP[5]			0.001(0.000-0.003)	**0.015**				
HFDP exposure			-0.03(-0.07-0.006)	0.095				
Maternal post-partum factors								
Exclusive breastfeeding					-0.01(-0.081-0.06)	0.779		
Exercise					0.008(-0.044-0.06)	0.757		
BMI difference					0.002(0.000-0.004)	**0.048**		
TG[6] level					0.042(0.000-0.085)	**0.047**		
HDL[7] level					-0.02(-0.089-0.037)	0.419		

Combined models					
Parity				-0.015(-0.060-0.035)	0.555
Education				-0.011(-0.187-0.165)	0.900
SES score				0.007(-0.002-0.016)	0.146
HIV status				0.041(-0.020-0.103)	0.187
Smoking				0.021(-0.046-0.089)	0.533
Family history CVD				0.000(-0.050-0.050)	0.973
Age				0.009(0.006-0.013)	**<0.001***
Initial BMI				0.002(-0.000-0.006)	0.099
Initial SBP				0.002 (0.000-0.003)	**0.018***
HFDP exposure				-0.014(-0.074-0.045)	0.632
Exclusive breastfeeding				-0.001(-0.069-0.066)	0.969
Exercise				0.016(-0.037-0.071)	0.540
BMI difference				0.006(0.002-0.105)	**0.001***
TG level				0.002(-0.004-0.047)	0.929
HDL level				0.011(-0.051-0.075)	0.712
	N=197 Adj. R^2 =-0.005	N=192 Adj. R^2 =0.23	N=156 Adj R^2 =0.02	N=147 Adj. R^2 =0.21	

[1]SES: socioeconomic asset score, [2]HIV: Human immunodeficiency virus [3] CVD: cardiovascular disease [4]BMI: body mass index, [5]SBP: systolic blood pressure , [6] TG: triglyceride, [7]HDL: high density lipogram

Table 5. 8 Definitions of diagnostic criteria for HFDP, maternal pregnancy factors and outcomes

Maternal variables	
Risk factors for HFDP	**Persistent glycosuria, family history of diabetes(first degree), previous unexplained perinatal losses, previous HFDP, history of a macrosomic baby.**
Pregnancy losses	Includes miscarriage, intrauterine death, ectopic pregnancy and termination of pregnancy and neonatal death
Maternal overweight	BMI ≥25 kg/m^2
Maternal obesity	BMI ≥30 kg/m^2
Nephropathy	Creatinine > 71ummol/L and MAC >30 mg/L
Anaemia	Haemoglobin levels < 11 g/dl
HIV-positive	Based on results from rapid or antibody ELISA tests either at index pregnancy or follow up visit
Hypertensive disorders of pregnancy	Categorized as: Pregnancy-induced hypertension (PIH): hypertension presenting >20 completed weeks Preeclampsia: hypertension with proteinuria Eclampsia: hypertension with proteinuria and seizures
Any obstetric complication	Miscarriage, PIH, pre-eclampsia, eclampsia, urinary tract infection, polyhydramnios, oligohydramnios, abruptio placentae, maternal death, and/or other.
Maternal measurements and outcomes	
i) Anthropometrics	
All Measurements	Measured and recorded three times using a non-elastic tape and final measure being a mean of the 3 readings to one decimal place
Waist circumference	Taken at midpoint between iliac crest and lowest part of rib cage in the mid-axillary line
Hip circumference Waist: hip ratio (WHR)	Taken around widest part of hips As there are no recommended cut-offs for African populations, WHR was categorised as follows: Normal <0.849 & Central obesity>0.85
BMI categories	BMI <25kg/m^2 is normal BMI>25 and <30kg/m^2 overweight BMI>30kg/m^2 obese
Fat mass index	Derived from DXA as estimated fat mass (kg) divided by height (m) squared.
ii) Glycaemic measures	

Diabetes categories (WHO 2006 criteria)	Diabetes FPG ≥ 7.0mmol/L or 2hr plasma glucose ≥11.1mmol/L or HbA1c ≥6.5% Impaired fasting glucose ≥ 6.1-6.9 Impaired glucose tolerance ≥7.0 and < 11.1mmol/L
Dysglycaemia	Fasting blood glucose level ≥ 5.6mmol/L
Insulin resistance	Assessed using the homeostatic model and calculated as fasting glucose (mmol/L) fasting insulin (mU/l)/22.5 HOMA score>1.95 defined as insulin resistance
iii) Cardiovascular parameters	
Blood pressure measurement	3 measurements were taken using Omron M6(Omron Kyoto, Japan) on right arm in a sitting position 1 minute apart after 5 minutes at rest, Average of the last 2 readings were recorded
Hypertension	Blood pressure of > 140/90 mmHg for at least two measurements
Carotid intima media thickness(cIMT)	Thickened cIMT defined as ≥0.8mm
Atherosclerotic plaque	Focal structure that extends at least 0.5 mm into the arterial lumen and/or measures 50% or more of the adjacent IMT value and/or has an IMT value greater than 1.5 mm
Cardiovascular disease	Self-reported history of coronary artery disease, stroke and or peripheral arterial disease
Dyslipidaemia	Dyslipidaemia was defined as either triglyceride ≥ 1.7 mmol/L and/or high-density lipoprotein cholesterol ≤ 1.30 mmol/L and/or LDL ≥ 3.0mmol/L
Neonatal outcomes	
Prematurity	GA <37 completed weeks
Low birth weight	Birth weight <2500 grams
Macrosomia	Birth weight ≥ 4000 grams

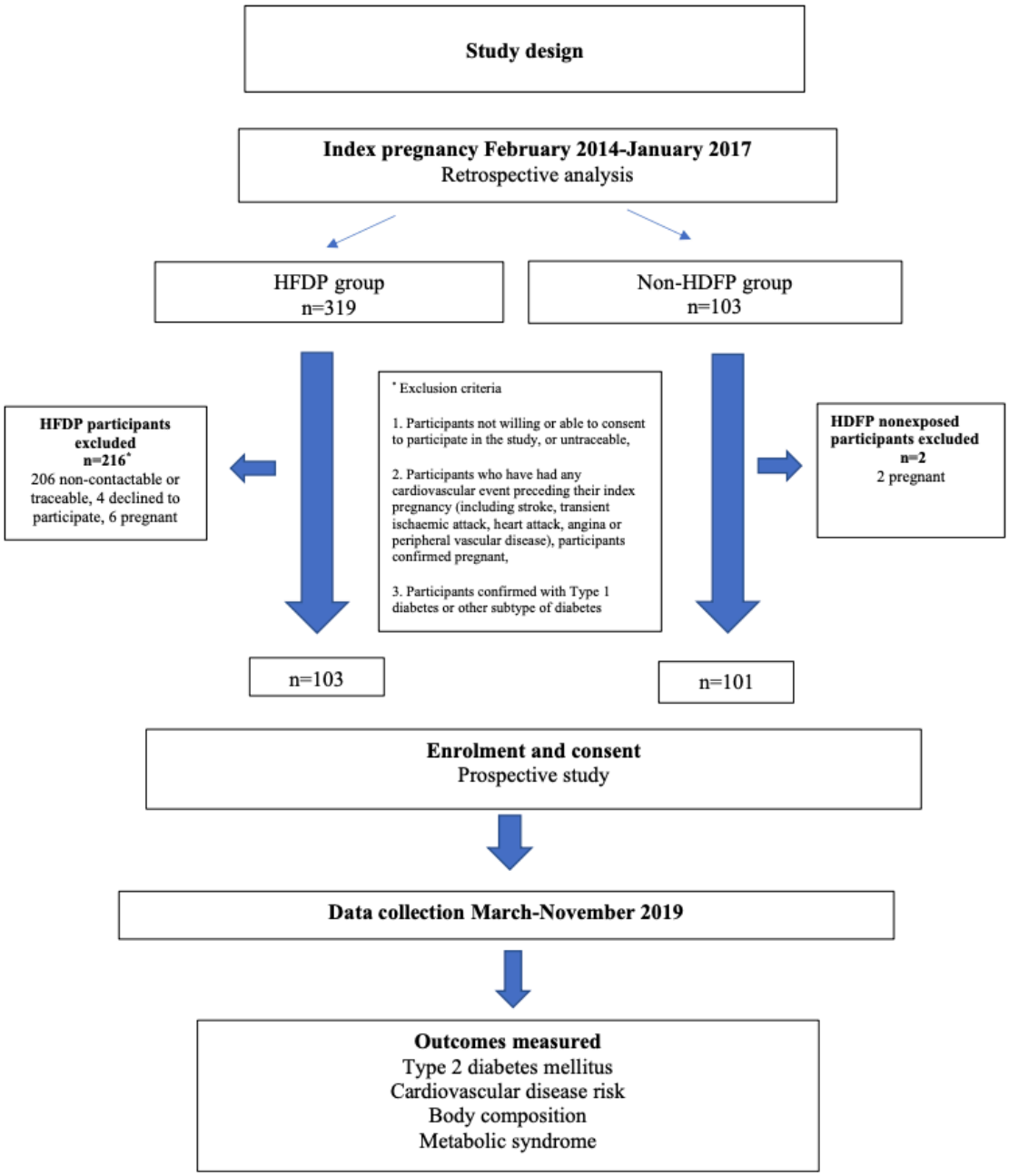


Figure 5. 1 Flow diagram of the study and eligible participants

SECTION 3
INTEGRATED DISCUSSION

"Start by doing what's necessary; then do what's possible; and suddenly you are doing the impossible."
Francis of Assisi

Chapter 6- Discussion and Conclusion

This chapter provides a summary of the key findings from the empirical papers presented in Chapters 3 to 5. It also discusses two main themes that have emerged from this research and how the findings add to the body of knowledge on HFDP from a global and national perspective. The strengths and limitations of the PhD study are highlighted and recommendations in the context of SA policies governing maternal healthcare are made. Finally, this chapter discusses potential research for the future and ends with an overall conclusion that describes the key take-home message.

6.1 Consolidated findings

This PhD thesis set out to investigate the short-term consequences in black SA women living in an urban setting following HFDP compared with a group of women who were not exposed to HFDP. The additional influence of HIV on these outcomes were explored. Body composition as assessed by DXA was reported for each group. Each objective was achieved through an empirical study presented as individual chapters within the thesis. The objectives of this thesis and related findings are summarised in Table 6.1

Table 6. 1 Summary table of objectives and findings

Number	Objectives	Chapter	Key Findings	Conclusion
1.	To describe maternal and neonatal outcomes in diabetic pregnancies including fetal miscarriage, preterm delivery, PIH, pre-eclampsia/eclampsia, CS rates and maternal death following diabetes in pregnancy	3	• Of the 1071 diabetic pregnancies reviewed, 43% had HFDP, 38.1% T2DM, 18.9% T1DM, • Within HFDP group: 51% overt GDM (DIP) vs. 49% GDM cases. • Obesity was highly prevalent across all three types of diabetes groups. • Miscarriage, maternal mortality and PNM rate highest amongst T1DM (6.9%, 1.5% and 6.7% respectively) • HDP(PIH and PE) 9.5 % for the entire group with highest rate amongst T1DM (13.8% followed by GDM 11.9%) • Caesarean section rate was high in all 3 groups at 72.2%, with failed induction of labour being the commonest indication. • Preterm delivery was high overall 30% (highest in T1DM 34.6%) • Diabetic control by 3rd trimester was >90% across all groups as measured by HbA1C • Maternal BMI was a significant predictor of macrosomia and LBW • Initial maternal HbA1c was a predictive factor for poor neonatal outcomes	Diabetic pregnancies, despite good glycaemic control demonstrate higher adverse outcomes compared with background population Initial maternal BMI, HbA1C and glycaemic control were significant predictors of poorer neonatal outcomes.
2.	To evaluate how HIV influenced these immediate outcomes amongst women with a diabetic pregnancy.	3	• HIV prevalence within cohort 24%, lowest in HFDP group (17.4%) • Maternal initial BMI, HTN rates and glycaemic control did not differ between HIV positive and negative women • Anaemia rates were significantly higher in HIV positive, diabetic pregnancies (29.2 % vs 9.7%, p<0.001) • Lower rate of macrosomia amongst HIV positive, HFDP group (0 vs 28 (10%) p=0.012)	The association of higher PNM with HIV warrants further exploration

			• PNM was higher in HIV-infected pregnancies (9.4%) than non-HIV-infected pregnancies (1.8% p<0.001)	
3.	Evaluate the role of OHA agents (metformin, glibenclamide) vs. insulin on these immediate outcomes following a diabetic pregnancy.	3	• No role of any of the oral agents on adverse maternal outcomes. • In terms of neonatal outcomes: Macrosomia rates were higher in those exposed to glibenclamide vs insulin alone groups (12.2% vs 0%, p=0.0025). LBW was higher (20% vs 8.4% p=0.032) and macrosomia rates were lower (1.2% vs 10.8% p=0.009) among HFDP exposed when comparing metformin vs insulin alone.	The safety and efficacy of OHA vs. the gold standard of insulin, were confirmed following a diabetic pregnancy except for higher macrosomia rates with glibenclamide.
4.	Examine the safety and efficacy of OHA on maternal and neonatal outcomes in the setting of HFDP exposure vs. insulin monotherapy amongst predominantly Black women, in an urban setting	4	• No differences in maternal or neonatal outcomes when comparing exposure to either agent (metformin monotherapy vs. glibenclamide exposure vs. insulin monotherapy). • Metformin monotherapy group had non-significantly lower incidence for prematurity, macrosomia and PNM when compared to insulin alone. • Glibenclamide exposure compared to insulin monotherapy; both maternal and neonatal outcomes were comparable. • OHA were effective at achieving good glycaemic control at term.	In the setting of HFDP, both OHA were found to be safe and efficacious when compared to the gold standard of therapy, insulin.
5.	. Establish the risk of dysglycaemia (impaired fasting glucose, impaired glucose tolerance and development of T2DM), CVD (pre-clinical and	5	• 45% of women with HFDP progressed to T2DM vs. 5% of controls. • OR (adjusted) for risk of progression to T2DM was 11. 1(95% CI 3.7-32.5). This remained significant within the HFDP subtypes. • Only 43% of women aware of their diabetic status. • Mediators of progression to diabetes included HFDP exposure, family history of T2DM, and an absence of exclusive breastfeeding	Women with a history of HFDP are at increased risk of all forms of dysglycaemia including T2DM, cardiovascular risk and MetS when compared to women

	clinical disease states) and MetS in women with a history of HFDP for a period three-six years post-partum and compare it to women non-exposed to HFDP for the same period.		• Prevalence of MetS was 41% amongst women exposed to HFDP vs. 6% among controls. OR (adjusted) was 6.3 (95% CI 2.2-18.1) which persisted within the subtypes of HFDP. • Mediators for MetS risk included HFDP exposure, maternal age and BMI at index pregnancy. • Higher CVD risk was found in women exposed to HFDP as assessed by FRS and cIMT, though this was mostly driven by factors at index pregnancy including age, systolic blood pressure and fasting plasma glucose .	non-exposed to HFDP in the post-partum period. Various maternal mediators played a role in augmenting these risks.
6.	To investigate the additional role of HIV during pregnancy on incident T2DM, CVD (pre-clinical and disease states) and MetS in women with a history of HFDP over a 6-year period	5	• There was no significant influence of treated HIV on either of the outcomes including FMI, T2DM, MetS, cardiovascular risk as assessed by both cIMT and FRS.	HIV was not found to play a role in any of the outcomes.
6.	Compare the body composition and fat mass distribution as measured by DXA in women with a history of HFDP compared to women non-exposed to HFDP.	5	• Body composition scores were significantly higher in women exposed to HFDP vs controls. • FMI was significantly higher 13.9 vs 12.3, p=0.0008 • Visceral fat was significantly higher in exposed women VAT: SAT ratio 0.2 vs. 0.16, p<0.001. • Body composition remained significant after correcting for confounders including age.	Body composition measures including obesity and in particular visceral adiposity, is significantly higher in women exposed to HFDP when compared to those non-exposed in the short-term following delivery

6.2 Hypotheses revisited

The current study supported and accepted the initial hypothesis which stated that a history of HFDP leads to a higher likelihood of progressing to T2DM, MetS and higher CVD risk in the short-term following the index pregnancy when compared to women who were not exposed to HFDP among black SA women. The theory that the co-existence of HIV infection and HFDP may augment the development of these cardiometabolic consequences following delivery compared to HIV negative women was rejected.

6.3 Emerging research themes

This section reflects upon themes that have emerged from the research conducted and is divided into two main areas, the first contemplates an empirical theme that arose and the second deals with a methodological theme that emerged.

6.3.1 Underestimation of cardiovascular risk in young women

Cardiovascular disease is the number one cause of mortality in women worldwide despite successes in prevention and treatment of CVD(134). A great proportion (85%) of annual CVD-related deaths occur in LMIC. Even more concerning is the increased mortality amongst young women aged 35-45 years which has been noted over the past two decades. CVD risk is underestimated by both women and their providers. A previous study showed that the CVD mortality in young women with risk factors was 88% higher than in women without any risk factors when followed over a 30-year period (278).

i) Cardiovascular disease risk factors in women

While traditional risk factors have long been known and led to the development of risk prediction models and to major developments in therapy, several risk factors unique to women have been identified which confer an increased risk for CVD. These include adverse pregnancy history (279–281)including HFDP, HDP, preterm delivery and recurrent miscarriages. Outside of pregnancy factors such as PCOS, rheumatologic and other inflammatory diseases, depression and later on menopause have been associated with a higher risk (282–284).

Appreciation of these unique risk factors became evident when a study (285)demonstrated that up to 20% of patients with coronary artery disease have no traditional risk factors and 40% have only one. Additionally, evidence suggests that pregnancy complications are not limited to the pregnancy, instead are independent risk factors for future CVD (286,287).

South Africa is not exempt from the cardiovascular disease burden among women, with its complex epidemiological transitions characterised by extensive urbanisation together with the lifestyle changes accompanying this, including decreased physical activity and calorie dense diets (288,289).This has led to a surge in cardiovascular risk factors such as obesity, HTN and diabetes which further adds to the existing burden of communicable diseases like TB and HIV (290).

The underlying mechanisms connecting adverse pregnancy outcomes and CVD risk- are not well understood, though the existence of similar pathophysiological pathways explaining the association between HFDP and future CVD and diabetes, has been proposed, better known as the "common soil hypothesis"(291).Whether adverse pregnancy outcomes merely unmask a woman's pre-existing CVD risk or are actually mediators of future CVD pathogenesis is unclear. Given that CVD is often neglected by health care professionals with no specific prevention efforts in place worldwide, targeting women is of major concern since they have worse outcomes in addition to their unique set of risk factors.

ii) Cardiovascular risk assessment tools

A major difficulty in the prevention of CVD is identifying the at-risk individual at an early enough stage to benefit from interventions, with cardiovascular risk prediction remaining an increasing clinical challenge. Furthermore, current CVD risk assessment tools underestimate risk for this age group (292). Other than the presence of traditional risk factors and biomarkers, vascular imaging and novel biomarkers are amongst other methods that we can employ to better predict risk in these women. The identification of clinically useful markers is necessary in this young group of women at risk for CVD.

Risk scoring tools

In order to identify patients who may benefit from aggressive lifestyle interventions and pharmacological therapy in the prevention of CVD, risk estimation analysis should be employed. Risk score models, such as the FRS(164), Systematic COronary Risk Evaluation (SCORE) (216), QRISK(215)and the Pooled Cohort Equation (PCE) (293) were created to

prognosticate risk and provide guidance for informed decision-making regarding initiation or intensification of preventive strategies. The limitations of these scores are that they potentially underestimate risk since they have been derived from older age cohorts where prevalence of risk factors differ. The score is calculated based on observations of the relative degree of importance of individual major risk factors with most of these models being limited in that they do not include unique factors such as pregnancy-related attributes or evidence of subclinical CVD. PCE, is one exception which is gender-specific and provides a 10-year atherosclerotic CVD risk and a lifetime risk for CVD. This is relevant in women who carry a higher lifetime risk for CVD which then allows for aggressive lifestyle changes and medications to reduce CVD. Each multivariate model carries with it, its own set of advantages and disadvantages, with no single risk model being more accurate at estimating CVD risk. To achieve a more precise estimation of CVD risk in young asymptomatic individuals would include using one of the models alongside laboratory results, family history of premature CAD and the presence of non-traditional risk factors.

Biomarkers

A biomarker is defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease," by the WHO (294). While traditional biomarkers in the field of cardiovascular health like lipids have always had a place in risk stratification models, no currently available biomarker is suitable for detecting early CVD. The increasing need to identify novel cardiovascular biomarkers have been the subject of more recent studies, though their clinical utility has not been fully elucidated. The different mechanisms at play in the progression of diabetes and associated cardiovascular complications such as abnormal adipocyte signalling, insulin resistance, endothelial dysfunction, and oxidative stress, leads to the alteration of different signalling molecules in blood and other body fluids, which offer potential biomarkers for the identification and early detection of disease (285). Certain inflammatory and endothelial markers have been explored in women with a prior history of HFDP, including VCAM, ICAM, hsCRP, IL-6 and were found to be associated with both higher cardiovascular risk and CVD (294,295). Studies on more novel biomarkers including GDF-15 (Growth differentiation factor 15, MR-proANP (mid-regional pro-atrial natriuretic peptide) CT-proANP (C-terminal pro-arginine vasopressin) are either scarce or lacking (296,297). Currently no validated markers of this vascular risk are identifiable before the onset of diabetes, MetS and CVD. . To

better appreciate the value of these markers, larger cohorts and longitudinal studies are needed. Going forward in order to better appreciate the global risk for CVD may necessitate the integration of multiple biomarkers reflecting the different pathological pathways involved in atherosclerosis.

Other assessment tools

Atherosclerosis is the multistep process in which the end result is the accumulation of cholesterol and calcium plaque within the arterial wall. The pathophysiologic process by which this occurs is complex with endothelial dysfunction being the initial stage. This can be accurately measured by several non-invasive techniques including large artery function which can be detected through FMD, structural changes which can be detected through cIMT and vascular compliance using pulse wave analysis. Pulse wave velocity (PWV), a measure of arterial stiffness, has been used to detect subclinical disease in hypertensive patients (298) and has been shown to be useful as a marker of future cardiovascular events (299) and improves classification of patients to higher risk categories (161) A few studies have demonstrated that PWV is significantly higher in women with prior HFDP compared to controls as early as 3-years post-delivery (162,163) however this was not seen in the early post-partum period (300). Flow-mediated dilatation (FMD) is another proposed surrogate marker for early detection of atherosclerosis which only recently has reached an adequate level of methodological standardisation to be proposed as a surrogate endpoint in clinical trials. Though only a few studies with varying FMD results have been conducted in women with prior HFDP, the correlation of FMD with cIMT has been established in cross-sectional studies (302–304). The measurement of cIMT is the most widely accepted marker of subclinical atherosclerosis (315) that strongly predicts heart disease and stroke, particularly amongst women (304).Numerous studies exist to support the role of HFDP in thicker IMT when compared to controls from as early as 2 years post-partum (149,305).

HIV infection confers an increased cardiovascular risk and events with a 2-fold increased risk relative to controls (306),though most of this research stems from HMIC. The proposed mechanism for this is the complex interplay of factors, including both traditional factors and those associated with immune activation, HIV-associated inflammation together with ART exposure (192). Since 25.6 million of the estimated 37.9 million people with HIV live in SSA, with 7.7 million living in SA (307) acknowledging this background burden when appreciating

cardiovascular risk among these young women with a history of HFDP is critical. Studies in SSA, though few, have explored the association of HIV and markers of subclinical atherosclerosis, in particular cIMT, and found higher cIMT amongst HIV positive patients when compared with controls (309,310). A more recent study (310)representing men and women in SSA however, showed a non-significant inverse correlation between HIV and lower cIMT which is in contrast to other findings. In support of this, another study from rural SA(311) where 5059 participants, aged 40 years and over, showed that HIV positive subjects had a lower prevalence of classical CVD risk factors in men and women. In our study, though all cardiovascular risk factors were lower amongst those with HIV, this was not significant between the groups. Plausible explanations for these findings are the increased access to ART in SA which has the largest ART roll-out program in the world (309) and application of treatment guidelines to screen people living with HIV for CVD risk, which may have prompted diagnosis and consequent treatment of identified risk factors, or changes in lifestyle. Paucity of data on the role of HIV and atherosclerotic CVD in Africa requires further studies.

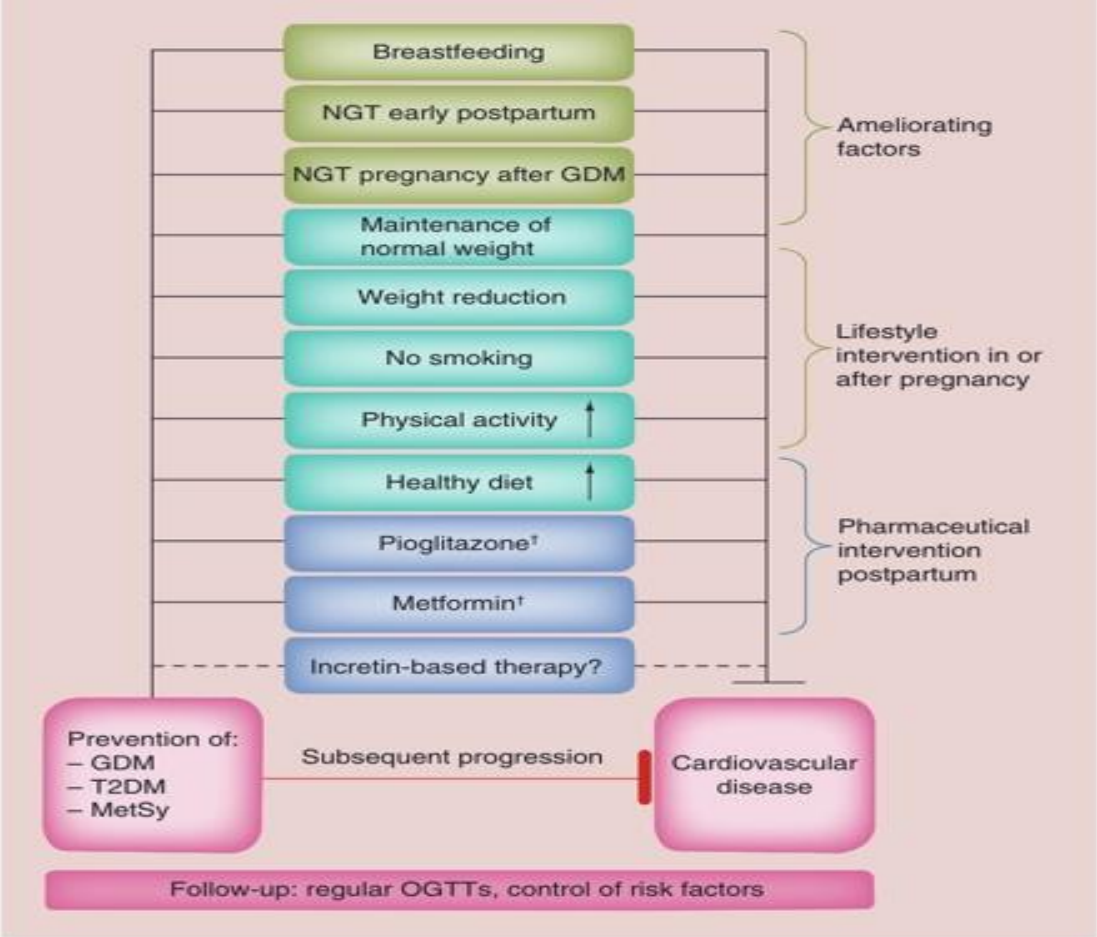
iii) Timing of screening

An important aspect to consider is the timing of screening for CVD in these at-risk women during their reproductive years. Ideally identifying risk factors as early as possible allows for earlier interventions. One study looked at screening during pregnancy (312)and used a simple scoring method including cardiometabolic risk measurements to determine 10-year risk of T2DM and CVD risk in women with HFDP. The study found that MetS like cluster (HTN, obesity, IGT and dyslipidaemia) during pregnancy identifies risk for T2DM and CVD risk at 10 years. Membership of the “high risk” cluster was a better predictor of progression to T2DM versus high fasting glucose >5.5mmol/L. This highlights that despite the well-versed hormonal changes and effects of pregnancy on CVD markers like lipids, measurement of traditional risk factors in conjunction with a family history of T2DM and CVD, in pregnant women with HFDP, may offer better opportunities to identify women with HFDP who are at increased risk of subsequent diabetes and CVD. Additionally, the post-partum period offers a unique window of opportunity to screen and offer lifestyle prevention programs for both mother and child alike since all family members face similar cardiometabolic risks. Investment in primary preventative approaches is not only favourable to those at risk but for health economies worldwide.

iv) Proposed way forward

The vicious cycle of cardiometabolic diseases with a " common soil" of risk factors ultimately giving rise to T2DM and CVD, exist along a continuum. Disease prevention seems to be a very appropriate way to break cycle. Throughout the literature, different approaches, with impressive and favourable results address this (313–319).

Within the SA context, some proposed solutions to improving screening and identification of CVD risk and disease amongst this vulnerable group include increasing funding for basic research and clinical trials that focus on CVD risk in younger women, augmenting health care systems and quality improvement initiatives to improve and increase health care providers rates of practice and consistency with guidelines for CVD risk, partnering among primary care providers and specialists in women health to assess and manage women's CVD risk, collaborating with public health and community organisations to lead initiatives for CVD risk reduction in young women. More specific medical approaches which have been investigated and have been found to modify CVD risk mostly through diabetes prevention include amongst others; lifestyle interventions (317,319) promotion of exclusive breastfeeding (320,321)and pharmacological interventions such as metformin (108), rosiglitazone (314) and pioglitazone (315,316). Incretin-based therapies might be of relevance in CVD prevention in the future (314) though further studies are needed. Figure 6.1 outlines preventative options for stopping the subsequent progression of CVD.



Abbreviations:†Randomized controlled trial available. GDM Gestational diabetes mellitus; MetS Metabolic syndrome; NGT Normal glucose tolerance; OGTT Oral glucose tolerance test; T2DM Type 2 diabetes mellitus.

Figure 6. 1 Prevention options in women with gestational diabetes mellitus (136).

In conclusion, better estimates of CVD incidence are needed to appropriately allocate resources for the emerging burden of HFDP and CVD on the background of HIV in LMIC. Disease prevention programmes are key to stopping disease progression in patients who are at risk and in turn this will save enormous costs for the economic systems. The well-known significant risk of developing diabetes in women exposed to HFDP further augments the CVD burden (135)acting as a double-edged sword. Given that pregnancy occurs early in a woman's life, well before the onset of clinically evident CVD, it serves as a unique opportunity to assess a women's later life CVD risk and initiate meaningful reduction strategies. Additionally, these measures will interrupt the intergenerational cycle of cardiometabolic diseases.

6.3.2. Strengthening health care systems in South Africa

The careful and thoughtful consideration given to the various methodologies adopted for the purpose of this study, though not ideal, was built around the current health and social context prevailing in SA. This in itself posed its own set of unique challenges. To overcome these, often adaptive strategies were warranted in order to retain the methodology and provide high-quality scientific evidence to potentially inform policies which in turn can be translated into practical operational programmes adopted by the health care sector. The challenges we encountered were multi-facetted reflecting the countries social, political and economic climate and inequalities. As a consequence of this, the main limiting step to the ideal methodological design was the fragmented health care infrastructure with its poor service delivery, a consequence of its unique history. Further issues outside the scope of this thesis pertain to the dynamic movement of the population group and inadequate funding for research. Ideally a prospective longitudinal design would have surpassed the limitations of our once-off cross-sectional analysis of women exposed to HFDP in the post-partum period.

i) South African healthcare system

In order to comprehend the extent of these problems it is important to understand the structure of the SA health system. Many of the problems within the SA health care system can be traced back to the Apartheid period (1948-1993) in which access to care for the majority of the population was mostly inequitable, mostly centralised and for the most part highly fragmented (322). The prevailing social inequality of apartheid was replicated in the health of the nation. Following the advent of democracy in 1994, the SA government put in place measures to strengthen the public health system performance and improve service delivery. In spite of this and significant annual expenditure, challenges still abound, and the public health system shortcomings continue to endanger the health and lives of South Africans. The disparities in the health system as a whole is reflected in the unjust expenditure of GDP with 4.1% of GDP spent on 84% of the population who are dependent on the public sector and 4.4% of GDP on 16% of the population who have insurance and utilise the private sector (323).

Given the quadruple burden of disease that we face as a country, including HIV and TB, maternal and child health issues, NCD as well as trauma and violence (324), interventions to address these are key given that much of the disease burden is preventable. Likewise, the dire consequences for maternal and neonatal health can be avoided with well-established maternal

health services. The vast area of Soweto, home to 2.3 million people is supported by the third largest hospital in the world, CHBAH, in which the aggravation of the broad social, economic and political changes in the SA are reflected here. A well-established maternity service with an average of 60, 000 maternity cases treated per year (205) is available to women with high-risk pregnancies, including diabetes in pregnancy for which a dedicated service was implemented in 1984. This specialised clinic is quintessence of how the integration of services to include a multidisciplinary team has improved the short-term outcomes for these women and their offspring alike. It was here that the seed for this research was planted and our quest to further improve the long-term outcomes of these women was initiated. In SA, the situation is much the same as in most other LMIC in which the need to strengthen health care infrastructure by addressing some of the key obstacles including staff shortages, cash-flow problems and overcoming fragmentation of services remains key .

ii) Obstacles encountered during the study

During the planning stages and conduction of the project various obstacles became evident.

a) Population dynamics and migration

Our participants were recruited from an area where basic infrastructure together with access to water and sanitation is often lacking, education and employment are a privilege and the migration of people in and out of the area is high (325). Tracing our participants highlighted these migration dynamics within the area, which is a product of various socio-economic factors, the scope of which is beyond this thesis.

b) Poor hospital record keeping

A major theme which was highlighted was the poorly kept paper-based records at the hospital. Of the women found to have HFDP for the period identified, we had difficulty retrieving file records for which contact details were often scant. We were only able to trace 25% of the participants from these records and site visits. The patient filing system at the hospital is organised and stored according to the medical divisions consulted and hence there is no single file where all patient records are kept. This fragmented approach makes accessing a patients historical medical history challenging. Many of these issues could be mitigated by introducing an electronic database which makes for easier recording of patient data and accessibility to patient information, which additionally benefits research (326). Implementation of such a

system will, however, require technical infrastructure, skilled personnel, funding, ethical consideration, organisation and sustainability (327). There are long-term benefits to establishing an electronic medical record system in low-resource settings. The implementation of such a system has proven to be successful and effective in a resource-poor antenatal clinic in Kenya(340). A study performed in Malawi demonstrated that from a financial perspective, the benefits of an electronic medical system outweigh the costs (328).

c) Underestimation of HFDP burden

Given the most recent prevalence of GDM for the Soweto region of 9%, based on a study which adopted universal screening (59) we appreciate that the number of patients seen at our specialist clinic is well below what would be expected. The reason here is threefold. One, the adoption of a risk-factor based screening approach for HFDP at a primary care level as opposed to the internationally recommended universal screening would certainly underestimate and miss cases, since numerous studies (75,329,330) have shown the poor predictive value of risk-factor based screening with a local study (58) showing that 10.6% of cases would be missed. A gross estimation, based on the population statistics (331), for the total number of women expected to be harbouring this condition in the Soweto region during their reproductive years, would be 36,000 women . Currently the clinic sees on average 100 women with HFDP yearly, clearly a gross underrepresentation. This has dire consequences for their pregnancies, their offspring and future generations to come. The adoption of universal screening in a resource strapped country though costly, will certainly be cost-effective in the long-term in mitigating future disease. Studies from HMIC have demonstrated the cost-effectiveness of universal screening (332,333), though no studies have been performed in LMIC to date. A further reason for the misrepresentation is the inefficient primary health care facilities which are fraught with difficulties, from accessibility to lack of continuity of care, to understaffing etc. This is generally the first point of contact for pregnant women and where screening should occur. Often lack of awareness, by both health care professionals and the women alike, to inadequate staffing and lack of resources are some of the reasons this is the case, despite the fact that it is nationally prescribed in policies and guidelines (60). Our primary health care system is designed such that selective based vertical care for diseases often funded by foreign aid is provided as opposed to comprehensive care that includes health promotion and disease prevention (334). The third reason is lack of awareness both at a health care provider level and in the population at large. Primary prevention at each level is key and necessary if we want to decrease the burden of HFDP and its short and long-term consequences. Identifying these at-

risk women prenatally and addressing major modifiable risk factors prior to conception is a major step in decreasing the growing burden of HFDP.

d) Availability of dedicated health care services

The availability of maternal services dedicated to the care of HFDP within the tertiary sector remains scarce in SA and is restricted to the three major tertiary hospitals in the country located in Gauteng and the Western Cape, home to a mere 10 million of the 55 million population. These centres have afforded excellent care and management over the years which has become evident in the improved outcomes for both mother and child (120,121). The need to expand the availability of these specialised and dedicated antenatal care services for women found to have HFDP is clearly imperative. Given that 66.8% of SA live in urban areas and a further 33% of SA live within rural SA(199), this demonstrates that the majority of these high-risk women with the disease do not have access to health care.

e) Limited post-partum screening and follow-up

Postpartum care for HFDP women appears to be poorly structured and misaligned with existing policy with follow-up often lacking and if available poorly attended (335) This is concerning given the findings of this study together with the fact that ongoing screening following delivery is key to preventing the development of these NCD. The HIV epidemic has brought about the development of medical platforms that could be expanded for use in treating NCDs. Integrating health care to address these multiple morbidities seems logical given the additional metabolic disease profile which has arisen from the addition of ART. Measures include counselling regarding lifestyle modifications, screening for dysglycaemia/T2DM, HTN, dyslipidaemia, obesity and initiating therapeutic interventions where necessary. Health and patient-related barriers have been identified as major obstacles within our setting resulting in poor compliance of follow-up. Among others, financial constraints, time constraints and misalignment of neonatal and maternal follow-up at primary care level, lack of education and awareness surrounding the importance of follow-up were reported(336).

With this background, it is clear that with our current health care system and prevailing economic and social climate, a well-constructed prospective longitudinal study starting during the pre-conception years following women throughout pregnancy and the years following the pregnancy would be challenging to say the least, though not impossible with the correct support and funding. The dual benefit of strengthening health care systems is that we are able to

comprehensively improve service delivery and improve platforms to conduct better research which ultimately can inform policy change.

6.4 Theoretical relevance

This section reflects upon the conceptual framework presented at the beginning of this study (Figure 6.2). The framework was designed based on our understanding that maternal health factors coexist alongside various environmental and societal factors. The complex interplay of these factors, which are present along a historical timeline, starting in the pre-pregnancy period and extending well into the post-partum period play a pivotal role in mediating chronic diseases in particular T2DM. On this premise, the major theme of this thesis to establish the role of HFDP exposure on the progression to T2DM was studied whilst acknowledging the background interplay of various factors, which is best studied in a longitudinal fashion. Data exploring modifiable risk factors in the progression from HFDP to T2DM is limited, especially among developing and LMIC. Therefore, based on the relevant literature mostly from HMIC, our overall research objective, key findings and emerging themes of this thesis, we confirmed that although HFDP exposure played a significant role in the progression to T2DM, other maternal factors present along a peripartum timeline was involved in moderating this outcome including a family history of T2DM and the presence of visceral fat. HIV was not found to play a role when incorporating all relevant factors, though this may be as a result of the small numbers who had the disease. In applying this framework, we see that our results contribute to confirming the continued use of the conceptual framework as a guide for exploring the determinants of NCD in this vulnerable group of women.

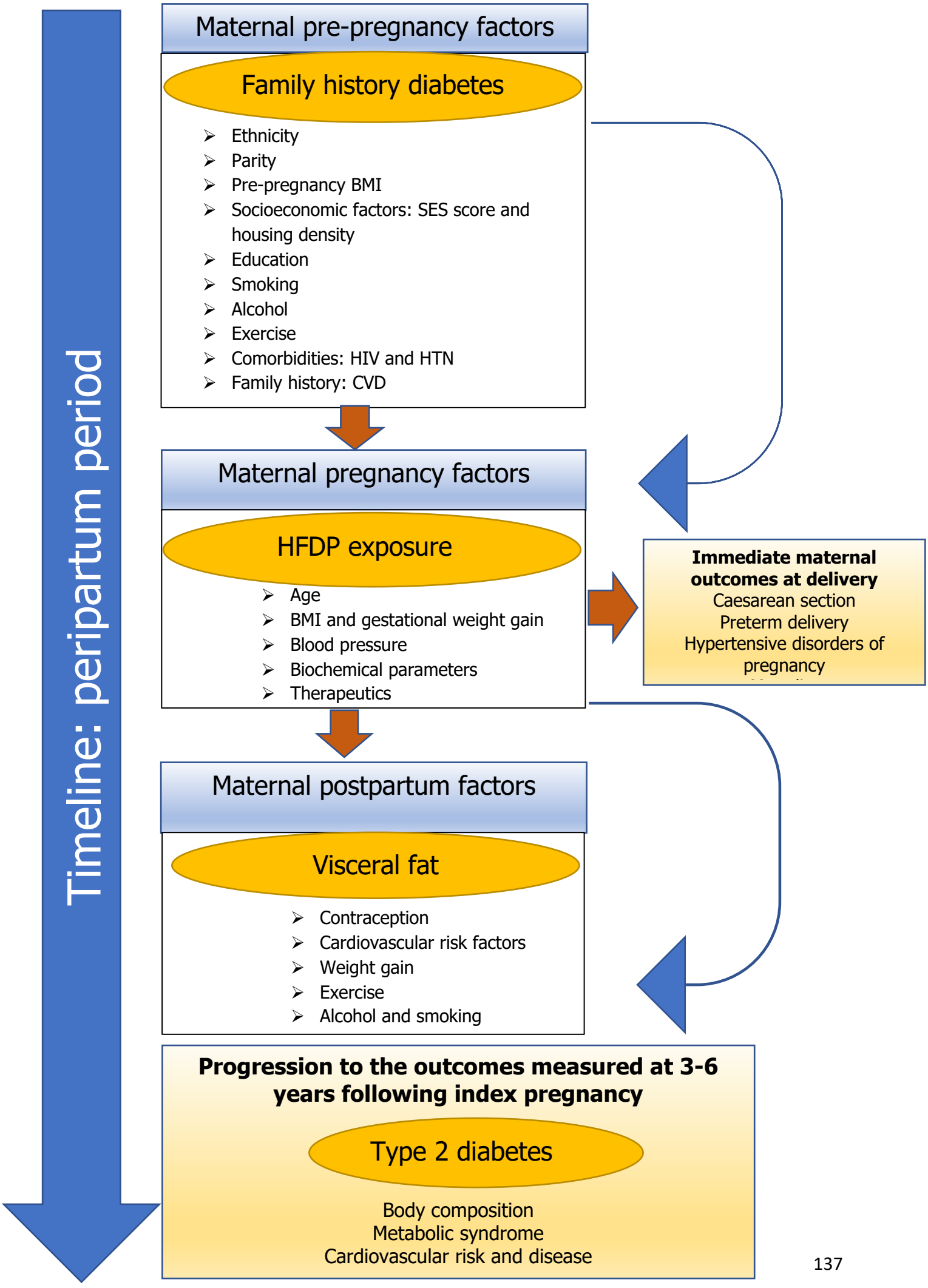


Figure 6. 2 Conceptual framework revisited

6.5 Implications

This thesis has highlighted some key factors which should be incorporated into health policies and systems in order to strengthen maternal health care and facilitate interventions following HFDP in order to curb the burden of NCD which are often overlooked.

- Acknowledging the important contribution of local research to both policy design and development, which in turn speaks to clinical practice through healthcare stakeholders. In other words, developing African specific policies and protocols tailored to an African-centric climate. Currently our guidelines are based on international evidence with a great need for local research to inform policy.
- Despite the adequate antenatal care which is in line with international norms, albeit at a few sites, a notable gap in preconception and postpartum care was evident through this research with a pressing need to address the fragmentation and disorganisation of health care services.
- A further problem in the field of HFDP is the lack of international consensus regarding terminology, best screening approach, diagnostic testing, intra-partum management and post-partum surveillance which has been to the detriment of clinicians and patients alike resulting in lack of uniformity within practice. These factors taken together augment the burden of this condition as well as its future consequences, both of which are underestimated and contribute significantly to unnecessary economic health costs and increased pressure on an already ailing system, most of which can be augmented and overcome by strengthening existing health care frameworks and policies to address these shortfalls.
- The introduction of a standardised life-course approach instead of a feto-centric approach, starting in the pre-conception period through to late post-partum period would aid in attenuating most of the short and long-term adverse outcomes by actively screening and intervening when necessary.
- To achieve this goal, measures can be put in place to expand the existing platforms for HIV, implement integrated primary health care services for mother and child and standardising postnatal care.
- Actionable recommendations arising from research like ours is key to delivering a successful, feasible and sustainable service to this vulnerable group of women.

- More research is warranted in order to obtain a more comprehensive and accurate picture of the epidemiology and consequences of HFDP in SA. This will require a substantial investment in a purposeful network of surveillance sites collecting standardized data across all parts of the country.
- At a community level, patient related barriers including lack of awareness, difficulty accessing health care facilities and economic constraints hamper the successful follow-up of these women beyond the pregnancy, all of which need to be addressed.
- In order to achieve this, systems should be in place to improve awareness, enhance education, create easier accessibility to integrated primary health care services in conjunction with the provision of social and economic support to encourage contact with the health services starting as early as pre-conception and extending well into their post-partum years.
- Post-partum interventions such as screening for chronic diseases and interventions to prevent these, such as, simple lifestyle measures including sustained exercise and dietary advice, encouraging breastfeeding and contraceptive counselling amongst others, remains important in alleviating the burden of NCD.
- In order to achieve this, strengthening existing primary health care structures together with equipping and training community health care workers to provide a holistic service with improved communications and referrals with clinicians at different levels of care.

6.6 Strengths and limitations

This section aims to address the major strengths and limitations of this thesis as a whole. Whilst individual strengths and limitations have been discussed in detail in each paper, additional ones will be highlighted here.

The major strength of this thesis lies in its novelty, it is the first study in Africa which incorporated a "control" group of women (non-exposed to HFDP) and compared them with a group of women who had been exposed to HFDP in order to examine the cardiometabolic outcomes on a background of high HIV burden among a predominantly black urban cohort. Additionally, SA's experience with the usage of OHA during pregnancy has been appreciated since the early 80's. Our study contributes to the existing body of knowledge worldwide in terms of the maternal safety and efficacy of these agents in the immediate term following delivery. The more comprehensive cardiovascular risk estimation incorporating a validated risk score together with cIMT measurement is unique in our setting and draws attention to the

cardiovascular vulnerability of these women soon after delivery with the need for urgent targeted screening and interventions. Additional blood specimens have been appropriately stored for future analysis of some of the novel cardiac biomarkers which will significantly contribute to the paucity of information that exists worldwide surrounding their usefulness in cardiovascular risk assessment and the identification of pre-clinical disease.

One significant limitation was the study design, which though prospective with a case-control design, was mostly a cross-sectional analysis following a retrospective evaluation of patient data during pregnancy. Ideally this study deserved a well-constructed longitudinal design with standardised data collection points starting as early as the pre-conception period and extending well into the post-partum period. Our chosen design was mostly determined by our resource limited setting and time constraints. This meant that missing data was a given and that initial measurements were not carried out within a research setting lacking standardised procedures and hence prone to systematic errors. Important pre-pregnancy and pregnancy variables not captured included amongst others BMI, cardiovascular risk assessment and post-partum OGTT results. This introduced difficulties when evaluating progression to the measured outcomes. A further limitation arose from a unique aspect of our study, the background epidemic of HIV, which in our cohort was a small sample size, and this hampered multivariate analysis in determining the role this factor had on the outcomes. Lastly cardiovascular risk may have been better assessed if we were able to incorporate the measurement of validated cardiovascular biomarkers and have evaluated these women at a longer time interval since their index pregnancy in order to appreciate the significant role of HFDP on this outcome.

6.7 Future studies

This study has highlighted several research areas for subsequent studies to focus on which will augment and compliment the work that has been done in SA thus far. Among others, future work should include expanding research on the prevalence of this condition, better defining and understanding maternal risk factors for HFDP to ensure optimal screening, consolidation of diagnostic tests and diagnostic thresholds utilised across the country, understanding the poor rate of follow-up in the post-partum period together with augmenting the limited research performed in exploring the short- and long-term post-partum maternal consequences.
Going forward, together with the emerging literature from abroad, we need to evaluate the following more specifically in our setting:

6.7.1. Universally screening for HFDP

Research in which universal screening for HFDP is performed as opposed to the current risk-factor based screening approach, will best establish the burden of this condition, particularly among our different ethnic groups. This will allow for better appreciation of the need to grow the health services for women with HFDP which currently is insufficient. The accuracy of risk-factor screening can also be established on this backdrop.

A project of this nature will entail nationwide participation at all levels of care which requires significant collaboration and engagement between various stakeholders. Comprehensive analysis of the existing policies and institutional mapping to project design and implementation through to incorporation of study findings back into policy and programmatic frameworks are necessary steps to be undertaken in order for a study of this magnitude to be successful. Cost constraints remain a major limitation to a project of this nature, however the morbidity burden and promotion of the intergenerational cycle of NCD far outweighs this.

6.7.2. Evaluating various lifestyle and or therapeutic interventions during the post-partum period following HFDP in slowing and or halting the progression to NCD's.

The emergence of a substantial body of literature regarding the post-partum cardiometabolic consequences following HFDP suggests that more work is needed regarding better ways of detecting and preventing the chronic disease burden in these young women. Additionally, the intergenerational effect ,which includes the perpetuation of chronic diseases in the offspring, creates a vicious cycle of disease burden being passed on from one generation to the next.

This being said, interrupting this cycle requires prevention of HFDP altogether for which the pre-conception period is ideal, but this requires a better understanding of the maternal risk factors associated with HFDP together with better access to health care, education and social upliftment. Current health systems are already in place to follow up these women and their children in the post-partum period. The post-partum period offers a window of opportunity to educate, screen and detect disease. In light of this, a longitudinal study recruiting women with HFDP from the gestational endocrine clinic at the CHBAH during their pregnancies and following these women up in the post-partum period is warranted. At their 6-week post-partum follow up visit, women will be screened for diabetes and other chronic diseases. Women who do not meet the criteria for diabetes will be assigned to one of three arms, control(no interventions), intensive lifestyle intervention, which would include a dietary programme together with an exercise regime and lastly various therapeutic interventions. At set follow up

intervals various anthropometric and biochemical parameters will be measured to ascertain disease burden among the three groups.

7.7.3. Larger study evaluating the role HIV and ART have as mediators on the outcomes given the well-versed metabolic complications associated with illness itself and its treatment.

In order to better delineate the role of HIV on the cardiometabolic outcomes following HFDP, a larger sample size needs to be recruited and hence a multisite study is necessary, engaging the major tertiary centres across the country. A longitudinal case control design could be employed in which women identified with HFDP during their pregnancies will be assessed for their HIV status together with important parameters surrounding this at baseline, like type of ART, viral load etc. Longitudinal follow-up of these women with ongoing assessments of both HIV-related parameters together with development of various NCD will be measured. This will allow for better evaluation and analysis of the role HIV has on these outcomes. This is of relevance in a country with the highest burden of HIV worldwide, more so among women of reproductive age. This has implications for policy development and protocols surrounding HFDP and may inform the type of drugs to be initiated in these women, i.e., cardio-metabolically neutral agents as well as the incorporation of regular screening for NCD's amongst these women.

6.8 Conclusion

HFDP represents a maternal phenotype at increased risk of developing T2DM, MetS and with time, overt CVD. The peri-partum period offers a window of opportunity to implement targeted screening, advice for lifestyle modification and potentially therapeutic interventions to preserve future maternal health and interrupt the intergenerational cycle of chronic diseases. This study has identified a vulnerable group of women, during their reproductive years, who not only are at increased risk of short-term consequences following a diabetic pregnancy, but additional long-term consequences which increase the morbidity burden on the country and perpetuate the intergenerational cycle of disease.

7.0 References

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“Scientists have become the bearers of the torch of discovery in our quest for knowledge”

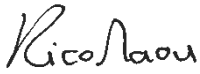
Stephen Hawking

8.0 Appendices

APPENDIX A: AUTHORS/CO-AUTHORS DECLARATION

Declaration: Students contribution to articles and agreement of co-authors

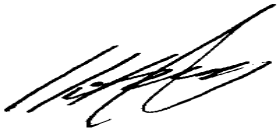
I, Veronique Nicolaou, student number 9702987E declare that this Thesis is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis. Signature of Student: Date: 01/12/2020
Name of Primary Supervisor: Professor Shane Norris
Signature of Primary Supervisor: Date: 01/12/2020

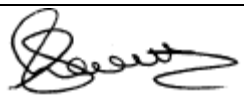
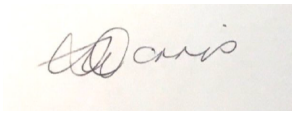


Signature of student **Date:** 11th January 2021

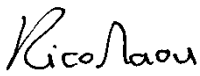
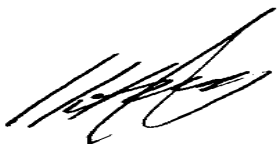
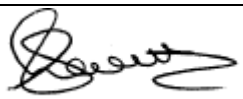
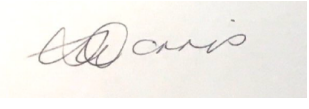
Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of his Thesis. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled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

Article 1: Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV. *International Journal of Gynaecology and Obstetrics* (*Published 3rd September 2019*). DOI: https://doi.org/10.1002/ijgo.12956
(Appendix H)

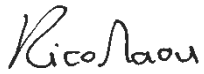
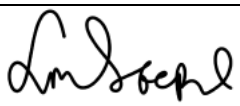
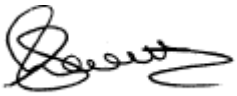
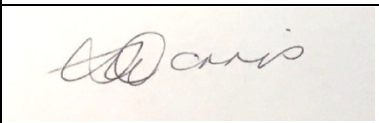
Author	Name	Signature	Date
1st Author	V. Nicolaou		11/01/2021
1st Author	L. Soepnel		11/01/2021
2nd Author	K.R. Huddle		11/01/2021
3rd Author	K. Klipstein-Grobusch		11/01/2021

4th Author	N.S. Levitt		11/01/2021
Last Author	S. Norris		11/01/2021

Article 2: Maternal and neonatal outcomes following the introduction of oral hypoglycaemic agents for gestational diabetes mellitus were comparable to insulin monotherapy in two historical cohorts. *South African Medical Journal* (*Published 29 January 2020*). DOI: http://dx.doi.org/10.7196/samj.2020.v110i2.14024 (Appendix I)

Author	Name	Signature	Date
1st Author	V. Nicolaou		11/01/2021
2nd Author	L.Soepnel		11/01/2021
3rd Author	K.R. Huddle		11/01/2021
4th Author	N.S. Levitt		11/01/2912
5th Author	K. Klipstein-Grobusch		11/01/2021
Last Author	S. Norris		11/01/2021

Article 3: Cardiometabolic outcomes of women exposed to hyperglycaemia first detected in pregnancy versus women not exposed at 3-6 years post-partum, in an urban South African setting. Submitted to *PLOS ONE Journal* on 31st July 2021 and has been provisionally accepted subject to minor revisions (Appendix J)

Author	Name	Signature	Date
1st Author	V. Nicolaou		11/01/2021
1st Author	L. Soepnel		11/01/2021
2nd Author	K.R. Huddle		11/01/2021
3rd Author	N.S. Levitt		11/01/2021
4th Author	K. Klipstein-Grobusch		11/01/2021
Last Author	S. Norris		11/01/2021

APPENDIX B: MATERNAL INFORMATION AND CONSENT SHEETS

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

Department of Paediatrics, School of Clinical Medicine
University of the Witwatersrand
Johannesburg

Information Sheet

THE IMPACT OF DIABETES MELLITUS IN PREGNANCY ON MATERNAL HEALTH OUTCOMES

My name is Dr Veronique Nicolaou, and I am a researcher at the Developmental Pathways for Health Research Unit (DPHRU). We want to gain better understand of whether a mother's diabetes during pregnancy has an impact on her long-term health. For this reason, my colleagues and I are inviting women who attended the Gestational Endocrine Clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) during pregnancy to an appointment at our research unit in the hospital. There, we will use a variety of measurements and activities to collect information about yourself, including various measurements and testing to establish the potential role diabetes in pregnancy has on your future risk of diabetes and cardiovascular disease.

Before you decide whether you will participate, we would like to tell you about the procedures involved.

Study procedures

If you agree to take part in this study, we will ask you to attend a single data collection appointment at DPHRU. The visit will take approximately 1.5-2 hours.

During this appointment, we will ask you to fill in a questionnaire with questions concerning your demographics, past illnesses, medications and your usual and past lifestyle habits etc.

We will measure your weight using scales, your height using a height board, your waist circumference using a tape measure, and we will also estimate how much of your body is made up of fat, as opposed to bone or lean tissue. We do this using a machine called DEXA, which uses x-rays to determine body composition.

Furthermore, we will measure your heart rate and blood pressure. This will be done by placing a cuff around your arm attached to a machine, which then automatically takes the measurements, three times. An ultrasound machine will be used to determine your carotid artery blood flow and noting whether any blood vessel disease is present. This is not invasive or painful.

A few bloods will need to be taken from a vein in your arm, (3-4 tubes of blood), which amounts to 4-5 teaspoons (20ml). We will process this to collect data regarding your health. Some of the blood collected will be stored for the possibility of future studies. One of the blood tests include doing an HIV test, only once we obtained consent from you. If you are known with HIV, we will not need to do this.
Then, using a small needle we will collect a single drop of blood from your finger, where we will test for the amount of sugar in your blood. If this result is normal, we will follow this with a sugar test, whereby you drink a sugary drink after fasting overnight for 8 hours (no water or food during this time). We will then draw blood from a vein on your arm at various intervals. If you fingerpick result is abnormal, we will not need to perform the sugar test and refer you to a diabetic centre at CHBAH for further management.

You will be asked to give us a small amount of your urine. Firstly, we will perform a pregnancy test and if you are found to be pregnant, you will not be allowed to continue with the study. The rest of the urine will be analysed for any kidney problems and some of the urine may be stored for potential future studies.

Participation in the study will not affect any routine care you have at your local clinic or at CHBAH.

Risks and benefits

There are no significant medical risks for you associated with participation in this study. Certain measurements taken, including the finger stick for blood sugar, blood drawing as well as lengthier measurements such as DEXA and the ultrasound for carotid Doppler may cause slight discomfort or restlessness for you. The use of x-rays in DEXA exposes you to a very small dose of radiation. If you are feeling unwell or unable to continue at any point, the data collection will be stopped or resume another time.
There are no direct benefits to participating in this study. Indirect benefits of participation include contributing to important research and access to basic development and health assessments for you during data collection.

Reimbursement

To compensate for any transport or incidental costs, you will be reimbursed with 150 rand at attendance of the data collection visit.

Referral

If any abnormal outcomes or factors come to light during the data collection appointment that might require medical attention or guidance, you will be referred to either your local clinic or the adult outpatient clinic at CHBAH for further assessment, diagnosis, or management.

Voluntary participation in research

You have the right to agree or refuse to participate in this research. If you decide to participate and later change your mind, you are free to stop at any time. Your refusal to participate will not result in any penalty or loss of benefits to which you are otherwise entitled.

Confidentiality

To ensure your privacy, the investigator will keep all of the personal information collected in locked documents or files. Any data collected will be coded with a participant number to ensure your privacy. If the results of this research appear in scientific publications, you will not be identified in any way.

Ethical approval

This study will be approved by the University of Witwatersrand's Human Research Ethics Committee (HREC).

Do you have any questions?

This study has been approved by the Human Research Ethics Committee (Medical) of the University of Witwatersrand, Johannesburg. A principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.

The investigator listed on the first page of this form is available to answer any of your questions about this research study. You may contact the investigator at any time on the following number: (011) 933-1122. You can also contact Professor Shane Norris, the Director of DPHRU, by e-mail at Shane.Norris@wits.ac.za.

If you require any further information or have any questions, comments, or complaints about the study, please contact the Human Research Ethics Committee of the University of the Witwatersrand: Chairperson Dr Clement Penny, who may be contacted on telephone number (011) 7172301 or by

email Clement.Penny@wits.ac.za. The telephone numbers for the committee secretariat are (011) 7171252/1234 or through e-mail at zanele.ndlovu@wits.ac.za and Rhulani.Mukansi@wits.ac.za

YOU WILL HAVE A COPY OF THIS INFORMATION SHEET TO KEEP

If you are willing to participate in this study, please read and sign the attached consent form.

Your signature on the consent form certifies that:

1. You have read and understood the information provided in this consent form
2. You have received answer to your questions about this research
3. You have freely decided to participate in this research
4. You understand that you are not giving up any of your legal rights.

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

Department of Paediatrics, School of Clinical Medicine
University of the Witwatersrand
Johannesburg

Maternal Consent Form

THE IMPACT OF DIABETES MELLITUS IN PREGNANCY ON MATERNAL HEALTH OUTCOMES

I understand that the study will involve a data collection appointment where my current health status, in terms of diabetes status and cardiovascular health will be determined. All the details and purposes of the study have been explained to me. I understand that I have the right to refuse participation in this study, and that I will not benefit directly from research done with information collected at the appointment.

I agree to be a participant in this study, on the condition that:

1. The procedures have been approved by the Human Research Ethics Committee of the University of Witwatersrand.

2. I have the right to refrain from answering any or all questions posed in the questionnaire and to not participate in any or all procedures of the study.

3. I may withdraw from the study at any time, and this will not result in any penalty or loss of benefits to which I would have otherwise been entitled.

4. All results will be treated with confidentiality to protect my privacy Only group results will be made public in scientific journals or the media.

5. The research team is committed to treating participants with respect and privacy throughout the procedures described above.

PARTICIPANT

Printed Name	**Signature / Mark or Thumbprint**	**Date and Time**

RESEARCH ASSISTANT

Printed Name	Signature	Date and Time

Maternal Consent Form- HIV testing

THE IMPACT OF DIABETES MELLITUS IN PREGNANCY ON MATERNAL HEALTH OUTCOMES

I understand that the study will involve a data collection appointment whereby I will be counselled and ask if I consent to testing my HIV status, only in the event my status is unknown. The test result will be disclosed in a confidential manner by a trained nurse and where necessary appropriate referral for further management will take place. All the details of the test have been explained to me and I understand that I have the right to refuse testing.

I agree to testing my HIV status in this study, on the condition that:

1. The procedures have been approved by the Human Research Ethics Committee of the University of Witwatersrand.

2. I may withdraw from the study at any time, and this will not result in any penalty or loss of benefits to which I would have otherwise been entitled.

3. All results will be treated with confidentiality to protect my privacy Only group results will be made public in scientific journals or the media.

4. The research team is committed to treating participants with respect and privacy throughout the procedure described above.

PARTICIPANT

Printed Name	**Signature / Mark or Thumbprint**	**Date and Time**

RESEARCH ASSISTANT

Printed Name	Signature	Date and Time

APPENDIX C: QUESTIONNAIRE

Maternal Questionnaire

Study Number	_____

Maternal Questionnaire

Date of Visit	_____

Maternal Questionnaire

Data Collector	_____

Maternal demographic information

Age (Years)	_____
What is your marital status?	○ Single ○ Married ○ Divorced ○ Other
Please describe	_____
Ethnic group	○ Black ○ Colored ○ Indian ○ White ○ Other
Please describe	_____
How many times have you been pregnant, regardless of the outcome?	_____ (Gravidity)
How many of your pregnancies have successfully gone beyond 20 weeks?	_____ (Parity)
How many pregnancies have you had following the index pregnancy?	_____
Pregnancy losses?	○ Yes ○ No

Type of loss	☐ miscarriage ☐ stillbirth ☐ ectopic ☐ termination ☐ neonatal death ☐ infant/childhood death
Any twin/multiple pregnancies?	◯ Yes ◯ No
Any pre-term deliveries? (< 37 completed weeks)	◯ Yes ◯ No
If preterm delivery, how many?	_____
Were you told you had high blood pressure during your pregnancy?	◯ Yes ◯ No (Not known with chronic hypertension)
If yes to above, how many times?	_____
Are you the primary caregiver for your child?	◯ Yes ◯ No (Concerning the child you brought to the data collection appointment today.)
Are you the biological mother of this child?	◯ Yes ◯ No
What number child is the child you brought in today?	_____ (eg., if they are the oldest child, this would be 1; if born 3rd this would be 3; regardless of any child losses.)

Maternal Education	
Have you had an education?	◯ No ◯ Yes ◯ Don't know
What is your highest level of education attained?	◯ Pre-school ◯ Primary school ◯ Secondary school ◯ Professional or technical training ◯ University ◯ Don't know

Socioeconomic Questionnaire Maternal

Study Number	_____

Socioeconomic Status

Household composition

Total number of people living in your home	_____
Number of children less than 5 years old living in your home	_____
Total number of sleeping rooms in your home	_____
How would you describe the home you are living in?	◯ House of brick/ concrete block structure on a separate stand or yard or on a farm ◯ Traditional dwelling / hut / structure made of traditional materials ◯ Flat or apartment in a block of flats ◯ Cluster house in complex ◯ Townhouse (semi-detached house in complex) ◯ Semi-detached house ◯ House/flat / room on your homestead ◯ Informal dwelling / shack in back yard ◯ Informal dwelling / shack not in back yard ◯ Caravan / tent ◯ Other
Specify	_____
What is the main source of drinking water for members of your household? (Please tick the box that best applies)	◯ Piped water (tap) in dwelling ◯ Piped water (tap) in site / yard ◯ Bottled water ◯ Water carrier/ tanker ◯ Rain water tank ◯ Borehole / well / spring ◯ Dam / river / stream ◯ Public / communal tap ◯ Other
Specify	_____

What is this household's main source of water for household use (other than for drinking)?	○ Regional / local water scheme (operated by municipality or other services provider) ○ Borehole ○ Spring ○ Rain-water tank ○ Dam / pool/ stagnant water ○ River / stream ○ Water vendor ○ Water tanker ○ Other
Specify	_____
What kind of toilet facilities does your household have?	○ Flush toilet (connected to sewerage system) ○ Flush toilet (with septic tank) ○ Chemical toilet ○ Pit toilet with ventilation (VIP) ○ Pit toilet without ventilation ○ Bucket toilet ○ Other ○ None
Specify	_____
Do you share this toilet with other households?	○ Yes ○ No

Which of the following do you have in your current home (place where you spent most nights in the past three months)? It does not matter who owns/pays for these things. (Answer all questions)

	Yes	No
Electricity	○	○
Fridge	○	○
Stove	○	○
Vacuum cleaner	○	○
Washing machine	○	○
MNet/DSTV/Satellite	○	○
DVD Player	○	○
Motorcar	○	○
Television	○	○
Telephone (landline)	○	○
Cell phone	○	○
Computer/Laptop/ Tablet	○	○
Internet access	○	○

Main fuel for cooking in house	○ Gas ○ Oil ○ Kerosene ○ Electricity ○ Charcoal ○ Wood ○ Straw or grass ○ Animal dung ○ None

Maternal And Child Medical Questionnaire

Study Number	_____

Current maternal morbidities	
Have you been diagnosed with HIV?	◯ Yes ◯ No
Year of diagnosis	_____ (Year)
Time of diagnosis in relation to index pregnancy	◯ Before index pregnancy ◯ During index pregnancy ◯ After index pregnancy (index pregnancy refers to the time you were pregnant with the child you brought in today.)
Which ARV was used during pregnancy?	_____
If HIV Positive, do you know your CD4?	◯ Yes ◯ No
CD4 value	_____
Do you know your viral load?	◯ Yes ◯ No
Viral load value	_____

Diabetes status	
Have you been diagnosed with diabetes mellitus following the index pregnancy?	◯ No ◯ Yes ◯ Don't know
Year of diagnosis	_____ (year)
Which subtype?	◯ Type 2 ◯ Type 1
What treatment are you currently on?	◯ Oral: Metformin or Glibenclamide or both ◯ Insulin ◯ Both orals and insulin ◯ Other ◯ Unknown

Which type of oral agent are you using?	_____
Please describe	_____
Did you remain on treatment for diabetes after delivery for the index pregnancy?	○ No ○ Yes ○ Don't know ( )
If yes to above, what treatment were you on?	○ Oral: Metformin or Glibenclamide or both ○ Insulin ○ Both orals and insulin ○ Unknown
Did you attend the postpartum visit following your index pregnancy for an oral glucose tolerance test (OGTT)?	○ No ○ Yes ○ Don't know
What was the result?	○ Diabetes ○ No diabetes ○ Unknown
Please describe	_____
What was your main reason for not attending?	☐ Not told or aware of appointment ☐ Too busy or no time to attend ☐ Did not know I had to come if I had no symptoms ☐ I was not told importance of the visit ☐ I had no transportation to the clinic ☐ Other
Overall, how many GDM pregnancies have you had (including the index pregnancy)?	_____

Have you been diagnosed with any of these conditions?

	No	Yes	Don't know
Have you been diagnosed with hypertension?	○	○	○
Have you been diagnosed with cholestrol problems?	○	○	○
Any other chronic medical illnesses?	○	○	○

Are you taking lipid lowering agents?	○ Yes ○ No
If yes, please specify	_____

Mother -CVD event			
	No	Yes	Don't know
Heart attack	○	○	○
Stroke	○	○	○
Peripheral vascular disease-gangrene/amputation of limb	○	○	○

Was it before or after index pregnancy?
- ○ before index pregnancy
- ○ after index pregnancy

Maternal and family lifestyle factors

Did you partake in any recreational (not work related) exercise following the index pregnancy?
- ○ Yes
- ○ No

If yes to above, how many times a week?
- ○ 1 (once)
- ○ 2 (twice)
- ○ 3 (thrice)
- ○ 4 (4 times)
- ○ 5 (5 times)
- ○ more than 5 times
- ○ Don't know

How many years?
- ○ < 1 year
- ○ 1-2 years
- ○ 3-5 years
- ○ >5 years

Do you consume any alcohol?
- ○ Yes
- ○ No

If yes to previous, how many times in a week?

_____

If yes, how many units at a time?

_____

Did you drink alcohol during your pregnancy with this child?
- ○ No
- ○ Yes
- ○ Don't know

How many times in a week?

_____

How many glasses at a time?

_____

Do you smoke cigarettes
- ○ Yes
- ○ No

If yes to previous, how many cigarettes per day?

_____

How many years?	_____
Did you smoke cigarettes during your pregnancy with this child?	◯ No ◯ Yes ◯ Don't know
How many cigarettes per day?	_____
Any family history (1st/2nd degree relatives) with a cardiovascular event/s? (heart attack or stroke)	◯ No ◯ Yes ◯ unknown
Do you have any family history of diabetes?	◯ No ◯ Yes ◯ Don't know
Do your mother, father, or any siblings have diabetes?	◯ 1) Yes ◯ 2) No ◯ 3) Don't know (2nd degree relative)
Do your grandparents, aunts, uncles, nephews or nieces, have diabetes?	◯ 1) Yes ◯ 2) No ◯ 3) Don't know (2nd degree relative)
Does your child's biological father have diabetes?	◯ 1) Yes ◯ 2) No ◯ 3) Don't know
Early nutrition	
Did you breastfeed following this pregnancy?	◯ No ◯ Yes ◯ Don't know
How long did you breastfeed for?	_____ (months)
Did you breastfeed exclusively following this pregnancy?	◯ No ◯ Yes ◯ Don't know
How long did you breastfeed exclusively for?	_____ (months)
Was you child hospitalized in the first two years of life?	◯ No ◯ Yes ◯ Don't know
How often was the child hospitalised in the first two years of life?	_____

What was the reason for hospitalization?	_____

Attendance of pre-primary

Does your child attend pre-school, pre-primary, creche, or a similar facility?	○ No ○ Yes ○ Don't know
How many hours per week does your child spend there?	_____ (Hours)
At what age did your child first attend the facility?	_____ (Months)

Contraception

Have you been on any pregnancy prevention (contraception) since the index pregnancy?	○ No ○ Yes ○ Don't know
Which form of contraception?	☐ Oral (pill) ☐ Injectable ☐ Other
If other, which?	_____
If oral, progesterone only oral contraception?	○ Yes ○ No ○ Unknown
Questionnaire completed?	○ Yes ○ No
If no to previous, please provide explanation for incompleteness.	_____

APPENDIX D: PROTOCOLS FOR BIOSPECIMEN COLLECTION



BIOSPECIMEN COLLECTION
STANDARD OPERATING PROCEDURES

SITE	DEVELOPMENT PATHWAYS HEALTH RESEARCH UNIT (DPHRU)		
SOP#	052		
TITLE	GDM BIOSPECIMEN COLLECTION, PROCESSING AND ANALYSIS		
WRITTEN BY	YUSUF GUMAN/VERONIQUE NICOLAOU		
APPROVAL	NAME	SIGNATURE	DATE

1 PURPOSE AND SCOPE OF THE STANDARD OPERATING PROCEDURE

1. 1. PURPOSE FOR THE SOP

This Standard Operating Procedure (SOP) describes the timing and the procedures for the collection, processing and storage of biological samples (venous and capillary blood, and urine) from women at specified times.

1.2. SCOPE OF THE SOP

This SOP covers the collection, processing, temporary and long-term storage of blood and urine. It does not cover how to conduct any assays performed with any of the samples after processing.

1.3. SAFETY PROCEDURES

All research personnel and technicians are expected to be trained and follow universal safety precautions when handling biological or hazardous materials and when performing any of the procedures described in this SOP, especially in the high-risk areas and populations for HIV infection.

The standard SOP approach assumes that all biological material is infective and potentially carrying HIV virus. Therefore, all possible precautions are to be adhered to – both those outlined in this SOP and those imposed by the affiliated institution.

In the event of an outbreak in the surrounding area of the study site of a highly contagious infectious disease like Ebola or a similar airborne or highly virulent pathogen, the project coordinators will initiate an immediate lock-down of the sampling site. This situation is to be reported to the Project Management team as soon as it arises. Biological sampling will be put on hold, study staff or participants not already in the building will not be able to enter and all persons in the building will remain in the building until obtaining advice from local public health teams. Biological samples will not be removed from storage during this period and sample analysis will not be undertaken. Individuals within the building at the time of the lockdown who display any symptoms of illness will be isolated in a separate room. All research activities will be suspended pending clearance from appropriately qualified and designated authorities.

2. BLOOD SAMPLE COLLECTION AND PROCESSING

Time of collection

Early morning upon arrival following informed consent

1. Baseline +/- 8am
2. OGTT +/- 8am and 10am-refer to OGTT SOP

2.1. MATERIALS NEEDED

The equipment and designated containers are listed below:

Table 2.1 Overview of the maternal venous blood sample collection and processing

Tubes	**Processing and aliquot assignment**	
1st Purple top 6 ml (Vacutainer) for whole blood	**EDTA tube** a) **1 x 1mL of whole blood** for immediate HbA1c analysis in a 2ml yellow top cryotube. b) Centrifuge remaining specimen c) Buffy coat aliquoted and store 1ml in yellow top cryotube and freeze for later analysis (genetics) d) Plasma aliquoted and store 1-2ml in yellow top cryotube	
2 x Yellow top 5ml (Vacutainer) for serum	**Serum separation gel tubes** (clotting for 30 minutes, then centrifugation) a)**2 x 2ml cryotubes** with 1 ml of aliquot (serum) in each, use yellow cap cryotube. Remaining sediment (centrifuged erythrocytes) is discarded	

OGTT **2x Grey top (4ml)**	**Grey sodium fluoride tubes** (clotting for 30 minutes, then centrifugation) a)**4 x 2ml cryotubes** with 500ul-1 ml of aliquot (plasma) in each, use yellow cap cryotube. Remaining sediment (centrifuged erythrocytes) is discarded See OGTT processing SOP	

a. **1 Sterile butterfly Safety Needle** 21G x 3/4" (Sarstedt 85.1638.235)
b. ***1 label*** *for whole blood*
c. ***4 labels*** *for serum*
d. ***4 labels*** *for glucose*

2.1.2. Other equipment and supplies

a. 1 purple top 6ml EDTA tubes (Lasec VGRV456043)
b. 2 yellow top 5mL serum tube (Lasec VGRV456018R)
c. 2 Grey top 4ml tubes (Lasec VGRV455051)
d. Tourniquet
e. Silica gel
f. Antiseptic wipes
g. Gauze sponges
h. Adhesive bandages
i. Sharps container
j. Gloves
k. Automated pipette and 5 filter tips (Sarstedt 70.762.211)
l. Racks for cryotubes
m. Rack for holding vacutainers

2.2. BLOOD SAMPLE COLLECTION

A trained phlebotomist, midwife or research technician should collect blood.

Procedure to follow when taking bloods:
Once all blood taking equipment is prepared and ready, identify which arm blood will be taken from.
Position tourniquet about 10cm above the antecubital fossa. If vein is not easily visible in the fossa, get patient to pump her fist.
Once vein identified, wipe the area clean with alcohol swap.
Wait 5 seconds and insert needle of butterfly or attached to the vacutainer into the vein.
Secure the needle position and insert relevant tubes for blood collection.

Once all bloods have been collected, remove the tourniquet, remove the needle and apply pressure with a piece of cotton wool and strap down with a band aid.
If you fail to successfully enter the vein, and hence you are not able to withdraw any blood, repeat steps above on the same arm, trying to identify a different vein in the antecubital fossa or try the opposite arm.
If you still fail, call the Principal investigator, Veronique Nicolaou

2.3 DISPOSAL OF WASTE

The phlebotomist should discard used materials in the biohazard waste bags.
Sharps should be discarded in a puncture resistant container that should never be allowed to be more than two thirds full.

2.4 DEALING WITH VENEPUNCTURE PROBLEMS:

2.4.1 How to prevent bleeding and bruising:

Do not apply pressure to the venipuncture site whilst the needle or cannula is still in the vein as this can be painful and cause damage to the wall of the vein.
Remove the needle or cannula from the vein and immediately apply pressure for 2 minutes to stop bleeding.

2.4.2 If a blood sample is not forthcoming, the following manipulations may be helpful.

Turn needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein. Move the needle slightly in hope of entering vein or pull back slightly in case you have gone through the vein. Do not probe. If not successful, release tourniquet and remove needle and apply pressure to the site. Explain to the participant that there are challenges with the blood draw and request him/her to allow you to use the second hand. A second attempt can be made on the other arm. The same technician should not attempt a venipuncture more than twice. If a third attempt is necessary, a different phlebotomist should attempt the venipuncture.
Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.

NEEDLE STICK, BLOOD HANDLING BREAKAGES OR OTHER BLOOD EXPOSURE INJURIES:

In the event of a staff member sustaining a needlestick injury or becoming exposed to blood, bleeding should be **encouraged,** and the wound washed in warm running water with soap. *Arrangements should be made for the exposed person to undergo an urgent assessment of the risk of acquiring blood-borne infection and advised about whether PEP should be initiated.* The assessment should include consideration of the circumstances and nature of the injury, whether appropriate first aid measures were undertaken, the probability of the 'donor' being infectious and the suitability of the exposed person for PEP. *If this risk assessment indicates there is a significant risk of HIV, post exposure prophylaxis (PEP) needs to be started as soon as possible, ideally within 1 hour and not beyond 72 hours. This reduces risk of transmission by 80%.*
Occupational exposure to HIV infected blood, either through needle stick, blood handling, or other blood exposure injuries, should be managed in accordance with the Unit's policy on *PEP. This should be reported on the appropriate systems as per the policy.*

In an emergency situation it may be appropriate, provided there is no significant major contraindication, *to give the first dose of PEP pending a fuller assessment after the HIV status of the 'donor' is verified. PEP currently consist of a 28-day course of treatment with a triple combination of antiretroviral drugs, has significant side effects, and needs careful follow up.*

Please refer to the Unit's policy on post-exposure prophylaxis for further information

Procedure to follow following successful blood collection

Attach a unique participant ID code label to each of the collection tubes and to the kit label
A certified phlebotomy technician should draw blood into each of the vacutainer tubes using the vacutainer needle and guide tube provided. The order of draw is blue top (4.5ml), yellow top (2 x 5ml), green top (4.5ml), purple top (2 x 6ml)



Vacutainer needle and guide tube



Serum separation gel (yellow top) tubes



EDTA (Purple top) tubes



Baseline **2-hour post baseline**

Glucose fluoride (grey) tubes

1. The yellow top tubes should be inverted gently 5 times slowly (not shaken) in order for the reagents to mix with the blood sample.

2. The purple top tubes should be inverted gently 8 to 10 times slowly (not shaken) in order for the reagents to mix with the blood sample.

3. The grey top tubes should be inverted gently 5 times slowly (not shaken) in order for the reagents to mix with the blood sample.

4. Check the time of collection has been entered by the nurse/phlebotomist on the biological sample processing sheet.

5. Set timer for 30 minutes as soon as the blood draw has been completed as a reminder to centrifuge the tubes.

6. Place blood samples in the zip lock bag and deliver the samples to the lab with the biological sample processing sheet.

2.5. PROCESSING OF THE SAMPLES

2.5.1 Whole blood samples – EDTA tube

1. It is important to track labels, specimens and documents of individual participants while processing.
2. Avoid all clots. Do not include blood clots in whole blood collection.
3. Pipette out 1ml of whole blood from EDTA tube and recap specimen.
4. Aliquot 1ml whole blood into 2ml cryotube and freeze at -20°C.
5. Place EDTA tube with remaining blood into centrifuge at 3000 rpm for 10 mins
6. Extract and Aliquot 1-2ml plasma and place into a 2ml cryotube
7. Extract 300ul buffy coat and aliquot into a single (2ml) cryotube
8. Store specimens at -20°C

2.5.2 Aliquoting of blood from the two yellow top tubes (serum)

1. Nurse gives two (2) labelled tubes of blood (yellow top) to the lab technician
2. Place the yellow capped tube in the rack to clot at room temperature for 30 minutes after blood draw.
3. Lab technician must create labels (at least 2 labels per tube) for aliquoting serum into 4x2ml cryotubes
4. Centrifuge blood samples at 3000 rpm for 10 minutes
5. Whilst samples are in the centrifuge, place the new labels onto the 2ml cryotubes
6. After centrifugation, two layers will be visible, serum and sediment (erythrocytes). Normally the serum will be golden in colour and semi-transparent.
    - If it is pink or red, then it is haemolysed, and this should be noted in the sample processing form.
    - If it is opaque or white in colour, it is lipemic and this should be noted in the data collection software.

Occasionally there is a lipid layer on the plasma surface. **Avoid** inclusion of the lipid layer at the top of the serum when taking aliquots.

1. Un-cap the 4 x 2ml cryotubes
2. Using a pipette, remove the 1ml serum for each cryotube from the top of the sample and dispense 1ml into each correctly labelled cryotube
    a. For every 5mls of blood spun one should get approximately 2.5mls of plasma
    b. Do this for every vacutainer
    c. **NB: ENSURE THAT THE LABELS ON THE CRYOTUBES IN WHICH THE SERUM IS DISPENSED MATCH WITH THE LABELS ON THE VACUTAINERS**
7. Fasten the caps onto the cryotubes and check that they are secure and that each tube has a specimen ID label.

8. Freeze specimens at -20C
9. Note the time of storage on the biological sample processing sheet and aliquot as described in Table 3.1.

2.5.3 *Aliquoting of blood from the two grey top tubes (plasma)*

10. Nurse gives three (3) labelled tubes of blood (grey tops) to the lab technician
    a. Tube A: 0 minutes (fasting blood glucose)
    b. Tube D: 120 minutes post glucose load

11. Lab technician must create labels (at least 4 labels per tube) for aliquoting serum into 4x 2ml cryotubes
    a. **0 minutes** sample: Label should be participant's study ID followed by **".a"**
       E.g. 14-10012**.a** or 3G2555661**.a**
    b. **120 minutes** sample: Label should be participant's study ID followed by **".d"**
       E.g. 14-10012**.d** or 3G2555661**.d**

12. Centrifuge blood samples at 3000 rpm for 10 minutes
13. Whilst samples are in the centrifuge, place the new labels onto the 4 x2ml cryotubes
14. Using a pipette, remove the plasma from the top of the sample and dispense 500ul-1ml into each correctly labelled cryotube
    a. For every 4mls of blood spun one should get approximately 2mls of plasma
    b. Do this for every vacutainer (0,60 & 120 minutes)
    c. **NB: ENSURE THAT THE LABELS ON THE CRYOTUBES IN WHICH THE PLASMA IS DISPENSED MATCH WITH THE LABELS ON THE VACUTAINERS**

Freeze the aliquots at -20°C. Ensure they are all stored in one tray in the correct order (a and d). Record which samples belonging to which participants are stored in which freezer.

2.6. STORAGE OF BLOOD SAMPLES

1. After blood, plasma, buffy coat and serum specimens have been processed, aliquoted and labelled date and time as well as number of aliquots should be **recorded onto the Laboratory Management System (LMS) daily.**
2. Except for DMSO - preserved whole blood where storage is detailed above, specimens must be stored, within 2 hours of collection at a minimum of -20°C for short term storage (<30 days), but preferably at -80°C until shipped to the core repository.

2.7. ANALYSIS OF BLOOD FOR HBA1C

2.7.1 CONTROLS

a. Reconstitute and store controls according to manufacturer's package insert.
b. Controls must be diluted before analysis.
c. Pipet 1.5ml of Wash/ Diluent Solution into 15.ml vial, followed by 5µL of reconstituted control.

d. Cap each control vial and mix thoroughly.

2.7.2 SPECIMEN PREPERATION

NORMAL TUBES

i. Allow sample tubes to reach room temperature (15 -30°C) before performing the assay.
ii. No sample preparation is required. Mixing has no impact on
iii. The sample tubes are loaded into the D10 sample rack and placed in the D10.
iv. Ensure that the sample barcodes are facing the back of the instrument.
v. Use special rack inserts for 12, 13 and 14mm diameter tubes.
vi. Remove all inserts for 16mm diameter tubes
vii. Place rack into analyser
viii. Click start

ABNORMAL TUBES

a. If the sample is an abnormal size/ type tube, or if the is less than 2.0ml of sample in the tube, then the sample must be prediluted.
b. Before pipetting, thoroughly mix the sample by gently inverting the tube
c. To predilute, pipet 1.5ml of Wash/Diluent Solution into a labelled 1.5ml vial, followed by 5µL of the whole blood sample
d. Cap the sample vial and mix thoroughly.
e. Use a sample vial adaptor for 1.5ml vials.
f. Place rack into analyser
g. Click start



D10 analyser



Vial adaptors for 1.5ml tubes

2.6. ANALYSIS OF PLASMA FOR OGTT GLUCOSE

Take one aliquot per time point (e.g. 1 cryotube of 0-minute sample, 1 cryotube of 60 minutes sample, & 1 cryotube of 120 minutes sample) out of the -20°C freezer.

1. Defrost at room temperature for approximately half an hour
2. Prepare the Randox RX Daytona machine. The control samples provided by Randox are as follows:

Assayed Human Sera Level 2 (HUM ASY CONTROL)					
Unit	Target	Low	High	1SD	2SD

mmol/l	6.36	5.51	7.45	0.49	0.97

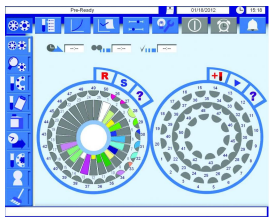
Assayed Human Sera Level 3 (HUM ASY CONTROL)					
Unit	Target	Low	High	1SD	2SD
mmol/l	16.30	13.7	18.5	1.20	2.40

GLUC-PAP GL8318		
Unit	Low	High
mmol/l	3	6

3. Scan the barcodes of the cryotubes you wish to run. A table on the Randox screen will be populated with barcodes. Ensure you place them in the machine to run in chronological order (a, b, c)
4. Run the samples (A maximum of 40 samples can be run which takes approximately 45 minutes)
5. Export results to an Excel Spread sheet
6. Look through the results and identify samples that failed to run. These will need to be re-run
7. Send results to Veronique Nicolaou
8. Store left-over samples in the fridge for a maximum of 5 days
9. Get feedback from Veronique Nicolaou regarding which samples need to be re-run
10. Re-run the samples (use what is left over from the run stored in fridge) or defrost another aliquot if there is not enough sample left)
11. Send results of re-runs to Veronique Nicolaou
12. Once results have been signed off by Veronique throw away the samples in the fridge
13. Veronique to give feedback regarding participants' results to nurses, if deranged so that participants can be contacted regarding referral







3. URINE SAMPLE COLLECTION, DIPSTICK ANALYSIS, PROCESSING AND STORAGE

Following consent and prior to bloods being taken

3.1. MATERIALS NEEDED

The equipment and designated containers are listed below.

3.1.2. Kit Contents

a.	**1 x 45 ml urine collection** container (screw cap lid, sterile)
b.	**1 x 1 ml transfer pipette**
c.	**1 x Roche combur 10 urine dipsticks**

3.2. URINE SAMPLE COLLECTION

3.2.1. For collection at facility/lab – preferred option



1. Provide woman with the participant ID-labelled urine collection cup, mark the minimum volume required (10ml) on the cup and explain to the woman this is the minimum we need.

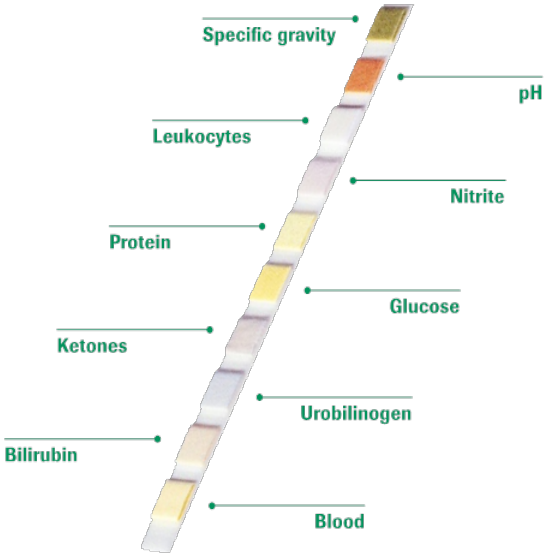
2. Ask the woman to provide a mid-stream urine sample:

    a. The participant should be given three cleansing towelettes with the urine collection cup.
    b. After washing her hands, the participant opens the sterile cup making sure to avoid touching the inside of the lids and containers to prevent contamination. Set the lid down with the inside of the lid facing up beside the matching cup.
    c. Make sure the cup is within easy reach of toilet.
    d. Using one hand the patient should separate the folds of the urinary opening. Using the first towelette, the patient should wipe one side of the inner fold front to back and discard towelette. Using the second towelette, the patient should wipe the opposite side, front to back, and discard. Using the third towelette, the patient should wipe down the centre, front to back and discard.
    e. The initial part of the urine stream should be voided into the toilet or another container.
    f. At approximately the middle of the urine flow, the container should be positioned to capture urine until about half full (or at least at the minimum marked line)
    g. The cup should be set down in a secure location. The remainder of the urine should be voided into the toilet.
    h. The specimen cup and lid should be re-capped tightly, and any splashed urine wiped from the outside of the cup.
    i. Participant should then present collected urine specimen to study coordinator and the date and time of collection should be recorded.

3. Scan the bar code on the collection cup to register the time of collection.

3.2.2. Testing of urine with dipstick

Once the participant has returned the container containing urine, ensure that the lid is tightly screwed on and gently invert the container to ensure adequate mixing. Then take a urine dipstick from the Roche Combur® 10 bottle and dip the stick into the urine for no longer than 1 second, ensuring that all the test pads on the stick are covered with urine. Remove the stick, gently tapping the edge of the stick on the side of the container to get rid of excess urine and place the stick flat on a piece of tissue paper on a flat surface. Wait 60 seconds (60-120 seconds for leucocytes) and then compare the reaction colour on the dipped stick with the colours on the bottle. Document whether there are any positive findings on the biochemistry sheet.



Urine must be tested as soon as possible. Urine that has been left to stand for longer than two hours may yield false results.

Do's	Don'ts
✔ Examine the test strip within two hours	✘ Allow contamination by residues of cleaning agents or disinfectants, as these can give false-positive findings for blood, protein and glucose
✔ Mix the specimen thoroughly prior to the test	✘ Freeze the urine specimen, as this will destroy leukocytes and erythrocytes and hence make it unusable for subsequent microscopic examinations
✔ If the tests cannot be done within two hours of urine collection, keep the specimen in a refrigerator at +4°C	✘ Centrifuge the specimen prior to test strip analysis
✔ At the time of testing, ensure the samples are at room temperature	✘ Expose the specimen to direct sunlight
✔ Close the test strip tube immediately after removing a test strip	
✔ Remember to label the urine container	

If the dipstick is positive for leucocytes or nitrites or both, then ask the participant whether they have any of the following symptoms: burning or pain when passing urine, lower back or flank pain or a fever. If they do, please refer the participant to their local clinic for management of a urinary tract infection.

If the dipstick has ≥ 3+ protein and/or blood please refer the participant to principal investigator Veronique Nicolaou or Gladys Morsi.
If the dipstick is positive for glucose, please do a fingerprick blood glucose test and enquire whether the participant is known to have diabetes or not and whether or not they are on medication. If the blood glucose is > 7 mmol/L and < 20 mmol/L and the participant is not known to have diabetes, please refer to the local clinic for confirmation and management of diabetes. If the participant is known to have diabetes, then enquire whether they have taken their diabetic medication that morning or not. If the blood glucose is ≥ 20 mmol/L please refer the participant to Ward 20 at the Baragwanath Hospital.

3.2.3. When the woman cannot produce any urine at the study facility

Urine collection by the woman at home IS A LAST RESORT OPTION in this study. Where for any reason, other than pathological, a woman is not able to provide urine during the visit, study teams should take the following actions:

1. Explain to the woman that the urine sample collection is an important part of the study and that the study team will provide them water to drink to help them fill their bladder and to be able to pass some urine.

2. Explain that it may require that they stay at the facility UNTIL they feel the urge to void.

3.3. URINE SAMPLE PROCESSING FOR URINE MICROALBUMINURIA/CREATININE RATIO

Specimen processing should be completed, and aliquots frozen within 2 hours of collecting urine from participants.

Table 4.1 Overview of the urine sample collection and processing

Processing and aliquot assignment	
a) Aliquot 1 ml of urine from the collection container into each of 2 pre-barcoded 2ml yellow-capped cryotubes.	

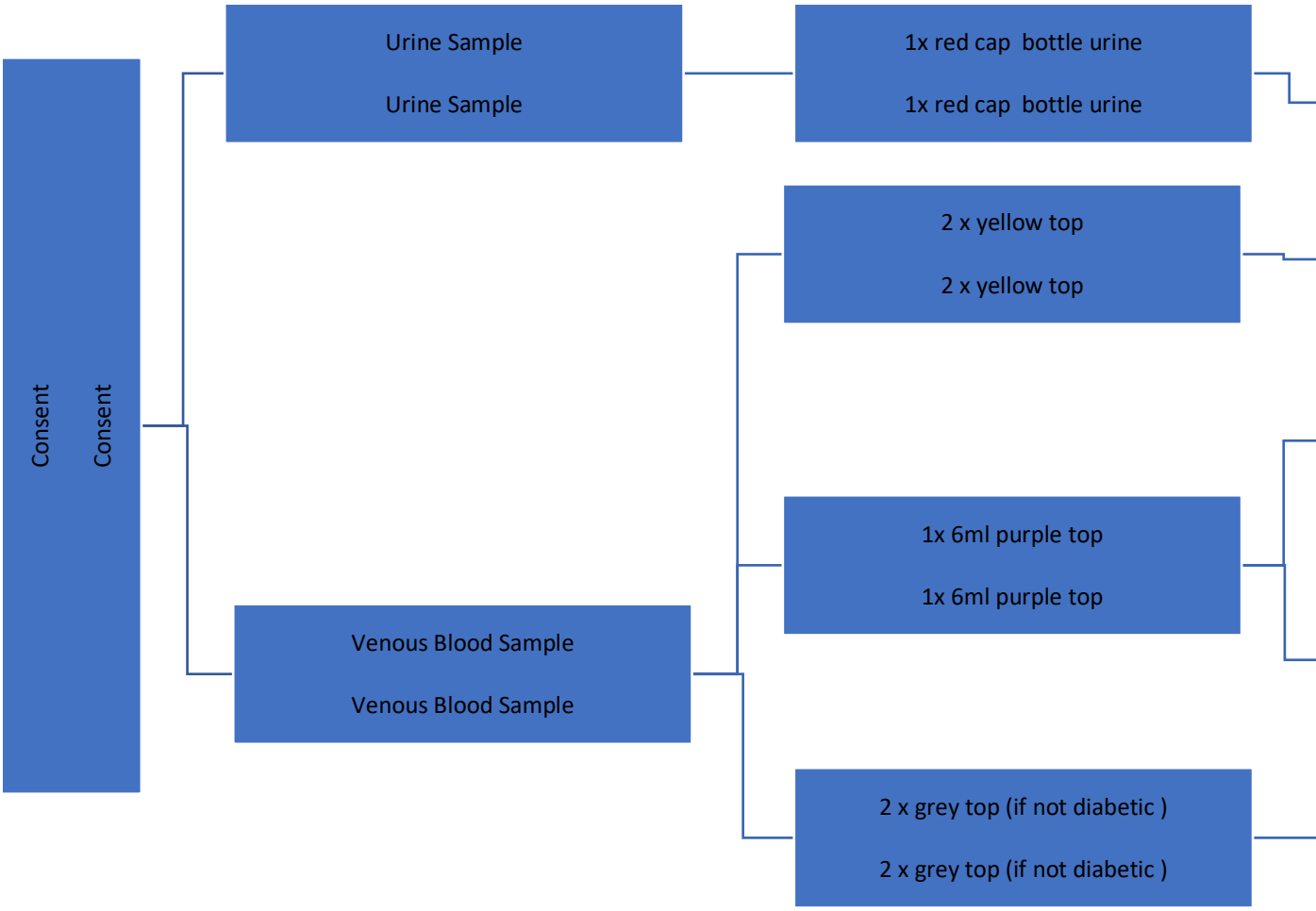
3.4. STORAGE OF URINE SAMPLES

1. Allocate the aliquots into freezer boxes noting their location within the freezer and the total number of aliquots in data management system, which will also register the timing.

2. Store aliquots in a specimen box at a minimum of -20°C for short-term storage (< 30 days), and preferably at -80°C for long term storage.

Once all of the urine specimens from the collection container have been processed, divided into aliquots, scanned into the database, and stored in the freezer, all of the information from the sample collection form should also be entered into the LMS.

3. Workflow Schema & biological sample processing sheet



Appendix 1

Patient Label

IMPACT OF DIABETES IN PREGNANCY ON MATERNAL HEALTH OUTCOMES STUDY

Biological Sample Processing Sheet

Patient ID:

Date: D D M M Y Y

Fasting: Y N

Time of Blood taking H

Sample	Y/N	Tubes	Cryotubes	Reason samples not taken/comments
				___
				___
				___
				___
				___
				___
				___
				___
Blood		2 x 5 ml Yellow top	4x2ml cryotubes with 1ml plasma in each-freeze	---------- ---------- ---------- ---------- ----------
Blood		6 ml Purple top	1x1ml of whole blood into 2ml cryotube-freeze 1x2ml cryotube-aliquot 300ul buffy coat-freeze 1x2ml cryotube-aliquot 1-2ml plasma-freeze	---------- ---------- ---------- ----------

Blood		2x 4ml Grey top	2x2ml cryotubes with 1ml serum in each- freeze

---

Completed By: _____

Bloods Processed By: _____

		H		

Time of blood storage

		H		

Time of centrifugation

Appendix 2

DPHRU
MRC/Wits Developmental Pathways for Health Research Unit

Urine collection and dipstick- Analysis

Patient Label

_____

Patient ID:

Date:

D	D		M	M		Y	Y	

Time of urine collection:_____

_

Time of reading Urine dipstick:_____

Urine		45ml screwcap specimen jar	1) 2x2ml cryotube

Comments

	1.000		1.005		1.010		1.015		1.020		1.025		1.030	
pH					5		6		7		8		9	
Leucocytes					neg		1+		2+		3+			
Nitrites					neg		pos							
Protein					neg		1+		2+		3+			
Glucose					norm		1+		2+		3+		4+	
Ketones					neg		1+		2+		3+			
Urobilinogen					norm		1+		2+		3+		4+	
Bilirubin					neg		1+		2+		3+			
Erythrocytes					neg		1+		2+		3+		4+	
Haemoglobin					neg		1+		2+		3+		4+	

Pregnancy test results: Positive OR Negative _____

Test done by:_____
Time of urine storage:
Urine processed by:_____

APPENDIX E: PROTOCOL FOR PERFORMING OGTT AND PROCESSING OF BLOODS

DPHRU
MRC/Wits Developmental Pathways for Health Research Unit

Standard Operating Procedure for performing OGTT and processing of blood samples for glucose testing

This SOP refers specifically for procedure implemented at DPHRU.
SOP Number:1
Version Number:1.0

	Name	Title	Signature	Date
Author	Veronique Nicolaou	Researcher		30-01-2019
Reviewer				
Authoriser				

Equipment needed

1. 75gm glucose sachet, or alternatively mix 75gm glucose powder (to weigh on scale) with 250ml tap water (150ml boiled water and 100ml cold water)
2. Glass cup for mixing and drinking solution
3. Two labelled grey top tubes
4. Blood taking equipment (see blood collection SOP)

Procedure of performing 75gm OGTT

1. Check that the participant has fasted for at least 8 hours prior to procedure

2. Check the finger prick glucose if fasting glucose>7.0 or random fingerpick glucose >11.1-no need to proceed to OGTT

3. If glucose values are BELOW the above-mentioned cut-off points and the patient HAS fasted, perform a formal fasting glucose blood (see below)-note time point ON WHITE BOARD
4. Get participant to drink the oral glucose solution, to ingest within 5 minutes-note time point-**THIS IS TIME POINT ZERO (A)**
5. Repeat formal glucose measurement 120mins following oral glucose ingestion - **THIS IS TIME POINT 120MINS (D)**

Procedure to follow in case the mother cannot tolerate the solution or vomits or has not fasted.
Do not proceed with OGTT (i.e., do not measure glucose at 120mins)

PERFORMANCE OF THR BLOOD SAMPLE COLLECTION

A trained phlebotomist, midwife or research technician should perform the oral glucose tolerance test.

Procedure to follow when taking bloods:

1. Once all blood taking equipment is prepared and ready, identify which arm blood will be taken from.
2. Position tourniquet about 10cm above the antecubital fossa. If vein is not easily visible in the fossa, get patient to pump her fist.
3. Once vein identified, wipe the area clean with alcohol swap.
4. Wait 5 seconds and insert needle of butterfly or attached to the vacutainer into the vein.
5. Secure the needle position and insert relevant tubes (2 grey top tubes) for blood collection.
6. Once all bloods have been collected, remove the tourniquet, remove the needle and apply pressure with a piece of cotton wool and strap down with a band aid.
7. If you fail to successfully enter the vein, and hence you are not able to withdraw any blood, repeat steps above on the same arm, trying to identify a different vein in the antecubital fossa or try the opposite arm.
8. If you still fail, call the Principal investigator, Veronique Nicolaou

Processing and Storing:

3. Nurse gives (2) labelled tubes of blood (grey tops) to the lab technician
   a. Tube A: 0 minutes (fasting blood glucose)
   b. Tube D: 120 minutes post glucose load

4. Lab technician must create labels (at least 4 labels per tube) for aliquoting serum into 5ml cryotubes (2 cryotubes per specimen, hence 4 in total per patient)
   a. **0 minutes** sample: Label should be participant's study ID followed by **".a"**
      E.g. 14-10012**.A** or 3G2555661**.A**
   b. **120 minutes** sample: Label should be participant's study ID followed by **".b"**
      E.g. 14-10012**.D** or 3G2555661**.D**

5. Centrifuge blood samples at 3000 rpm for 10 minutes

6. Whilst samples are in the centrifuge, place the new labels onto the 5ml cryotubes

7. Using a pipette, remove the serum from the top of the sample and dispense 500µl into each correctly labelled cryotube
   a. For every 5mls of blood spun one should get approximately 2mls of serum
   b. Do this for every vacutainer (0,60 & 120 minutes)
   c. **NB: ENSURE THAT THE LABELS ON THE CRYOTUBES IN WHICH THE SERUM IS DISPENSED MATCH WITH THE LABELS ON THE VACUTAINERS**

8. Freeze the aliquots at -20'C. Ensure they are all stored in one tray in the correct order (a and d). Record which samples belonging to which participants are stored in which freezer.

Running:

14. Take one aliquot per time point (e.g. 1 cryotube of 0-minute sample, & 1 cryotube of 120 minutes sample) out of the -70'C freezer.

15. Defrost at room temperature for approximately half an hour

16. Prepare the Randox RX Daytona machine. The control samples provided by Randox are as follows:

Assayed Human Sera Level 2 (HUM ASY CONTROL)					
Unit	Target	Low	High	1SD	2SD
mmol/l	6.48	5.51	7.45	0.49	0.97

Assayed Human Sera Level 2 (HUM ASY CONTROL)

Unit	Target	Low	High	1SD	2SD
mmol/l	6.72	5.71	7.73	0.51	1.01

Assayed Human Sera Level 3 (HUM ASY CONTROL)

Unit	Target	Low	High	1SD	2SD
mmol/l	16.1	13.7	18.5	1.20	2.40

GLUC-PAP GL3815			
Unit	Low	High	
mmol/l	3	6	

17. Scan the barcodes of the cryotubes you wish to run. A table on the Randox screen will be populated with barcodes. Ensure you place them in the machine to run in chronological order (a and d)

18. Run the samples (A maximum of 40 samples can be run which takes approximately 45 minutes)

19. Export results to an Excel Spread sheet

20. Look through the results and identify samples that failed to run. These will need to be re-run

21. Send results to Veronique Nicolaou

22. Store left-over samples in the fridge

23. Get feedback from Veronique Nicolaou regarding which samples need to be re-run

24. Re-run the samples (use what is left over from the run stored in fridge) or defrost another aliquot if there is not enough sample left)

25. Send results of re-runs to Veronique Nicolaou

26. Once results have been signed off by Veronique throw away the samples in the fridge

27. Veronique to give feedback regarding participants' results to nurses, if deranged so that participants can be contacted regarding referral

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APPENDIX F: PROTOCOL FOR DXA MEASUREMENT

Standard Operating Procedure for DXA measurements
Abbreviated SOP: DXA

This SOP describes the procedures personnel will use in conducting DXA measurements while fulfilling ethical responsibilities for protecting the rights and welfare of participants. While this must align with procedures at other study centres (if a multi-site study), the SOP refers specifically to procedures employed at DPHRU.

SOP Number	**DXA-001-v1**
Version Number	**1.0**

	NAME	TITLE	SIGNATURE	DATE
Author	Thamsanqa Mchunu	Radiographer		19 April 2018
Reviewer	Veronique Nicolaou	Researcher		14 February 2019
Authoriser	Shane Norris	Unit Director		14 February 2019

PURPOSE AND SCOPE
To describe procedures for DXA WHOLE BODY composition, and calibration of DXA equipment.

RESPONSIBLITIES AND SAFETY
It is the responsibility of all staff involved in DXA, to adhere to the standard operating procedures as outlined in this document. All DXA staff are expected to review this document at regular intervals to ensure that they are up to date with current practices.
Since the study depends on the voluntary participation of participants, every effort must be made to make the entire procedure as easy and painless as possible for them.
The study team must remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The best way to achieve this is for the survey staff to be thoroughly knowledgeable about all aspects of the procedures.
Universal safety precautions must be carried out at all times. These include the use of gloves at all times, sanitising all equipment before use and between each participant.
It is the responsibility of the project to replenish all consumables used for DXA.
It is the responsibility of all staff conducting DXA to report any broken missing equipment.

EQUIPMENT
The following equipment is necessary for DXA
DXA machine
printer
lead screen protector

computer
anthropomorphic spine phantom

The following consumables are necessary for DXA
Gloves
Sanitising spray, non-alcoholic
Tissue paper
Gowns

PROCEDURES
1. Lies patient supine on the table, head 1-2 cm from the top of the scanning area, feet loosely held together. Instruct the patient to breath normally and remain still.
2. Instruct the patient to look at the ceiling to maintain head position.
3. Check the patient's position on the table pad ensuring that the following conditions are met: -
Body straight on the table pad using the centre lines at the head and foot ends of the table pad as the gauge.
Body, including the feet, is positioned entirely within the scan limit border line.
Arms are at the sides, with the palms down, separated from the trunk of the body but within the scan limit border.
Feet are pointing up, rotate the patient's legs inward 25 degrees until the toes touch, and then place tape around the feet to maintain position.
If patient is too large then d-centre the patient to the left so that the right arm fits within the scanning area, scan as per usual but change left arms measurements with the right arm measurement for analysis.
Two scans need to be done if it is not possible to fit right arm and left hip on same scan.
One with the patient centred and both arms cut off the scanned image and the other with the patient de-centred, capturing the right arm.

Maximum table weight is 120 kg
START UP

Press button on hard drive tower.
QDR platform comes up
Press **DAILY QC.** A spine phantom is scanned daily to ascertain the reliability of the machine.
Read prompt screen after the table moves to position, **(something table already in position so you might find that the table does not move),** the cross on the phantom should be at the base of the bed, and the laser cross should be positioned over it; the vertical line should run along the phantom spine and should be central.
Press continue.
Once the QC has been done, the machine will stop, and a prompt will appear.
Press the plot button and have a look at the graphs that appear. If the solid green dot appears between the two broken red lines, it is acceptable (the values have to correspond with the recommended values of the manufacturer, which can be found on the phantom.
But if it does not then re-run the QC and making sure that the positioning of the phantom is correct. Any deviations from the normal values have to be reported immediately following a second phantom scan and if the results are the same as the first scan.
Should there be a discrepancy with the phantom, ideally patients should not be scanned until the problem is sorted out by the manufacture's technician. **NB:** if there was a power failure

during the evening, the machine will not come on. Go to the bottom of the scan table and at the back of the table there is a switch, flick the switch, then press the button on the tower. **NB:** once a week a prompt will appear asking you to perform a **Radiographic Uniformity Test.** Follow the instructions, once finished it will give you two values, these values should be under 2.0, if one of the values are more than 2, then the test needs to be repeated. Go to QC-Body Composition-run the body composition with the spine phantom and then you will be requested to run the **Radiographic Uniformity Test.**

Shut down

Press exit on the bottom far right corner
A prompt will appear
Exit QDR with shutdown. Click ok and the machine will shut down itself. Make sure windows are closed and lights off.

CREATING PATIENTS BIOGRAPHY

Patient's biography is the information concerning the patient that is stored in a database on the computer. This information is entered into the computer on the patient's first examination and can be retrieved on subsequent visits.
First click on **Perform Exam** then click the **New Patient** button to add a new patient record. The window will guide you on what to enter e.g. name and surname, date of birth, sex, race, weight, height, etc.
To retrieve the patient's biography on the next visit you will need to
Click the **Perform Exam** button
Click on the patient's name. Patients name will be highlighted.
Click **OK,** the **Patient Confirmation** window will be displayed.

PERFORMING SCANS

Make sure the patient has removed all jewellery, money, hair accessories, piercings and clothing that have metal.
Positioning is much more emphasized than the actual analysis

Useful tips

All scans are done on array mode except for AP/Lateral spine which is done on array fast array.
On the old scanner (room 1) all scans are done on fast array mode
When repositioning and table will not move go to AP reposition and follow the prompt thereafter.

DATA MANAGEMENT

Data Erasing

Go to Utilities- Database tools- Patient Management- select the Patient- Manage scans- select scans- Erase.

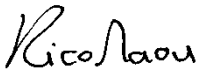
Retrieving Data

Go to Locate scans- select the relevant patient- locate scans again- select the scans to be transferred- restore scans- then go to analyse scans to view/analyse the images

APPENDIX G: PROTOCOL FOR CAROTID AND FEMORAL INTIMA MEDIA THICKNESS ASSESSMENT

Standard Operating Procedure for Ultrasound Measurement of Carotid and Femoral intima media thickness

SOP number:1
Version number 1.0

	Name	Title	Signature	Date
Author	Veronique Nicolaou	Researcher		30-01-2019
Reviewer				
Authoriser				

Ultrasound Machine
Siemens Acuson P500 Ultrasound machine, model number 10785577, serial number 390126

Settings
B-mode
Examination: carotid
Linear probe-frequency 12 MHz
Depth of field 30-40mm

Enter the following:
Press "Patient" on control panel
Patient Name/study number and DOB
Press register shown on left hand side of screen
Press exit and then pre-set button on keyboard(bottom left)
Select probe/transducer
Select Vas(vascular)and wait for new platform then select carotid.
Prepopulated settings are now selected to begin scanning.

Measurement of carotid intima media thickness (cIMT)
Ask participant to lie down supine, head partially extended with a wedge pillow inserted under head to gain better field access
Right carotid is scanned first, turn participants head towards their left
Apply enough gel to neck.
Start scanning in a longitudinal plane and find common carotid artery (CCA)
Identify carotid bifurcation, ECA, ICA and CCA (Fig. 1)



Figure 1: Anatomy right CCA as seen on longitudinal view with ECA, ICA and CCA

The CCA wall is composed of three layers, the intima, media and adventitia(Fig 2)
Measurement of intima media layer (cIMT) should occur distal to the bifurcation and utilising posterior wall of CCA(see Fig. 2)
Both anterior and posterior walls should be well visualised.
Freeze pane upon acquiring adequate image, scroll back and identify the clearest boundaries free of plaque at least 10mm distal to bifurcation
Set calliper to a point identified along the top part of the intima layer and drag cursor along top of intima layer line for approximately 10mm and click calliper again. A box will be highlighted on the segment in which IMT will be automatically assessed
Click measurement, autoIMT
Readings should appear in bottom left of screen and capture these into REDCap file labelled "maternal carotid and femoral artery doppler template" , including IMT post average, IMT post maximum, IMT post minimum, IMT SD, IMT points
Now repeat measurement at a further site distal to initial measurement, free of plaque and repeat above steps.

NOW
Ask patient to turn head to right side and repeat above steps for measuring left CCA IMT measurements
Capture measurements into REDCap

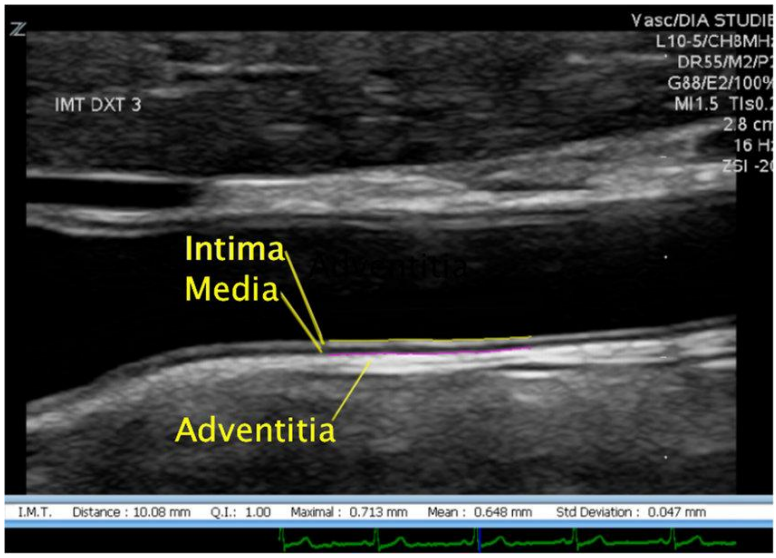


Figure 2. The 3 layers of the CCA wall

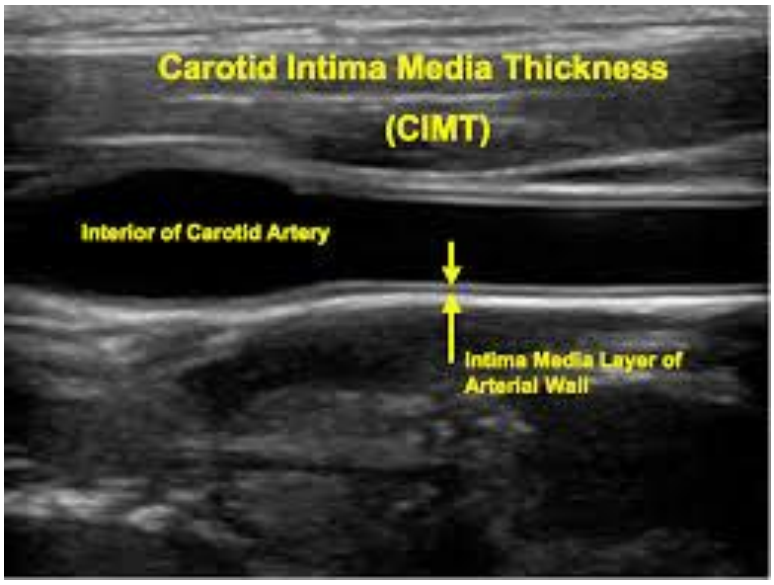


Figure 3. Longitudinal view of CCA demonstrating the posterior intima media layer

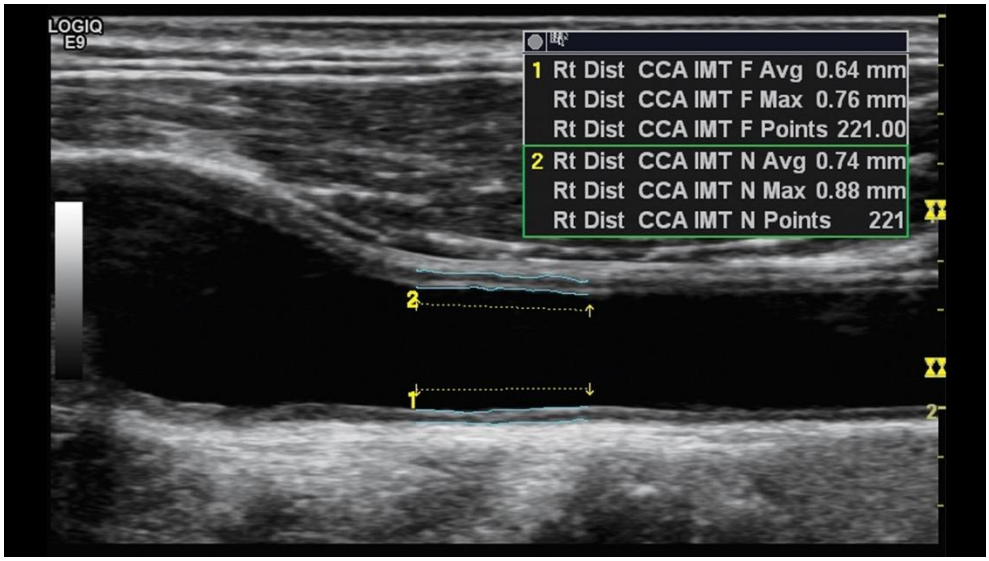


Figure 4. Measurement of the cIMT

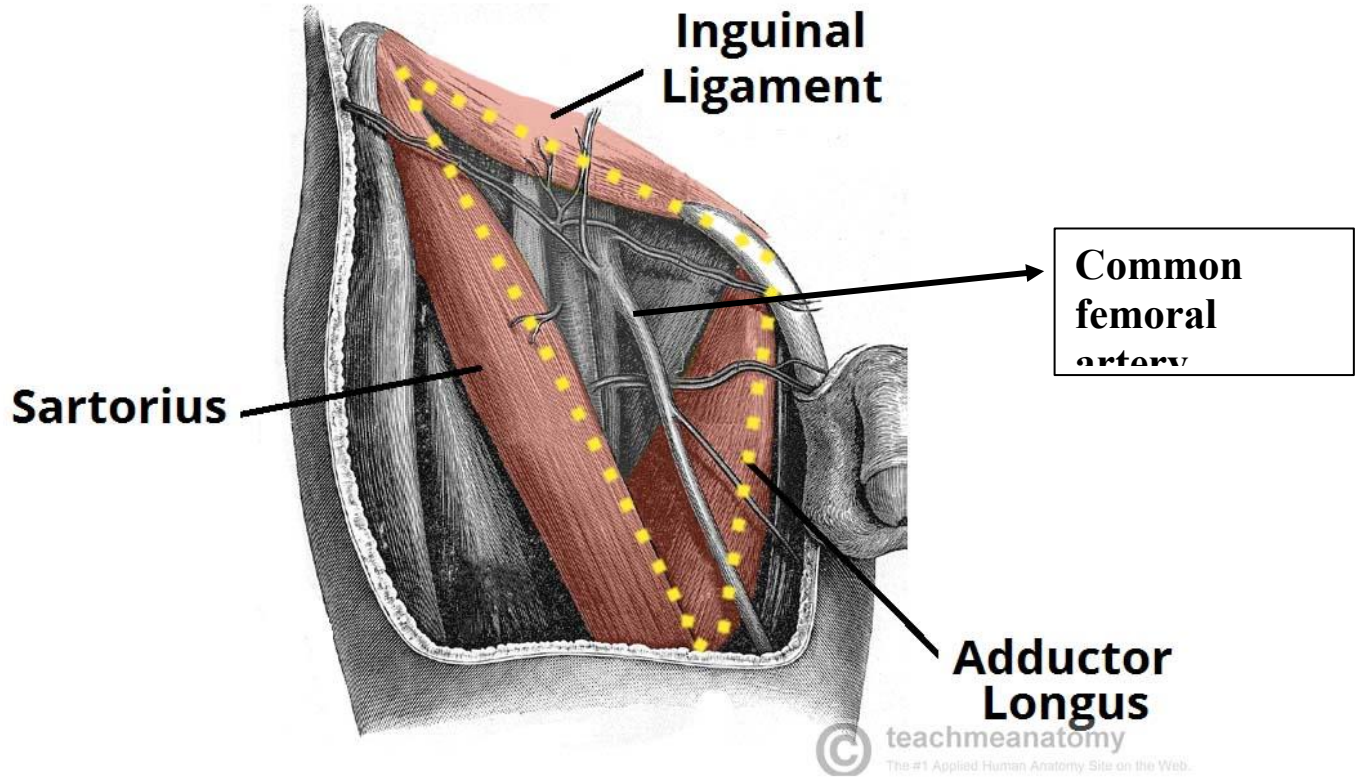
Identification of plaque

Procedure

Scan the entire length of the neck from the supraclavicular fossa (clavicle) to the submandibular angle (angle of the jaw), visualizing the carotid artery on either side, in the longitudinal view, looking for any plaque/s.
Firstly, note the number in REDCap (under maternal carotid doppler template) if identified. If noted, freeze image and measure thickness by placing calliper at the highest point of the plaque and measuring down to the intimal layer. Measure in both longitudinal and transverse view and note in Recap. Also note the echogenicity of the plaque as either hypoechoic, mixed or hyperechoic. Note these parameters for each plaque identified.

Measurement of femoral artery IMT(fIMT)

Patient to continue lying supine
Settings of machine remain the same
Abduct the leg to obtain optimum positioning of the probe
Expose both Inguinal areas (groin) and place linear probe in femoral region in a longitudinal position
Identify the femoral artery and visualize entire length from their point of entry into the Scarpa triangle till the bifurcation in superficial and deep femoral arteries and horizontalize the vessel optimizing both boundaries, and freeze image.



Scarpa triangle

Measurement of x2 IMT readings on each side, 5-10mm mm apart, starting proximally at the vessels point of entry and moving distally, using same technique as for cIMT measurement.
Capture measurements Into RedCap
Plaque identification: follow procedure as above

APPENDIX H: ETHICS CLEARANCE CERTIFICATES

RETROSPECTIVE STUDY ETHICS CLEARANCE: THE IMPACT OF DIABETES IN PREGNANCY ON MATERNAL AND FETAL HEALTH

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R14/49 Dr Veronique Nicolaou

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180204

NAME: (Principal Investigator)	Dr Veronique Nicolaou
DEPARTMENT:	Paediatrics DPHRU Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	The Impact of Diabetes in Pregnancy on Maternal and Foetal Health
DATE CONSIDERED:	23/02/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shane Norris
APPROVED BY:	Prof C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/04/2018

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

PHD PROSPECTIVE STUDY ETHICS CLEARANCE: THE IMPACT OF DIABETES IN PREGNANCY ON MATERNAL HEALTH OUTCOMES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Veronique Nicolaou

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180316

NAME: (Principal Investigator)	Dr Veronique Nicolaou
DEPARTMENT:	Paediatrics MRC/Wits Developmental Pathways for Health Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	The Impact of Diabetes Mellitus in Pregnancy on Maternal Health and Outcomes
DATE CONSIDERED:	06/04/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shane Norris
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/06/2018

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

03 February 2020

Dr Veronique Nicolaou
MRC/Wits DPHRU

Sent by email to: docvic9@gmail.com

Re: **Study no:** M180316
Project Title: The Impact of Diabetes Mellitus in Pregnancy on Maternal Health and Outcomes
Principal Investigator: Dr Veronique Nicolaou
Protocol Amendment

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has approved protocol amendments as detailed in your letter dated 05 December 2019.

Thank you for keeping us informed and updated.

Yours Sincerely,

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
03 FEB 2020
RESEARCH OFFICE HREC (Medical)
JOHANNESBURG

Ms Zanele Ndlovu
Administrative Officer
Human Research Ethics Committee (Medical)

Research Office Secretariat: Faculty of Health Sciences, Phillip Tobias Building, 3rd Floor, Office 301, 29 Princess of Wales Terrace, Parktown, 2193 Tel +27 (0)11-717-1252 /1234/2656/2700 Private Bag 3, Wits 2050, email: zanele.ndlovu@wits.ac.za Office email: hrec-medical.researchoffice@wits.ac.zaWebsite:

APPENDIX I: PUBLISHED PAPER I

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DOI: 10.1002/ijgo.12956

CLINICAL ARTICLE
Obstetrics

WILEY GYNECOLOGY OBSTETRICS FIGO

Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV

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[4]Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

[5]Diabetic Medicine and Endocrinology, Groote Schuur Hospital, University of Cape Town, Cape Town, South Africa

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Funding Information
The South African Medical Research Council; University of the Witwatersrand

Abstract

Objective: To characterize the demographics, comorbidities, management, and outcomes of pregnant women with pre-gestational and gestational diabetes (GDM), including overt and true GDM, taking into account HIV infection and the influence of exposure to oral hypoglycemic agents (OHAs).

Methods: A review of medical records of 1071 diabetic pregnancies (between 2012 and 2018) at a tertiary hospital in South Africa.

Results: Of the women, 43% had GDM, 19% had type 1 diabetes (T1DM), and 38% had type 2 diabetes (T2DM). Each group had a mean initial body mass index (BMI) >25 kg/m^2. Despite poor initial HbA1c for pre-gestational groups, over 90% of the cohort achieved glycemic control by the time of delivery. The rate of prematurity was 30.9%. Perinatal mortality (PNM) was 5.1% for the pre-gestational group and 1.8% for GDM. Of the cohort, 23.9% was HIV infected. PNM was higher in the HIV-infected pregnancies (9.4%) than non-HIV exposed pregnancies (1.8%, P<0.001). The macrosomia rate was higher in the glibenclamide-exposed group than the insulin-alone group (12.2% vs 0%, P=0.025).

Conclusion: Obesity is a significant predictor for macrosomia and was high in all groups. In a low-/middle-income country setting with a high prevalence of HIV and high usage of OHAs as an alternative to insulin therapy, HIV might be associated with higher PNM and glibenclamide with increased rates of macrosomia, which warrants further exploration.

KEYWORDS
Diabetes; Gestational; Glibenclamide; HIV; Maternal; Metformin; Neonatal; Pregnancy

1 | INTRODUCTION

The International Federation of Gynecology and Obstetrics (FIGO) has called for hyperglycemia in pregnancy to be a global health priority due to its rising prevalence, including in low- and middle-income countries (LMICs), and the serious associated consequences for mother and child.[1,2] These include pregnancy-induced hypertension (PIH), macrosomia, and stillbirth.[3] Moreover, mothers with gestational diabetes mellitus (GDM) have a higher chance of developing type 2 diabetes (T2DM) and cardiovascular complications postpartum, and metabolic consequences for the child contribute to an intergenerational cycle of disease.[4,5]

Data about diabetes in pregnancy in sub-Saharan Africa (SSA) are limited. A recent review showed that merely 11% of countries had published prevalence data of GDM.[6] Reported rates of GDM prevalence for Africa are in the range of 8.8%–9.3%.[6–8] Within South Africa,

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varying rates of prevalence could be due to differences in population, screening practices, and diagnostic methods.[8,9] Recent international recommendations for universal screening and the lower International Association of Diabetes and Pregnancy Study Groups (IADPSG) diagnostic criteria, distinguishing between overt and true GDM, are not uniformly implemented in South Africa, partly due to resource constraints.[1,10] These discrepancies contribute to the under-reporting and under-managing of diabetes in pregnancy. A 20-year audit at a large urban hospital in South Africa between 1983 and 2002 showed the positive impact of management on maternal and neonatal outcomes for pre-gestational and GDM.[11] Since this publication, the same hospital has introduced oral hypoglycemic agents (OHAs), universal HIV monitoring and treatment, and lower diagnostic criteria for GDM.

OHAs are not currently licensed for use during pregnancy due to lack of long-term safety data, but use of metformin and glibenclamide is endorsed by major South African guidelines and is preferable to insulin in LMICs due to ease of use and affordability.[12] Data from South Africa have shown a higher rate of macrosomia, neonatal hypoglycemia, and perinatal mortality (PMR) in patients treated solely with glibenclamide.[13,14] However, it is not clear whether these findings are a direct drug effect, and generalizability across South Africa is problematic due to disparities in ethnicity, diagnostic tests and criteria, and definitions of glycemic control.

Additionally, understanding the metabolic risk of HIV-infected patients remains critical, since the overall prevalence of glucose metabolism disorders in HIV-infected individuals on antiretroviral therapy (ART) is as high as 25%[15]; pregnancy is an additional provoking factor.[16] Treatment with ART has dramatically improved outcomes in HIV-infected pregnancies. However, the combined effect of hyperglycemia, HIV, and ART exposure on maternal and neonatal outcomes remains unexplored.

Using data from one of the few specialized gestational endocrine clinics in South Africa, the aim of the present study was to investigate maternal and neonatal outcomes of type 1 diabetes (T1DM), T2DM, and GDM, and to explore the impact of HIV infection and exposure to OHAs on these outcomes, in order to create an informed platform to improve care for diabetes in pregnancy in LMICs.

2 | MATERIALS AND METHODS

We retrospectively analyzed medical records of women attending the Gestational Endocrine Clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) for diabetes in pregnancy between 2012 and 2018. CHBAH is a 3000-bed teaching hospital with 1400–1600 deliveries per month in the large urban township of Soweto, with an antenatal HIV prevalence of 30%. The population attending the clinic is predominantly black African. Figure S1 shows a timeline of clinical practice after referral to the clinic. Participants with overt GDM were initiated on insulin based on a clinical decision where the degree of hyperglycemia and presence of complications were taken into account. Excluding this, the first choice of pharmacological treatment for GDM participants was metformin, which was escalated, if necessary, by either adding glibenclamide or transitioning to insulin, a decision based on levels of hyperglycemia and the presence of diabetic complications. Those with T2DM on existing regimes were switched, where necessary, to either metformin and/or glibenclamide; otherwise, treatment was escalated as above. Only women with singleton pregnancies treated for T1DM, T2DM, and GDM diagnosed according to the definitions in Table S1A were included in the present study. See Table S1B for definitions of maternal and neonatal outcomes. The Human Research Ethics Committee, University of the Witwatersrand (M180204) approved this study.

Data were captured directly into an electronic database (REDCap 9.3.1, Vanderbilt University, Nashville, TN, USA), and subsequently imported into STATA 13 (StataCorp, College Station, TX, USA). Baseline characteristics were described using number and percentage for categorical variables and, depending on normality, mean and standard deviation or median and interquartile range (IQR) for continuous variables. To test for normality, a skewness-kurtosis test was performed if the mean and median were substantially different. When analyzing the role of OHAs, participants that presented after a gestational age of 36 weeks or received less than 2 weeks of medical intervention were excluded.

For statistical significance, a Student *t* test or nonparametric Mann-Whitney *U* test was used for continuous outcomes, and chi-squared statistic or Fisher exact test was used for categorical outcomes. For analysis across more than two groups, analysis of variance (ANOVA) or the nonparametric Kruskal-Willis test was used. Statistical significance was set at a two-sided *P* value of <0.05. For statistically significant outcomes, logistic regression analysis was performed using backwards selection following univariate analysis at a significance level of $P<0.20$, considering the following variables: maternal age, initial body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters), weight gain over pregnancy, gestational age at initial presentation, initial glycated hemoglobin (HbA1c), presence of chronic hypertension, HIV, or anemia, multiparity, glycemic control by the third trimester, and exposure to OHAs. We reported those that reached statistical significance by $P<0.05$ in the final model. A positive outcome per variable ratio of 10 was adhered to for regression models.

3 | RESULTS

There were 1071 diabetic pregnancies during the study period: 461 (43.0%) GDM; 408 (38.1%) T2DM; and 202 (18.9%) T1DM (Table 1). Overt GDM constituted 235 (51.0%) of GDM cases. The median gestational age at initial presentation was 15.6–16.1 weeks for those with pre-gestational diabetes and 28.0–29.5 weeks for those with GDM. The T1DM group was younger (mean 26.5 ± 5.6), had a higher initial HbA1c (median 8.3, IQR 7–10.2), higher frequency of retinopathy and nephropathy 17 (8.4%) and 4 (4.7%), respectively, and a lower BMI (29.3 ± 6.6) compared to the GDM and T2DM groups. For T2DM, the median initial HbA1c was 7.6 (IQR 6.4–9.1), for overt GDM it was 7.2 (IQR 6.5–8.8) and for true GDM it was 5.8 (IQR

TABLE 1 Demographic, obstetric, comorbidity, and diabetes characteristics of participants with T1DM, T2DM, and GDM.

					GDM			
	n=202	T1DM	n=408	T2DM	n=235	Overt	n=226	True
Maternal characteristics at initial presentation								
Maternal age (mean, SD)	202	26.9 (5.6)	403	34.0 (5.2)	235	32.4 (5.3)	226	32.9 (6.1)
GA, weeks (median, IQR)	200	15.6 (9.8–23.1)	407	16.1 (11.0–24.3)	235	28.0 (21.7–32)	224	29.5 (26.6–33.0)
BMI (mean, SD)	112	29.3 (6.6)	207	35.1 (8.1)	116	36.6 (7.9)	105	35.5 (8.5)
BP (mean, SD)	190	123/74.7 (16.0–11.5)	396	125.9/76.5 (15.0–11.6)	223	123.0/73.6 (14.0–10.9)	213	120.5/71.1 (14.2–9.6)
Obstetric history								
Primigravida (n, %)	200	69 (34.5)	407	21 (5.2)	235	20 (8.5)	226	22 (9.7)
Previous pregnancy losses (n, %)	200	49 (24.5)	407	147 (36.1)	235	94 (40.0)	226	99 (43.8)
Comorbidities at presentation								
Chronic hypertension (n, %)	202	27 (13.4)	408	136 (33.3)	234	35 (15.0)	225	36 (16.0)
HIV positive (n, %)	153	45 (29.4)	320	95 (29.7)	212	25 (11.8)	203	47 (23.1)
Anemia (n, %)	199	46 (23.1)	403	56 (13.9)	455	25 (10.7)	455	34 (15.4)
Characteristics of diabetes								
Age at diagnosis, y (median, IQR)	196	21 (16–25)	383	31 (27–34)		NA		N/A
Initial Hba1c, % (median, IQR)	191	8.3 (7–10.2)	393	7.6 (6.4–9.1)	215	7.2 (6.5–8.8)	207	5.8 (5.4–6.3)
Control at initial presentation (n, %)	86	48 (55.8)	133	63 (47.3)	136	NA	78	NA
Control at third trimester[a] (n, %)	147	141 (95.9)	351	335 (95.4)	207	187 (90.3)	196	191 (97.5)
Target organ damage (n, %)								
Retinopathy	202	17 (8.4)	407	15 (3.7)	234	3 (1.3)	226	2 (0.9)
Nephropathy	85	4 (4.7)	168	1 (0.6)	81	0	85	0
Therapy								
Exposure to metformin (n, %)	202	14 (6.9)	408	351 (86.0)	235	120 (52.8)	226	171 (75.7)
Exposure to glibenclamide (n, %)	202	1 (0.5)	408	151 (37.0)	235	80 (34.0)	226	76 (33.6)
Exposure to insulin (n, %)	202	202 (100.0)	408	270 (66.2)	235	134 (57.0)	226	25 (11.1)

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; GDM, gestational diabetes; SD, standard deviation; GA, gestational age; IQR, interquartile range; BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; MBG, mean blood glucose.
[a]Control in third trimester=MBG <7.1 in third trimester.

5.4–6.3). All four groups were overweight by mean BMI at presentation. Overall, 234 (21.8%) women in the cohort had hypertension, with the T2DM group accounting for the highest prevalence at 136 (33.3%).

All patients with T1DM received insulin. A total of 261 (56.6%) participants with GDM and 347 (85.1%) participants with T2DM were exposed to OHAs. Of these, 31 (11.9%) in the GDM group and 217 (62.5%) in the T2DM group were switched to insulin for insufficient glycemic control. Of all groups, 854 (94.9%) participants were controlled by the third trimester, with the lowest control in the overt GDM group at 187 (90.3%).

In terms of maternal outcomes, loss to follow-up was highest in the T1DM (n=37, 18.3%) and true GDM (n=47, 20.8%) groups (Table 2). The number of cesarean deliveries was 641 (72.2%) and the rate was highest in the T2DM group at 275 (78.4%). The rate of PIH was 9.2% (n=98) across all groups; the combined rate of pre-eclampsia and eclampsia was 2.7% (n=29). The rates of miscarriage and maternal mortality were highest in the T1DM group at 14 (6.9%) and 3 (1.5%),

TABLE 2 Neonatal and maternal outcomes of participants with T1DM, T2DM, and GDM.[a]

					GDM				
	n	T1DM	n	T2DM	n	Overt	n	True	*P* value
Lost to follow-up	202	37 (18.3)	408	51 (12.5)	235	31 (13.2)	226	47 (20.8)	0.020[b]
Maternal outcomes									
CD	155	104 (67.1)	351	275 (78.4)	199	142 (71.4)	177	120 (67.8)	0.015[b]
Miscarriage	202	14 (6.9)	408	12 (2.9)	235	4 (1.7)	226	2 (0.9)	0.001[b]
PIH	202	23 (11.4)	408	31 (7.6)	235	27 (11.5)	226	17 (7.5)	0.198
Pre-eclampsia/ eclampsia	202	5 (2.5)	408	13 (3.2)	235	2 (0.9)	226	9 (4.0)	0.749
Maternal mortality	202	3 (1.5)	408	0 (0)	235	1 (0.4)	226	0 (0)	0.017[b,c]
Neonatal outcomes									
Prematurity	165	57 (34.6)	358	117 (32.9)	212	65 (31.9)	195	41 (22.8)	0.065
Macrosomia	141	12 (8.5)	333	28 (8.4)	195	24 (12.3)	174	7 (4.0)	0.042[b]
LBW	141	41 (29.1)	333	59 (17.7)	195	26 (13.3)	174	23 (13.2)	0.001[b]
Major congenital anomaly	115	2 (1.7)	282	2 (0.7)	172	2 (1.2)	147	0 (0)	0.450[c]
PNM	149	10 (6.7)	346	15 (4.3)	201	4 (2.0)	178	3 (1.7)	0.053[c]
Stillbirth	149	8 (5.4)	346	11 (3.2)	201	4 (2.0)	178	3 (1.7)	0.225

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; GDM, gestational diabetes; CD, cesarean delivery; PIH, pregnancy-induced hypertension; LBW, low birth weight; PNM, perinatal mortality.
[a]Values are given as number (percentage).
[b]Statistical significance ($P<0.05$).
[c]Fisher exact test.

respectively. Initial HbA1c was the significant predictor for miscarriage when performing regression analyses (odds ratio [OR] 1.2, 95% confidence interval [CI] 1.13–1.55, $P=0.001$). When investigating "any obstetric complication" (the combined rate of miscarriage, hypertensive disorders of pregnancy, urinary tract infection, polyhydramnios, oligohydramnios, maternal death), no significant differences were found between the groups.

Of offspring, 747 (92.6%) had an APGAR score ≥7 at 1-minute postpartum (Table 2). The rate of prematurity (n=237, 30.9%) was significantly lower in the true GDM group (n=41, 22.8%) than the other groups, but not when correcting for initial HbA1c (OR 1.17, 95% CI 1.08–1.26, $P<0.001$) and lack of glycemic control in the third trimester (OR 2.36, 95% CI 1.24–4.47, $P=0.009$). Macrosomia, with a prevalence of 71 (8.4%) in the whole cohort, was highest in the overt GDM group (n=24, 12.3%) and significantly lower in the true GDM group (n=7, 4.0%). For both low birth weight (LBW), which was highest in the T1DM group, and macrosomia, the difference between the groups was no longer significant when adjusting for maternal BMI (OR 0.95, 95% CI 0.91–0.98, $P=0.002$; OR 1.05, 95% CI 1.00–1.09, $P=0.027$, respectively, for LBW and macrosomia), with multiparity (OR 0.61, 95% CI 0.37–0.99, $P=0.047$) and hypertension (OR 1.85, 95% CI 1.04–3.29, $P=0.036$) being the other two significant predictors for LBW. Perinatal mortality (PNM) was significantly higher in the T1DM group (n=10, 6.7%) when compared to T2DM and the GDM group in its entirety ($P=0.019$), although HIV was the main statistical contributor in our model (OR 5.00, 95% CI 2.06–12.15, $P<0.001$). The T1DM group also had the most major congenital anomalies at 1.7% (n=2). Additional neonatal outcomes in the cohort included hypoglycemia (n=10, 1.2%), respiratory distress syndrome (n=8, 1.0%), and jaundice (n=4, 0.5%), with no significant differences between groups.

When considering predictive factors for poor neonatal outcome (prematurity, LBW, macrosomia, PNM) per type of diabetes, initial HbA1C was found to significantly predict poor neonatal outcome for T1DM. In the GDM group, initial HbA1C (OR 1.17, 95% CI 1.00–1.37, $P=0.044$), gestational age at presentation (OR 0.95, 95% CI 0.91–0.98, $P=0.005$), and lack of glycemic control by the third trimester (OR 3.12, 95% CI 1.21–9.08, $P=0.019$) were significant predictors for poor neonatal outcome. Within the T2DM group, no significant predictors were found.

When comparing outcomes for the group exposed to OHAs (metformin monotherapy and glibenclamide exposure) to insulin monotherapy (Table 3), the characteristics with significant differences between groups were weight gain over pregnancy and initial HbA1c (both higher in the insulin group), and the number achieving glycemic control by the third trimester (lower in the insulin group). In addition, BMI at first presentation in those with T2DM was significantly higher in the glibenclamide group versus the insulin monotherapy group (34.6 vs 31.6; IQR 31.6–41.8, 27.6–36.2). No significant differences were found in maternal outcomes between treatment groups.

TABLE 3 Comparison of baseline characteristics in metformin monotherapy and glibenclamide-exposed groups vs insulin monotherapy.

	n	Exposed to metformin monotherapy[a] (n=106)	n	Exposed to glibenclamide[b] (n=296)	n	Exposed to insulin monotherapy (n=332)	*P* value
T2DM	408	29 (7.1)		100 (24.5)		46 (11.3)	<0.001[a], <0.001[b]
GDM	461	106 (23.0)		124 (26.9)		106 (23.0)	
Age at presentation (median, IQR)							
T2DM	26	35 (28–38)	99	34 (30–37)	46	35 (31–38)	0.518[a], 0.391[b]
GDM	106	33 (39–37)	124	33 (29–37)	106	34 (29–38)	0.660[a], 0.593[b]
BMI at first presentation (median, IQR)							
T2DM	12	32.95 (27.3–42.1)	55	34.6 (31.6–41.8)	29	31.6 (27.6–36.2)	0.626[a], 0.021[b,c]
GDM	51	33.8 (30.3–40)	55	36.7 (31.6–41.5)	50	35.35 (31.2–41.1)	0.250[a], 0.674
Weight gain over pregnancy (median, IQR)							
T2DM	28	3.5 (0.45–6.4)	97	3.3 (1–7)	43	7.1 (2.5–11)	0.008[a,c], 0.003[b,c]
GDM	100	0.95 (−0.2 to 2.5)	117	2.5 (0–6)	101	5 (1.9–9.8)	<0.001[a,c], <0.001[c]
Initial HbA1c (median, IQR)							
T2DM	27	6.2 (5.5–7.5)	97	6.7 (5.9–7.6)	45	7.9 (6.6–9.5)	0.002[a,c],
GDM	99	5.8 (5.4–6.2)	119	6.2 (5.8–6.9)	94	8.55 (7–10.1)	<0.001[b,c], <0.001[a,c], <0.001[b,c]
Number controlled at third trimester[d] (n, %)							
T2DM	23	22 (95.7)	92	92 (100)	40	36 (90.0)	0.644[a], 0.008[b,c]
GDM	96	96 (100)	119	117 (98.3)	96	86 (89.6)	0.002[a,c], 0.007[b,c]

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; IQR, interquartile range; HbA1c, glycated hemoglobin; MBG, mean blood glucose.
[a]*P* value for comparison between metformin monotherapy and insulin monotherapy groups.
[b]*P* value for comparison between glibenclamide and insulin monotherapy groups.
[c]Statistical significance (*P*<0.05).
[d]Control in third trimester=MBG <7.1 in third trimester.

T2DM: For the participants with T2DM, glibenclamide exposure was associated with a significantly higher prevalence of macrosomia in the T2DM group when compared to the insulin monotherapy group (n=10 [12.2%] vs 0, *P*=0.030) (Fig. 1). Participants with T2DM who were on glibenclamide but were switched to insulin (n=51, 12.5%) also had higher rates of macrosomia compared to insulin monotherapy (n=9 [19.6%] vs 0, *P*=0.003). PNM did not significantly differ between the groups.

GDM: In the GDM metformin-exposed group, a higher number of offspring had LBW (17 [20.0%] vs 7 [8.4%], *P*=0.032) and a lower number presented with macrosomia than in the insulin monotherapy group (1 [1.2%] vs 9 [10.8%], *P*=0.009) (Fig. 1). There were too few cases of LBW and macrosomia in the GDM group to pursue regression analysis. As with T2DM, PNM was not significantly different between groups.

HIV infection was found in 212 (24.0%) women in the cohort, the highest in the T1DM group with 45 (29.4%) participants and the lowest in the GDM group with 72 (17.4%) participants. Maternal weight and BMI at first presentation, hypertension, gestational age at presentation, and glycemic control in the third trimester were similar among HIV-infected and uninfected participants. However, the HIV-infected group had a significantly higher rate of anemia (61 [29.2%] vs 65 [9.7%], *P*≤0.001) and a lower initial HbA1C (median 6.7, IQR 5.6–8.5 vs median 7.1, IQR 6.1–8.7, *P*=0.013). A lower rate of macrosomia was observed in the HIV-infected GDM group (0 vs 28 [10.0%], *P*=0.012) (Fig. 2). However, PNM was significantly higher in the HIV-infected T1DM (5 [14.7%] vs 0, *P*=0.002) and GDM groups (4 [7.0%] vs 3 [1.1%], *P*=0.017).

4 | DISCUSSION

In this large retrospective study: (1) initial HbA1c, BMI, and glycemic control were important predictors of maternal and neonatal outcomes; (2) initial BMI was high in all diabetes groups, including T1DM; (3) glibenclamide exposure was associated with higher macrosomia rates; and (4) HIV infection was associated with higher PNM.

The rates of key adverse outcomes including birth weight (macrosomia and LBW), prematurity, fetal anomalies, hypertensive disorders of pregnancy, maternal mortality, and PNM were similar to a previous audit from 1992 to 2002 at our clinic[11] and to another recent South African audit.[17] However, macrosomia, PNM, and prematurity remain inferior to respective rates in South Africa for non-diabetic pregnancies.[18–22] The rate of cesarean delivery (n=641, 72.2%) was much higher in the present study than the estimated rate of cesarean delivery at CHBAH of 39.8% in non-diabetic pregnancies,[23] but only

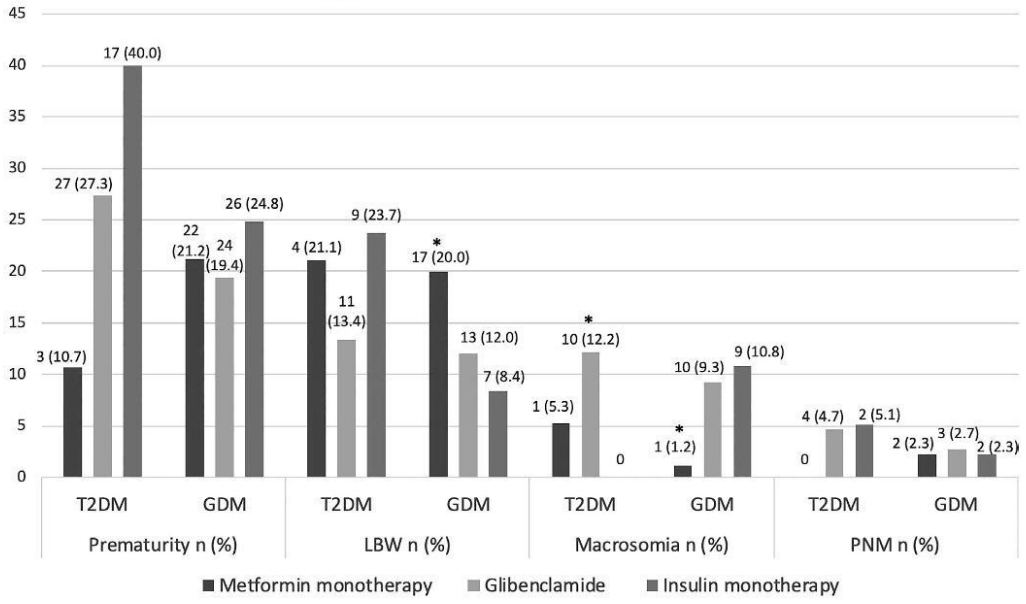


FIGURE 1 Neonatal outcomes in metformin monotherapy and glibenclamide-exposed groups vs insulin monotherapy in participants with T2DM and GDM. T2DM, type 2 diabetes; GDM, gestational diabetes; LBW, low birth weight; PNM, perinatal mortality.

moderately higher than the 1992–2002 audit of diabetic pregnancies (62%), a difference that could be due to an overall increase in rates of cesarean delivery over this period.

When compared to two recent studies from the UK, rates of cesarean delivery and PNM, which were in the range of 1.2–2.7%, were higher in our pre-gestational groups.[24,25] However, comparisons to high-income countries (HIC) as a whole are complicated by differences in population, study design, diagnosis, and management.[26,27]

Obesity, so common in our cohort and in South African women in general, not only augments the metabolic burden of pregnancy, it also increases the risk of adverse outcomes.[28,29] In our cohort, BMI was found to be a significant predictor for macrosomia. Even the T1DM group had a high BMI at presentation, in contrast to the traditional set of characteristics attributed to T1DM. This indicates that the rising public health burden of metabolic syndrome is impacting even unexpected population groups.

The T1DM group showed higher rates of fetal anomaly, maternal mortality, miscarriage, and PNM than the other groups. This is most likely driven by the severity of hyperglycemia, a trend that is consistent with the international literature.[17,30] However, our high rates of PNM, compared to international studies[24,25] and South African background rates, reveals a critical area for improvement for women with pre-gestational diabetes, especially considering the unacceptably high initial HbA1c for both T1DM and T2DM and the lack of glycemic control in 111 (49%) participants at initial presentation. This highlights the need for improved counselling and family planning for women of childbearing age with diabetes, for optimization of glycemic control.

Notably, initial HbA1c and outcomes including prematurity and stillbirth were similar for the overt GDM and T2DM groups, but rates of macrosomia, PIH, and congenital anomalies were higher in the former, indicating that the overt GDM group most likely consisted of undetected T2DM. This highlights the importance of increased preconceptional detection of T2DM through strengthening of diabetic health systems in LMICs. The true GDM group had fewer adverse outcomes than the overt GDM group, which consisted of more than 50% of GDMs. This low rate of adverse outcomes and the 97.5% glycemic control attained indicates the successful treatment of this group, predominantly with metformin (n=171, 75.7%). The discrepancy with overt GDM reiterates that severity of initial hyperglycemia is an important predictor for worse outcomes and lack of glycemic control.

The HIV prevalence rate in our cohort of 212 (24.0%), though high in an international context, is similar to regional estimates of 28%.[31] The lower HbA1C in the HIV-infected population was unexpected, as this group had higher rates of anemia, which results in higher HbA1C if caused by iron deficiency anemia.[32] However, this relationship is complex, since the cause of anemia in our cohort was unknown and HbA1C has previously been found to underreport hyperglycemia in HIV-positive patients.[33] On the other hand, the observation that first visit mean blood glucose (MBG) was also lower in our HIV-infected group

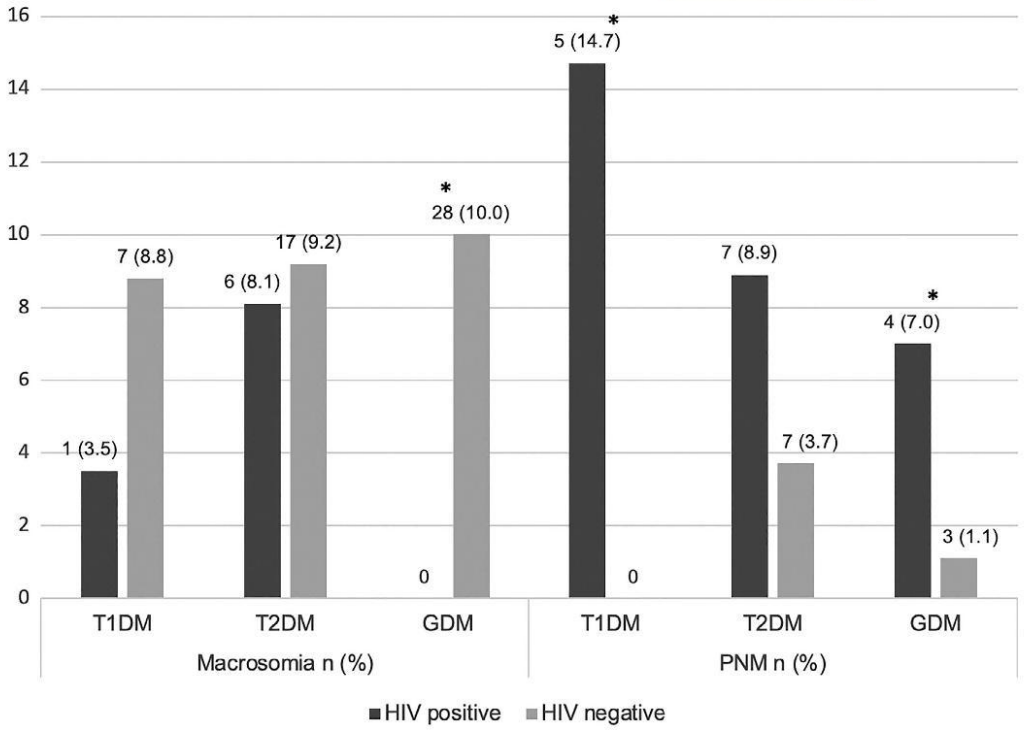


FIGURE 2 Neonatal outcomes in HIV-infected and uninfected participants with T1DM, T2DM, and GDM. T1DM, type 1 diabetes; T2DM, type 2 diabetes; GDM, gestational diabetes.

tends to affirm better control in this group. One possible explanation is that participants with known HIV infection may have increased contact with health services, improving lifestyle measures,[34] although this was not reflected in earlier presentation for the HIV-infected group.

We found a higher PNM rate of 9.1% (16 cases) in HIV-infected participants. Although a background rate for PNM in HIV-infected patients on ART is not known, this was higher than the South African national PNM estimate for the general population (3.3%).[18] Previous studies, in both HICs and LMICs, have found an increased PNM in HIV-infected pregnancies.[35,36] When considering the consequences of HIV infection, the contribution of chronic generalized inflammation, indirect HIV-associated conditions, and side-effects of ART are difficult to unravel.[37] The higher rates of anemia in the HIV-infected group may have contributed to the high PNM rates, given that anemia is associated with higher neonatal morbidity and mortality.[38]

All of the HIV-infected participants would have likely been placed on a lifelong ART regimen, in adherence with "test and treat" guidelines.[39] However, a lack of documented information on both severity of HIV and exact use of ART prohibited us from further analyses in these areas. Regardless, the high risk of PNM and higher rates of anemia in this combined diabetic and HIV-infected population marks them as a group of patients that could benefit from close monitoring and further research.

An unusually high number of GDM patients required pharmaceutical treatment to achieve glycemic control, which may be attributable to the high proportion of overt GDM women and the use of risk-factor based screening. Importantly, the use of OHAs was adequate to achieve glycemic targets in 261 (88.1%) GDM pregnancies, which is relevant in an LMIC setting due to their convenience and affordability. Evidence regarding safety is therefore highly important.

For both T2DM and GDM, maternal outcomes were not significantly different between patients exposed to glibenclamide and metformin compared to the gold standard of insulin. However, rates of macrosomia were higher among those exposed to glibenclamide (n=10 [12.2%] vs 0) despite a significantly lower initial HbA1c and better glycemic control in the third trimester in this group. This was in line with results from a recent meta-analysis observing glibenclamide to be inferior to both metformin and insulin in terms of rates of macrosomia and neonatal hypoglycemia.[13] It has been suggested that the effect of

glibenclamide may be due to a direct drug effect via placental transfer, but the relationship could also be due to the higher initial BMI in the glibenclamide group.[21]

Reassuringly, we found no significant relationship between use of OHAs and prevalence of congenital anomalies, but a limited number of patients with T2DM (n=115, 25.8%) and GDM (n=9, 2.6%) were exposed to the drugs in the first trimester. Neither OHA negatively influenced PNM when compared to insulin. This contributes to the evidence that these agents may be a safe alternative to insulin in our setting.[13,40]

Limitations of our study are related to the retrospective study design, resulting in missing data due to under-reporting in files, including BMI, HIV status and treatment details, possible rates of pre-eclampsia/eclampsia, and some neonatal outcomes (hypoglycemia, jaundice, and respiratory distress syndrome). Moreover, we did not have data on pre-pregnancy BMI and the use of BMI at initial presentation unadjusted for gestational age could have resulted in an overestimation of rates of obesity and overweight. It is important to note that the rate of miscarriage is likely an underestimation due to a lack of information on miscarriages in early gestation, since participants commonly presented during the second and third trimesters. Regression analyses could not be performed for all outcomes regarding HIV and treatment groups because of low positive outcomes. Loss to follow-up of 166 (15.4%) participants may have resulted in bias, although the only significant differences in baseline characteristics were younger age at presentation and higher primiparity rates in the group lost to follow-up.

The high rate of obesity and the significant impact of initial hyperglycemia and glycemic control on adverse outcomes emphasizes the need for improved pre-conception care, including increasing awareness of both obesity and diabetic health risks, earlier detection of T2DM, implementation of lifestyle interventions, and, in the case of pre-gestational diabetes, improved pre-conceptional glycemic control. HIV-infected women with diabetes in pregnancy demonstrated a higher rate of PNM, which warrants further exploration. In addition, a greater understanding of the reasons for the increased rate of macrosomia associated with glibenclamide exposure is especially relevant in a LMIC setting, where the affordability and accessibility of OHAs make them an attractive alternative to insulin. These insights address gaps in diabetes health care in pregnancy that are meaningful for other LMICs where the double burden of infectious and non-communicable disease is impacting maternal and child health.

AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization of the study. LMS and VN contributed equally to planning, data capturing and analysis, and manuscript writing and editing. KKG, NSL, and SAN contributed to planning, interpretation of data, and manuscript writing and editing. KH contributed to the database, and manuscript writing and editing.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Flow chart of clinical practice for patients attending the gestational endocrine clinic. GA, gestational age; GDM, gestational diabetes; OGTT, oral glucose tolerance test.

Table S1. (A) Definition and criteria for diagnosis of diabetes in pregnancy, including T1DM, T2DM, and GDM. (B) Definition of maternal and neonatal outcomes.

APPENDIX J: PUBLISHED PAPER II

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SAMJ RESEARCH

Maternal and neonatal outcomes following the introduction of oral hypoglycaemic agents for gestational diabetes mellitus were comparable to insulin monotherapy in two historical cohorts

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Background. Gestational diabetes mellitus (GDM), a disorder of glucose intolerance first encountered during pregnancy, has far-reaching implications for both mother and child. Insulin therapy remains the 'gold standard' of care, with oral hypoglycaemic agents (OHAs) increasingly being viewed as potential alternatives.
Objectives. To compare maternal and neonatal outcomes in two cohorts of women with GDM exposed to either insulin monotherapy or OHAs.
Methods. A retrospective medical record review at Chris Hani Baragwanath Academic Hospital in South Africa was conducted for women with GDM diagnosed using the 100 g oral glucose tolerance test and/or random capillary blood glucose >11.1 mmol/L in 2010 - 2014. The findings were compared with a previous audit at the same clinic for the period 1992 - 2002. Variables of interest included maternal demographics, maternal comorbidities, glycaemic indices, treatments used during pregnancy, and obstetric and neonatal outcomes.
Results. A total of 192 women with GDM were identified for 2010 - 2014, and there were 348 women in the previous audit (1992 - 2002). Baseline characteristics and outcomes of women in the two cohorts were similar apart from earlier presentation (mean (standard deviation) gestational age (GA) 27 (7.5) weeks v. 28.3 (6.4) weeks; p=0.04), lower GA at delivery (36.3 (3.6) weeks v. 37 (1.6) weeks); p=0.008) and lower macrosomia rates (12.5% v. 4.9%; p=0.011) in the later cohort. When comparing the individual OHAs against insulin in the later cohort, both agents were comparable to insulin in terms of maternal and neonatal outcomes.
Conclusions. This study contributes to the paucity of data on the safety of OHAs in GDM pregnancy in terms of maternal and neonatal outcomes. OHAs were shown to be an effective alternative to insulin for women with GDM in whom lifestyle measures fail, particularly in a resource-poor setting.

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The landscape of gestational diabetes mellitus (GDM) has remained dynamic, with the prevalence, definition, screening protocols, diagnostic criteria and therapeutic modalities continuously changing from as early as the 1960s. Most recently, GDM has been defined as a disorder of glucose intolerance first encountered during pregnancy that is not clearly overt diabetes.[1] GDM has far-reaching and well-known ramifications for both mother and infant.[2] Global prevalence estimates are 16.9%,[3,4] with recent studies in South Africa (SA) showing prevalence figures ranging from 9.1% to 25.8%.[5,6]

Traditionally, GDM is initially managed with dietary and lifestyle interventions alone. However, insulin is introduced if glycaemic goals are not achieved.[7-9] Oral hypoglycaemic agents (OHAs) are an attractive alternative if lifestyle measures fail, particularly in resource-poor countries, because of their low cost and ease of administration. However, their use for GDM is surrounded by much debate regarding efficacy and safety, and they are recommended by some guidelines[9-11] and omitted in others.[12] One meta-analysis that included 13 studies utilising various diagnostic tests and criteria for GDM, with representation from both high- and low-income countries, investigated the safety of OHAs compared with insulin when treating GDM.[13] It demonstrated a higher preterm birth rate (relative risk (RR) 1.51) in patients exposed to metformin monotherapy, with a reduced risk of both gestational hypertension (RR 0.54) and lowered postprandial glucose levels. A further meta-analysis of 15 studies showed an increased risk of macrosomia and neonatal hypoglycaemia in patients exposed to glibenclamide.[14] Research from SA has generally shown reassuring safety outcomes in terms of fetal anomalies and maternal glycaemic control for both

metformin and glibenclamide in patients with both type 2 diabetes in pregnancy and GDM.[15,16] However, a recent study showed notably increased perinatal mortality (PNM) in type 2 diabetes patients exposed to glibenclamide, which could not be explained.[15]

Objectives

To compare the fetal and maternal outcomes of two cohorts of patients with GDM treated at Chris Hani Baragwanath Academic Hospital (CHBAH) in Johannesburg, SA, a decade apart, where the first cohort (1992 - 2002) was exposed to insulin monotherapy and the more recent group (2010 - 2014) to one or more treatment combinations including OHA monotherapy (metformin alone), OHA dual therapy (metformin plus glibenclamide), or insulin monotherapy. Furthermore, the outcomes in the 2010 - 2014 cohort were compared between the insulin monotherapy group v. those exposed to OHAs.

Methods

Study population

This study retrospectively compared two cohorts of women attending the gestational endocrine clinic at CHBAH for GDM, the first between 1992 and 2002 and the second between 2010 and 2014. Characteristics and outcomes of the 1992 - 2002 cohort, where patients were exclusively exposed to insulin monotherapy, have been published previously[17] and are included for the purposes of the present study. A secondary analysis in the later cohort was performed with a medical record review of women exposed to OHAs v. insulin monotherapy, and their outcomes were compared.

CHBAH, a 3 000-bed teaching hospital with between 1 400 and 1 600 deliveries per month, serves the population of the sprawling township of Soweto, SA, ethnically predominantly black. All patients with singleton pregnancies treated at CHBAH for GDM between 1992 and 2002 and between 2010 and 2014, either diagnosed by random capillary blood glucose ≥11.1 mmol/L or fulfilling National Diabetes Data Group (NDDG) criteria following a 3-hour 100 g oral glucose tolerance test (OGTT), were included in the study. Both cohorts had similar screening strategies for GDM. Participants with twin pregnancies and other subtypes of diabetes, including pregestational diabetes, pancreatic diabetes and steroid-induced diabetes, were excluded. A control group was defined as patients who presented after 36 weeks' gestational age (GA) or received <2 weeks of medical intervention, and these patients were excluded for the purposes of the study. The study protocol was approved by the Human Research Ethics Committee of the University of the Witwatersrand (ref. no. M180316).

Definitions

Diabetes was considered gestational if the diagnosis was first made during pregnancy in women referred because of the presence of risk factors for diabetes: persistent glycosuria, first-degree family history of diabetes, previous unexplained perinatal losses, previous GDM, or history of a macrosomic baby. Selective screening for GDM was performed between 24 and 28 weeks' GA. GDM was diagnosed using a 100 g 3-hour OGTT and NDDG criteria, which required two or more of the following plasma glucose levels for the diagnosis of GDM: fasting plasma glucose (FPG) ≥5.8 mmol/L, 1-hour post-glucose load ≥10.6 mmol/L, 2-hour post-glucose load ≥9.2 mmol/L, or 3-hour post-glucose load ≥9.2 mmol/L. GDM was classified as 'overt' if the baseline FPG was ≥7.0 mmol/L.

Maternal characteristics and outcomes were defined as follows. GA was determined using an early ultrasound scan. Glycaemic control: mean blood glucose (MBG) <7.1 mmol/L for the third trimester, calculated as a mean of daily self-monitored six-point profiles, including both fasting and 1-hour post-prandial. Dietary failure: failing to achieve glycaemic control after 2 weeks of dietary intervention. Diabetic nephropathy: microalbuminuria, with a microalbumin-creatinine ratio >30 mg/mmol on repeated testing, in the absence of urinary tract infection and other renal disease. Body mass index (BMI): the patient was considered obese if the BMI was >30 kg/m^2 at the first visit. Anaemia: haemoglobin concentration <11 g/dL. HIV-positive: based on results from rapid or antibody tests before or at the time of initial presentation. Obstetric complications included premature labour, caesarean section (CS), miscarriage, hypertensive disorders of pregnancy (HDP), urinary tract infection, polyhydramnios, oligohydramnios and maternal death. Hypertension was diagnosed when blood pressure readings were elevated (>140/90 mmHg) for at least two measurements. This was further categorised into HDP and chronic hypertension. HDP was further classified into pregnancy-induced hypertension (PIH), where hypertension developed after 20 weeks of pregnancy, pre-eclampsia, where PIH was found in the presence of proteinuria on more than one occasion, and eclampsia, where PIH was found in the presence of proteinuria and seizures. Chronic hypertension was defined as hypertension diagnosed prior to the pregnancy. Maternal hypoglycaemia was defined as one or more episodes of hypoglycaemia severe enough to necessitate the administration of intravenous dextrose.

Neonatal outcomes were defined as follows. Prematurity/preterm birth: delivery at <37 completed weeks' GA. Miscarriage: loss in pregnancy occurring at <28 completed weeks' GA. Stillbirth: loss in pregnancy occurring at ≥29 weeks' GA. Early neonatal death: neonatal death occurring in the first week. PNM: the number of stillbirths and early neonatal deaths out of the total number of deliveries (successful deliveries and stillbirths). Low birth weight (LBW): birth weight <2 500 g. Macrosomia: birth weight ≥4 000 g. Major congenital anomalies: spinal, cardiac, central nervous system, renal or digestive system anomalies.

Definitions applied to both study cohorts except for MBG and nephropathy, where the MBG in the earlier cohort was calculated as a mean of the fasting and 1-hour plasma glucose levels, and nephropathy was defined as dipstick urinalysis positive for proteinuria (protein >0.3 g/L) on repeated testing.

Clinical practice

The specialist clinic at CHBAH was established in 1983 and is staffed by a physician, an obstetrician, a paediatrician and a diabetes nurse educator. The initial visit comprised a full clinical assessment with ultrasound and treatment initiation. Initial ultrasound was used to assess GA and to rule out any major fetal anomalies. All patients were screened for target organ damage, including retinopathy and nephropathy. Newly diagnosed women with GDM (as per the NDDG criteria) were hospitalised for glycaemic stabilisation. Patients were taught blood glucose monitoring by the nurse educator. Fingerprick capillary blood samples were obtained using lancets, and blood glucose levels were read using various glucometers for the later cohort. In the earlier cohort, glucose levels were read visually using Haemo-glukotest 20-800R strips (Roche Diagnostics, Germany). Dietary advice was provided on an ongoing basis by a dietician and/or a nurse educator, with a 2-week trial of dietary monotherapy being prescribed where the fasting glucose level was <7.0 mmol/L. For the period 1990 - 2010, insulin monotherapy was prescribed in the event of dietary failure. From 2010 onward, OHAs (metformin and/or glibenclamide) were introduced as follows: metformin monotherapy was prescribed in the event of dietary failure, then therapy was

escalated with either the addition of glibenclamide or a switch to insulin monotherapy. Insulin was initiated at the outset in patients whose fasting glucose exceeded 7.0 mmol/L or if maternal or fetal complications were present. Glycaemic targets were <5.3 mmol/L for fasting glucose and <7.8 mmol/L for a 1-hour postprandial reading for the later cohort, levels between 4 and 7 mmol/L being accepted for the earlier cohort. Combined MBG, which included an average of the fasting and 1-hour postprandial readings of <7.1 mmol/L, was included to assess glycaemic control as per institutional practice. Patient-monitored daily six-point profiles were also assessed based on Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) criteria.[11,18]

Following the initial assessment, patients were seen fortnightly or sooner if indicated. At each visit, patients were clinically assessed, the results of home blood glucose monitoring were noted, and treatment was adjusted if necessary. Ultrasound scans were repeated at intervals to assess fetal growth and anomalies. A point-of-care glycated haemoglobin (HbA1c) test was performed monthly using the DCA Vantage Analyzer (Siemens, Germany), which had been standardised and was traceable to the Diabetes Complications and Control Trial. All patients had an HbA1c test within a month prior to delivery. After 32 weeks' GA the visits were weekly until 37 - 38 completed weeks, when labour was induced. Time and mode of delivery were dictated by obstetric factors and glycaemic control, with the goal of 38 completed weeks of pregnancy unless complications arose. During labour, glucose levels were monitored hourly and controlled using dextrose-insulin infusions. Oral and/or insulin therapy was stopped postpartum, and only reinitiated based on results from inpatient self-glucose monitoring. All GDM patients not requiring ongoing therapy were asked to return for a 6-week 75 g OGTT at the maternity clinic. All neonates were assessed for clinical or biochemical abnormalities by paediatric staff in the neonatal unit and managed accordingly.

The control group defined above was excluded for the purposes of this study

Statistical analysis

Data were captured and entered directly into an electronic database (REDCap, USA), cleaned and imported into Stata 13 (StataCorp, USA) for statistical analysis. Comparison of the 1992 - 2002 v. 2010 - 2014 cohort was expressed as numbers and percentages for categorical variables and means and standard deviations (SDs) for continuous variables. Comparisons between the two cohorts were made using an online statistical calculator, MedCalc (version 16.4.3; www.medcalc.org), where a χ^2 test was used for categorical outcomes and a *t*-test was employed for the purpose of continuous variables, to determine statistical significance.

Baseline characteristics of the 2010 - 2014 cohort were described using numbers and percentages for categorical variables and, depending on normality, means and SDs or medians and interquartile ranges (IQRs) for continuous variables. To test for normality, a skewness-kurtosis test was performed if the mean and median were substantially different. Statistical significance testing in the 2010 - 2014 group was done using Student's *t*-test or the non-parametric Mann-Whitney *U*-test for continuous outcomes, and a χ^2 statistic or Fisher's exact test for categorical outcomes. For analysis across more than two groups, analysis of variance (ANOVA) or the non-parametric Kruskal-Willis test was used. Statistical significance was set at $p<0.05$.

Results

Comparison of the two cohorts of GDM patients

Maternal profiles

Table 1 presents maternal profiles of the 348 GDM patients in the period 1992 - 2002 and the 192 GDM patients in the period 2010 - 2014. The two cohorts were comparable in terms of maternal age at presentation, overall rates of hypertension (20.7% v. 14.4%; $p=0.06$) and the significant decrease from HbA1c at first booking to HbA1c within a month of delivery (earlier cohort mean (SD) 7.1% (1.7) to 6.6% (1.2); $p=0.0001$ and later cohort 7.8% (1.8) to 7.1% (1.4);

Table 1. Comparison of maternal characteristics, glycaemic control and outcomes between 1992 - 2002 and 2010 - 2014 gestational diabetes mellitus cohorts diagnosed by 100 g oral glucose tolerance test and random capillary blood glucose

	1992 - 2002 cohort (*N*=348)	2010 - 2014 cohort (*N*=192)	*p*-value
Maternal characteristics			
Maternal age at initial presentation (years), mean (SD)	33.9 (5.2)	33.0 (5.2)	0.06
GA at first presentation (weeks), mean (SD)	28.3 (6.4)	27.0 (7.5)	0.04*
Hypertension,† *n* (%)	50 (14.4)	39 (20.7)	0.06
Target organ damage, *n* (%)			
Retinopathy	5 (1.4)	2 (1.05)	0.72
Diabetic nephropathy	1 (0.3)	0	-
Glycaemic control and therapeutics			
HbA1c (%), mean (SD), at			
First booking	7.1 (1.7)	7.8 (1.8)	-‡
Within a month of delivery	6.6 (1.2)	7.0 (1.2)	-‡
Controlled by third trimester (by MBG), *n* (%)	-	139 (85)	-
Dietary therapy alone, *n* (%)	17 (4.9)	4 (2.1)	0.182
OHA exposure, *n* (%)			
Metformin	n/a	84 (43.7)	-
Glibenclamide	n/a	46 (23.4)	-
Switched from OHA to insulin	n/a	15 (7.8)	-
Controlled by delivery on OHA alone, *n* (%)	n/a	59 (95)	-
Insulin monotherapy, *n* (%)	331 (95.1)	99 (52.6)	<0.0001*

GA = gestational age; SD = standard deviation; HbA1c = glycated haemoglobin; MBG = mean blood glucose; OHA = oral hypoglycaemic agent; n/a = not applicable.
*Statistically significant ($p<0.05$).
†Includes all forms of hypertension noted in pregnancy.
‡*p*-value omitted given different methodologies utilised for measuring HbA1c between the two groups.

p=0.000). In the later cohort, first presentation was earlier (mean (SD) 27 (7.5) weeks v. 28 (6.4) weeks; p=0.04), and >85% of all patients in this cohort achieved their glycaemic goals as measured by their MBG for the third trimester, with >95% of those on oral agents alone achieving good control by delivery. Failure rates for dietary monotherapy were high for both cohorts (97.9% v. 95.1%). The need for insulin monotherapy was lower for the later cohort than for the earlier cohort (52.6% v. 95.1%).

Maternal and neonatal outcomes (Table 2)

GA at delivery in the recent cohort was lower (mean (SD) 36.4 (3.65) weeks v. 37 (1.6) weeks; p=0.008), with a higher number of preterm deliveries. Birth weight and macrosomia rates were statistically lower in the most recent cohort (3 017 (648) g v. 3 204 (657) g; p=0.002 and 12.5% v. 4.9%; p=0.01, respectively). PNM was comparable between the cohorts (4.2% v. 4.9%; p=0.72), with fewer stillbirths and a non-significant increase in early neonatal deaths in the recent cohort.

Effect of oral agents on maternal and neonatal outcomes in the 2010 - 2014 cohort

In the 2010 - 2014 cohort, 'overt' GDM was diagnosed in 43.8% of patients based on a baseline FPG ≥7.0 mmol/L. The mean (SD) FPG at first visit for the cohort was 7.3 (2.14) mmol/L. Ninety-nine patients (53.8%) required insulin monotherapy at first diagnosis, with 45.0% requiring OHAs and 2.1% dietary monotherapy. Of those initiated on diet alone, 97.8% failed and required a switch to OHAs. Eighty-four patients (43.7%) were exposed to metformin and 45 (23.4%) to glibenclamide, with 7.8% of the OHA group requiring a switch to insulin. Within the cohort treatment groups, no significant differences were observed in maternal age and BMI in the groups exposed to OHA compared with insulin monotherapy. For the insulin- v. glibenclamide-exposed groups, initial HbA1c (median (IQR) 8.9% (7.2 - 10.1) v. 6.7% (6.3 - 7.1); p=0.001) and weight gain during pregnancy (median 5 (2.2 - 9.6) kg v. 2 (0 - 4) kg; p=0.00) were significantly higher in the insulin group.

Of the 192 files recorded, outcomes were available for 162 patients, given a 16.7% loss-to-follow-up rate. There were no differences in maternal and neonatal outcomes in terms of each oral agent v. insulin. When comparing metformin with insulin, the incidences of prematurity, macrosomia, LBW and PNM were all non-significantly lower with metformin therapy. Outcomes for the glibenclamide- v. insulin-exposed groups were comparable, including rates of macrosomia (11.4% v. 3.3%; p=0.19) and LBW (22.2% v. 14.6%; p=0.35). CS rates, although not different among the groups, were high (78.6%, 77.7% and 64.7%, respectively), with failed induction of labour being the commonest indication. No maternal mortality was reported for the cohort.

Discussion

In this comparative study: (*i*) lower macrosomia rates and earlier GA at delivery were found in the later cohort exposed to both OHA and insulin therapy (2010 - 2014) v. the earlier cohort (1992 - 2002) exposed to insulin monotherapy; (*ii*) effectiveness of OHAs was evident in terms of the low number of patients who needed to be switched to insulin; and (*iii*) maternal and neonatal outcomes were comparable in the group exposed to OHAs and those exposed to insulin alone for the 2010 - 2014 cohort.

When comparing the historical cohorts, the introduction of OHAs in the later cohort was effective in achieving good glycaemic control at delivery, with favourable and comparable maternal and neonatal outcomes, including maternal hypoglycaemia, CS rates, macrosomia, major congenital anomalies and PNM, in women treated with OHAs. This finding is of public health relevance in SA, where limited resources and access to specialised care for GDM often translate into adverse outcomes, because it provides healthcare professionals with a cost-effective and less complex alternative to the 'gold standard' of care, insulin, with a similar safety profile.

Of interest were the lower birth weights and macrosomia rates in the cohort exposed to OHAs. One plausible explanation is the earlier GA at delivery, which may have numerous contributing factors. Metformin has been found to be protective against macrosomia in some studies,[19,20] although this was not evident in our study when metformin monotherapy was compared with insulin alone; however, numbers were small for this comparison. Our study did not corroborate a finding from a robust meta-analysis, including seven randomised controlled trials, of a significant relationship between glibenclamide exposure and higher birth weights, macrosomia rates and neonatal hypoglycaemia compared with insulin therapy.[14]

Lastly, PNM did not differ between the two cohorts (4.2% v. 4.9%; p=0.72), and no higher PNM was found in the OHA-exposed group. This PNM figure, while higher than the national rate of 3.3%[21] for all pregnancies, compares favourably with a recent audit of diabetic pregnancies from Cape Town[22] and those from elsewhere in Africa, which range from 5.2% to 25.4%.[23]

In the 2010 - 2014 cohort, GDM was detected earlier, possibly as a result of improved awareness both among the public and in the public health system through enhanced screening protocols at both primary- and secondary-level clinics. However, the high mean HbA1c at booking raises concern that many of the women had pre-gestational diabetes that had not been diagnosed before pregnancy, highlighting a potential gap in the diabetes screening programmes. Furthermore, this burden of type 2 diabetes mellitus occurs alongside a rising prevalence of obesity,[24] as is appreciated in the recent cohort, with 81.4% of the women having a BMI >30 kg/m^2. Despite this high

Table 2. Maternal and neonatal outcomes for the 1992 - 2002 and 2010 - 2014 gestational diabetes mellitus cohorts

	1992 - 2002 cohort (*N*=348)	2010 - 2014 cohort (*N*=164)	*p*-value
GA at delivery (weeks), mean (SD)	37.0 (1.6)	36.4 (3.6)	0.008*
Live births, *n* (%)	344 (98.8)	161 (98.3)	0.65
Caesarean section, *n* (%)	215 (61.8)	113 (68.7)	0.10
Maternal hypoglycaemia, *n* (%)	3 (0.9)	1 (1.0)	0.87
Birth weight (g), mean (SD)	3 204 (657)	3 017 (648)	0.002*
Macrosomia, *n* (%)	43 (12.5)	7 (4.9)	0.011*
Stillbirths, *n* (%)	10 (2.8)	3 (1.8)	0.49
Early neonatal deaths, *n* (%)	5 (1.4)	5 (3.1)	0.193
PNM, *n* (%)	15 (4.2)	8 (4.9)	0.72

GA = gestational age; SD = standard deviation; PNM = perinatal mortality.
*Statistically significant (p<0.05).

rate of obesity, most of our patients attained good glycaemic control by the third trimester (>85%), with similar outcomes to studies both internationally and locally.[19,20]

In the recent cohort, over half of the patients (53.8%) required insulin from their first visit owing to the severity of their initial dysglycaemia, explained by the fact that close to 50% of the cohort had 'overt' diabetes. In the patients initiated on OHAs, the effectiveness of these agents in this group was evident in the small number of patients who needed to be switched from OHA to insulin (7.8%), and the good glycaemic control at delivery. Notably, both cohorts demonstrated high dietary failure rates compared with figures elsewhere (95.1% and 97.9% v. 20.0 - 30.0%),[12] which is probably due to the presence of more severe cases of dysglycaemia (as indicated by the high initial HbA1c) because of risk factor-based screening rather than universal screening. Furthermore, financial challenges encourage patients to consume a carbohydrate-rich diet as opposed to fully embracing the proposed diet. Lastly, this discordance in the percentage of patients failing dietary measures may be attributed to differences in definitions of glycaemic control, population characteristics and the population responses.

Study limitations and strengths

Limitations of the current study include its retrospective nature and small numbers of patients in the cohorts. The diagnostic criteria employed, although relevant at the time, are no longer applicable and may limit reproducibility of the study. Furthermore, comparison of certain variables between the two cohorts was limited. We did not have access to the earlier cohort's database and hence could not merge the insulin monotherapy group with the later cohorts for the purposes of analysis. In addition, the definitions of nephropathy and the MBG calculations were different. Different assays for HbA1c were used at the two time points, with an immunoassay (Roche Diagnostics, Germany) being performed on whole blood by the National Health Laboratory Service in the earlier cohort and point-of-care testing being employed in the later cohort, making comparison of HbA1c between the cohorts problematic. As the prevalence rate of anaemia in the later cohort was high (11%), decreased reliability of this test cannot be ruled out. Comparison of maternal and neonatal outcomes between cohorts was restricted to the variables obtained for the earlier cohort. This prevented us from exploring whether the lower GA at delivery in the recent cohort may have translated into more cases of prematurity. Various confounders for outcomes were either poorly reported, like BMI, or not at all, like HIV status, in one or both of the cohorts. Strengths of the study include the comparison of patients from the same clinic 10 years apart, diagnosed utilising the same test and criteria, where the only difference was the introduction of oral hypoglycaemic agents.

Conclusions

After the introduction of OHAs at the CHBAH gestational endocrine clinic, patients achieved similar levels of glycaemic control at delivery and showed similar outcomes compared with patients receiving insulin therapy. This is reassuring, particularly for low- and middle-income countries, where the OHAs are cheaper, more accessible, easier to use and eliminate the availability and storage issues associated with insulin therapy. Further prospective studies with adequate sample sizes are needed to confirm our findings and to determine the impact of OHA on pregnancy outcomes, both in the short and long term.

Declaration. This publication was part of the requirements for VN's PhD degree.

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Author contributions. All authors contributed to conceptualisation of the study. VN contributed to planning, data capturing and analysis, and manuscript writing and editing. LS, KK-G, NL and SAN contributed to planning, interpretation of data, and manuscript writing and editing. KRH contributed to manuscript writing and editing.

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Conflicts of interest. None.

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APPENDIX K: SUBMITTED PAPER III

Cardiometabolic outcomes of women exposed to hyperglycaemia first detected in pregnancy at 3-6 years post-partum in an urban South African setting

Short title: Diabetes in pregnancy and cardiometabolic outcomes

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Abstract (301 words)

Background: Hyperglycaemia first detected during pregnancy(HFDP) has far-reaching maternal consequences beyond the pregnancy. Our study evaluated the cardiometabolic outcomes in women with prior HFDP versus women without HFDP 3-6 years post-partum in urban South Africa.

Design and Methods: A prospective cohort study was performed of 103 black African women with prior HFDP and 101 without HFDP, 3-6 years post-partum at Chris Hani Baragwanath Academic Hospital, Soweto. Index pregnancy data was obtained from medical records. Post-partum, participants were re-evaluated for anthropometric measurements, body composition utilizing dual energy X-ray absorptiometry(DXA) and biochemical analysis (two-hour 75gm OGTT fasting insulin, lipids, creatinine levels and glucose levels). Cardiovascular risk was assessed by Framingham risk score(FRS). Carotid intima media thickness(cIMT) was used as a surrogate marker for subclinical atherosclerosis. Factors associated with progression to cardiometabolic outcomes were assessed using multivariable logistic and linear regression models.

Results: Forty-six(45.1%) HFDP women progressed to diabetes compared to 5(4.9%) in non HFDP group($p<0.001$); only 20(43.4%) were aware of their diabetic status in the whole group. The odds(OR, 95% confidence interval(CI)) of progressing to type 2 diabetes(T2DM) and metabolic syndrome(MetS) after correcting for confounders in the HFDP group was 10.5(95% CI 3.7-29.5) and 6.3(95%CI 2.2-18.1), respectively. All visceral fat indices were found to be significantly higher in the HFDP group after adjusting for baseline body mass index. Ten-year estimated cardiovascular risk(FRS) and mean cIMT was statistically higher in the HFDP group(8.46 IQR 4.9-14.4; 0.48 mm IQR 0.44-0.53 respectively) compared to the non-HFDP group(3.48 IQR 2.1-5.7; 0.46mm IQR 0.42-0.50) respectively and this remained significant for FRS but was attenuated for cIMT after correcting for confounders. HIV did not play a role in progression to any of these outcomes.

Conclusion: Women with a history of HFDP have a higher risk of cardiometabolic conditions within 6 years post-partum in an urban sub-Saharan African setting.

Introduction

The non-communicable diseases(NCD) burden remains the leading cause of death worldwide, with diabetes and cardiovascular diseases(CVD) accounting for almost half the burden. In South Africa, diabetes and CVD are the second and third leading cause of death since 2014. In high-income countries, hyperglycaemia first detected during pregnancy(HFDP) has been associated with a sevenfold higher overall incidence of type 2 diabetes mellitus(T2DM) within the first decade following delivery(1) and an increased risk of CVD(2) and the metabolic syndrome(MetS)(3).

Prevalence figures for HFDP in Africa remain limited, with a few studies in South Africa demonstrating that 9-25% of women have HFDP(4–6). These figures may underrepresent the disease burden since risk-factor based screening and varying diagnostic tests and strategies are currently employed. Whilst numerous studies have explored the short term maternal and neonatal outcomes following HFDP, long-term outcomes for the African continent remain limited to a mere three studies. One study performed amongst a predominantly mixed ethnic ancestral group in Cape Town, South Africa demonstrated a 48% progression rate to T2DM at 5-6 years following the index pregnancy(7), although no control group was included. A high CVD risk and prevalence of MetS(60.9%) was demonstrated in the same cohort(8). A further smaller study amongst the same ethnic group from the same region established that the prevalence of T2DM at six-weeks post-partum was 27%(9).

Though racial and ethnic disparities for progression to T2DM following HFDP is well known with African American women being particularly vulnerable(10) owing to acculturation, lifestyle factors arising from social determinants in addition to genetic susceptibility and traditional risk factors(11), to our knowledge, the long-term CVD and metabolic impact of HFDP has not previously been explored in Black African women or compared to women in this setting without a history of HFDP.

Obesity, a well-known risk factor of HFDP, T2DM and CVD, is commonly encountered amongst black South African women(40.9%) and accounts for an estimated 87% of their diabetic risk(12,13). Obesity and weight-related characteristics including pre-pregnancy body mass index (BMI), post-pregnancy BMI and weight gain following the pregnancy, all have been shown to increase the risk of progression to T2DM following HFDP(14). The pathophysiological mechanism behind this lies in the differential impact of regional fat deposition, with upper body(android and visceral fat) and lower body fat (gynoid and leg fat) showing directionally opposite associations with these risks(15) which too has been appreciated in black SA women(16,17). However, the impact of body composition characteristics on progression to T2DM, MetS and CVD following an HFDP pregnancy has yet to be explored in this setting.

Moreover, little is known about the impact of human immunodeficiency virus (HIV), which the healthcare sector in Africa is faced with, in addition to the growing burden of NCDs, on CVD and metabolic risk in the context of HFDP. The additional metabolic risk attributable to HIV is debatable, with an overall prevalence of glucose metabolism disorders in HIV-infected individuals on antiretroviral therapy(ART) in Africa ranging from 3-33.5%(18). A meta-analysis of 5 case-control studies in Africa did not demonstrate a significant relationship between HIV and exposure to ART and the prevalence of T2DM, as encountered in other studies in Europe and North America.

Given the growing prevalence of HFDP in South Africa and its well-known role in the intergenerational transmission of NCD, we sought to explore its impact on maternal CVD risk, development of T2DM and MetS in a group of Black African women with and without a

prior history of HFDP. Secondary aims of our study were to explore how body composition differs between the two groups and if HIV-infection impacted the development of these outcomes following pregnancies with and without HFDP. To our knowledge this is the first study of its kind in Africa.

Study design and population

The study setting was the Chris Hani Baragwanath Academic Hospital(CHBAH), located in urban Soweto, South Africa. Between March and November 2019, we conducted a prospective cohort study in women previously diagnosed with HFDP (HFDP group) and women who tested negative for HFDP (non-HFDP group) using the same diagnostic test and criteria between February 2014 to January 2017. Both groups of women were derived from the same specialist clinic though were identified differently. The HFDP group were selected first and consisted mostly of women who were identified by risk-factor based screening and had attended a specialised gestational endocrine clinic at CHBAH for HFDP with their pregnancy characteristics and outcomes have been previously published(19). A subgroup of the HFDP women were referred to the specialist clinic as a result of universal screening being performed by a research study (5). The "control group", were women who tested negative for HFDP for the same time period and had undergone universal screening as part of a previous study (5). Women were diagnosed using a 75-gram 2-hour oral glucose tolerance test(OGTT) with International Association of Diabetes in Pregnancy Study Group(IADPSG) criteria. HFDP comprised of GDM and DIP.

Participants were recruited telephonically and if unsuccessful traced by visiting their home address. Of the initial 319 HFDP cases identified, 206 were non-contactable/traceable, 4 declined to participate, 6 were pregnant. Difficulties tracing participants following delivery was mostly due to relocation or change of contact details. There were 845 women who screened negative for HFDP identified from the database of the previous study of which 103

women were contacted in a random order until the number of mothers were the same as those in the HFDP group. Two hundred and four participants were enrolled at follow up, of which 103 had confirmed HFDP and 101 did not.

Sample size calculation

Sample size calculations were calculated for each of the three main outcomes(T2DM, MetS and CVD risk) using a two-sample proportion test based on population parameters using a 5% margin of error, a confidence interval of 95% and power of 80% to detect the effect. Given the reported estimated risk of developing T2DM following a HFDP pregnancy of 20-60% and 12%(1,20) in the background population a sample size of 15 per group was calculated. The minimum total sample size calculated for the outcome MetS was 64 given the risk of 40% following HFDP(3) and background prevalence of MetS of 10%. A size of 98 per group was needed based on a reported estimated risk of developing CVD of 17.6%(2) following HFDP vs. 7% in the background population. The sample size needed to establish CVD risk informed our final sample size.

Data collection

Questionnaire

A self-reported questionnaire was captured at the follow-up visit and incorporated maternal demographics, marital status, various socioeconomic parameters (SES), obstetrical history, maternal complications and outcomes and postnatal factors (history of CVD risk factors, any vascular event/s, risk factors for the development of T2DM including recurrent HFDP pregnancies, breastfeeding following the index pregnancy, family history of diabetes or the presence of diabetes). Use of cholesterol-lowering or antihypertensive medication, smoking status and pack year history and ethanol consumption(ml/day) based on the quantity and frequency and physical activity was captured. Contraceptive use was self-reported and was

categorized as none, oral contraceptives or injectable contraceptives and type. HIV status and therapy were noted where applicable. (See Supplementary Table 1 for relevant definitions of maternal variables noted). The questionnaire was informed by the literature and adapted from several existing standard and recognised sources(21) in order to incorporate relevant factors.

Supplementary Table 1. Definitions of maternal and neonatal variables and outcomes

Maternal variables	
Risk factors for HFDP	Persistent glycosuria, family history of diabetes(first degree), previous unexplained perinatal losses, previous HFDP, history of a macrosomic baby.
Pregnancy losses	Includes miscarriage, intrauterine death, ectopic pregnancy and termination of pregnancy and neonatal death
Maternal overweight	BMI ≥25 kg/m^2
Maternal obesity	BMI ≥30 kg/m^2
Nephropathy	Creatinine > 71ummol/L and MAC >30 mg/L
Anaemia	Haemoglobin levels < 11 g/dl
HIV-positive	Based on results from rapid or antibody ELISA tests either at index pregnancy or follow up visit
Hypertensive disorders of pregnancy	Categorized as: Pregnancy-induced hypertension (PIH): hypertension presenting >20 completed weeks Preeclampsia: hypertension with proteinuria Eclampsia: hypertension with proteinuria and seizures
Any obstetric complication	Miscarriage, PIH, pre-eclampsia, eclampsia, urinary tract infection, polyhydramnios, oligohydramnios, abruptio placentae, maternal death, and/or other.
Maternal measurements and outcomes	
i) Anthropometrics	
All Measurements	Measured and recorded three times using a non-elastic tape and final measure being a mean of the 3 readings to one decimal place
Waist circumference	Taken at midpoint between iliac crest and lowest part of rib cage in the mid-axillary line
Hip circumference	Taken around widest part of hips
Waist: hip ratio (WHR)	As there are no recommended cut-offs for African populations, WHR was categorised as follows: Normal <0.849 & Central obesity>0.85
BMI categories	BMI <25kg/m^2 is normal BMI>25 and <30kg/m^2 overweight

Fat mass index	BMI>30kg/m^2 obese Derived from DXA as estimated fat mass (kg) divided by height (m) squared.
ii) Glycaemic measures	
Diabetes categories (WHO 2006 criteria)	Diabetes FPG ≥ 7.0mmol/L or 2hr plasma glucose ≥11.1mmol/L or HbA1c ≥6.5% Impaired fasting glucose ≥ 6.1-6.9 Impaired glucose tolerance ≥7.0 and < 11.1mmol/L
Dysglycaemia	Fasting blood glucose level ≥ 5.6mmol/L
Insulin resistance	Assessed using the homeostatic model and calculated as fasting glucose (mmol/L) fasting insulin (mU/l)/22.5 HOMA score>1.95 defined as insulin resistance
iii) Cardiovascular parameters	
Blood pressure measurement	3 measurements were taken using Omron M6(Omron Kyoto, Japan) on right arm in a sitting position 1 minute apart after 5 minutes at rest, Average of the last 2 readings were recorded
Hypertension	Blood pressure of > 140/90 mmHg for at least two measurements
Carotid intima media thickness(cIMT)	Thickened cIMT defined as ≥0.8mm
Atherosclerotic plaque	Focal structure that extends at least 0.5 mm into the arterial lumen and/or measures 50% or more of the adjacent IMT value and/or has an IMT value greater than 1.5 mm
Cardiovascular disease	Self-reported history of coronary artery disease, stroke and or peripheral arterial disease
Dyslipidaemia	Dyslipidaemia was defined as either triglyceride ≥ 1.7 mmol/L and/or high-density lipoprotein cholesterol ≤ 1.30 mmol/L and/or LDL ≥ 3.0mmol/L
Neonatal outcomes	
Prematurity	GA <37 completed weeks
Low birth weight	Birth weight <2500 grams
Macrosomia	Birth weight ≥ 4000 grams

a) Anthropometrics

Subjects underwent a physical examination for weight, height, waist and hip circumference and blood pressure measurements utilising standardised methods by qualified trained research assistants. Height (cm) was recorded to one decimal place using a wall mounted Holtain stadiometer(Crymych, UK) with subjects standing on a flat surface at a right angle to the vertical board of the stadiometer. Weight(kg) was measured on a SECA digital scale (Hamburg, Germany), to the nearest 0.1kg, which was calibrated and standardised using a weight of known mass. Participants wore light clothing and were asked to remove their shoes and socks. Blood pressure(mmHg) measurements were taken. Details pertaining to each measurement is outlined in Supplementary Table 1.

Dual–energy x–ray absorptiometry (DXA)(Hologic Discovery-A (S/N83145), Bedford, MA, USA) was used to determine whole-body composition since BMI alone is not an accurate indicator of body composition. This included subtotal(whole-body minus head) fat mass and fat-free soft tissue mass. Regional body fat, namely trunk, arm, leg, android, and gynoid fat mass(expressed in kg and as a percentage of subtotal fat mass, (% FM) were measured using DXA cut-off lines positioned at standard anatomical positions, as defined in the software (software version apex 4.2.0). FMI (kg/m^2) was calculated using height and body fat mass which offers superiority over BMI as a marker of obesity since the index is based on fat mass, not body weight, which is a combination of fat and lean components. In addition, abdominal visceral adipose tissue(VAT) and subcutaneous adipose tissue(SAT) were estimated using algorithms included in the DXA software, which have been shown to perform as well as clinical computed tomography(22). During data collection, a phantom scan was performed each morning to) determine the coefficient of variance of the DXA machine and the coefficient of variance (CV) was less than 0,5% for all parameters. CVs for DXA parameters were <2 % for total fat mass, and 1% for fat–free soft tissue mass(FFSM).

a) Biological samples and OGTT

Point of care testing was performed for haemoglobin(Hemocue^{r} Hb 201) and HIV(Homemed HIV1/2 rapid test kit). An early morning midstream urine sample was collected from each participant to exclude pregnancy and to perform bedside testing with a urine dipstick(Roche Combur) screening for glycosuria, albumin/protein, evidence of infection or renal disease.

A finger prick(OneTouch) fasting capillary glucose was performed at baseline and the OGTT was commenced irrespective of the result. At baseline, blood samples were drawn by a trained nurse after an 8-hour overnight fast for measurement of serum creatinine, lipogram, Hba1c, fasting insulin and glucose. This was followed by ingestion of 75g glucose in 250ml water, Blood samples for glucose were drawn at baseline and 2 hours. Those with self-reported diabetes diagnosis, which was confirmed by either medical card record or drugs in-use only had fasting bloods drawn. . Specimens were centrifuged and stored at -80^{0} C within 30 min of being drawn. Categories of glucose intolerance were defined applying the 2006 WHO criteria(23). (Supplementary Table 1).

b) Carotid and femoral imaging

Ultrasonographic assessment of the common carotid artery(CCA) and femoral artery(CFA) was performed(Linear-Array 12L-RS transducer with a B-mode Logic E Ultrasound machine, GE healthcare, CT, USA), to assess for intima media thickness(IMT) and for the presence of plaque. The IMT measurement was then performed on the posterior wall of the common carotid artery and common femoral artery in an area free of plaque, defined as the distance between two echogenic lines represented by the lumen-intima interface and media-adventitia interface of the arterial wall. The ultrasound machine software then detected the intima-lumen and the media-adventitia interfaces and calculated the minimum, maximum,

and mean common carotid IMT(cIMT) and femoral IMT in millimetres and to 2 decimal places(24,25). All patients were positioned supine with the neck slightly hyperextended and rotated in the opposite direction to the probe. A 45-degree angle wedge pillow was used to standardize lateral rotation. Measurements were performed by one observer with intra-observer variability 1.1%.

c) Biochemistry and lab analyses

Plasma glucose was measured using Randox Rx Daytona chemistry analyser using enzymatic methods(Randox Laboratory Ltd, London, UK) glycosylated haemoglobin a1c(HbA1c) was measured using the Bio-Rad D-10™ Haemoglobin analyser using the HPLC method (catalogue number, 2200101) (Bio-Rad Laboratories, Inc. CA, USA). The precision and trueness of the Randox Rx Daytona chemistry analyser were verified using the clinical and laboratory standards institute document EP15. Coefficients of variation calculated from running 30 separate samples at 3 different times were 0.7% for glucose and 1.8% for HbA1c. Lipids including HDL, low-density lipoprotein(LDL), triglycerides(TG), total cholesterol concentrations were analysed on the Randox Rx Daytona chemistry analyser using enzymatic colorimetric (catalogue number, CH8311(HDL), CH8312(LDL), TR8332(trigs), CH8310(Chol)) Randox Laboratories Ltd., London, UK). Enzymatic colorimetric assays were used to measure TG, total cholesterol and HDL cholesterol using the Roche modular auto analyser, while low-density lipoprotein cholesterol was calculated using direct methods/Friedewald formula. Fasting serum insulin concentrations were measured on the Immulite® 1000 Immunoassay system using the chemiluminescent method(catalogue number lkin1/ catalogue number, 10381429) (Siemens) chemiluminescent healthcare GMBH, Henkestr, Germany). Serum creatinine concentrations were analysed on the Randox Rx Daytona chemistry analyser using enzymatic methods (catalogue number, CR8317) (Randox

Laboratories Ltd., London, UK). CVs calculated from running 40 separate samples in duplicate were, 0.8% for HDL and total cholesterol, 1.19% for TG and 3.9% for insulin and 0.7% creatinine.

Outcomes

The primary outcome was progression to and time to developing T2DM between women with HFDP(sub-categorised; GDM and DIP) and women without HFDP following their index pregnancy. Secondary outcomes included comparison of body composition measures, progression to MetS and CVD risk(utilising two surrogate measure, Framingham Risk Score(FRS) and cIMT between the groups. MetS was defined using the harmonised criteria(26). Gender specific prediction for 10-year CVD risk was calculated using the modified Framingham risk score(FRS)2008(27) (See Supplementary Table 1 for definitions).

Ethics

This study was approved by the Human Research Ethics Committee at the University of the Witwatersrand(M180316). Informed consent, both verbal and written, was obtained from participants prior to enrolment in the study.

Statistical analysis

Data was captured using REDCap(28) (Vanderbilt University, Nashville, USA) and analysed using Stata software 13.0(College station, USA)(29). Sensitivity analysis to address potential bias of missing data was performed. Mean and standard deviations were reported for normally distributed continuous variables(anthropometric parameters) and medians and interquartile ranges for non-normally distributed measured data (all other continuous variables). Number and percentages for categorical variables(chronic hypertension, family history of diabetes etc). Statistical differences between three groups(control, GDM and DIP), were tested using Analysis of Variance(ANOVA) or Kruskal-Wallis test. For categorical

variables, Chi-squared test and Fischer's exact(small frequencies) was utilised. The statistical significance level was set at two-sided p-value <0.05.

Crude odds ratio(OR 95% CI) and multivariable adjusted odds ratio(aOR 95% CI) for T2DM, MetS, and 10-year cardiovascular risk calculated using FRS were estimated from logistic regression models. Covariates evaluated as potential confounders based on a priori hypotheses are included in Table 3. Covariates were excluded as confounders if they were not associated with both the dependent variables and independent variable (exposure to HFDP), p<0.05.

Further multivariable models were designed to explore the relationship of maternal factors associated with the relevant outcomes adopting a chronological approach in which maternal factors present either at pre-pregnancy(distal model 1), index pregnancy(intermediate model 2) and post-partum(proximal model 3) were assessed using logistic or linear regressions. The final model combined all variables from the three models. The outcomes explored in these multivariate models were fat mass index FMI(continuous, as surrogate for adiposity), T2DM(binary) and cIMT(continuous). The independent variables were identified using univariate analysis for each outcome. HFDP, an independent variable for all models, was categorised according to degree of dysglycaemia as GDM and DIP. Both BMI and WHR were used as continuous variables for the purposes of the models. For logistic regression model diagnostics, results are expressed as OR and 95% confidence intervals(CI) and we assessed the following: linearity assumption using the Lowes graph, multicollinearity using variance inflation factors, model specification using the C-statistic, and confirmed the fit of the model using the Hosmer-Lemeshow goodness-of-fit test. We also checked for outliers. For linear regressions cIMT was log-transformed to increase normality of residuals. Results are expressed as beta coefficient and 95% CI. In order to assess the influence of HIV on the

outcomes, it was included as binary variable into each of the multivariate models exploring maternal factors and outcomes.

Results

There were 103 women recruited in the HFDP group and 101 in the non-HFDP group after all exclusions were applied. There was <1% missing data and no biases detected through the sensitivity analyses. Details are shown in the study flow diagram (Figure 1)

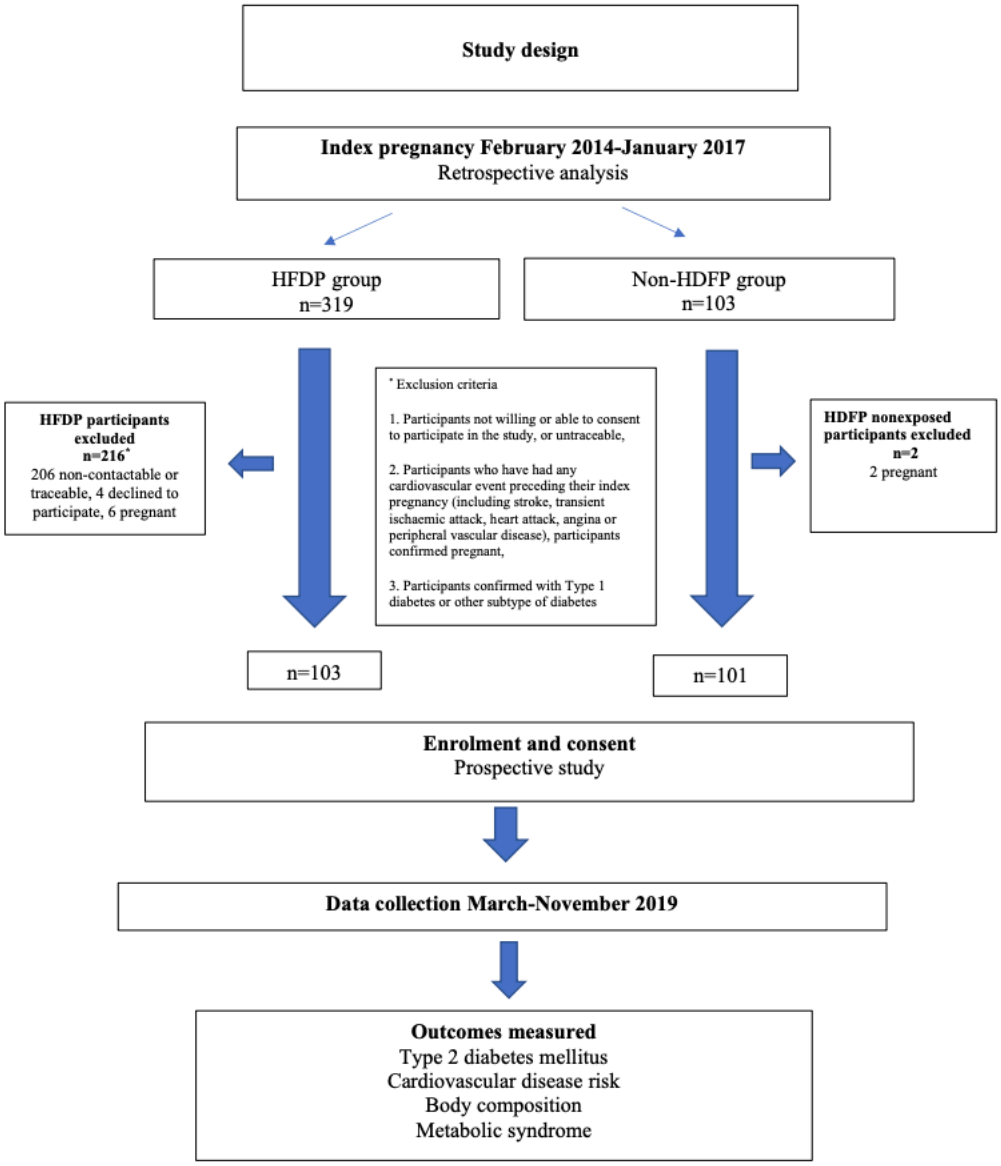


Figure 1. Flow diagram of the study and eligible participants

Baseline and follow up demographics, maternal factors, anthropometrics, and biochemical parameters between the groups

The majority of the cohort was of Black African ancestry (n=198, 97%). Relevant baseline characteristics during the index pregnancy and at follow-up for the HFDP group and non-HFDP groups are shown in Table 1. Of the participants with prior HFDP, 45(43.7%) had "overt" diabetes in pregnancy (DIP) with the remaining 58(56.3%) classified as "true" GDM (GDM). When comparing the HFDP vs. non-HFDP groups at first booking during their index pregnancy, the median age was higher for the HFDP group, 32.5(29-38 IQR) vs. 29.5 (25-34 IQR) respectively, with a median follow-up period of 3 years (IQR3-4) and their median BMI were 35.2(IQR30.6-39.8) and 29.5(IQR 25.0-32.9). Baseline prevalence of HIV during pregnancy was lower in the HFDP group 13(12.7%) vs. 20(19.8%) amongst the non-HFDP group and this remained the case at follow-up. All women with HIV were on fixed dose combination of treatment whilst the majority(80%) were diagnosed at or before their index pregnancy. Forty-five(43.7%) of the women in the HFDP group experienced an obstetric complication vs.16(15.8%) in the non-HFDP group. When comparing the DIP and GDM groups, the only variables significantly different at baseline were glucose values on OGTT testing and exposure to therapeutic agents, with more in the DIP group being exposed to insulin 22(48.9%) vs. 8(14.8%).

At follow-up, the HFDP group remained obese (32.8 (29.1-39.2)) with elevated anthropometric measures, higher blood pressure measurements and glucose profiles when compared to the non-HFDP group. Their overall socioeconomic status was lower than those for the non-HFDP group.

Table 1. Maternal demographics, characteristics and outcomes at index pregnancy and follow-up.

Index pregnancy		**HFDP group**			**Non-HFDP group**
		Total group n=103	GDM n=58	DIP n=45	n=101
Age(years)	(median/IQR†)	32.5 (29-38)	33(29-38)	32(30-37)	29.5 (25-34)
Primigravida	n (%)	8 (7.8)	5(8.8)	3(6.8)	54 (53.4)
GA[1] at presentation (weeks)	(median/IQR†)	30(26-33)	30(27-33)	30(24-32)	26(25-27)
BMI[2] at first visit	(median/IQR†)	35.2 (30.6-39.8)	34.3(30.5-38.2)	37.2(31.6-40.9)	29.5(25.0-32.9)
BP[3], (mmHg) Systolic BP DBP BP	(median/IQR†)	 121(112-129) 73(64-79)	 124(112-129) 71(62-77)	 119(113-129) 74(66-79)	 109(102.5-114.5) 69(64-73)
HIV[4] positive	n (%)	13(12.7)	11(18.9)	2(4.4)	20(19.8)
OGTT[5] results, mmol/L Fasting plasma glucose 60 mins 120 mins	(median IQR†)	n=95 6.1(5.3-7.4) 10.6(8.6-11.9) 10.0(8.0-11.9)	n=55 5.6(5.16-6.1) 9.25(8.1-10.65) 9.0(7.4-10)	n=40 7.6(6.5-9.3) 12.2 (11.4-14.3) 12.2 (11.35-15.05)	n=101 4.0(3.6-4.4) 5.8 (5.0-6.7) 5.2(4.5-6.3)
GA at delivery weeks Delivery <37 weeks	(median/IQR†) n (%)	n=100 38(37-38) 24(24)	n=55 38(37-38) 12(21.8)	n=45 37(36-38) 12(26.7)	n=90 39(37-40) 13(14.4)

Obstetric complications Caesarian section HDP[6]	n (%)	45(43.7) 67(66.3) 12(11.6)	21(36.2) 39(68.4) 6(10.3)	24(53.3) 28(63.6) 6(10.3)	16(15.8) 59(58.4) 11(10.9)
Exposure to OHA[7] Metformin Glibenclamide Exposure to insulin	n (%)	n=99 65(65.7) 31(31.3) 34(33.0)	n=54 38(70.4) 12(22.2) 8(14.8)	n=45 27(60) 19(42.2) 22(48.9)	 N/A N/A N/A
Neonatal outcomes Birth weight LBW[8] Macrosomia Anomalies	(median/IQR†) n (%)	n=99 3065 (2665-3430) 18(18.1) 5(5.0) 3(2.9)	n=56 3042.5(2650-3345) 11(19.6) 3(5.3) 2(3.5)	n=43 3140(2765-3685) 7(16.3) 2(4.6) 1(2.2)	n=100 3040(2792.5-3357.5) 17(17) 5(5) 2(2.1)
Demographic, anthropometric and biochemical status at 3-6 years after the index pregnancy		**HFDP group**			**Non- HFDP group**
		Total group n=103	GDM n=58	DIP n=45	n=101
Age (years)	(median/IQR†)	37.3 (33.1-42.7)	38.1(32.8-42.7)	37.1(33.9-41.7)	34.0(29.2-37.3)
Primigravida	n (%)	6(5.8)	3(5.2)	3(6.7)	13(12.8)
Time at follow-up (years)	(median/IQR†)	3(3-4)	3(3-4)	3(3-4)	3(3-4)

SES[9]					
1)Marital status, married	n(%)	38(36.9)	20 (34.4)	18(40)	15(14.8)
2) Education Secondary and higher		66(65.3)	40(71.4)	26(57.8)	73(23)
3) Household items	(mean SD ‡)	7.9(2.1)	7.8(2.0)	7.9(2.3)	8.3(1.9)
4) Housing density	(median/IQR†)	2.5(2-3)	2.5(2-4)	2.5(2-3)	2.0(1.5-3)
BP[3], (mmHg)					
Systolic BP	(median/IQR†)	115.1(108-126.6)	115(106.6-125)	117.5(112.-131.8)	113.7(104.6-120.3)
Diastolic BP		82.6(76.6-90.6)	81(73.3-89.6)	84.8(77.6-90.6)	79(73.3-86.6)
HIV positive	n(%)	19(18.6)	14(24.1)	5(11.1)	25(24.7)
Anthropometrics					
Waist circumference	(mean/SD ‡)	102(15.0)	100.2(14.4)	104.2(15.5)	90.9(16.6)
Hip circumference		115.1(12.2)	115.3(11.8)	114.9(12.8)	110.6(15.0)
BMI[2]	(median/IQR†)	32.8(29.1-39.2)	31.9(28.8-36.1)	33.8(29.5-39.7)	29.2(24.2-33.4)
BMI diff		-1.7(-3.7-0.3)	-1.5(-3.7-1.3)	-2.08(-3.2-(-0.56)	0.45(-2.7-3.3)
Weight diff		-3.6(-9.6-0.3)	-1.85(-9.6-3.3)	-5.2(-8.5-(-1.4)	-0.4(-8.7-10)
Glucose profiles					
Fasting glucose	(median /IQR†)	5.9(5.1-9.0)	4.9(4.6-5.2)	7.5(5.7-11.8)	4.9(4.6-5.3)
120 mins glucose		7.1(5.6-9.1)	5.5(4.8-6.1)	8.4(6.3-13.2)	5.5(4.8-6.1)

†IQR: interquartile range ‡SD: Standard deviation [1]GA: gestational age. [2]BMI: body mass index [3]BP: blood pressure. [4]HIV: human immunodeficiency virus.[5] OGTT: 75gm 2-hour oral glucose tolerance test [6]HDP: Hypertensive disorders in pregnancy including gestational hypertension, pre-eclampsia, eclampsia. [7]OHA: oral hypoglycaemic agents [8]LBW: low birth weight. [9]SES: socioeconomic status. [10]IFG: impaired fasting glucose. [11]T2DM: type 2 diabetes [12] IGT: impaired glucose tolerance [13]FPG: fasting plasma glucose

Stratified analysis of maternal outcomes including diabetes, cardiovascular risk and metabolic syndrome by HFDP subtypes: DIP and GDM (Table 2)

Of the HFDP group, 46(44.6%) progressed to diabetes compared with 5(4.9%) in the non HFDP group($p<0.001$). Only 20(42.5%) of the entire group were aware of their diabetes status and the average time to event was 30 months (SD +/-1.32). Both dysglycaemia (57 (55.9%) vs.14 (13.9%)) and insulin resistance (92 (90.2%) vs. 69 (68.3%), $p<0.001$) were significantly higher in the HFDP group. Within the HFDP group, all measures of dysglycaemia, insulin resistance and progression to T2DM were higher among the DIP group compared to the GDM group.

All CVD risk factors were higher in the HFDP group including diabetes 46(44.6%), hypertension 27(26.1%), dyslipidemia 85(82.5%), family history of CVD 20(19.4%), history of ever smoking 10 (9.7%) and central obesity 66(64.1%), though smoking and family history of CVD were not significantly different between the groups. Overall, the calculated FRS was significantly higher in the HFDP group 8.46(4.9-14.4) vs. 3.48(2.1-5.7) $p<0.001$, with an intermediate to high risk score being present in over 40% of the HFDP individuals 42 (42%) vs. 8 (8%) $p<0.001$. Within the HFDP subtypes, DIP displayed a significantly higher intermediate-high risk category cardiovascular score of 26 (60.4%) vs. 16 (28%) in the GDM group. The overall median cIMT measurement, though within normal limits in all participants, was significantly higher in the HFDP group, with a cIMT of 0.48 (IQR 0.44-0.53) vs. 0.46 (IQR 0.42-0.5) $p=0.037$, though this was no longer significant after adjusting for age. No atherosclerotic plaque was noted at either carotid or femoral sites in any of the participants and there was only one reported event of CAD and one of cerebrovascular accident. Though these measured outcomes may be attenuated after correcting for differences in maternal age and BMI at index pregnancy, this has been explored in the subsequent regression models (Tables 5,6 &7).

The prevalence of the MetS was higher in the HFDP group, 42(40.8%) vs. non-HFDP group 6(5.9%) p<0. 001.Between the two subtypes of HFDP, more women in the DIP arm had MetS 25 (55.6%) vs 17(29.3%).

Table 2. Stratified analysis of maternal outcomes by HFDP groups.

Outcomes at follow-up		**HFDP group**			**Non-HFDP group**	**p-value**
		Total group n=103	GDM n=58	DIP n=45	n=101	
Diabetes status						
Type 2 diabetes mellitus	n(%)	46(44.6)	16(27.6)	30(66.7)	5(4.9)	*a0.0001 *b0.016
Status unknown		27(57.4)	13(81.2)	14(45.2)	4(100)	*a<0.001 *b<0.001
Dysglycaemia		57(55.9)	23(39.6)	34(77.3)	14(13.9)	*a<0.001 *b<0.001
Insulin resistance		92(90.2)	50(87.7)	42(93.3)	69(68.3)	*a<0.001 *b<0.001
Cardiovascular						
Cardiovascular risk factors						
Historical						
1.Age	median(IQR)	37.3(33.1-42.7)	37.1(33.9-41.7)	38.1(32.8-42.7)	34.0(29.2-37.3)	*a <0.001*b0.001
2.Smoking						
Smoker-current/ever	n(%)	10(9.7)	6(10.3)	4(8.9)	8(7.9)	#a0.65 #b0.901
3. Family history CVD[1]		20(19.4)	10(17.2)	10(22.2)	17(17.1)	a0.65. b0.90
Blood pressure						
Chronic HTN[2] ≥ 140/90	n(%)	27(26.1)	10(17.2)	17(37.8)	12(11.9)	*a0.009 *b0.001
Known HTN		22(21)	8(13.8)	14(31.1)	11(10.9)	*a0.03 *b0.005
Newly diagnosed		5(4.8)	2(3.5)	3(6.7)	1(0.9)	*#a0.02 *#b0.005
Biochemistry						
1. Proportion with dyslipidaemia		85(82.5)	46(79.3)	39(86.7)	73(72.2)	a0.080 *b0.146
2. Triglycerides	n(%)	0.8(0.6-.1.14)	0.79(0.6-1.1)	0.9(0.6-1.2)	0.58 (0.44-0.8)	*a0.000 *b<0.001
3. High density lipoprotein	median(IQR)	1.1(0.9-1.3)	1.1(0.9-1.3)	1.1(1-1.2)	1.1(0.91-1.36)	a0.894 *b0.8770

4. Total cholesterol 5. Low density lipoprotein		4.2(3.6-4.8) 2.7(2.1-3.1)	4.2(3.6-4.8) 2.7(1.9-3.2)	4.2(3.8-4.8) 2.7(2.2-3.1)	3.6(3.1-4.4) 2.2(1.7-2.7)	*a0.002 *b0.002 a0.003 *b0.002
Cardiovascular risk a) Framingham risk score (FRS) Categories	median(IQR)	n=100 8.46(4.9-14.4)	n=57 7.17(4.4-10.7)	n=43 11.7(5.8-16.7)	n=101 3.48(2.1-5.7)	*a<0.001 *b<0.001
• High risk(>20%) • Intermediate(10-20%) • Low(<10%)	n(%)	13(13) 29(29) 58(58)	5(8.7) 11(19.3) 41(71.9)	8(18.6) 18(41.8) 17(239.5)	0 8(7.9) 93(92.1)	*a<0.001 *b<0.001
Non-lab based FRS score	median(IQR)	43.9(25>30-62.8)	35.1(21.1-48.9)	53.2(35.5-70.2)	20.7(12.0-32.7)	*a<0.001 *b<0.001
b) Carotid intima media thickness(cIMT)						
• Thickened IMT (>0.8mm)	n(%)	0	0	0	0	--
• Overall mean CIMT • Right CCA[6] • Left CCA	median(IQR)	0.48(0.44-0.53) 0.47(0.43-0.51) 0.49(0.44-0.54)	0.48(0.44-0.52) 0.47(0.42-0.52) 0.5(0.45-0.54)	0.49(0.43-0.55) 0.46(0.42-0.55) 0.49(0.44-0.55)	0.46(0.42-0.50) 0.46 (0.42-0.51) 0.46(0.43-0.51)	*a0.0371 b0.113 a0.35 b0.59 *a0.007 *b0.0274
3. Metabolic syndrome						
Metabolic Syndrome Harmonized criteria	n(%)	42(40.8)	17(29.3)	25(55.6)	6(5.9)	a<0.001 *b<0.001
BMI[3] ≥ 30 Waist circumference ≥ 80cm Waist: hip ratio (>0.85)	n(%)	71(68.9) 95(92.2) 66(64)	39(67.2) 54(93.1) 33(56.9)	32(71.1) 41(91.1) 33(73.3)	46(45.5) 77(77) 26(26)	*a <0.001 *b<0.003 *a<0.001 *b<0.001 *a<0.001 *b<0.010

[a]p-value for control vs. hyperglycemia detected in pregnancy; [b]p-value for control vs GDM and DIP. *indicates significance <0.05. [#] indicates chi exact test. [†]IQR: interquartile range [‡]SD: Standard deviation [1]CVD: cardiovascular disease. [2] HTN: hypertension. [3] BMI: body mass index. [4] CCA: common carotid artery.

Body composition measures between the groups (Table 3)

Ninety-nine HFDP and 96 of the non HFDP women(n=195) had a DXA scan performed. All body composition variables, except total percent body fat, were significantly higher in the exposed vs. nonexposed groups, including the fat mass index (FMI) 13.9(11.7-16.3) vs.12.3(8.9-14.6) p=0.0008 and VAT:SAT ratio 0.20 vs.0.16. p<0.001. In particular, measures of visceral adipose tissue(VAT) were elevated in HFDP women. VAT volume was 517cm^3(IQR372.6-610.2) vs. 322 cm^3(IQR 219.2-469.2) p <0.001 and percentage trunk fat of 39.8%(IQR 35-43.6) vs 36.5%(IQR 29.4-42.5) p=0.0066 with android:gynoid ratio at 0.93(0.86-1.02) vs 0.86(0.75-0.95) p <0.0001.After adjusting for BMI at baseline, only visceral adiposity indices remained significant between the groups.

Table 3. Maternal body composition outcomes by exposure to HFDP vs. nonexposure to HFDP group.

Follow up		**HFDP group**			**Non-HFDP group**	**p-value**
		Total n=99	GDM n=55	DIP n=44	n=96	
Fat-free soft tissue mass (FFSM)/kg	median(IQR)	44.9(39.2-51.0)	44.1(38.7-49.2)	45.5(41.6-53.7)	41.9(36.2-46.1)	* [a]0.0006*[b]0.0007
Total body fat mass/kg	mean(SD)	36.1(9.6)	34.9(9.2)	37.5(10.1)	31.1(10.7)	[*a] 0.0008 [*b] 0.0011
Total lean body mass/kg	mean(SD)	48.1(7.2)	46.9(6.2)	49.7(8.3)	44.1(6.9)	[*a] 0.0001 [*b] 0.0005
Total body fat %		44.1(40-46.1)	44.3(40.2-47.1)	43.7(40.3-45.3)	43.5(38.9-46.9)	[a]0.42 [b] 0.6146
Android fat/kg	median(IQR)	2.6(2.0-3.4)	2.4(1.9-3.4)	2.9(2.1-3.7)	1.9(1.3-2.7)	* [a]<0.001 [*b] 0.0001
Visceral fat/kg	median(IQR)	1.5(1.2-1.9)	1.3(1.2-1.9)	1.6(1.3-2.0)	1.1(0.7-1.6)	[*a]<0.001 [*b]0.0001
Visceral fat %	median(IQR)	39.8(35-43.6)	38.4(35.4-43.6)	40.2(35.8-43.2)	36.5(29.4-42.5)	[*a] 0.0066 [*b]0.0237
Arm(right) fat mass/kg	median(IQR)	2.1(1.8-2.7)	2.1(1.8-2.6)	2.4(2.0-2.9)	1.8(1.3-2.2)	* [a] <0.001 [*b]0.0001
Leg(right) fat mass/kg	median(IQR)	7.4(5.7-9.1)	7.4(5.6-9.0)	7.1(5.8-8.9)	6.9(5.2-8.5)	[a] 0.13 [b]0.3178

VAT[1] (volume) cm^3	median(IQR)	517.6(372.6-610.2)	510.6(361-594)	570.5(407.9-655.5)	322(219.2-469.2)	*[a]<0.001 *[b] 0.0001
SAT[2](volume) cm^3	median(IQR)	2334.6(2005.1-3003.6)	2316.2(1995.4-2949.2)	2492.6(20240.8-3121.8)	1943.9(1430-2678.6)	*[a]0.0003 [b]0.0001

Ratios	**HFDP group**	GDM	DIP	**Non-HFDP group**	**p-value**
VAT:SAT	0.20(0.16-0.24)	0.20(0.15-0.24)	0.21(0.17-0.24)	0.16(0.12-0.20)	*<0.001
Android: Gynoid	0.93(0.86-1.02)	0.93(0.85-1.02)	0.98(0.86-1.02)	0.86 (0.75-0.95)	[a]*<0.001
Trunk: limb fat mass	0.82(0.73-0.98)	0.81(0.70-0.94)	0.86(0.76-1.01)	0.72(0.60-0.83)	[a]*<0.001
FMI[3] (Fat mass/height[2])	13.9(11.7-16.3)	13.4(11.3-15.9)	14.6(12.2-18.0)	12.3(8.9-14.6)	[a]*0.0008
Lean mass//height[2]	18.7(17.1-20.8)	18.4(17.1-20.3)	19.6(17.0-21.8)	17.1(16.7-18.9)	[a]*<0.001

*indicates significance <0.05 [a]p-value for control vs. hyperglycemia detected in pregnancy; [b]p-value for control vs GDM and DIP [1]VAT: visceral adipose tissue. [2]SAT: subcutaneous adipose tissue [3]FMI: fat mass

Strength of association of prior HFDP and progression to diabetes, MetS and CVD risk (Table 4)

The crude OR(15.5(CI 5.8-41.3, p<0.001)) for progressing to T2DM in the HFDP group remained significant(adjusted aOR 10.5(CI 3.7-29.5) p<0.001) after correcting for confounders. This significant risk persisted within the subtypes of HFDP, with a greater aOR 27.6(8.7-87.4) in the DIP group. The crude OR for prior HFDP and its association with MetS was 10.9(4.3-27.1 p<0.001) vs. aOR of 6.3(CI 2.2-18.1), which remained significant (p=0.004) after adjusting for factors present at the index pregnancy including maternal age, parity, systolic blood pressure(SBP) and BMI. Additionally, the aOR within the HFDP subtypes and MetS risk remained significant . In the HFDP group, the association with an intermediate-high 10-year estimated CVD risk based on FRS was significant for crude OR 8.4(3.6-19.1) p<0.001; and when adjusting for confounders including factors at index pregnancy including age, BMI, SBP(aOR of 4.3(1.6-11.5, p=0.003) and this significance was retained in the DIP group.

Table 4. Frequency of the outcome's diabetes, metabolic syndrome and CVD event as predicted by FRS and the adjusted odds ratio for the association with a history of HFDP.

	Diabetes			**Metabolic syndrome**			**Predicted cardiovascular risk (FRS)[b]**		
	Crude OR[a] (95% CI)	**Adjusted OR[#] (95% CI)**	**p-value**	**Crude OR[a] (95% CI)**	**Adjusted OR[†] (95% CI)**	**p-value**	**Crude OR[a] (95% CI)**	**Adjusted OR[‡] (95% CI)**	**p-value**
HFDP	15.5(5.8-41.3)	10.5(3.7-29.5)	[*]<0.001	10.9(4.4-27.9)	6.3(2.2-18.1)	<0.001	8.4(3.6-19.1)	4.3(1.6-11.5)	[*]0.003
GDM	7.3(2.5-21.3)	4.6(1.5-14.4)	0.008	6.5(2.4-17.8)	3.9(1.2-12.5)	[*]0.021	4.5(1.7-11.4)	1.7(0.52-5.55)	0.373
DIP	38.4(12.9-114.4)	27.6(8.7-87.4)	[*]<0.001	19.7(7.2-54.5)	10.5(3.3-32.8)	[*]<0.001	17.7(6.9-45.7)	1.5(0.23-10.0)	[*]<0.001

[a] p-values for all unadjusted OR was p <0.001
[b] FRS categorized as intermediate and high (10-year risk of >10% risk for CVD event)
[#]Confounders: Post-partum variables: Maternal age, BMI, Parity and family history diabetes
[†]Confounders: Index pregnancy variables: Maternal age, parity, SBP, BMI
[‡]Confounders: Index pregnancy variables: Maternal age, SBP, BMI

Factors associated with FMI and progression to type 2 diabetes and cardiovascular risk using multiple variable logistic and linear regression

i) Factors associated with FMI

Linear regression models of relevant independent maternal factors present at pre-pregnancy(distal), pregnancy(intermediate) and post-partum(proximal) associated with log FMI is shown in Table 5. In the distal model only multiparty was significantly associated with FMI. In the intermediate model, BMI measured at first visit in pregnancy was significantly associated with the outcome, with BMI difference (post-partum BMI- pregnancy BMI) being the only significant factor in the proximal model. In the final model, only initial BMI in pregnancy and BMI difference was significantly associated with the outcome log FMI.

Table 5. Linear regression model for log-transformed FMI. Values are standardized regression coefficients (β) with 95% confidence interval, and p-values.

Outcome: Fat mass index	**M1: Distal model**		**M2: Intermediate model**		**M3: Proximal model**		**M4:M1+M2+M3**	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Maternal pre-pregnancy factors								
Parity-multiparous	0.18(0.07-0.29)	**0.001**						
Education: secondary and higher	0.03(-0.46-0.53)	0.890						
SES[1] score	0.004 (-0.02-0.03)	0.718						
Smoking	-0.04(-0.21-0.13)	0.654						
Alcohol	0.08(-0.04-0.21)	0.182						
HIV[2] positive	-0.04(-0.16-0.07)	0.476						
Maternal pregnancy factors								
Age			-0.001(-0.008-0.005)	0.748				
Initial BMI[3]			0.031(0.02-0.03)	**<0.001**				
Prior HFDP[4]			0.019(-0.07-0.11)	0.683				
Maternal post-partum factors								
Exclusive breastfeeding					-0.086(-0.27-0.10)	0.374		
BMI difference[#]					0.009(0.003-0.016)	**0.005**		
Exercise					-0.086(-0.224-0.062)	0.266		
Combined models								
Parity							0.023(-0.065-0.112)	0.599
Education: secondary and up							0.167(-0.139-0.474)	0.283
SES score							0.002(-0.015-0.020)	0.818

Smoking Alcohol HIV positive Age Initial BMI Prior HFDP Exclusive breastfeeding BMI difference Exercise				0.053(-0.066-0.172) 0.001(-0.892-0.091) 0.092(-0.012-0.198) -0.000(-0.006-0.006) 0.045(0.039-0.051) 0.029(-0.06-0.124) 0.033(-0.085-0.151) 0.043(0.034-0.051) -0.030(-0.126-0.065)	0.379 0.976 0.082 0.982 **<0.001** *0.539 0.581 **<0.001** *0.527
Adj. R^2 =0.02 N=191		Adj R^2 =0.36 N=187	Adj. R^2 =0.04 N=151	Adj. R^2 =0.66 N=144	

[1]SES: socioeconomic asset score, [2]HIV: Human immunodeficiency virus, [3]BMI: body mass index, [4 5]HFDP: hyperglycaemia first detected in pregnancy
[#]BMI diff: the difference between BMI measured at post-partum visit minus BMI at pregnancy

ii) Progression to T2DM (Table 6)

Multivariate logistic regression models examining the association between relevant maternal risk factors and progression to diabetes found that higher parity, family history of T2DM, and positive HIV status were significant in the distal model. Prior HFDP was significant in the intermediate model, with absence of exclusive breastfeeding being significant in the proximal model. However, in the combined final model only prior history of HFDP, a family history of T2DM and an elevated VAT:SAT ratio were independently associated with risk of progression to T2DM.

Table 6. Multivariate analysis of factors associated with risk of progression to diabetes. Values are standardized odds ratios (OR) with 95% confidence interval, and p-value.

Outcome Diabetes	**M1: Distal model**		**M2: Intermediate model**		**M3: Proximal model**		**M4: M1+M2 +M3**	
	OR (95%CI)	p value	OR (95% CI)	p value	OR (95%CI)	P value	OR (95%CI)	P value
Maternal pre-pregnancy factors								
Parity	5.14(1.82-14.50)	**0.002**						
Education: secondary and higher	0.20 (0.09-4.31)	0.309						
SES[1] score	0.88(0.74-1.05)	0.180						
Alcohol	0.60(0.211-1.71)	0.341						
Smoking	0.40(0.09-1.68)	0.213						
HIV[2]	0.29(0.108-0.82)	**0.019**						
Family history diabetes	4.67(2.15-10.13)	**<0.001**						
Maternal pregnancy factors								
Age			1.01(0.94-1.08)	0.704				
Initial BMI[3]			1.04(0.98-1.09)	0.151				
Initial SBP[4]			0.99(0.96-1.02)	0.886				
Prior HFDP[5]			12.9(4.33-38.45)	**<0.001**				
Maternal post-partum factors								
Exclusive breastfeeding					0.141(0.04-0.48)	**0.002**		
Exercise					2.28(0.86-6.05)	0.097		
Progesterone only contraception					1.11 (0.49-2.53)	0.794		
VAT:SAT[6] ratio					10.70(2.53-45.25)	**<0.001**		

Combined models Parity Education: secondary and up SES score Alcohol Smoking HIV Family history diabetes Age Initial BMI Initial SBP Prior HFDP Exclusive breastfeeding Exercise Progesterone only contraception VAT:SAT ratio				0.94(0.19-4.55) 0.32(0.01-8.50) 0.75(0.56-1.01) 0.79(0.18-3.51) 0.31(0.04-2.07) 0.40(0.07-2.29) 4.37(1.80-15.80) 0.96(0.88-1.06) 1.03(0.94-1.13) 0.99(0.95-1.04) 7.84(1.37-44.6) 0.23(0.05-0.96) 1.00(0.25-4.01) 0.96(0.33-2.81) 7.98(1.09-57.94)	0.944 0.498 0.062 0.763 0.230 0.307 **0.010*** 0.482 0.407 0.840 **0.020*** 0.057 0.998 0.955 **0.004***
N=200 Adj. R^2 =0.18		N=193 Adj. R^2 =0.23	N=151 Adj R^2 =0.15	N=142 Adj. R^2 =0.37	

[1]SES: socioeconomic asset score, [2]HIV: Human immunodeficiency virus, [3]BMI: body mass index, [4]SBP: systolic blood pressure, [5]HFDP: hyperglycaemia first detected in pregnancy, [6]VAT:SAT ratio: visceral adipose tissue: subcutaneous adipose tissue ratio

iii) Carotid intima media thickness (Table 7)

Linear regression models examining maternal factors associated with log cIMT in the various models are displayed in Table 7. These analyses show that of the variables included in the distal model, no variables were significant. In the intermediate model; maternal age, initial SBP remained significant with triglyceride levels and BMI difference being significant in the distal model. In the combined final model, maternal age and SBP at pregnancy and BMI difference were significantly associated with cIMT thickness.

Table 7. Linear regression model for log-transformed carotid intima media thickness (cIMT). Values are standardized regression coefficients (β) with 95% confidence interval, and p-value.

Outcome CIMT	**M1: Distal variables**		**M2: Intermediate model**		**M3: Proximal model**		**M4: M1+M2 +M3**	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Maternal pre-pregnancy factors								
Parity								
Education: secondary and higher	0.02(-0.011-0.070)	0.163						
SES[1] score	0.02(-0.175-0.207)	0.868						
HIV[2] status	0.003(-0.005-0.013)	0.432						
Smoking	0.035(-0.011-0.081)	0.142						
Family history CVD[3]	-0.019(-0.085-0.046	0.557						
	0.009(-0.039-0.059)	0.695						
Maternal pregnancy factors								
Age			0.01(0.007-0.013)	**<0.001**				
Initial BMI[4]			0.002(-0.002-0.004)	0.075				
Initial SBP[5]			0.001(0.000-0.003)	**0.015**				
Prior HFDP			-0.03(-0.07-0.006)	0.095				
Maternal post-partum factors								
Exclusive breastfeeding					-0.01(-0.081-0.06)	0.779		
Exercise					0.008(-0.044-0.06)	0.757		
BMI difference					0.002(0.000-0.004)	**0.048**		
TG[6] level					0.042(0.000-0.085)	**0.047**		
HDL[7] level					-0.02(-0.089-0.037)	0.419		

Combined models					
Parity				-0.015(-0.060-0.035)	0.555
Education				-0.011(-0.187-0.165)	0.900
SES score				0.007(-0.002-0.016)	0.146
HIV status				0.041(-0.020-0.103)	0.187
Smoking				0.021(-0.046-0.089)	0.533
Family history CVD				0.000(-0.050-0.050)	0.973
Age				0.009(0.006-0.013)	**<0.001***
Initial BMI				0.002(-0.000-0.006)	0.099
Initial SBP				0.002 (0.000-0.003)	**0.018***
Prior HFDP				-0.014(-0.074-0.045)	0.632
Exclusive breastfeeding				-0.001(-0.069-0.066)	0.969
Exercise				0.016(-0.037-0.071)	0.540
BMI difference				0.006(0.002-0.105)	**0.001***
TG level				0.002(-0.004-0.047)	0.929
HDL level				0.011(-0.051-0.075)	0.712
N=197 Adj. R^2 =-0.005		N=192 Adj. R^2 =0.23	N=156 Adj R^2 =0.02	N=147 Adj. R^2 =0.21	

[1]SES: socioeconomic asset score, [2]HIV: Human immunodeficiency virus [3] CVD: cardiovascular disease [4]BMI: body mass index, [5]SBP: systolic blood pressure , [6] TG: triglyceride, [7]HDL: high density lipoprotein

a) HIV influence on outcomes

The prevalence of HIV within our cohort was 21.6%(n=44), of which 18.5%(n=19) were HIV reactive in the HFDP exposed group. The independent influence of HIV on the measured outcomes assessed in multivariate regression models was not found to be significant between women with a prior history of HFDP vs those without (Table 5,6 &7).

Discussion

We found that Black African women with compared to those without a history of HFDP have a (a) 10.5-fold increased risk for developing T2DM(4.6 and 27.6-fold for GDM and DIP groups respectively) (b) 6-fold increased risk of having MetS together with higher visceral adiposity; and (c) higher cardiovascular risk. HIV infection did not influence any of these outcomes.

The rate of progression to T2DM we found was similar to that reported from Cape Town (48% at 5-6 years post-partum) in predominantly mixed-ancestral women using the same diagnostic criteria (7). This is over 3-fold higher than the background prevalence rates of T2DM for black South African women (30), and identifies a highly vulnerable population. This risk was significantly higher both in women with GDM, the less severe form of dysglycaemia in pregnancy, as well as women with DIP. Comparisons of our findings with international studies are challenging, as different study designs, lengths of follow-up, definitions and diagnostic criteria are employed. Nevertheless, a recent meta-analysis (31) identified the time period with the highest risk of progression as 3 to 6 years following the pregnancy, which aligns with our findings.

The modifiable risk factors associated with progression and risk of progression to T2DM have best been explored in high income countries(HIC)(32–34) with limited data for low to

middle-income countries(LMIC) and very little African representation(35). Identification of these factors play a key role when implementing strategies to delay or prevent the onset of T2DM. The post-partum period offers a critical window of opportunity to implement such strategies and screen for diabetes in these high-risk individuals. In our study, though a history of HFDP and family history of T2DM and visceral adiposity were significantly associated with progression to T2DM , only adiposity is modifiable. Though these factors have been associated with progression in previous studies, two other studies in South Africa found that a range of measures of the extent of dysglycaemia to be significant predictors: fasting and 2-hr plasma glucose on OGTT in pregnancy diagnosis of HFDP before 24 weeks indicative of undiagnosed/unrecognized pregestational diabetes, and exposure to insulin and OHA in pregnancy (7,9), all of which are non-modifiable. The protective role of breastfeeding for T2DM (36), was not found to be significant in our study. The high number of women with HFDP who were unaware of their diabetes status indicates that postnatal assessment for diabetes is necessary even in an already overwhelmed LMIC health infrastructure.

Our study highlighted that, in addition to the high risk of progression to T2DM, women with a history of HFDP are more vulnerable to CVD as evidenced by their higher CVD risk scores and greater cIMT. Though their risk scores remained significantly elevated after correcting for obvious confounders, their cIMT, a surrogate marker for identifying pre-clinical atherosclerosis and hence often used as a surrogate CVD measure (37) was independent of prior HFDP and rather influenced by age, SBP and BMI difference. Though no cardiovascular events were reported in our study, their higher cardiovascular risk is likely to translate into CVD events with time as our cohort was young with a relatively short period of follow-up. Consequently our study did not corroborate the findings of a recent metanalysis(2)

demonstrating that women exposed to HFDP have a two-fold higher risk of cardiovascular events independent of the development of T2DM 10-25 years post-delivery.

The rates of obesity were high across both groups during the index pregnancy and follow-up, in keeping with high rates of obesity amongst Black women in SA(30). However, fat distribution differed in those with and without a history of HFDP; with the former having significantly greater upper body fat distribution, in particular, the visceral area. Visceral adiposity is known to be a strong predictor of diabetes and CVD independent of overall fatness(17,38). South African studies have demonstrated that visceral depots are generally lower in black African women compared to their white female counterparts, despite the higher insulin resistance and high rate of obesity in this group (30,39,40). Pregnancies complicated by both obesity and HFDP are known to independently influence immediate and long-term maternal outcomes. In our study, initial BMI at index pregnancy and weight gain post-pregnancy were not significantly associated with progression to diabetes or cardiovascular risk outcomes, but visceral adiposity was a significant predictor of progression to T2DM. A better appreciation of the role of obesity and body composition on our measured cardiometabolic outcomes would have been possible if it weren't for our lack of knowledge relating to fat distribution pre- and during pregnancy and BMI measurement at pre-pregnancy and early post-delivery, known to be independent risk factors for progression to T2DM(30). It is therefore plausible that our study confirmed a high prevalence of MetS in women with HFDP with a 6-fold increased risk after adjusting for age, parity, BMI and SBP. Overall, altered body composition favouring visceral adiposity, together with the increasing burden of HFDP and its cardiometabolic consequences is the setting for the perfect storm in which the coexistence of these entities may perpetuate a vicious cycle fueling the NCD burden.

Strengths of our study were the inclusion of a control group of women with confirmed normoglycaemia on OGTT from the same time period and setting as the HFDP group. The sample was adequately powered for establishing both the primary and secondary outcomes for our study. The use of DXA to assess maternal body composition at 3-5 years post-partum has not previously been reported from Africa. Lastly, exploring the impact HIV had on these outcomes which was insignificant contributes to the limited existing data.

A major limitation of our study was that this was a single centre study. Further limitations included missing pre-pregnancy BMI values for participants leading to difficulty in exploring this variable as a confounder. Difficulty tracing participants resulted in a small sample size and hence less power for sub-group comparisons in particular within the HFDP group (GDM vs. DIP). Self-reporting bias was a potential problem in the administered questionnaire. Cardiovascular risk and body composition outcomes were not measured in pregnancy for longitudinal comparison. However, these parameters are often influenced by the normal physiological adaptations of pregnancy, making interpretation difficult. The use of FRS to calculate cardiovascular risk in these women may have underestimated their risk since the formula doesn't take into account other unique potential risk factors relating to HFDP such as recurrent HFDP and hypertensive disorders of pregnancy. The time of diabetes diagnosis is unclear as women did not have a 6-week post-partum OGTT, in some cases, HFDP may have never resolved post-partum. The small number of women who were HIV positive may have accounted for the lack of effect it had on the measured outcomes. The lack of data surrounding ART regimes was a further limitation as well as the fact that this was a single centre study.

The ever-growing epidemic of diabetes, obesity, and CVD, particularly amongst LMIC populations, poses a significant public health burden, occurring alongside the burden of chronic infectious diseases. Our study identifies a group of young women at high risk of cardiometabolic outcomes, in whom the postpartum period offers a window of opportunity to implement targeted screening, counseling and lifestyle and/or pharmacologic interventions. Future prospective studies are needed to explore the best timing and impact of these interventions in curtailing the adverse outcomes and improving cardiometabolic health in this vulnerable group of women.

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Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

SAN, KKG, NL, VN, and LMS contributed to the research conceptualisation. VN and LMS contributed to the study project coordination and data collection. VN wrote the initial version of the manuscript, and all the authors contributed to interpretation of data, editing, and revising the manuscript.

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APPENDIX L: PLAGIARISM DECLARATION

Plagiarism declaration

FACULTY OF HEALTH SCIENCES UNIVERSITY OF WITWATERSRAND

I, **Veronique Nicolaou** (student number 9702987E), am a student registered for the degree of **DOCTOR OF PHILOSOPHY**, in the academic year 2021.

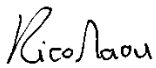
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- I have followed the required conventions in referencing the thoughts and ideas of others
- I understand that the University of Witwatersrand may take disciplinary action against me if there is a belief that this is not my own work or that I have failed to acknowledge the source of the ideas or words in my writing

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