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GLUCOSE METABOLISM AND PREGNANCY IN SOUTH AFRICAN WOMEN

Shelley Macaulay

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

Johannesburg, 2018

DECLARATION

I, Shelley Macaulay, declare that this thesis is my own work. Where others have contributed and assisted, they have been duly acknowledged. This thesis is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this, or any other university.



Signed on the 31st day of July 2018

DEDICATION

I dedicate this thesis to my sister, Casey; my best friend, my sounding board, my rock.

"The applause of a sister means so much more than that of any crowd. For they see your achievement. She sees all that led up to it."(Anonymous)

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

SCIENTIFIC SEMINARS

Macaulay, S. *Glucose metabolism and pregnancy in South African women*. Postgraduate Lunchtime Talks, Faculty of Health Sciences, University of the Witwatersrand, Chris Hani Baragwanath Academic Hospital, 20 June 2013.

Macaulay, S. *The diabetic pregnancy: a bitter-sweet state*. Department of Obstetrics and Gynaecology's Seminar Series, Chris Hani Baragwanath Academic Hospital, 30 July 2013.

Macaulay, S. *The diabetic pregnancy: a bitter-sweet state*. Centre for Exercise Science and Sports Medicine, School of Therapeutic Sciences, University of the Witwatersrand, 12 March 2014.

Macaulay, S. *"Boys live dangerously in the womb*." Division of Human Genetics, Clinical Academic Meeting, University of the Witwatersrand and National Health Laboratory Service, 7 September 2017.

Macaulay, S. *Gestational diabetes: prevalence and impact on fetal growth and neonatal outcomes*. Gestational Diabetes Mellitus Symposium, School of Public Health, University of the Witwatersrand, 25 July 2018.

CONFERENCE PROCEEDINGS

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PEER REVIEWED PUBLICATIONS ARISING FROM THIS RESEARCH PROJECT

The research findings arising from this PhD study were written up as four separate manuscripts that were submitted to international peer-reviewed scientific journals. Three manuscripts have been accepted for publication and have been published by the respective journals, and one manuscript has been reviewed, revised (as per reviewers' recommendations) and resubmitted to the journal. The original publications presented in this thesis are listed below and the student's contribution to the publications follows. The signed co-author agreement for the use of each publication within this thesis can be found in Appendix A.

1. **Macaulay, S.**, Dunger, D.B. & Norris, S.A. (2014) Gestational diabetes mellitus in Africa: A systematic review. *PLoS One*, 9 (6): e97871. doi:10.1371/journal.pone.

2. **Macaulay, S.**, Ngobeni, M., Dunger, D.B. & Norris, S.A. (2018) The prevalence of gestational diabetes mellitus amongst black South African women is a public health concern. *Diabetes Research and Clinical Practice*, 139: 278-287. doi:10.1016/j.diabres.2018.03.012.

3. **Macaulay, S.**, Munthali, R.J., Dunger, D.B. & Norris, S.A. (2018) The effects of gestational diabetes mellitus on fetal growth and neonatal birth measures in an African cohort. *Diabetic Medicine.* Advanced online publication. doi: 10.1111/dme.13668.

4. **Macaulay, S.,** Buchmann, E.J., Dunger, D.B. & Norris, S.A. The reliability and validity of last menstrual period for gestational age estimation in a low-middle-income setting. Submitted to the *Journal of Obstetrics and Gynaecology Research.* Reviewed, revised and resubmitted.

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.

RELATED PEER REVIEWED PUBLICATIONS

1. Khan, T., **Macaulay, S**., Norris, S.A., Micklesfield, L. & Watson, E.D. (2016) Study Protocol: Physical activity and the risk for gestational diabetes mellitus amongst pregnant women living in Soweto. *BMC Women's Health*, 16:66.

2. Watson, E.D., **Macaulay, S**., Lamont, K., Gradidge, P. J-L., Pretorius, S., Crowther, N.J. & Libhaber, E. (2017) The effect of lifestyle interventions on maternal body composition during pregnancy in developing countries: a systematic review. *CardioVascular Journal of Africa*, 28(6): 397-403.

3. Norris, S.A., Kagura, J., Koethe, J.R., **Macaulay, S**., Larske, S., Wedi, C.O.O., Hemelaar, J., Hulgan, T., Aronoff, D., Adam, Y., Gray, G., Klipstein-Grobusch, K., Kennedy, S. & Dunger, D.B. The co-morbidity of HIV infection and obesity has a compounding risk for the development of gestational diabetes mellitus among pregnant women. *Lancet Diabetes and Endocrinology.* Submitted and under review.

GRANTS AND AWARDS

- 2017: Recipient of a South African National Research Foundation (NRF) PhD six month Sabbatical Grant (R100 000).
- 2017: Recipient of a Staff Bursary for PhD fees through the University of the Witwatersrand
- 2016: Recipient of a University of the Witwatersrand Faculty Research Committee (FRC) Individual Research Grant (R8 000).
- 2016: Recipient of a Staff Bursary for PhD fees through the University of the Witwatersrand.
- 2015: Recipient of a Staff Bursary for PhD fees through the University of the Witwatersrand.
- 2014: Recipient of a University of the Witwatersrand Faculty Research Committee (FRC) Individual Research Grant (R8 000).
- 2014: Recipient of a Staff Bursary for PhD fees through the University of the Witwatersrand.
- 2014: Macaulay S, Dunger DB & Norris SA. (2014) Gestational diabetes mellitus in Africa: A systematic review. *PLoS One*, 9 (6): e97871. doi:10.1371/journal.pone selected as one of the top articles for the MRC Celebrates Science E-Newsletter.
- 2013: Co-recipient with Professor Shane Norris of a World Diabetes Foundation Grant (R1.5 million)
- 2013: Recipient of a University of the Witwatersrand Faculty Research Committee (FRC) Individual Research Grant (R10 000).
- 2013: Recipient of a Staff Bursary for PhD fees through the University of the Witwatersrand.

NON-SCIENTIFIC PUBLICATIONS

- Article entitled *"The diabetic pregnancy: a bitter-sweet state"* written for PedMed (Issue 2, 2013), a magazine on Pediatric and Adolescent Medicine.

ABSTRACT

Background

Gestational diabetes mellitus (GDM) refers to diabetes with first onset during pregnancy. A diagnosis of GDM has serious implications for the affected woman and her unborn child. Women with GDM are at risk of developing Type 2 diabetes mellitus (T2DM) and children are at risk of being born large for gestational age, becoming overweight or obese, and developing T2DM later in life. The prevalence of T2DM and GDM is increasing worldwide and particularly within low-to-middle income countries (LMICs). However, there is a lack of data on GDM prevalence and the effects of GDM-exposure amongst African populations. Like other LMICs, South Africa's public healthcare system is heavily burdened and under-resourced. In terms of antenatal care, South Africa utilises a selective screening approach for GDM whereby only women with certain risk factors are investigated further, and prenatal ultrasound services are not readily available to all. These two factors make the identification of women with GDM, the monitoring of fetal growth, and clinical decisions around gestational age at delivery, difficult. Evidence-based data is required in order to propose changes to current policies governing maternal health.

Aims

The overarching aim of this study was to investigate GDM amongst black South African women. The study set out to discern what GDM prevalence figures exist for the African continent, determine the prevalence of GDM amongst women living in urban Soweto, Johannesburg, assess the effects of GDM exposure on fetal growth and neonatal birth measures, and evaluate the reliability and validity of using last menstrual period (LMP) dates to estimate gestational age.

Methods

Firstly, a systematic review was performed to determine what GDM prevalence figures exist for Africa. Secondly, a cross-sectional screening study was performed to ascertain the GDM prevalence amongst black South African women living in Soweto. Pregnant women were recruited from the Chris Hani Baragwanath Academic Hospital in Johannesburg. Inclusion criteria were; black South African females, ≥18 years of age, residing in Soweto, ≤20 weeks pregnant with singleton pregnancies. A total of 3 656 women who fulfilled the inclusion criteria were briefed on the study and invited to access free GDM screening when they were

24-28 weeks pregnant. A total of 2 009 women underwent a two-hour 75 g oral glucose tolerance test (OGTT) at 24-28 weeks gestation and a diagnosis of GDM was made using the World Health Organization's 2013 criteria. Of those 2 009 women, 1 909 had complete and conclusive OGTT readings and formed the study sample for the 'GDM screening' component of this study. Thirdly, a subgroup (n=1 017) of the 3 656 women formed the Soweto First 1000 Days study (S1000); a longitudinal pregnancy cohort study. These pregnant women were followed up from early in their pregnancies with repeated fetal ultrasounds and neonatal birth measures were taken at delivery. A total of 741 women from the S1000 study underwent an OGTT and had conclusive glucose results. These women formed the 'fetal growth and neonatal birth measures' component of this study whereby GDM-exposed fetuses were compared to unexposed fetuses. Furthermore, amongst these 741 women, gestational age was determined by last menstrual period (LMP) and ultrasonography. Comparisons between the two methods were made. Multiple statistical analyses were performed.

Results

Only six of the 54 African countries had reported data on GDM prevalence. At the time the systematic review was performed, South Africa had four reported studies of which only two involved black women. Based on the limited number of African studies, the GDM prevalence across Africa was estimated to be around 5%.

The GDM screening study revealed a 9.1% (95% confidence interval (CI) 7.9, 10.5) (174/1906) GDM prevalence. Compared to the women without GDM, those with GDM were significantly heavier with higher body mass indexes (BMIs), older, and of higher household socioeconomic status. A family history of diabetes and a diagnosis of anaemia were also more common amongst the women with GDM. Being ≥35 years, having a BMI ≥30 kg/m^2 (obese) and a family history of diabetes were found to be significant GDM risk factors. Furthermore, the fasting plasma glucose reading had a high sensitivity (83.3% (95% CI 77.0, 88.5)) in diagnosing GDM.

The longitudinal cohort study involving the 741 women who underwent repeated fetal ultrasounds showed that GDM exposure was associated with an increase in fetal growth measures, especially abdominal circumference which was already seen at 16-18 weeks gestation. When stratified by sex, male fetuses showed a significant association between GDM exposure and increased abdominal circumference (p=0.009) but this was not observed amongst female fetuses (p=0.286). There was no difference in birth measures between the

GDM-exposed and unexposed neonates. Gestational age dating by LMP overestimated gestational age by 0.2 days. Women with discrepancies between their LMP-based and ultrasound-based estimates were of significantly lower weight and household socioeconomic status that those without discrepancies. Whilst there was substantial agreement between the two methods, LMP had poor sensitivity in identifying late-term (41 weeks 0 days - 41 weeks 6 days gestation) and post-term (≥42 weeks gestation) pregnancies (29.0% (95% CI 14.2, 48.0) and 33.3% (95% CI: 4.33, 77.7) respectively).

Conclusion

Only 11% of the African continent reported GDM prevalence figures. The GDM screening component of this study represents the largest GDM prevalence study in South Africa to date. A GDM prevalence of 9.1% amongst black South African women living in urban Soweto is concerning and warrants further discussion around current GDM screening policies. Whilst universal screening for GDM may be unrealistic in South Africa's heavily burdened public healthcare system, the use of a fasting plasma glucose screen was shown to be highly sensitive in identifying women with GDM and should be considered as a possible screening tool.

Additionally, repeated ultrasound measures identified the effects of GDM as early as 16-18 weeks gestation, with GDM-exposed male fetuses having lager abdominal circumferences than unexposed fetuses. This highlights that sexual dimorphism in relation to *in utero* exposure to GDM exists with male fetuses being particularly susceptible to the hyperglycaemic environment and abdominal circumference being an indicator of increased fetal growth. A low rate of macrosomia and large-for-gestational age neonates was observed amongst the GMD-exposed group of neonates compared to historical GDM-exposed populations. In the absence of ultrasound, LMP is a reliable alternative for gestational age dating during early pregnancy. However, LMP estimates should not be relied upon to make clinical decisions regarding elective Caesarean sections or induction of labour for supposed prolonged pregnancies. In the case of GDM, fetal ultrasonography appears important for fetal sexing and the monitoring of fetal growth, as well as for informing clinical decisions around delivery. Health systems strengthening through increased availability of ultrasound services and detection of GDM should be considered in order to improve maternal and child health in South Africa.

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TABLE OF CONTENTS

DECLARATION ii
DEDICATION iii
PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT iv
PEER REVIEWED PUBLICATIONS ARISING FROM THIS RESEARCH PROJECT v
RELATED PEER REVIEWED PUBLICATIONS vi
GRANTS AND AWARDS vii
NON-SCIENTIFIC PUBLICATIONS viii
ABSTRACT ix
ACKNOWLEDGEMENTS xii
TABLE OF CONTENTS xiv
LIST OF FIGURES xx
LIST OF TABLES xxii
LIST OF ABBREVIATIONS xxiv
PREFACE xxvii
SECTION 1 BACKGROUND AND STUDY CONTEXT 1
CHAPTER 1 LITERATURE REVIEW 2
1.1 The Developmental Origins of Health and Disease 3
1.1.1 Developmental Plasticity 3
1.1.2 The Role of Epigenetics 6
1.1.3 Transgenerational Effects of the Developmental Origins of Health and Disease 8
1.1.3.1 Fetal Adaptation to Under- and Overnutrition 9
1.2 The Nutrition Transition 10
1.2.1 The Impact of the Nutrition Transition on the Risk for Diabetes 11
1.3 Homeostatic Regulation of Glucose 12
1.3.1 Type 2 Diabetes Mellitus 14
1.3.1.1 The Pathophysiology of Type 2 Diabetes Mellitus 14
1.3.1.2 The Prevalence of Type 2 Diabetes Mellitus 15
1.3.2 Glucose Metabolism during Pregnancy 16
1.3.2.1 The Role of Hormones in Glucose Metabolism during Pregnancy 17
1.3.2.2 The Role of Adipokines in Glucose Metabolism during Pregnancy 19
1.4 Gestational Diabetes Mellitus 19
1.4.1 Risk Factors for Gestational Diabetes Mellitus 22

1.4.1.1 Older Age 22
1.4.1.2 High Weight and Body Mass Index 22
1.4.1.3 High Parity 23
1.4.1.4 Previous Macrosomic Infant 24
1.4.1.5 Family History 24
1.4.1.6 Ethnicity 24
1.4.2 Fetal Exposure to Gestational Diabetes Mellitus 25
1.4.2.1 Altered Fetal Growth 26
1.4.2.2 Macrosomia/ Large for Gestational Age 27
1.4.2.3 Congenital Abnormalities 27
1.4.2.4 Other Short-Term Complications 28
1.4.2.5 Obesity and Type 2 Diabetes Mellitus 29
1.4.3 Maternal Morbidities Associated with Gestational Diabetes Mellitus 31
1.4.3.1 Gestational Hypertension and Pre-eclampsia 31
1.4.3.2 Birth Complications and Decision around Delivery 32
1.4.3.3 Recurrent Gestational Diabetes Mellitus 33
1.4.3.4 Type 2 Diabetes Mellitus 33
1.4.4 Screening Methods and Diagnostic Criteria for Gestational Diabetes Mellitus 34
1.4.4.1 The Oral Glucose Tolerance Test 34
1.4.4.2 Diagnostic Criteria for Gestational Diabetes Mellitus 35
1.4.4.3 Universal versus Selective Screening for Gestational Diabetes Mellitus 37
1.4.5 An Increase in the Global Prevalence of Gestational Diabetes Mellitus 38
1.4.6 Management of Gestational Diabetes Mellitus 39
1.5 Gestational Diabetes Mellitus in the South African Context 41
1.5.1 South Africa's Obesity Epidemic 42
1.5.2 The Burden of Diabetes in South Africa 45
1.5.3 Maternity Care in South Africa 46
1.5.3.1 Screening for Gestational Diabetes Mellitus in South Africa 47
1.6 Summary of the Literature Review 49
1.7 Gaps in the Literature and the Motivation for the Study 50
1.8 Aims and Objectives 51
1.9 Study Hypotheses 51
1.10 The Study in Relation to the Gestational Diabetes Mellitus Conceptual Framework 52

CHAPTER 2 STUDY CONTEXT AND METHODS 54
2.1 Soweto, Johannesburg, South Africa 55
2.2 Chris Hani Baragwanath Academic Hospital 57
2.2.1 Current Screening and Management of Gestational Diabetes Mellitus in Soweto 57
2.3 The MRC/Wits Developmental Pathways for Health Research Unit 59
2.4 Participant Recruitment 60
2.5 Data Collection 61
2.5.1 Soweto First 1000 Days Study 61
2.6 Methodology 63
2.7 Sample Size 64
2.8 Data Analysis 65
2.9 Ethical Approval 65
SECTION 2 EMPIRICAL PAPERS 66
CHAPTER 3 GESTATIONAL DIABETES MELLITUS IN AFRICA: A SYSTEMATIC REVIEW 67
3.1 Introduction 68
3.2 Methods 70
3.2.1 Protocol and Registration 70
3.2.2 Information Sources and Search Strategy 70
3.2.3 Data Extraction 71
3.2.4 Assessment of Reporting Quality and Risk of Bias 72
3.3 Results 72
3.3.1 Study Selection 72
3.3.2 Reporting Quality and Risk of Bias 73
3.3.3 Study Characteristics 75
3.4 Discussion 81
CHAPTER 4 THE PREVALENCE OF GESTATIONAL DIABETES MELLITUS AMONGST BLACK SOUTH AFRICAN WOMEN IS A PUBLIC HEALTH CONCERN .. 86
4.1 Introduction 87
4.2 Participants, Materials and Methods 88
4.2.1 Participants 88
4.2.2 Anthropometry 89
4.2.3 The Oral Glucose Tolerance Test 90
4.2.4 Questionnaires 91
4.2.5 Management and Follow-up 91

4.2.6 Statistical Analyses 92
4.2.7 Ethical Approval 92
4.3 Results 93
4.4 Discussion 99
4.5 Conclusion 102
CHAPTER 5 THE EFFECTS OF GESTATIONAL DIABETES MELLITUS ON FETAL GROWTH AND NEONATAL BIRTH MEASURES 103
5.1 Introduction 104
5.2 Participants and Methods 105
5.2.1 Study Design 105
5.2.2 Study Participants 105
5.2.3 Ethical Approval 105
5.2.4 Data Collection 105
5.2.4.1 Ultrasonography 106
5.2.4.2 The Oral Glucose Tolerance Test 106
5.2.4.3 Anthropometry 107
5.2.5 Statistical Analyses 108
5.3 Results 109
5.3.1 Fetal Growth Measures 112
5.3.2 Management of Women with Gestational Diabetes Mellitus 115
5.3.3 Delivery Outcomes 115
5.4 Discussion 118
5.5 Supplementary Tables 121
CHAPTER 6 THE RELIABILITY AND VALIDITY OF GESTATIONAL AGE DATING BY LAST MENSTRUAL PERIOD IN A LOW-MIDDLE-INCOME SETTING 127
6.1 Introduction 128
6.2 Methods 129
6.2.1 Participants 129
6.2.2 Questionnaires 129
6.2.3 Gestational Age Dating Methods 130
6.2.3.1 Last Menstrual Period 130
6.2.3.2 Ultrasonography 130
6.2.3.3 Discrepancies in Gestational Age Dating Methods 130
6.2.4 Anthropometry 131
6.2.5 Statistical Analyses 132

6.2.6 Ethical Approval 133
6.3 Results 133
6.3.1 Participants' Characteristics 133
6.3.2 Gestational Age Distribution 137
6.3.3 Level of Agreement of Gestational Age Dating by Last Menstrual Period and Ultrasonography 138
6.3.4 Reliability of Gestational Age Dating by Last Menstrual Period 139
6.3.5 Validity of Gestational Age Dating by Last Menstrual Period 140
6.4 Discussion 141
SECTION 3 INTEGRATED DISCUSSION 144
CHAPTER 7 DISCUSSION AND CONCLUSION 145
7.1 Consolidated Findings 146
7.2 Hypotheses Revisited 148
7.3 Emerging Research Themes 149
7.3.1 Consequences of Prenatal Exposure to Gestational Diabetes Mellitus 149
7.3.1.1 Sexual Dimorphism 149
7.3.1.2 Postnatal Risks 151
7.3.2 Health Systems Strengthening 152
7.3.2.1 Prenatal Ultrasound Services 152
7.3.2.2 Hospital Record Keeping 153
7.3.2.3 Screening for Gestational Diabetes Mellitus 154
7.3.2.4 Nutritional Counselling and Health Education 154
7.4 Contextual Relevance 156
7.4.1 The African and South African Context 156
7.4.1.1 Recommendations Arising From This Research 158
7.5 Strengths and Limitations 159
7.6 Future Studies 160
7.7 Conclusion 162
REFERENCES 163
APPENDICES 197
APPENDIX A: AUTHORS' AGREEMENT 198
APPENDIX B: INFORMATION AND CONSENT SHEETS 200
APPENDIX C: PHD DATA COLLECTION SHEETS 209
APPENDIX D: ORAL GLUCOSE TOLERANCE TEST PROCEDURE 219
APPENDIX E: ETHICS CLEARANCE CERTIFICATES 221

APPENDIX F: PRIMSA GUIDELINES FOR SYSTEMATIC REVIEWS 224
APPENDIX G: LIST OF AFRICAN COUNTRIES 226
APPENDIX H: STROBE CHECKLIST 227
APPENDIX I: RISK OF BIAS TOOL FOR PREVALENCE STUDIES 229
APPENDIX J: PUBLISHED PAPERS 230
APPENDIX K: PLAGARISM DECLARATION & TURNITIN REPORT 260

LIST OF FIGURES

Figure 1.1 The effects of sub-optimal fetal nutrition on organogenesis and the associations with non-communicable diseases 5

Figure 1.2 The effects of epigenetic changes induced by environmental cues during development plasticity 7

Figure 1.3 The effects of maternal under- and overnutrition on fetal programming resulting in a dual burden of childhood malnutrition and overweight 9

Figure 1.4 The most critical stages (Three, Four and Five) of the nutrition transition 11

Figure 1.5 The various steps involved in glucose metabolism in the human 13

Figure 1.6 Trends in prevalence of diabetes, 1980-2014, by country income group 15

Figure 1.7 Changes in glucose homeostasis during pregnancy 17

Figure 1.8 Hormonal changes that influence glucose homeostasis during pregnancy 18

Figure 1.9 A conceptual framework for gestational diabetes mellitus (GDM). 21

Figure 1.10 Short-term complications of exposure to maternal diabetes *in utero* 28

Figure 1.11 The perpetuating cycle of diabetes from *in utero* exposure to gestational diabetes mellitus (GDM) to Type 2 diabetes (T2DM) and obesity in later life. 29

Figure 1.12 Mean BMI curves for youth both exposed and unexposed to maternal diabetes *in utero* from 27 months of age to 13 years, adjusted for sex, race/ethnicity 31

Figure 1.13 A global map showing the age–standardized prevalence of obesity (BMI ≥30) in 2013 amongst males aged ≥20 years 43

Figure 1.14 A global map showing the age–standardized prevalence of obesity (BMI ≥30) in 2013 amongst females aged ≥20 years 44

Figure 1.15 The conceptual framework for gestational diabetes mellitus (GDM) in relation to the PhD study [Informed by 72]. The areas that this PhD study focuses on are indicated by the red dashed-line boxes and red text. 53

Figure 2.1a Map of South Africa showing Johannesburg in the Gauteng province 56

Figure 2.1b A map of Soweto spanning 200 km^2 South-West of Johannesburg 56

Figure 2.2 The life course framework at the MRC/Wits Developmental Pathways for Health Research Unit.....59

Figure 3.1 Flow diagram illustrating the number of included and excluded studies in the systematic review on gestational diabetes mellitus in Africa.....74

Figure 4.1 Study participation in the GDM prevalence study. OGTT denotes oral glucose tolerance test; ANC, antenatal clinic. 94

Figure 5.1 Sample size for the study investigating the effects of gestational diabetes mellitus (GDM) on fetal growth and neonatal birth size.....110

Figure 5.2 Head circumference (a), biparietal diameter (b), abdominal circumference (c) and femur length (d) of fetuses exposed to gestational diabetes mellitus (GDM) (n=83; red dashed line) versus unexposed fetuses (n=658; solid black line) against gestational age in weeks. The asterisk (*) denotes a statistically significant difference ($p<0.05$) with GDM-exposed fetuses being larger than unexposed fetuses.....113

Figure 6.1 The distribution of gestational age at enrolment determined by prenatal ultrasound and last menstrual period. 137

Figure 6.2 A Bland-Altman plot comparing gestational age estimation by ultrasound and last menstrual period (LOA; limits of agreement).....138

LIST OF TABLES

Table 1.1 Some of the common risk factors for gestational diabetes mellitus 22

Table 1.2 The different diagnostic criteria available for the diagnosis of gestational diabetes mellitus 36

Table 1.3 Risk factors for gestational diabetes mellitus according to the National Guidelines for Maternity Care in South Africa 48

Table 2.1 Inclusion and exclusion criteria of study participants 60

Table 3.1 Reporting quality and risk of bias assessments 75

Table 3.2 Prevalence of gestational diabetes mellitus (GDM) in Africa 79

Table 4.1 Characteristics of the study participants 95

Table 4.2 Glucose readings and gestational diabetes mellitus prevalence according to body mass index category 96

Table 4.3 Analysis of the World Health Organization's 2013 criteria in diagnosing gestational diabetes mellitus 97

Table 4.4 Multiple logistic regression analysis for risk factors associated with gestational diabetes mellitus 98

Table 5.1 Characteristics of the women participating in the S1000 Study 111

Table 5.2 Linear mixed modelling results showing fixed effects on abdominal circumference size (cm) *in utero* 114

Table 5.3 Delivery outcomes and birth anthropometry for neonates exposed to gestational diabetes mellitus (GDM) and unexposed neonates 116

Table 5.4 Birth outcomes of male and female neonates exposed to gestational diabetes mellitus compared to unexposed male and female neonates 117

Table S5.1 Fetal measurements by fetal sex at five time points during pregnancy 121

Table S5.2 Fetal growth measurements in gestational diabetes mellitus (GDM)-exposed versus unexposed fetuses 122

Table S5.3 Fetal measurements by maternal body mass index (BMI) categories at five time points during pregnancy 123

Table S5.4 Fetal measurements by maternal body mass index (BMI) and gestational diabetes mellitus (GDM) status at five time points during pregnancy 124

Table S5.5 Delivery outcomes and birth anthropometry of male versus female neonates 126

Table 6.1 The American College of Obstetrician and Gynecologists' recommendations for defining deliveries according to gestational age 131

Table 6.2 Demographic characteristics of women with known versus unknown last menstrual period (LMP) dates 134

Table 6.3 Characteristics of the study participants with reported last menstrual period dates and ultrasound assessments 135

Table 6.4 Multiple logistic regression analysis for risk factors associated with having discrepancies† between gestational age dating by last menstrual period versus ultrasound 136

Table 6.5 Birth categories according to gestational age estimates by ultrasound, last menstrual period, and hospital records 139

Table 6.6 Preterm, term, and post-term deliveries according to gestational age estimates by early prenatal ultrasound (gold standard) and last menstrual period (n=536) 140

Table 6.7 Validity of gestational age estimates according to last menstrual period† in classifying birth categories 140

Table 6.8 Validity of gestational age estimates according to last menstrual period† in determining critical gestational age cut-offs 141

Table 7.1 Consolidated findings from the four empirical studies 147

LIST OF ABBREVIATIONS

ADA	The American Diabetes Association
AGA	appropriate for gestational age
ANC	antenatal care
BANC	basic antenatal care
BMI	body mass index
Bt20	Birth to Twenty cohort
CHBAH	Chis Hani Baragwanath Academic Hospital
CI	confidence interval
CpG	cytosine phosphate guanine
CRL	crown-rump-length
DALYs	disability-adjusted life years (number of lost years of healthy life)
DNA	deoxyribonucleic acid
DOHaD	developmental origins of health and disease
DPHRU	Developmental Pathways for Health Research Unit
DBP	diastolic blood pressure
FANC	focussed antenatal care
FLT1	*Fms-like tyrosine kinase 1* gene
GAD	glutamic acid decarboxylase
GCT	glucose challenge test
GDM	gestational diabetes mellitus
HAPO	The Hyperglycemia and Adverse Pregnancy Outcome Study
Hb	Haemoglobin
HbA1C	glycated haemoglobin A1C
hcS	human chorionic somato mammotropin
HIV	human immunodeficiency virus
hPGH	human placental growth hormone

hPL	human placental lactogen
HREC	Human Research Ethics Committee
IGF1	*Insulin-like Growth Factor 1* gene
IGF2	*Insulin-like Growth Factor 2* gene
iMMR	institutional Maternal Mortality Ratio
IADPSG	International Association of the Diabetes in Pregnancy Study Groups
LGA	large for gestational age
LMICs	low- middle-income countries
LMP	last menstrual period
MVM	maternal vascular malperfusion
MOUs	midwife run obstetric units
MRC	Medical Research Council
N	absolute number
NDDG	National Diabetes Data Group
NCDs	non-communicable diseases
OGTT	oral glucose tolerance test
P value	probability value
PhD	Doctor of Philosophy
PRIMSA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
REDCap	Research Electronic Data Capture
RNA	ribonucleic acid
S1000	Soweto First 1000 Days study
SEMDSA	Society for Endocrinology, Metabolism and Diabetes of South Africa
SES	socioeconomic status
SFH	symphysis-fundal height
SGA	small for gestational age
SBP	systolic blood pressure
T1DM	Type 1 diabetes mellitus

T2DM	Type 2 diabetes mellitus
TNF-α	tumour necrosis factor alpha
UI	uncertainty interval
USA	United States of America
WHO	World Health Organization
Wits	University of the Witwatersrand
YLD	years lost due to disability

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
α	alpha
β	beta
$	United States of America Dollar
cm	centimetre
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
ZAR	South African Rand

PREFACE

Prior to embarking on this journey of pursuing a PhD, my work and research fell purely within the field of Human Genetics, with a particular interest and fondness for maternal and child health.

My MSc (Med) in Human Genetics involved investigating the genetic susceptibility towards fetal alcohol syndrome (FAS), a condition that is highly prevalent in specific regions of South Africa. The study involved several field trips to Upington in the Northern Cape where FAS is a serious problem. During these field trips I was privileged enough to sit in on consultations between the medical geneticists/genetic counsellors, the affected children and their caregivers. This is where my passion for genetic counselling started, as well as my interest in maternal habits, maternal health and the effects thereof on the unborn child.

I then pursued an MSc (Med) in Genetic Counselling and am a registered genetic counsellor with the Health Professional Council of South Africa. As a practicing genetic counsellor my day-to-day activities involve consulting with patients and their families, including pregnant women and their unborn babies, about genetic conditions. Some of my clinical cases have involved fetal exposure to teratogens or maternal disease, including diabetes. Unlike most single gene disorders, fetal affects from teratogens and maternal illness can be avoided. For this reason, these prenatal cases resonate with me; as a healthcare professional there is a responsibility and duty to educate the pregnant patients and ensure they receive optimal management and care for the two lives at stake.

An opportunity arose for me to further my interest in maternal and child health and move from researching single gene disorders to developing a better understanding of non-communicable diseases and their transgenerational effects. I joined the MRC/Wits Developmental Pathways for Health Research Unit, within the Department of Paediatrics at the University of the Witwatersrand in 2013 to pursue a PhD. I chose to investigate gestational diabetes mellitus (GDM), a condition I had come across in the prenatal clinics but for which there is very little national data, and yet, if detected early enough, can be well-managed. The healthcare system of my country, South Africa, is already heavily burdened and non-communicable diseases, like GDM, compound the burden. I am a firm believer in evidence-based medicine and without clinical epidemiological studies describing the extent of the problem, interventions to assist cannot be designed or implemented. Data on the

prevalence of GDM and how the condition affects women and their babies are much needed in South Africa.

Pursuing a PhD has always been a goal of mine. As a woman in science and a healthcare professional I believe it is essential to further one's academic ability and become involved in research that has the potential to make a difference to the service we provide our patients. The results generated from my PhD add to the global body of literature on the subject of GDM but are also unique in that they represent the black South African population; a population in which little to no research on GDM has been performed. It would be my hope and dream for my work to inform stakeholders and assist in developing public health policies that promote and improve the health and care of pregnant women and their unborn babies in South Africa.

This PhD thesis is presented in the 'integrated' format which includes four manuscripts. Three manuscripts have been accepted for publication (two are published in the respective journals and one is currently available as an advanced online publication) and one manuscript has been reviewed, revised and resubmitted to the journal.

The thesis is divided into three sections that are further divided into seven chapters:

- **Section 1: Background and Study Context**
  This section describes the literature around the topic, the aims and objectives of the study, the study context and the approach taken to conduct the research.
  - Chapter 1: Literature Review
  - Chapter 2: Study Context and Methods

- **Section 2: Empirical Papers**
  This section consists of four empirical papers that report on the findings of the PhD study.
  - Chapter 3: Gestational Diabetes Mellitus in Africa: A Systematic Review
  - Chapter 4: The Prevalence of Gestational Diabetes Mellitus amongst Black South African Women is a Public Health Concern
  - Chapter 5: The Effects of Gestational Diabetes Mellitus on Fetal Growth and Neonatal Birth Measures in a South African Cohort
  - Chapter 6: The reliability and validity of last menstrual period for gestational age estimation in a low-middle-income setting

- **Section 3: Integrated Discussion**

  This section consists of an integrated discussion that consolidates the findings in all four manuscripts and ends with an overall conclusion.

  - Chapter 7: Discussion and Conclusion

SECTION 1

BACKGROUND AND STUDY CONTEXT

CHAPTER 1

LITERATURE REVIEW

This chapter provides an overview of the literature encompassing the study topic to date. Background information on key concepts pertaining to the research, including the Developmental Origins for Health and Disease hypothesis, the nutrition transition and general glucose metabolism are described broadly. In the section on gestational diabetes mellitus, its associated risk factors, screening methods, and consequences are described in more detail. As this is a South African study, there is a section in the literature review that describes the current situation in the country with regard to obesity and diabetes. In addition, details around the South African public healthcare system and antenatal care policies are described. Finally, the motivation behind the study, the overarching aim and the specific objectives, are described.

1.1 The Developmental Origins of Health and Disease

Professor David Barker was a pioneer in describing how chronic diseases, which generally become apparent in adulthood, are associated with early life exposures. The "Barker hypothesis," also known as the "Fetal Origins of Adult Disease" theory, describes how the *in utero* environment may permanently alter fetal programming and induce changes to developmental mechanisms, physiology and metabolism [1-3].

Barker's original research revolved around ischaemic heart disease in adult life and its correlation with poor nutrition during early life [4]. He observed that the rates of death from ischaemic heart disease were highest amongst men who had low weights at birth and at one year of age [5]. Barker and colleagues then went on to describe how Type 2 diabetes mellitus (T2DM) in later life is associated with poor nutrition during the fetal period and infancy [6]. Since then several cohort and animal studies have reported similar findings. The range of non-communicable diseases (NCDs), also referred to as "diseases of lifestyle", associated with an inadequate early-life environment is continuously growing [2, 7, 8]. This area of research has given rise to a field that encompasses biomedical science, public health and epidemiology, and is referred to as the "Developmental Origins of Health and Disease (DOHaD)" [9].

1.1.1 Developmental Plasticity

During human development, there are critical periods of plasticity where organs and physiological processes can be influenced and permanently changed by extrinsic factors. This provides the rationale behind which an unfavourable early environment is able to alter human programming. Human development occurs in the first 1000 days of life which includes the fetal period and extends up to two years of age. Whilst most organs will have developed *in utero*, some, including the brain, still continue to develop into the postnatal period. In addition, development of organs *in utero* is hierarchical; in a nutrient-poor environment a fetus will give preference to the development of important organs over less important organs. For example, development of the brain is prioritised over the kidneys because during fetal life the placenta performs most functions of the kidneys. This causes a permanent physiological change [3] resulting in an alternative phenotype (physical characteristics of an individual) induced by environmental signals [10].

Whilst developmental plasticity allows for adaptation in response to the current environment, the adaptation assumes the individual will be exposed to the same environment later in life. For example, during a famine a pregnant woman is likely to be undernourished and so her fetus would therefore adapt to a nutrient-poor environment. Should such an environment persist postnatally the fetus would be at an advantage as it would be biologically programmed to cope in such circumstances. However, more often than not the extrauterine environment differs from the *in utero* environment. This mismatch results in the individual being at increased risk for NCDs [11]. In the case of reduced kidney development *in utero,* caused by poor fetal nutrition and the prioritisation of the development of more essential organs, such as the brain, an extrauterine environment of adequate or overnutrition would require normal kidney function. If kidney function is suboptimal due to a reduction in renal volume and number of nephrons an individual would be at risk of high blood pressure and subsequent stroke. Similarly, a reduction in pancreatic development *in utero* would have an effect on beta cell (β-cell) mass and consequently insulin secretion (β-cells secrete insulin) which in turn could predispose an individual to developing T2DM [12]. These are some examples that illustrate how a poor *in utero* environment induces fetal physiological changes. Figure 1.1 describes how sub-optimal fetal nutrition can affect organ development and subsequently increase an individual's risk of developing NCDs.

Whilst initial research focussed on the consequences of fetuses and young children being malnourished, further research has shown that overnutrition is just as detrimental. Fetuses of obese mothers and mothers with diabetes are at risk of altered fetal programming [9, 13]. Overnutrition *in utero* results in fetal hyperglycaemia and therefore alters fetal pancreatic function by increasing β-cell volume and insulin secretion [14]. In addition, hyperglycaemia tends to cause an accumulation of fetal fat and a decrease in skeletal muscle mass [15, 16] (Figure 1.1). Optimal maternal nutrition before and during pregnancy is therefore essential to produce healthy offspring that are not at increased risk for NCDs later in life.

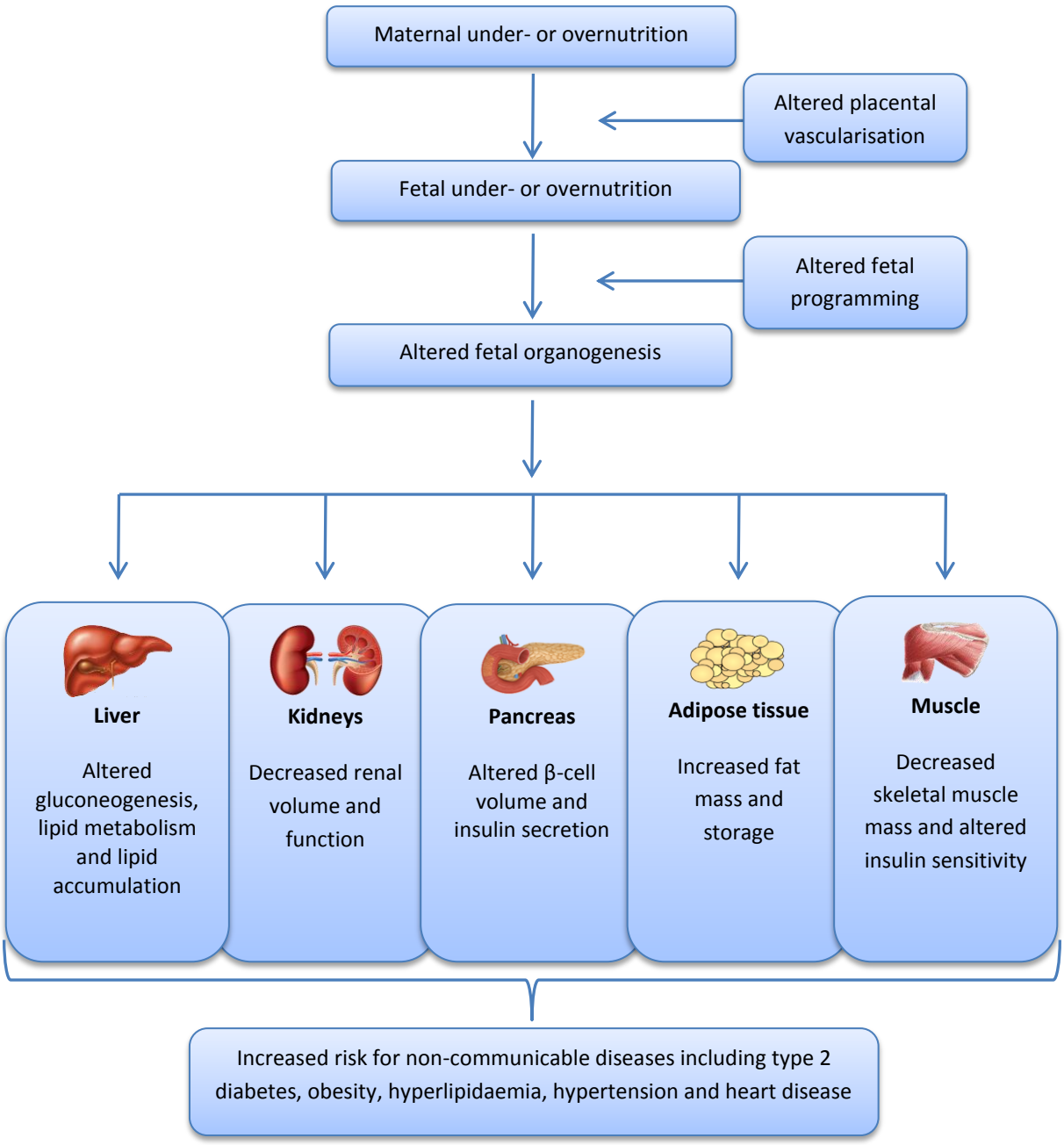


Figure 1.1 The effects of sub-optimal fetal nutrition on organogenesis and the associations with non-communicable diseases (Adapted from [12, 15]).

1.1.2 The Role of Epigenetics

One of the mechanisms behind the induced changes in fetal organ development and altered phenotype is epigenetic modification. Epigenetics refers to changes in gene expression as opposed to actual changes in the gene sequence of one's deoxyribonucleic acid (DNA). There are three epigenetic mechanisms that can affect gene expression; DNA methylation, histone modification and non-coding ribonucleic acid (RNA) molecule interference. Whilst all three epigenetic mechanisms are thought to be involved in DOHaD, DNA methylation is the most well described and understood mechanism. DNA methylation mainly involves the addition of methyl groups to cytosine phosphate guanine (CpG) sites in the genome resulting in cytosine being converted to 5-methylcytosine. This process affects transcription which in turn results in the down-regulation or silencing of gene expression. Conversely, demethylation by means of the removal of a methyl group from areas in the DNA can also occur thus upregulating gene expression [17, 18].

Changes in DNA methylation patterns have been observed in both humans and animals after exposure to unfavourable early-life circumstances, including poor nutrition and *in utero* alcohol exposure [18]. These findings indicate that DNA methylation is responsive to environmental cues. The connection between epigenetics and DOHaD involves the unfavourable early-life environment causing certain genes to become methylated or demethylated which in turn affects specific organ development and physiological processes [17-19]. Two examples of fetal genes that become methylated in the presence of maternal undernutrition are the *Insulin-like Growth Factor 1* (*IGF1*) and *Insulin-like Growth Factor 2* (*IGF2*) genes which are involved in fetal growth and adipose tissue development. Animal studies have shown that in the presence of maternal undernutrition there is a decrease in expression of these genes thus leading to less adipose tissue in the fetus [20].

As mentioned previously, whilst these epigenetic changes occur in order to allow adaptation to the developmental environment, the likelihood that the postnatal/later-life environment is identical to the developmental environment is slim (Figure 1.2).

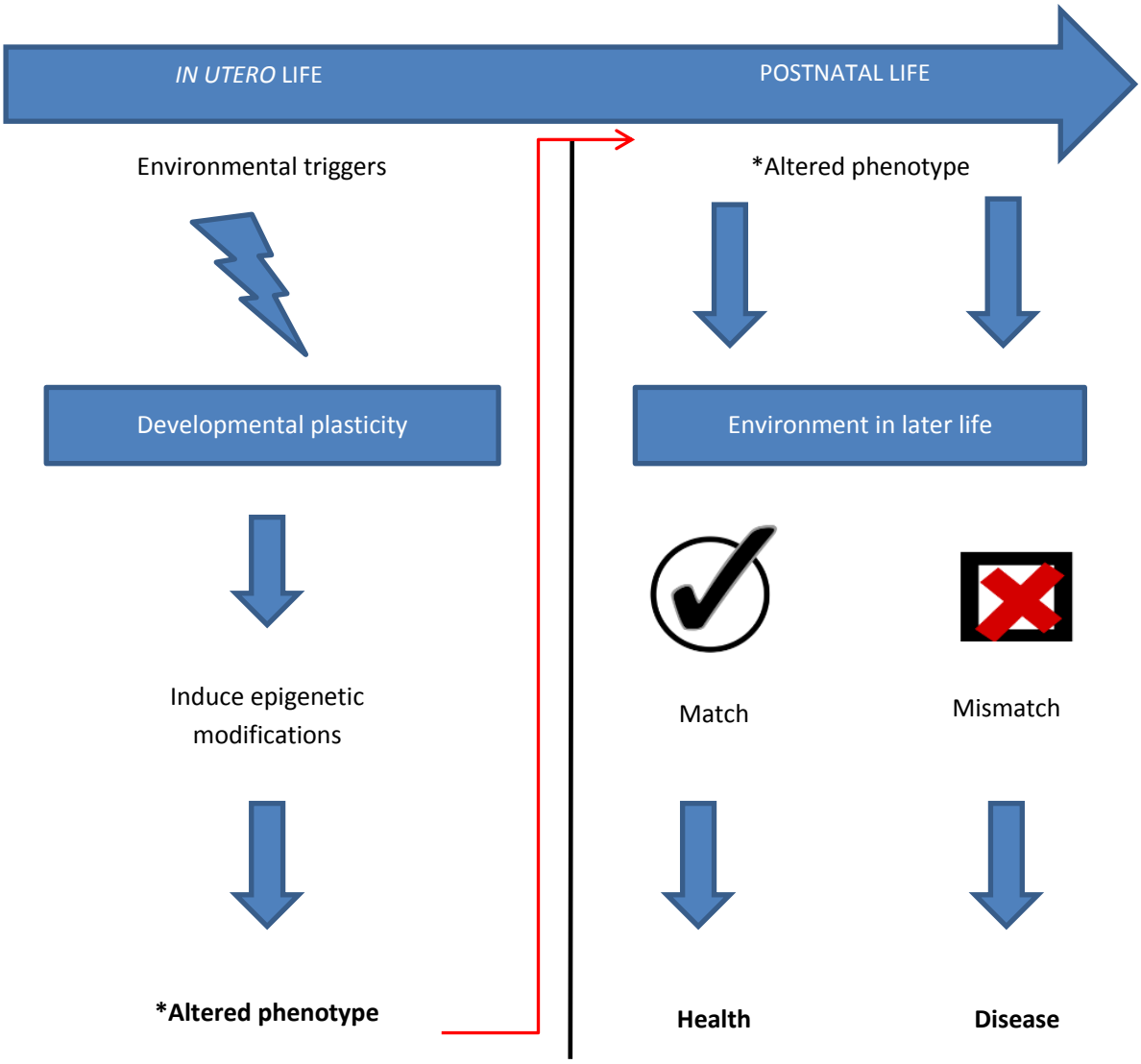


Figure 1.2 The effects of epigenetic changes induced by environmental cues during development plasticity (Adapted from [11]).

1.1.3 Transgenerational Effects of the Developmental Origins of Health and Disease

The altered phenotype that arises from the epigenetic and physiological changes induced by the unfavourable *in utero* environment cannot be reversed. As a result, a female with an altered phenotype would then be at risk of exposing her own offspring to a suboptimal *in utero* environment. This, in turn, would predispose the next generation to NCDs thus perpetuating the cycle [21].

There is evidence pointing towards epigenetic changes being inheritable and therefore being passed on from one generation to the next [22, 23]. This relates to the parent-of-origin effect whereby certain characteristics in the offspring are inherited from either the maternal or the paternal genome. Genomic imprinting is the epigenetic mechanism behind this pattern of inheritance. Imprinting refers to the silencing of an allele (one variant of a pair of genes) from one parental genome. The implication is that the expression of that allele arises only from the other non-silenced parental genome resulting in mono-allelic expression in the offspring. For example, regarding fetal growth, studies have shown that paternally expressed genes generally increase growth whereas maternally expressed genes generally suppress growth [24]. Offspring's size at birth, has been shown to be related to maternal weight (including current and birth weight, parity, and length of gestation) suggesting a significant contribution of maternal genes [25]. Investigators who looked at the genetic and environmental contributions to birth size in an intergenerational study found that maternal genetic factors were responsible for 22% of the variation in birth weight and 19% of the variation in birth length [26].

There is an additional level of complexity in that the *in utero* environment can also be impacted by variations (polymorphisms) in the fetal DNA. Polymorphic variation within fetal genes has been shown to affect maternal physiology and increase risk for adversities. For example, DNA variation within the paternally inherited *IGF2* gene of the fetus has been associated with increased maternal glucose concentration in late pregnancy [27]. Similarly, a genome wide association study identified variants near the *Fms-like tyrosine kinase 1* (*FLT1*) gene of the fetus as being associated with preeclampsia [28]. These examples highlight the complex interactions between the parental and fetal genomes and the *in utero* environment. This illustrates that DOHaD effects are not only transmitted from parents to offspring but that there may be bidirectional interplay.

1.1.3.1 Fetal Adaptation to Under- and Overnutrition

Whilst many maternal stressors can induce fetal adaptation *in utero*, maternal under- and overnutrition has received a lot of interest, especially in LMICs where a double burden of childhood malnutrition and obesity is starting to emerge. Whilst traditionally childhood malnutrition and stunting predominantly burdened LMICs, a decrease in these morbidities has been observed and an increase in overweight has become apparent [29].

The "dual burden" of malnutrition and overweight/obesity that is plaguing LMICs fits within the DOHaD framework and points towards a shift in diet during postnatal life. As mentioned previously, in instances where a pregnant woman is poorly nourished her fetus would adapt to such an environment and traditionally, the postnatal environment would also be one of poor nutrition. However, there appears to be a transition from a postnatal life of malnutrition to one of excess or normal nutrient intake. This transition is causing an increase in childhood weight gain which is increasing the risk for NCDs in later years (Figure 1.3) This interaction between under- and overnutrition observed in LMICs is largely due to what is termed the "nutrition transition" [30].

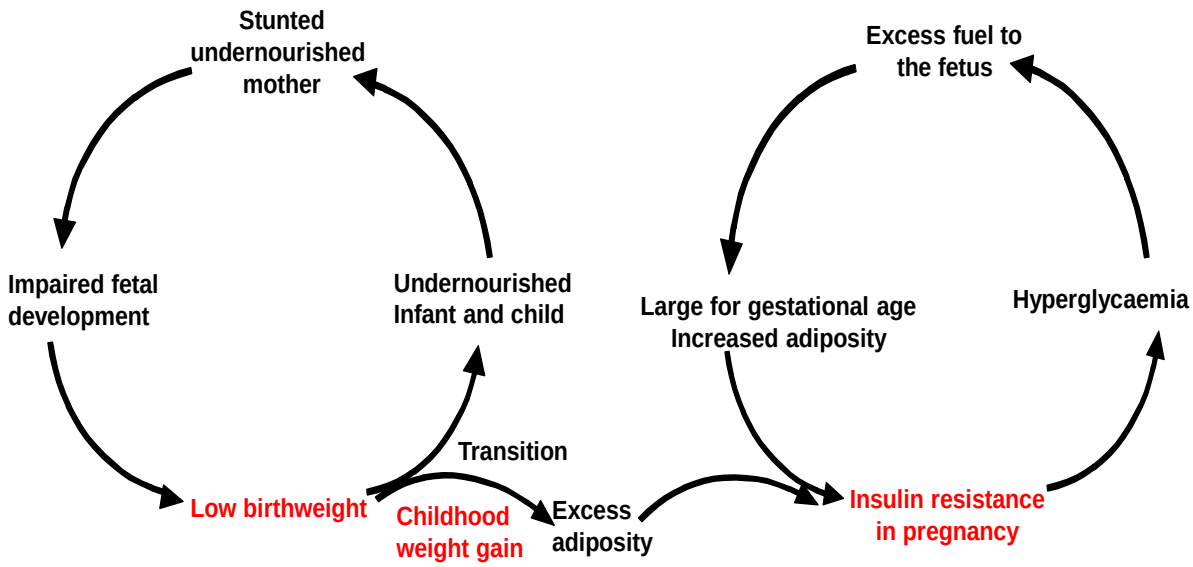


Figure 1.3 The effects of maternal under- and overnutrition on fetal programming resulting in a dual burden of childhood malnutrition and overweight (Adapted from [31, 32])

1.2 The Nutrition Transition

The effects of urbanisation have not only had a significant impact on countries' economies but also on public health. The transition from a rural to urban way of life results in changes in eating habits and a decrease in physical activity; both of which directly impact body mass composition. The adoption of Westernised diets results in more consumption of fats, sugars and refined carbohydrates. This shift is referred to as the "nutrition transition" and is described as occurring simultaneously, or following on from, demographic and epidemiological transitions which occur as a result of industrialisation and urbanisation [33]. A demographic transition involves shifts in fertility and mortality trends from high to low. A country undergoing an epidemiological transition will move from experiencing a high prevalence of infectious diseases and poor nutrition, to experiencing a high prevalence of chronic and progressive diseases and potentially obesity [34].

Popkin [35] describes the nutrition transition as consisting of five stages; Stage One involves the collection of food by means of hunter-gatherers and Stage Two involves emerging famine as a result of scarcity of food. This is followed by Stage Three which involves an improvement in a country's economy through a rise in revenue, and consequently, a decrease in famine. Stage Four involves dietary changes comprising of a more Westernised diet high in fats, sugars and processed food. Together with this dietary shift comes a shift in physical activity, for example reduced energy expenditure in the workplace as a result of access to technology. A combination of a diet high in fat and sugar and reduced physical activity increases one's risk of developing NCDs. Stage Five involves behavioural changes to try and counteract the effects of the preceding stages. Stages Three, Four and Five are of particular interest and represent the nutrition transition that is currently seen in many LMICs (Figure 1.4).

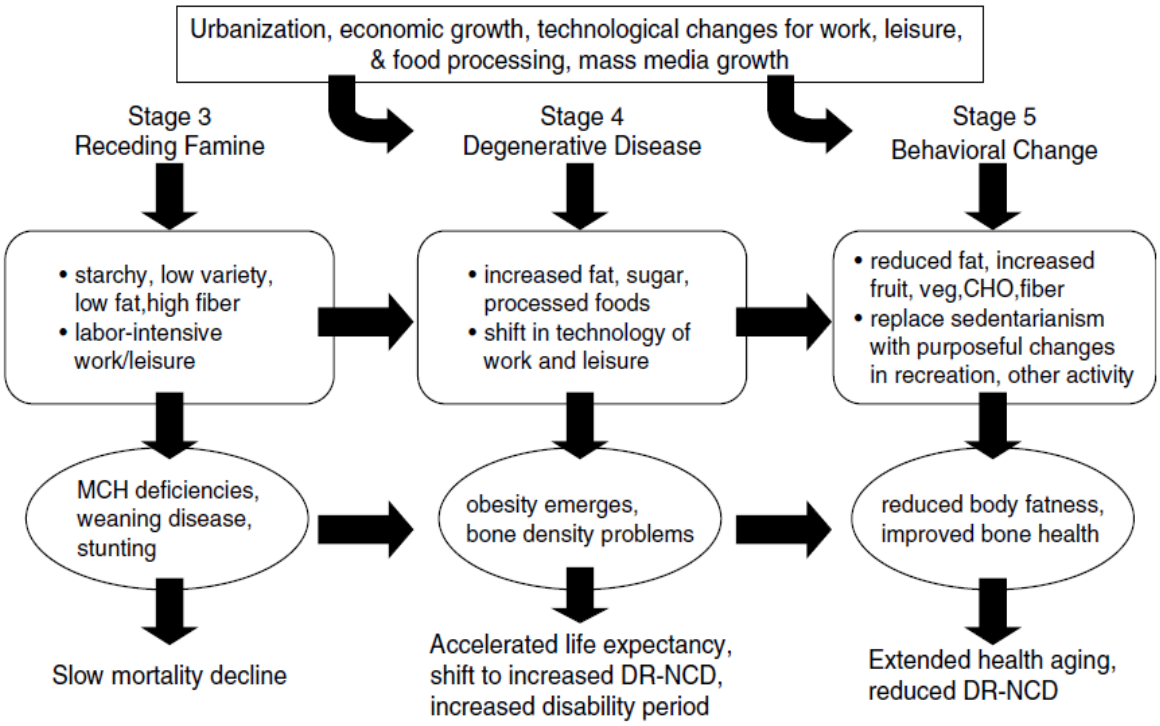


Figure 1.4 The most critical stages (Three, Four and Five) of the nutrition transition (Reproduced with permission from Nature Publishing Group [33])

1.2.1 The Impact of the Nutrition Transition on the Risk for Diabetes

The nutrition transition began in the early 1990s in LMICs and is fuelling overweight and obese populations through the consumption of increased amounts of fat, refined carbohydrates and sugar [36]. Along with the rapid increase in overweight and obesity is the increase in the number of people affected by T2DM [37, 38].

As illustrated in Figure 1.3 maternal undernutrition can result in a low birth weight infant and having a low birth weight increase ones risk of developing T2DM in middle-age [39, 40]. Over the past few years an increase in overweight and obesity has been noted amongst adult women in LMICs [41]. This raises concern around the risk of insulin resistance, aberrant glucose metabolism and hyperglycaemia during pregnancy, which in turn, would result in a suboptimal *in utero* environment for a fetus to develop in. Whilst glucose is an essential source of cellular energy too much of it has serious consequences for both the child and mother (Figure 1.3) [31].

1.3 Homeostatic Regulation of Glucose

The regulation of blood glucose is an endocrine process involving the pancreas, liver and glucoregulatory hormones. The islets of Langerhans are clusters of several types of pancreatic cells including alpha and β-cells. Alpha cells (α) secrete the hormone glucagon, whereas β-cells secrete the hormone insulin. These two hormones are essential in regulating blood glucose homeostasis. Normal blood glucose concentration should be between 3.9 mmol/l and 6.1 mmol/l [42]. Excess blood glucose causes the β-cells of the pancreas to release insulin and low levels of glucose triggers the α-cells of the pancreas to release glucagon. There are three ways in which glucose enters and circulates the blood system; through absorption from the intestinal tract after the ingestion of food, or through a process known as glycogenolysis, or via a process known as gluconeogenesis [43].

The process of glucose homeostasis and the essential roles of the liver and pancreas are illustrated in Figure 1.5. The diagram further describes the process of glucose metabolism after eating which involves ingested carbohydrates being broken down into the monosaccharide glucose, after which absorption into the blood stream from the digestive tract occurs. As blood glucose levels rise pancreatic β-cells secrete insulin. Insulin stimulates the majority of somatic cells to absorb glucose in the liver and muscle cells. Muscle cells convert glucose into glycogen for storage. Excess glucose is absorbed by adipose tissue and stored as fat (triglycerides). When blood glucose levels drop, the α-cells of the pancreas release glucagon. Glucagon promotes the breakdown of glycogen reserves in the liver and muscle cells to glucose which is then released into the blood stream; this is called glycogenolysis. Glycogenolysis occurs during the first eight to 12 hours of fasting [43].

In the prolonged fasting state, when glycogen reserves are depleted, the liver can also produce new glucose through a process known as gluconeogenesis that utilises lactate, pyruvate, glycerol, and amino acids [43, 44]. In addition, adipose tissue releases triglycerides as free fatty acids for use as an energy source by other tissues when glucose availability is low [45].

The inability of the pancreas to produce and secrete insulin due to the autoimmune destruction of β-cells leads to Type 1 diabetes mellitus (T1DM) which usually has an early onset. In contrast, T2DM is not an autoimmune disorder. It is caused by a combination of insulin resistance and decreased insulin secretion, often in association with obesity [45, 46].

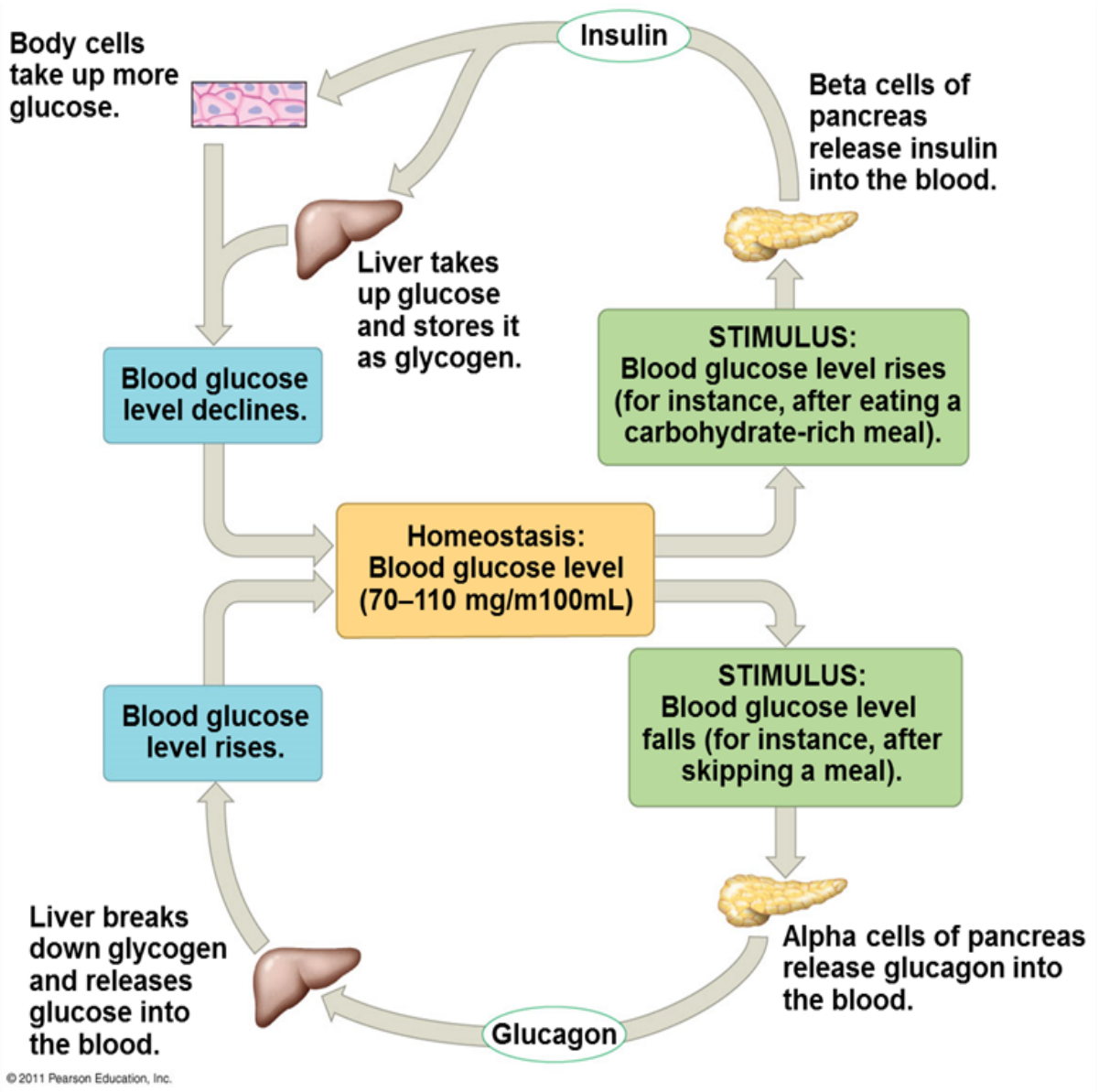


Figure 1.5 The various steps involved in glucose metabolism in the human (Reproduced with permission from Pearson Education [42]).

1.3.1 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is the most common form of diabetes, accounting for approximately 90% of people with diabetes worldwide [37, 46]. It is caused by insulin resistance and poor insulin secretion with a generally late onset [47]. However, once considered an adult onset disease, T2DM is now being diagnosed in adolescents. From a genetic perspective, monogenic causes of T2DM do exist but are very rare. The vast majority of T2DM cases are considered multifactorial whereby several genetic factors may collectively contribute towards a predisposition (susceptibility) together with environmental factors [48]. To date, more than 120 distinct genetic loci have been associated with the development of T2DM [49, 50]. Whilst genetics and older age are involved in T2DM predisposition, the majority of risk factors are lifestyle dependent; overweight and obesity, smoking and physical inactivity. Being overweight, particularly having a larger waist circumference and body mass index (BMI), is the most significant risk factor for T2DM in most populations [46].

1.3.1.1 The Pathophysiology of Type 2 Diabetes Mellitus

Insulin resistance and β-cell dysfunction result in impaired insulin secretion with insulin resistance usually being detected first [48, 51]. Insulin secreted by the pancreas is unable to stimulate the muscle and fat cells in absorbing glucose and unable to prevent glycogenolysis and gluconeogenesis in the liver. As a result, plasma glucose concentrations rise resulting in fasting hyperglycaemia. Consequently, the pancreatic β-cells secrete more insulin to compensate for the rise in blood glucose; this leads to hyperinsulinaemia. Over time, progressive β-cell dysfunction begins to occur causing ineffective insulin secretion. This then creates an environment of hyperglycaemia and elevated insulin resistance and a lack of glycaemic control resulting in T2DM. An individual can be in the insulin-resistant and impaired glucose tolerance state for several years before the condition progresses through β-cell deterioration and results in a diagnosis of T2DM [48].

Poorly managed T2DM causes several medical complications with serious short- and long-term effects. Cardiovascular disease, diabetic retinopathy and renal failure are some of the serious consequences of uncontrolled diabetes. The condition is therefore a significant public health concern that impacts significantly on a country's healthcare system and economy [46].

1.3.1.2 The Prevalence of Type 2 Diabetes Mellitus

The World Health Organization (WHO) estimated that 422 million adults were living with diabetes (of which the majority had T2DM) in 2014. This figure is four times that of the figure derived in 1980 when 108 million people were thought to have the condition. The global prevalence of diabetes in 1980 was 4.7% and in 2014 was reported to be 8.5% [46]. The rise in T2DM is as a result of the increase in urbanisation, the nutrition transition and people leading less active lives, and is in parallel with the global rise of obesity [52].

In 2012, 3.7 million deaths globally were attributable to hyperglycaemia or diabetes of which 43% occurred in people under the age of 70 years. The majority of these deaths under 70 years of age were of people living in LMICs. Whilst the prevalence of diabetes has increased globally, LMICs have shown the most rapid rise in prevalence (Figure 1.6) [46].

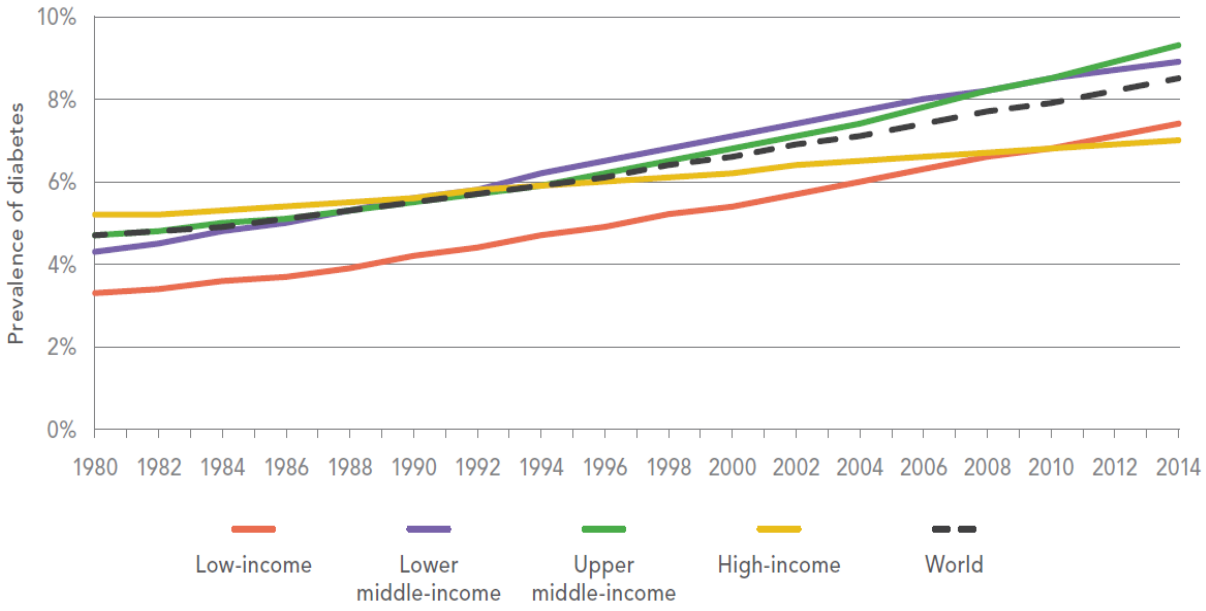


Figure 1.6 Trends in prevalence of diabetes, 1980-2014, by country income group (Reproduced with permission from WHO [46]).

1.3.2 Glucose Metabolism during Pregnancy

There are several adaptations related to maternal transfer of nutrients to the fetus for fetal growth that occur during pregnancy. As pregnancy progresses, an increase in insulin resistance is naturally observed, peaking during late pregnancy [53, 54]. 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% [53, 55]. As glucose readily crosses the placenta [56] the insulin-resistant state prevents or reduces maternal somatic tissues from absorbing the blood glucose thus shunting it to the fetus. 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 [53].

The developing fetus requires a continuous supply of glucose for energy and growth. Therefore, as the mother becomes more insulin resistant, fasting glucose may be stable but hepatic glucose production (by means of glycogenolysis) and new glucose production from lactate and amino acids (gluconeogenesis) increases to supply the glucose demands for both the mother and fetus. Endogenous glucose production by the liver increases by at least 16% in the third trimester in the fasting state [57]. The liver produces glucose despite the increased insulin concentrations which indicates that the liver becomes insensitive to insulin [58]. In addition, postprandial glucose levels are elevated and peak for a longer period of time in later pregnancy [59, 60]. Figure 1.7 illustrates how maternal insulin sensitivity decreases as pregnancy progresses. This results in more insulin production which facilitates glucose being shunted to the fetus. 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 [61].

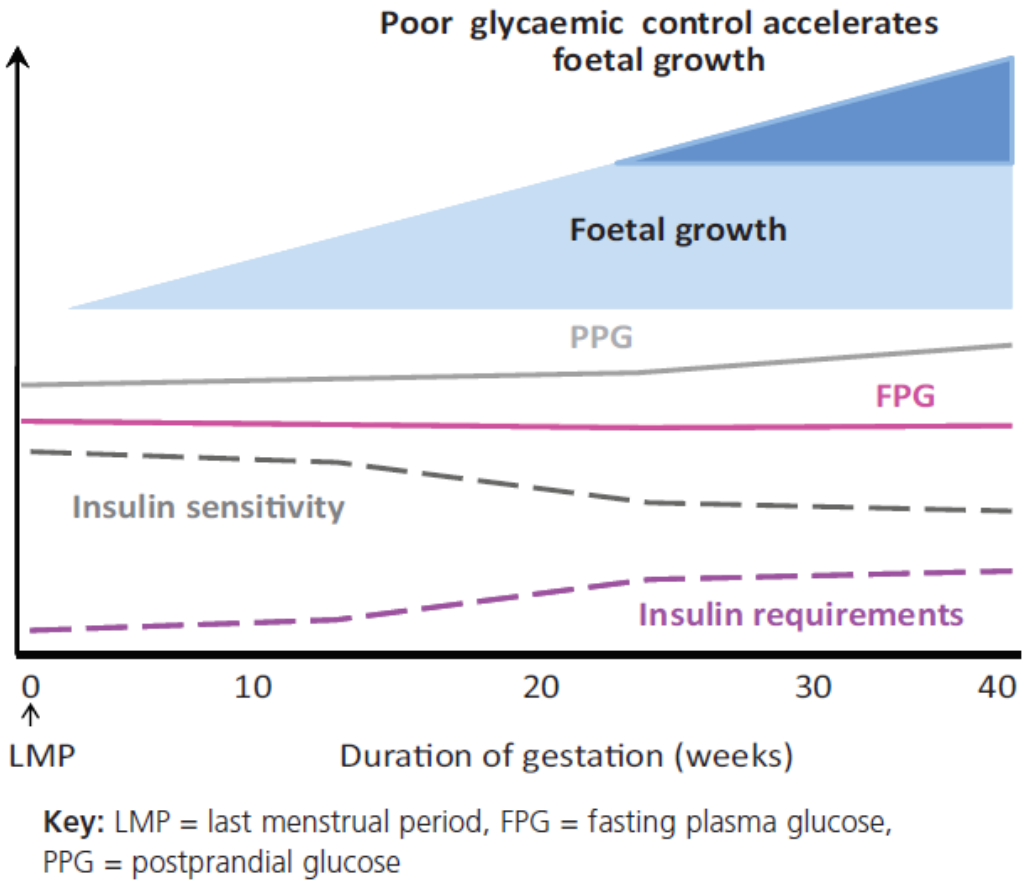


Figure 1.7 Changes in glucose homeostasis during pregnancy (Reproduced with permission from C. Day and C.J. Bailey [58]).

1.3.2.1 The Role of Hormones in Glucose Metabolism during Pregnancy

Several hormonal changes occur during pregnancy; as pregnancy progresses levels of oestrogen, progesterone, cortisol and human placental growth hormone (hPGH), amongst other hormones, increase (Figure 1.8). The increase in these hormones tends to occur at the same time as insulin sensitivity starts to decrease. Whilst they may only have indirect influences on insulin resistance and their exact involvement in the process is not yet fully understood, these hormones are all thought to be involved in the process of mediating insulin resistance during pregnancy [55, 58].

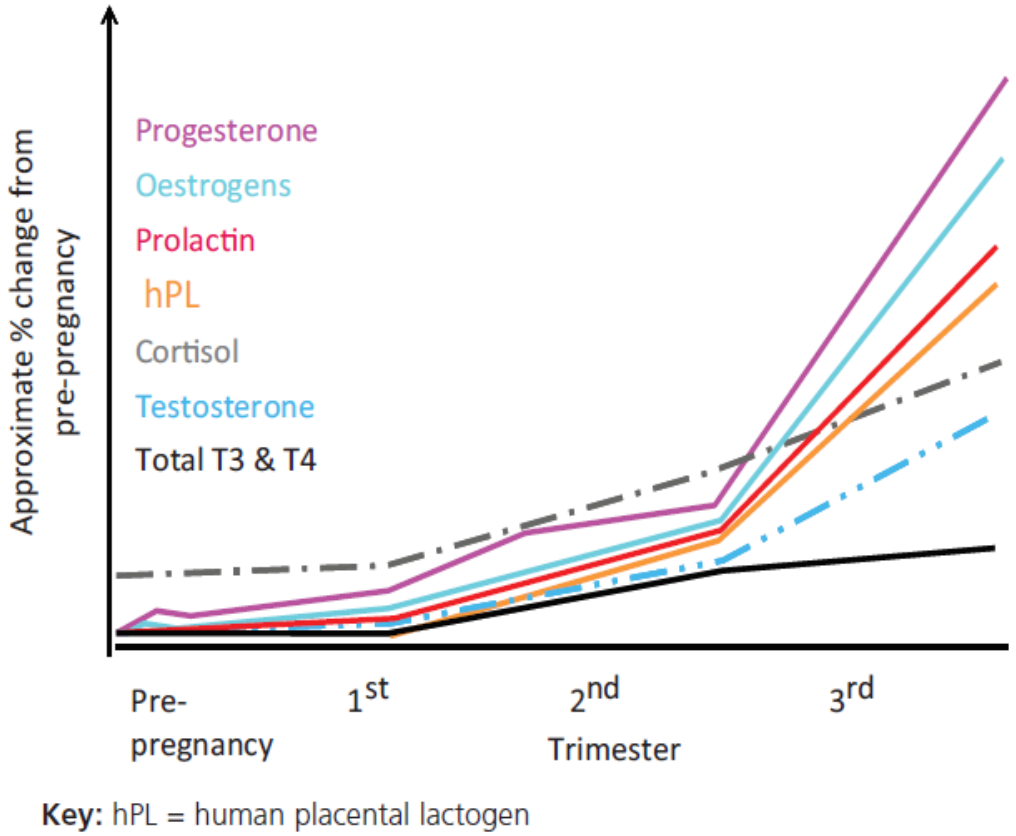


Figure 1.8 Hormonal changes that influence glucose homeostasis during pregnancy (Reproduced with permission from C. Day and C.J. Bailey [58]).

An increase in triglycerides, free fatty acids, and cholesterol is also seen as pregnancy progresses [62]. Lipid metabolism is altered as a result of the rise in oestrogen levels and insulin resistance. The hormone, human placental lactogen (hPL), is involved in lipid metabolism during pregnancy. The placenta utilises cholesterol to synthesis steroids and fatty acids are used to form membranes. Early and mid-pregnancy favour the accumulation of maternal fat stores, whereas the secretion of the hormone, hPL, by the placenta encourages lipolysis (breakdown of fat) in late pregnancy thus promoting the use of fat as the maternal energy source and allowing the glucose to be utilised by the fetus [60, 63].

1.3.2.2 The Role of Adipokines in Glucose Metabolism during Pregnancy

In part, a set of cytokines (proteins) secreted by adipose tissue, are also believed to be involved in facilitating insulin resistance during pregnancy. Amongst these proteins are tumour necrosis factor alpha (TNF-α), adiponectin, resistin, adipsin, leptin and plasminogen activator inhibitor-1 (PAI-1) [55, 62]. Of note is TNF-α and adiponectin. TNF-α is secreted by both adipose tissue and the placenta although the primary source during pregnancy is thought to be the placenta. During late gestation the concentration of circulating TNF-α increases and is strongly correlated with increased insulin resistance [64]. One study found that adiponectin levels decreased by 17% from the first to the second trimester, and then by 25% from the second to the third trimester. The results are in keeping with changes in adiposity; storage of fat in early pregnancy and utilisation of fat reserves in later pregnancy [62].

1.4 Gestational Diabetes Mellitus

In most cases, pregnant women compensate for the natural insulin resistance by increasing β-cell function, however, some women are unable to increase insulin secretion resulting in poor glycaemic control and consequently, a condition called gestational diabetes mellitus.

Gestational diabetes mellitus (GDM) is defined as being any degree of glucose intolerance diagnosed for the first time in a woman during pregnancy [65]. The International Association of Diabetes and Pregnancy Study Groups (IADPSG) and the WHO added to this definition by including the stipulation that the level of hyperglycaemia first detected in pregnancy should not fall in the overt diabetes range. “Overt diabetes” is also known as “diabetes mellitus in pregnancy” and refers to pre-existing diabetes that is only identified for the first time during pregnancy [66, 67]. The diagnostic criteria to distinguish GDM from overt diabetes are discussed in Section 1.4.4.2

The most common aetiology of GDM is multifactorial involving a combination of environmental and genetic factors which together predispose an individual to developing the condition. Usually women with GDM have chronic insulin resistance present before pregnancy. As late pregnancy naturally induces an insulin-resistant state (Figure 1.7), a pre-existing insulin resistance compounds the problem. Therefore, it is thought that most women

with GDM have two forms of insulin resistance; a chronic form that is present before pregnancy and an acquired form induced by late pregnancy. As a result of this coupled insulin resistance, pancreatic β-cells are unable to meet the insulin demands of pregnancy and insulin secretion is suboptimal resulting in hyperglycaemia [68].

In addition, in line with the DOHaD hypothesis, β-cell function may be impaired in the mother due to altered β-cell programming which occurred in response to early life stressors during the fetal stage. Fetal β-cell mass is affected by maternal hyperglycaemia, undernutrition and overnutrition [69] and so this type of adaptation in early life would put a female at increased risk of developing GDM when she is pregnant. Subsequently, the offspring of a woman with GDM would be at risk of defective β-cell mass and functioning and so the cycle continues [70].

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. Despite this, a diagnosis of GDM poses short- and long-term risks for both mother and child [71].

The development of GDM is complex and involves several biological, physiological, socioeconomic and lifestyle factors. Figure 1.9 is a conceptual framework that describes the various factors influencing the development of GDM and the consequences thereof. The remaining sections of this chapter will discuss the various aspects described in the conceptual framework.

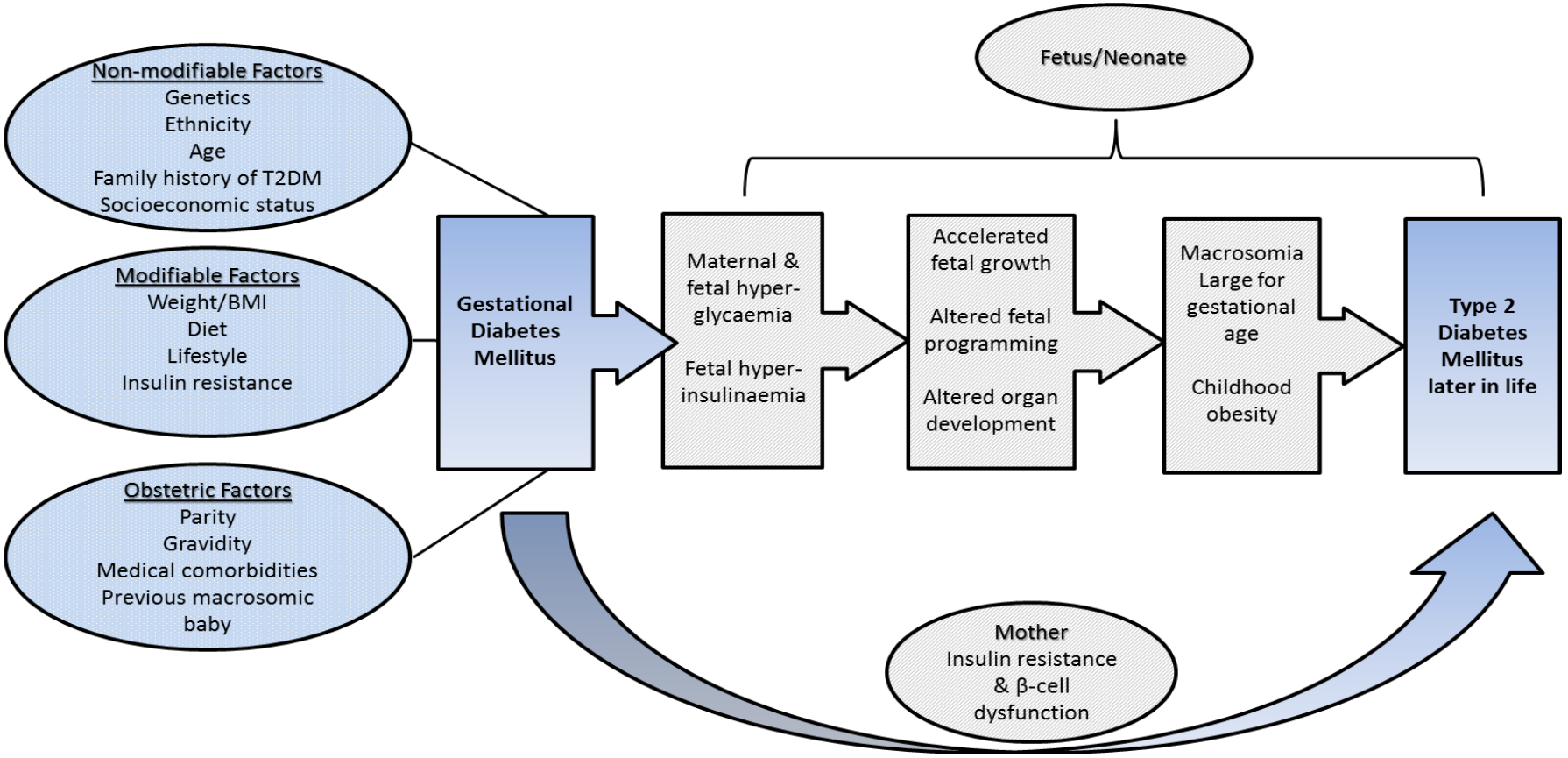


Figure 1.9 A conceptual framework for gestational diabetes mellitus (GDM). The figure illustrates the exposures associated with GDM development and the consequences of the condition on both the mother and child [Informed by 72].

1.4.1 Risk Factors for Gestational Diabetes Mellitus

There are several reported risk factors that are thought to increase a woman's chance of developing GDM. Some of the more common and well-described risk factors are listed in Table 1.1 and discussed below.

Table 1.1 Some of the common risk factors for gestational diabetes mellitus [73]

Risk Factors for Gestational Diabetes Mellitus (GDM)
Older age
High weight
A pre-pregnancy BMI of ≥27 kg/m^2
High parity
Having previously given birth to a baby weighing 4 kg or more
A family history of T2DM
A previous diagnosis of GDM
Belonging to a particular ethnic group (Hispanic, African, African American, Asian)

BMI; body mass index; T2DM, Type 2 diabetes mellitus

1.4.1.1 Older Age

Age is an important risk factor for the development of GDM because from a physiological perspective β-cell function and mass decrease with increasing age [74]. The American Diabetes Association suggests being ≥25 years of age as a risk factor for GDM [75]; this has been supported by other investigators [76].

1.4.1.2 High Weight and Body Mass Index

As with T2DM, a high BMI is an extremely significant risk factor for the development of GDM. Being overweight or obese increases one's risk of becoming insulin resistant [77] and as described previously, pre-existing insulin resistance coupled with the insulin-resistant state of late pregnancy gives rise to most cases of GDM. In addition, elevated levels of

triglycerides during pregnancy have been shown to be associated with GDM [78]. Several studies have proven an association between high BMI and GDM; a South African study found that overweight and obese pregnant women were more likely to develop GDM than women with BMIs in the normal range [79]. An Australian study found that the likelihood of developing GDM increased with increasing BMI; overweight women had an adjusted odds ratio of 1.78 (95% confidence interval (CI) 1.25, 2.52) of developing GDM whereas obese women had an adjusted odds ratio of 2.95 (95% CI 2.05, 4.25) and morbidly obese women had an adjusted odds ratio of 7.44 (95% CI 4.42, 12.54) of developing GDM [80]. Similarly, a meta-analysis performed by Chu et al. [81] reported that the unadjusted odds ratios of developing GDM were 2.14 (95% CI 1.82, 2.53), 3.56 (95% CI 3.05, 4.21), and 8.56 (95% CI 5.07, 16.04) amongst overweight, obese, and morbidly obese women respectively compared with women who had normal weights.

Interestingly, using BMI to identify women at risk of developing GDM is more effective in certain population groups than others. Shah et al. [82] found that a BMI of $\geq$25.0 kg/m^2 (overweight) as a GDM screening factor effectively detected more than 76% of African-American women with GDM, 58% of Latino women, and 46% of Caucasian women, but only 25% of Asian women (p<0.001). The predictive value of BMI for health risks amongst Asian populations is poor as studies have highlighted that Asian individuals with T2DM and cardiovascular disease tend to have lower BMIs compared to similarly affected individuals from European populations [83].

1.4.1.3 High Parity

Parity refers to the number of times a woman has given birth, regardless of whether or not the baby was born alive, but where the pregnancy reached 20 weeks gestation or more. Gravidity refers to the number of pregnancies a women has had [84]. The rationale behind a higher number of births increasing one's risk of GDM is based on the fact that a woman has undergone repeated exposure to insulin resistance and increased insulin secretion brought on by normal pregnancy. This repeated exposure could cause metabolic stress and result in β-cell exhaustion which in turn could cause insufficient insulin secretion and later, diabetes [85]. However, the role of parity as a risk factor for GDM is controversial, with some studies supporting it and others not. A study that examined the effects of parity on the development of diabetes in a non-pregnant population of Chinese women found a significant association between parity and T2DM [85]. Another study reported that parity is not directly linked to the

deterioration of insulin sensitivity during pregnancy or GDM development, but rather it is indirectly linked through the consequences of aging and progressive weight gain [86].

1.4.1.4 Previous Macrosomic Infant

Macrosomia, defined as a birth weight of ≥4 kg [87], is a well-known adverse outcome of fetal exposure to GDM. This is further discussed in Section 1.4.2.2. Approximately 20-30% of GDM-affected pregnancies result in macrosomia [88]. Therefore, if a woman had previously delivered a macrosomic infant there is a possibility that she had undiagnosed GDM in that pregnancy. Anzaku et al. [89] found that a previous history of fetal macrosomia is an independent risk factor for GDM amongst Nigerian women. Similarly, Ali et al. [90] found it to be a dependent risk factor for GDM amongst women living in Yemen but Kragelund-Nielsen et al. [91] did not find it to be a significant risk factor amongst Indian women.

1.4.1.5 Family History

Gestational diabetes mellitus and T2DM are considered to be multifactorial conditions that have both genetic and environmental risk factors which overlap. Therefore, from a genetic susceptibility perspective, a diagnosis of T2DM in a family member would place one at an increased risk for T2DM and GDM [73]. A study that assessed family history of diabetes in Chinese women found that it was a significant risk factor but only in women over 30 years of age. In younger women, family history alone did not confer an increased risk of GDM [92]. The study highlights the interaction of two GDM risk factors, age plus genetics, in disease development.

1.4.1.6 Ethnicity

The interaction of BMI and maternal age with ethnic group has been deemed important in the development of GDM; older age and higher BMI increased GDM risk particularly amongst African and South Asian women as opposed to white European or black Caribbean women [93]. This highlights the role of ethnicity in the development of GDM. As mentioned in Table 1.1, it is well described that certain ethnic groups are more susceptible to developing GDM than others. This is likely to be due to a genetic predisposition caused by specific gene

mutations or variations. Genetic association studies have found genes which are thought to contribute to the development of GDM. For example, the rs7903146 variant in the *Transcription factor 7-like 2 (TCF7L2)* gene [94] and the rs10830963 variant in the *Melatonin receptor 1B (MTNR1B)* gene [95] have been associated with significantly increasing one's risk of developing GDM. In addition, cultural factors concerning dietary practices amongst different ethnic groups also influence GDM development [96].

The studies assessing risk factors for GDM mentioned above highlight that the use of any one particular risk factor is not sufficient in the detection of at-risk women. Rather, more effective screening can be achieved through grouping multiple risk factors together [93]. However, considering the discrepant findings that the various studies report, risk-factor screening has its limitations [97]. Further discussion around screening for GDM follows in Section 1.4.4.

1.4.2 Fetal Exposure to Gestational Diabetes Mellitus

A cascade of events occurs as a result of a fetus being exposed to GDM. In 1954 Jorgen Pedersen hypothesised that maternal glucose readily crosses the placenta whereas insulin does not. As the fetal islet cells are incapable of secreting insulin before 20 weeks gestation, exposure to maternal hyperglycaemia before this time results in the fetus becoming hyperglycaemic which can be teratogenic. After 20 weeks gestation the fetus' pancreas is able to secrete insulin and overcompensates for its hyperglycaemic state by producing excessive amounts of insulin. This in turn results in accelerated fetal growth, in particular more fat accumulation, and therefore weight gain [56].

One of the pivotal studies looking at the effects of maternal hyperglycaemia on infant outcome was the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. The main objective of the HAPO study was "to clarify the risk of adverse outcome associated with degrees of maternal glucose intolerance less severe than overt diabetes during pregnancy" [98]. The study was conducted during 2000-2006 and involved nine countries, 15 field sites and a final total of 23 316 pregnant women from various ethnic backgrounds. Maternal glucose levels were determined at 24-32 weeks gestation and at birth neonatal anthropometric measures were taken on the infants [98]. The study confirmed a linear and continuous relationship between maternal glucose levels, birth weight, body fat percentage in newborns and fetal insulin levels [99, 100].

1.4.2.1 Altered Fetal Growth

Fetal growth has been shown to differ between GDM-exposed and unexposed fetuses. Disproportionate growth has been reported amongst fetuses of women with GDM when compared to fetuses of women with normal glucose profiles. In early pregnancy, the fetus of a woman with GDM may experience intrauterine growth restriction due to placental insufficiency [101]. However, in later pregnancy when maternal insulin resistance increases, fetuses of women with GDM tend to show accelerated growth [102]. This accelerated growth results in GDM-exposed fetuses being larger in comparison to unexposed fetuses of the same gestational age. In addition, the GDM-exposed fetuses tend to have higher fat mass/lean mass ratios with a significantly larger amount of total tissue [102].

Hammoud et al. [103] reported that at term GDM-exposed infants had larger abdominal circumferences than unexposed infants but head circumference and femur length did not differ between the two groups. This finding supports how an increase in adiposity is associated with GDM-exposure. Of all fetal biometry measures, abdominal circumference is known to be an effective predictor of fetal growth and can be used as a proxy for the development of disease or pathology in the fetus, referred to as 'fetopathy' [103]. Jazayeri et al. [104] reported an abdominal circumference measurement of ≥35 cm (obtained within two weeks of delivery) predicted 93% of macrosomic infants. Hammoud et al. [103] stated that an abdominal circumference of <90th centile for gestational age in the third trimester confers a <5% risk of macrosomia. Monitoring of fetal abdominal circumference every two to four weeks from the second and early third trimester has been suggested as a way of monitoring the fetal response to maternal hyperglycaemia and as a means of helping obstetricians decide how best to manage the pregnancy and treat the mother. An abdominal circumference measurement of <75th percentile for gestational age indicates normal growth, anything above that would indicate possible macrosomia or large for gestational age (LGA) (>90th percentile for gestational age) [68].

1.4.2.2 Macrosomia/ Large for Gestational Age

The HAPO Study [100], and many others have supported Pedersen's hypothesis by proving that elevated maternal glucose levels result in increased neonatal birth weight and adiposity [103, 105, 106]. As a result, babies born to mothers with GDM are more likely to be macrosomic or LGA [58, 106, 107]. The actual cut-off weight for the definition of macrosomia is debatable. Some define macrosomia as ≥4 kg and others define it as ≥4.5 kg [108, 109]. Nonetheless, being pregnant with a large fetus complicates the birthing process; shoulder dystocia caused by cephalopelvic disproportion is one of the major risks [110].

Shoulder dystocia causes obstructed labour whereby the fetal anterior shoulder becomes wedged against the maternal pubic bone and normal vertex delivery cannot proceed. This occurs due to the fetus being disproportionately large compared to the birth canal. Shoulder dystocia can cause serious injuries to the fetus, including nerve damage (most commonly brachial plexus palsy), clavicle fracture, hypoxia and fracture of the humerus. It can also result in fetal death [111]. The risk of shoulder dystocia increases linearly with birth weight. It has also been reported that fat mass in the mid-upper arm of GDM-exposed fetuses is greater than that of unexposed fetuses particularly from the 29th week of pregnancy. This area of fat deposition would increase the risk of shoulder dystocia [102]. Even in situations where birth weight of GDM-exposed infants does not differ significantly from unexposed infants, the GDM-exposed infants have been shown to have greater fat mass [105].

1.4.2.3 Congenital Abnormalities

The subject of congenital abnormalities being associated with GDM has been contentious. Some studies report that as with maternal T2DM, there is an increased risk of congenital abnormalities with GDM. Huddle [112] reported major congenital anomalies amongst 2.3% of babies born to mothers with GDM at Chris Hani Baragwanath Academic Hospital in Soweto, Johannesburg, South Africa, and an Italian study reported twice as many congenital abnormalities amongst women with GDM when compared to the general population [113]. A systematic review and meta-analysis performed on the topic concluded that there is a slightly increased risk of congenital malformations amongst women with GDM but that the risk is not as high as that associated with pre-existing diabetes [114]. Most GDM management guidelines now recommend that pregnant women with GDM be considered at risk of having a

child with a congenital abnormality, albeit a lower risk than that associated with pre-existing diabetes [115, 116].

1.4.2.4 Other Short-Term Complications

There are several other reported short-term neonatal complications of exposure to maternal diabetes (Figure 1.10). These include hypoglycaemia, hypoxia, cardiomyopathy, polycythaemia, hyperbilirubinaemia and respiratory distress syndrome. Many of these are more prevalent in pregnancies affected by pre-existing diabetes rather than GDM as the severity of the diabetes and degree of glycaemic control influences the prevalence of the complications [117]. Nonetheless, these are risks that need to be considered when managing a pregnant woman with any form of diabetes [118].

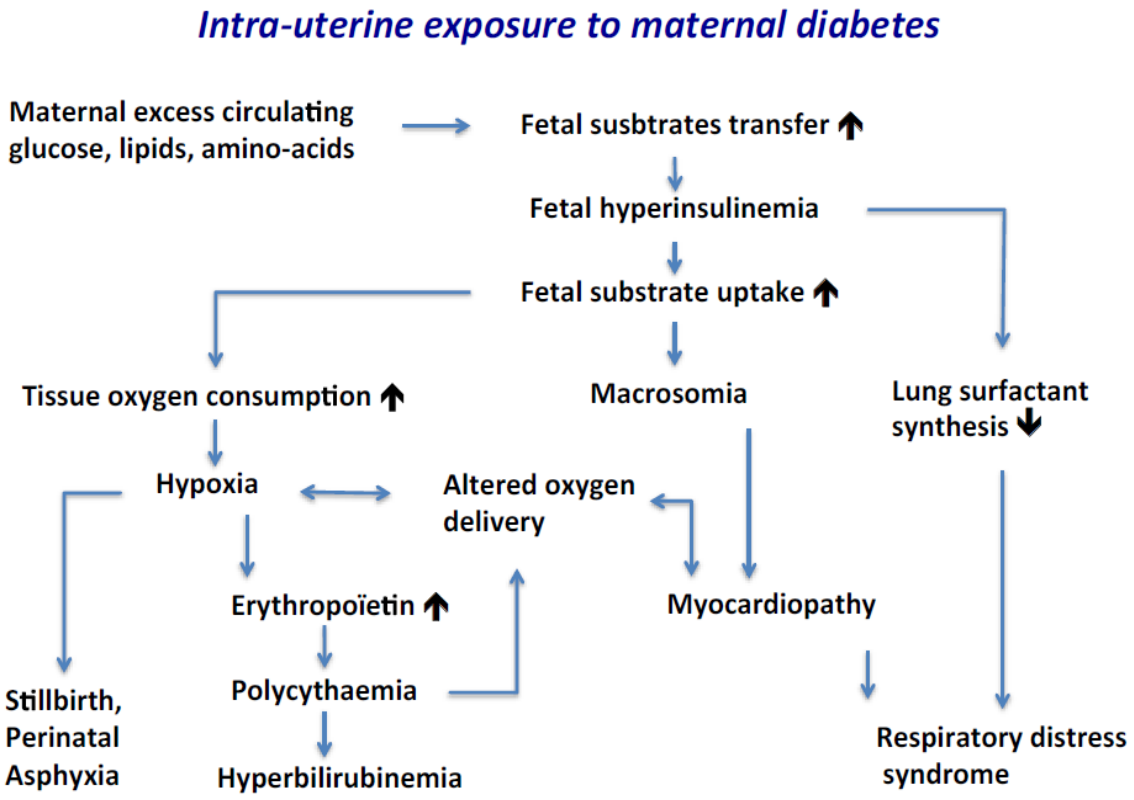


Figure 1.10 Short-term complications of exposure to maternal diabetes *in utero* (Reproduced with permission from Elsevier [118])

1.4.2.5 Obesity and Type 2 Diabetes Mellitus

A potential concern for children born to mothers with GDM is the long-term effects caused by fetal overnutrition and consequently, altered fetal programming. Altered gene expression in the placenta, particularly involving lipid and glucose metabolism pathways, has been observed in GDM cases [119]. As discussed, children of mothers with GDM are at risk of developing obesity, insulin resistance and consequently T2DM later in life [120] thus perpetuating the vicious cycle of diabetes (Figure 1.11).

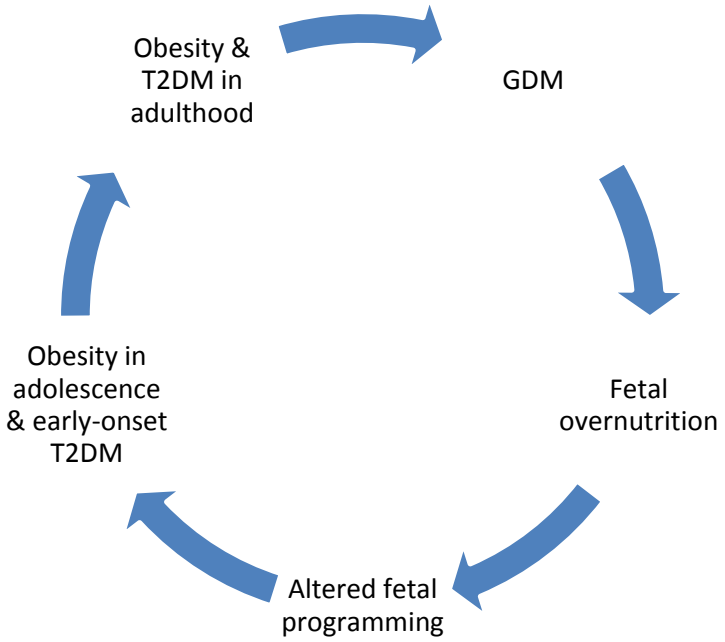


Figure 1.11 The perpetuating cycle of diabetes from *in utero* exposure to gestational diabetes mellitus (GDM) to Type 2 diabetes (T2DM) and obesity in later life.

Much research on this transgenerational effect has been performed on the Pima Indians living in Arizona in the United States of America (USA); a population with an extremely high prevalence of T2DM and GDM. Early research on this population revealed a T2DM prevalence of 42% amongst individuals aged 25 years and older, and a prevalence of 50% amongst individuals aged 35 years and older [121]. The prevalence of T2DM in Pima Indian children was assessed over a 30 year period (1967 to 1996). Boys aged 15-19 years had a prevalence of T2DM of 2.4% in 1967-1976 which increased to 3.8% during 1987-1996 and girls aged 15-19 years were found to have a T2DM prevalence of 2.7% in 1967-1976 and 5.31% in 1987-1996. The prevalence of T2DM more than doubled over the 30 years. During

this period an increase in weight was also observed and the percentage of children exposed to hyperglycaemia *in utero* increased from 18% to 35%. Whilst genetic factors must contribute to the high prevalence of TD2M in this population group, the actual exposure to hyperglycaemia *in utero* is largely accountable for the increase in T2DM and obesity in Pima Indian children [122].

Similarly, a study performed in Denmark on Caucasian individuals found that more than 20% of offspring born to mothers with diet-treated GDM had T2DM/pre-diabetes by the time they were 22 years of age compared to only 4% of the general 22 year old population [123].

A retrospective study that assessed children aged six to 13 years from various ethnic groups who were exposed to GDM *in utero* and compared them to unexposed children found that the GDM-exposed children had significantly higher BMIs, more subcutaneous abdominal fat and larger waist circumferences [124]. In addition, they also had increased subscapular to triceps skinfold thickness ratios. Such results indicate an overall increase in fat mass but with central fat deposition being predominant in children exposed to GDM. Furthermore, the long term effects of fetal overnutrition are thought to present around late childhood only becoming more noticeable around puberty (Figure 1.12) [125]. More longitudinal studies are needed to prove or refute this point. The HAPO Follow-Up Study (HAPO FUS) is currently underway and will hopefully provide important insight into the effects of GDM-exposure on childhood metabolic risks [126].

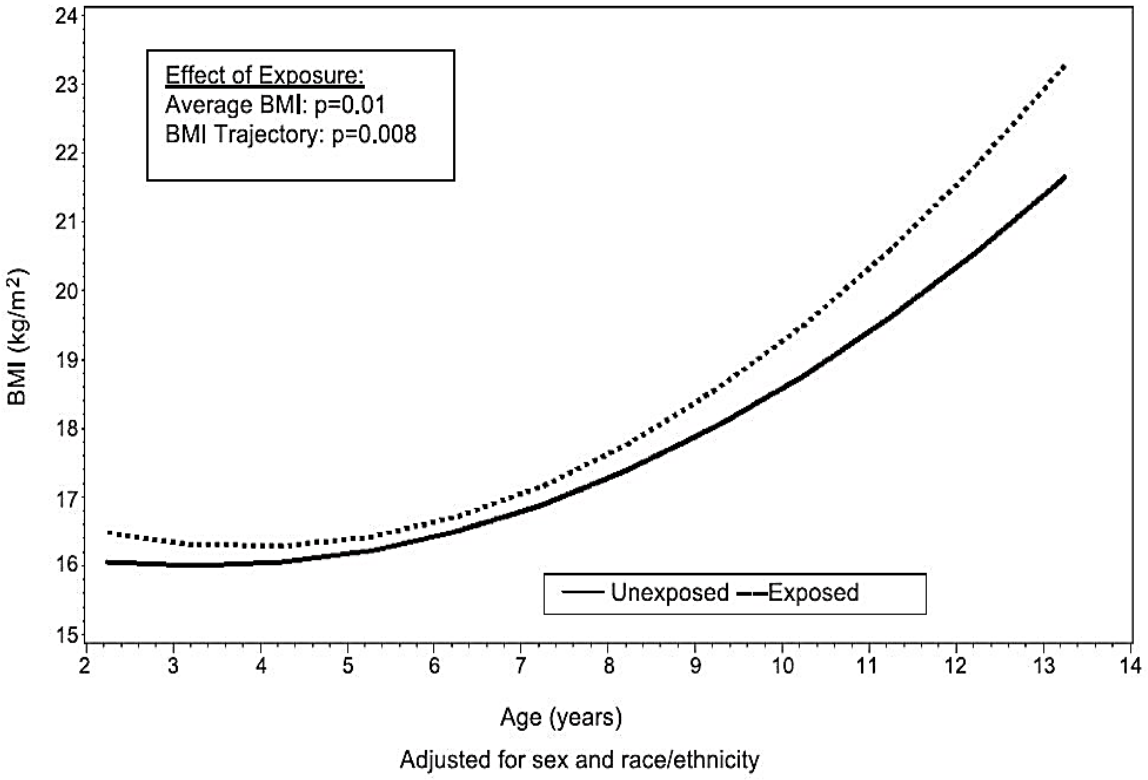


Figure 1.12 Mean BMI curves for youth both exposed and unexposed to maternal diabetes *in utero* from 27 months of age to 13 years, adjusted for sex, race/ethnicity (Reproduced with permission from Elsevier [125])

1.4.3 Maternal Morbidities Associated with Gestational Diabetes Mellitus

There are a number of maternal complications that can arise as a result of a woman having GDM. Some are short-term complications which arise during the pregnancy or around the time of giving birth and others are long-term which affect a woman in later years. Long-term effects occur despite normal glucose metabolism returning after delivery in the large majority of GDM cases [71].

1.4.3.1 Gestational Hypertension and Pre-eclampsia

Gestational hypertension (blood pressure of at least 140/90 mmHg occurring for the first time after mid-pregnancy) and pre-eclampsia, defined as gestational hypertension plus proteinuria (≥0.3 g of excreted protein in the urine over 24 hours), are thought to be more frequent in

women affected with GDM [63]. When compared to women unaffected by GDM, those with GDM have up to a 50% risk of pre-eclampsia [63].

Radaelli et al. [127] analysed the placentas of GDM and non-GDM pregnancies and found that expression profiles of genes involved in regulating inflammatory responses and endothelial reorganisation were altered in the GDM placentas. These findings indicate that GDM induces chronic inflammation and vascular dysfunction. Consequently, poor vascularisation results in poor oxygen flow to and from the placenta. Physical changes to the placentas of women with GDM have been observed with 30.5% of women with GDM having maternal vascular malperfusion (MVM) lesions caused by hypoxic-ischaemic damage. These lesions increase risk of hypertension [128].

1.4.3.2 Birth Complications and Decision around Delivery

The impact of GDM on obstetric care and timing of delivery is of importance. Women with GDM need to be closely monitored and their glucose levels regularly assessed. Traditionally, induction of labour or Caesarean section was recommended for women with GDM. This was advised in order to prevent birth complications associated with excessive fetal growth as well as to reduce the risk of stillbirths [129]. However, newer guidelines suggest early delivery may not be necessary in all women with GDM. In women whose glucose levels are poorly controlled, delivery should be planned for around 37-38 weeks gestation but in well-controlled women delivery can continue until 39-40 weeks gestation [130, 131].

However, delivering a LGA or macrosomic baby is problematic in terms of the actual delivery process. Shoulder dystocia can result in postpartum haemorrhaging, fourth-degree lacerations and uterine rupture in the mother [111]. In such cases Caesarean sections are required. One study reported a two fold increase in Caesarean sections in mothers with high glucose concentrations compared to mothers with low glucose concentrations [132]. A study in Portugal reported a significant difference in the percentage of non-elective Caesarean sections amongst women with GDM; 19.5% of women with GDM underwent Caesarean sections compared to 13.5% of women without GDM [133]. A fairly recent multicentre study revealed an overall Caesarean section delivery rate of 13% amongst women with GDM [134], however, the rate has been reported to be higher in different regions of the world. In Soweto,

South Africa, Huddle [112] reported that more than 60% of women with GDM deliver by Caesarean section.

Caesarean sections carry their own risks associated with maternal morbidity. The most common of these are infection, haemorrhaging and thromboembolism. Bladder laceration and bowel damage can also occur. In addition, maternal hospitalisation and recovery time is longer for delivery by Caesarean section than for normal vaginal delivery [84] making it more costly for the health system.

1.4.3.3 Recurrent Gestational Diabetes Mellitus

Women diagnosed with GDM have a risk of developing GDM in subsequent pregnancies. This risk increases with the number of previous GDM-affected pregnancies. In a study by Getahun et al. [135], 41.3% of women who were diagnosed with GDM in their first pregnancies developed GDM in their second pregnancies compared to 4.2% of women who developed GDM in the second pregnancies but were not diagnosed with the condition in their first pregnancies. The authors also showed that the risk increased in the third pregnancy when both the first and second pregnancies were affected by GDM. Nohira et al. [136] reported an even higher risk (65.5%) of GDM recurrence and found that maternal age, pre-pregnancy BMI, increased weight gain between pregnancies and a short interval between pregnancies were associated risk factors for recurrence.

1.4.3.4 Type 2 Diabetes Mellitus

Kjos et al. [137] assessed the prevalence of diabetes two to eight weeks postpartum in a group of women who had GDM and only 9% of them were found to have hyperglycaemia in the diabetic range, the rest had reverted to normal glucose metabolism. However, much like their offspring, these women are at risk of T2DM in later years. Various studies have supported this theory although risks for the development of T2DM may differ between studies depending on the length of follow-up. A systematic review performed by Kim et al. [138] looked at the reported risks of progression to T2DM after GDM diagnosis. They found that the incidences of T2DM ranged from 2.6% to 70% in studies that assessed women from six weeks to 28 years postpartum with 50% of women developing T2DM within ten years postpartum. They also reported that the risk for T2DM peaks five years following the GDM-

affected pregnancy and plateaus after ten years. A recent study looking at Sri Lankan women reported that within ten years of the index pregnancy 61.3% of women who had GDM developed T2DM compared to only 5.8% of women who did not have GDM (p<0.001) [139].

1.4.4 Screening Methods and Diagnostic Criteria for Gestational Diabetes Mellitus

Screening for GDM, including the approach, techniques and diagnostic criteria, differ from country to country, and even within countries. The topic of GDM screening is continuously under scrutiny with several unsuccessful attempts having been made to try to reach a global consensus on the subject [140]. Unlike detecting diabetes in a non-pregnant patient, testing of glycated haemoglobin A1C (HbA1C; whereby haemoglobin is glycosylated with circulating blood glucose) is ineffective in diagnosing GDM. This is due to red blood cell turnover increasing during pregnancy and naturally reducing HbA1C levels particularly in early and late pregnancy [116, 141]. Furthermore, HbA1C screening for the detection of diabetes is unreliable in populations with a high prevalence of haemoglobinopathies, such as sickle cell anaemia in central African countries and thalassaemia in Mediterranean countries as the genetic variants can decrease HbA1C levels [142].

Some centres use urine dipstick analysis to screen for GDM. An abnormal amount of glucose in the urine, referred to as glycosuria or glucosuria, is defined as glucoses levels of more than 1.39 mmol/l in a random fresh urine sample [143]. However, due to its low sensitivity, random urine analysis is not recommended for the screening of GDM [144, 145].

The gold standard diagnostic test for GDM is the oral glucose tolerance test (OGTT) which is usually performed between 24 to 28 weeks gestation [146].

1.4.4.1 The Oral Glucose Tolerance Test

The OGTT involves a pregnant woman ingesting a specific amount of glucose (usually 75 g or 100 g) dissolved in a cup (approximately 250 ml) of water after an overnight fast. Venous blood is drawn prior to administering the drink (to test the fasting glucose level) and post-glucose load blood samples are taken at specific times over either two or three hours. The 75 g glucose load is measured over two hours whereas the 100 g glucose load is measured over

three hours. Blood is drawn at one hour post-glucose load and two hours post-glucose load and in the case of a three hour OGTT another blood sample is taken at three hours post-glucose load [147]. Interpreting the results is dependent upon the diagnostic criteria one uses.

Some screening protocols make use of an OGTT alone (one-step approach) whereas others employ a two-step approach that involves a 50 g glucose challenge test (GCT) followed by an OGTT if necessary. The GCT does not require a woman to have fasted; she is given a drink of 50 g of glucose and one hour later the venous glucose levels are assessed. An indication for further investigation by means of an OGTT is a glucose reading of between 7.2-7.8 mmol/l post-GCT [147, 148] .

1.4.4.2 Diagnostic Criteria for Gestational Diabetes Mellitus

Over the years several screening regimes and diagnostic criteria for GDM have been proposed (Table 1.2). The more recent International Association for Diabetes and Pregnancy Study Group (IADPSG) diagnostic criteria were developed as a result of the findings of the HAPO study. The HAPO study demonstrated a continuous relationship between maternal glucose concentration and infant outcomes with no specific threshold for increased risk being identified. A consensus panel was called to interpret the findings of the HAPO study for clinical application and the IADPSG diagnostic criteria for the diagnosis of GDM were developed [66].

The IADPSG criteria involve a two hour 75 g OGTT where one or more of the glucose readings must equal or exceed the threshold values; fasting glucose of 5.1 mmol/l, one hour post-glucose load of 10 mmol/l and two hours post-glucose load of 8.5 mmol/l. These values were chosen because they predict an increased risk of adverse pregnancy outcomes (defined as a 75% higher chance of adverse outcomes versus normal glucose values). The IADPSG diagnostic criteria differentiate GDM from overt diabetes; a fasting blood glucose level of ≥ 7.0 mmol/l is diagnostic of overt diabetes [66]. Subsequently, the World Health Organization adopted the IADPSG diagnostic criteria for GDM but in addition to a fasting glucose of ≥7.0 mmol/l being indicative of overt diabetes, they added a two hour post-glucose load reading of ≥11.1 mmol/l [67, 149].

Table 1.2 The different diagnostic criteria available for the diagnosis of gestational diabetes mellitus [150]

Group/Organisation/Reference	Screening Test	Diagnostic Criteria (Blood glucose levels)
American Diabetes Association [151]	One step: 2 hr 75 g OGTT OR Two step: 1) 1 hr 50 g (non-fasting) 2) 3 hr 100 g OGTT	At least one of the following must be met: Fasting: ≥5.1 mmol/l 1 hr: ≥10.0 mmol/l 2 hr: ≥8.5 mmol/l OR If 1 hr: ≥10.0 mmol/l proceed with step 2 3 hr: ≥7.8 mmol/l
Carpenter and Coustan [152]	3 hr 100 g OGTT	At least two of the following must be met: Fasting: ≥5.3 mmol/l 1 hr: ≥10.0 mmol/l 2 hr: ≥8.6 mmol/l 3 hr: ≥7.8 mmol/l
Diabetes Pregnancy Study Group (DPSG) of the European Association for the Study of Diabetes (EASD) [153]	2 hr 75 g OGTT	Fasting: >5.2 mmol/l OR 2 hr: >9.0 mmol/l
International Association of Diabetes and Pregnancy Study Groups (IADPSG) [66]	2 hr 75 g OGTT	At least one of the following must be met: Fasting: ≥5.1 mmol/l 1 hr: ≥10.0 mmol/l 2 hr: ≥8.5 mmol/l
National Diabetes Data Group [154]	3 hr 100 g OGTT	At least two of the following must be met: Fasting: ≥5.8 mmol/l 1 hr: ≥10.6 mmol/l 2 hr: ≥9.2 mmol/l 3hr: ≥8.0 mmol/l
World Health Organization (1985) [155]	2 hr 75 g OGTT	Fasting: ≥7.8 mmol/l OR 2 hr: ≥7.8 mmol/l
World Health Organization (1999) [47]	2 hr 75 g OGTT	Fasting: ≥7.0 mmol/l OR 2 hr: ≥7.8 mmol/l
World Health Organization (2013) [67]	2 hr 75g OGTT	At least one of the following must be met: Fasting: 5.1-6.9 mmol/l 1 hr: ≥10.0 mmol/l 2 hr: 8.5-11.0 mmol/l

Several hospitals in the USA tend to use the two-step approach involving a 50 g GCT followed by a three hour 100 g OGTT that is interpreted using the Carpenter and Coustans criteria or the National Diabetes Data Group (NDDG) criteria (Table 1.2). However, the American Diabetes Association have also adopted the IADPSG criteria in their GDM screening guidelines proposing that either a one-step approach using the IADPSG criteria is used, or a two-step approach using the 50 g GCT followed by a 100 g three hour OGTT is used [151]. There is much debate around whether the two-step procedure is in fact necessary and effective as the 50 g GCT has been reported to miss approximately 25% of GDM cases [147].

The drawback to having different diagnostic criteria is that GDM prevalence figures will differ depending on what criteria are used. Although the IADPSG criteria and the adoption of them by other groupings is a step in the right direction in terms of achieving global consensus on GDM screening, concern has been raised regarding how the lower glucose threshold values may result in an increase in the number of women diagnosed with GDM. Several studies in different countries have shown that the IADPSG criteria do in fact diagnose more women with GDM than previously used criteria [156-159]. This has both pros and cons; diagnosing and managing more women prevents the adverse outcomes of GDM on mother and child, however, it also burdens a healthcare system [158].

1.4.4.3 Universal versus Selective Screening for Gestational Diabetes Mellitus

Universal screening for GDM involves all pregnant women being tested whereas selective screening for GDM involves only high-risk women, or women with certain risk factors (Table 1.1), being tested. Whilst universal screening is ideal and recommended by several organisations including the IADPSG [66] and the WHO [67], this approach has cost implications that cannot be ignored, particularly in resource-poor settings. The consumables and equipment for the OGTT are costly and the procedure is time consuming [160]. However, selective screening is thought to miss between 17-50% of GDM cases [160, 161]. In many LMICs pregnant women do not receive any screening for GDM and those who do are often insufficiently screened; this is concerning considering LMICs account for approximately 80% of the global burden of diabetes [162].

Given the long-term effects of GDM on both mother and child, universal screening where a diagnosis allows for intervention may in fact be more cost effective in the long run considering the costs involved in managing T2DM and its comorbidities. Alternatively, there is a need for improved biomarkers that have good sensitivity and specificity and allow for early detection of GDM which may eliminate the need for universal using an OGTT [163].

1.4.5 An Increase in the Global Prevalence of Gestational Diabetes Mellitus

Over the years an increase in the prevalence of GDM has been observed in several countries. This increase appears to be in parallel with the increasing prevalence of T2DM and obesity. Whilst GDM prevalence is influenced by ethnicity and genetic susceptibility, the nutrition transition is a significant contributing factor towards the increased prevalence [164, 165]. 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 and 6.2% involved pre-existing diabetes diagnosed before pregnancy [166]. These figures illustrate how GDM is the primary cause of hyperglycaemia during pregnancy.

Dabelea et al. [167] found that the prevalence rate of GDM doubled amongst women from various ethnic groups living in Colorado, USA, during 1994-2002. Any increase in GDM prevalence should be viewed as a public health concern given the adverse effects it has on mother and child, particularly in terms of perpetuating the cycle of diabetes [164]. However, in order for health policies and GDM screening guidelines to be implemented in a country, the extent of the problem needs to be well understood; this is where prevalence figures are essential.

1.4.6 Management of Gestational Diabetes Mellitus

The general worldwide approach to the treatment and management of GDM is to start with dietary advice, preferably by a registered dietician, in combination with self-monitoring of glucose levels. Whilst there are several different ideas about the ideal diet for GDM, the consensus is on limiting excessive carbohydrate intake and ensuring that carbohydrates are equally dispersed throughout the day. In addition to a change in diet, exercise is also recommended [68, 116, 145]. A systematic review investigating diet strategies for the treatment of GDM found a low glycaemic index (GI) diet to be the most effective at managing maternal glucose levels and reducing birth weights of offspring [168]. Furthermore, a low GI diet with additional dietary fibre has been shown to reduce the risk of macrosomia even further than a low GI diet alone [169]. Lifestyle modification alone has been shown to be effective in controlling blood glucose levels in 70-85% of women with GDM [116]. Regarding exercise, 30 minutes of planned physical activity per day is recommended; brisk walking and arm exercises whilst seated have been suggested [68]. However, the recent UK Pregnancies Better Eating and Activity Trial (UPBEAT) study assessed the outcomes of diet and physical activity interventions amongst obese pregnant women. The results indicated that diet and physical activity alone in this high-risk group were not effective enough in preventing GDM [170].

As HbA1C is not effective in diagnosing GDM, neither is it effective in monitoring glucose control in an already diagnosed woman. In addition, monitoring for glycosuria in a woman with GDM is ineffective, although urine analysis for ketones is recommended in order to detect hypertensive disorders [75] Therefore, self-monitoring of capillary blood glucose before and after meals using a glucometer is recommended. The targeted capillary blood glucose levels for women with GDM, as recommended by the American Diabetes Association, are as follows [116]:

- Pre-prandial or fasting: 5.3 mmol/l or lower
- One hour after meal: 7.8 mmol/l
- Two hours after meal: 6.7 mmol/l

If a woman with GDM is unable to control her blood glucose levels through diet and exercise alone, medication is required. Insulin has been the original drug of choice for treating GDM

because it does not cross the placental barrier, it is not teratogenic, and long term safety has been established [116]. The drawbacks to using insulin are it has to be injected and is therefore invasive, women need to be trained on when and how to administer it safely and correctly, and it can cause weight gain [171].

Oral antihyperglycaemic agents, metformin and glyburide, are commonly recommended as alternatives to insulin therapy for the treatment of GDM. These drugs are less invasive than insulin injections. Neither drug has been shown to have any harmful effects on the fetus although long-term effects remain unclear. Occasionally though, when glucose levels are uncontrolled, a combination of an antihyperglycaemic agent, lifestyle changes and insulin may be required [145].

The pharmacological action of glyburide is to stimulate insulin secretion directly. Maternal weight gain and hypoglycaemia are adverse effects associated with this drug [172]. Overall, glyburide appears to be less effective in managing GDM than insulin and metformin [173, 174].

Metformin acts by reducing gluconeogenesis in the liver [172]. It has been associated with less weight gain in women compared to insulin and also with lower rates of pregnancy induced hypertension [175]. In a South African study, the combination of lifestyle modification plus metformin was effective in managing 88% of women with GDM, with insulin only being needed in the remaining 12% of cases [176].

A randomised control trial that compared metformin to insulin use in women with GDM found that study participants preferred metformin over insulin. A total of 76.6% of women on metformin therapy said they would choose to be treated with it again whereas only 27.2% of women on insulin therapy said they would want to use insulin again ($p<0.001$). The investigators found no significant difference in the metformin versus insulin-treated group in terms of secondary outcomes and no adverse effects from metformin use were observed [177]. The same authors performed a follow-up study when the children of the treated women were two years old. They found that children whose mothers were treated with metformin had more subcutaneous fat but their general body fat percentage was the same as that of children whose mothers were treated with insulin [178].

Another randomised control trial evaluating metformin versus insulin-treated GDM pregnancies showed that there was no difference in size at birth between neonates exposed to metformin compared to those exposed to insulin. However, follow-up of these infants revealed that those born to women treated with metformin were significantly heavier at one year of age and heavier and taller at 18 months of age than those born to women treated with insulin [179]. Whilst treatment has been shown to improve short-term outcomes, the effect of treatment on fetal programming is unknown [180] and further longitudinal studies are required to understand this.

1.5 Gestational Diabetes Mellitus in the South African Context

South Africa is a diverse country with a population of 55.91 million people comprised of several ethnic groups; 80.7% black African, 8.8% Coloured (mixed ancestry), 2.5% Indian/Asian and 8.1% white [181]. It is considered a developing country and is classified by the World Bank as having an upper-middle-income economy which is defined as a gross national income per capita of $3 956 to $12 235 [182]. There is a 27.7% unemployment rate in South Africa [183]. From a morbidity and mortality perspective, South Africa's life expectancy is 59.7 years for males and 65.1 years for females and the infant mortality rate is estimated at 33.7 per 1 000 live births. The country consists of nine provinces but the province with the greatest number of individuals is Gauteng which is home to 13.5 million (24%) people [181]. The majority of South Africans rely on the public healthcare system with only 17.4% of the population having private medical aid cover [184].

South Africa is undergoing an epidemiological transition influenced by factors including, demographic, social, economic, technological, political, and cultural changes [185]. An increase in migration and urbanisation has been brought about by people moving from rural areas into the large cities, predominantly in Gauteng and the Western Cape, in search of work [181]. With urbanisation comes a shift in dietary patterns and thus a nutrition transition. As discussed previously, such a transition brings about an increase in the prevalence of chronic and progressive diseases and should result in a decrease in infectious diseases [34]. However, in South Africa, together with the nutrition transition there is in fact an increase in infectious disease. The human immunodeficiency virus (HIV) plagues the country with approximately

12.7% of the total South African population reportedly living with HIV in 2016. This total has increased since 2002 when 10.3% of the population was infected [181].

In addition to the burden of HIV, South Africa is experiencing a rapid increase in NCDs. Of the top ten causes of death amongst females aged ≥65 years in South Africa 62.5% are NCDs. For males aged ≥65 years, 48% of the top ten leading causes of death are attributable to NCDs [186]. Like other developing countries, South Africa is witnessing an increase in overweight, obesity and T2DM [187] which suggests a likely increase in GDM. This in turn, will weigh heavily on the public healthcare system.

1.5.1 South Africa's Obesity Epidemic

Obesity is proving to be a serious public health concern in the world and South Africa is certainly not exempt from this problem. A systematic review performed in 2013 found a substantial increase in obesity rates amongst children and adolescents (2-19 years) in both developed and developing countries. The rates of overweight/obesity in developed countries increased from 16.9% (16.1-17.7) amongst boys and 16.2% (15.5-17.1) amongst girls in 1998 to 23.8% (22.9-24.7) amongst boys and 22.6% (21.7-23.6) amongst girls in 2013. Similarly, over the same time period, developing countries saw an increase in overweight/obesity with the prevalence increasing from 8.1% (7.7-8.6) to 12.9% (12.3-13.5) amongst boys, and from 8.4% (8.1-8.8) to 13.4% (13.0-13.9) amongst girls [188]. Focusing specifically on Africa, the continent has observed an increase in obesity from 1980 to 2014; the mean BMI (standardised for age) for African men increased from 21.0 kg/m^2 (20.3–21.7) to 23.0 kg/m^2 (22.7–23.3) and that of African women increased from 21.9 kg/m^2 (21.3–22.5) to 24.9 kg/m^2 (24.6–25.1) [189].

In terms of South Africa, Pienaar [190] looked at rates of overweight and obesity amongst children aged six to nine years in the Northwest Province. The findings of the study showed that overall obesity increased from 12.5% at baseline to 16.7% during follow-up. The increase was seen significantly more in boys but girls had an overall higher prevalence of obesity (18.5%). Lundeen et al. [191] found that overweight and obesity increased across childhood (1-18 years of age) in South African girls whereas the rates declined in boys. The obesity prevalence amongst adult males (20 years or older) in South Africa in 2013 was reported to be only 13.5% (12.6-14.5) (Figure 1.13) [188].

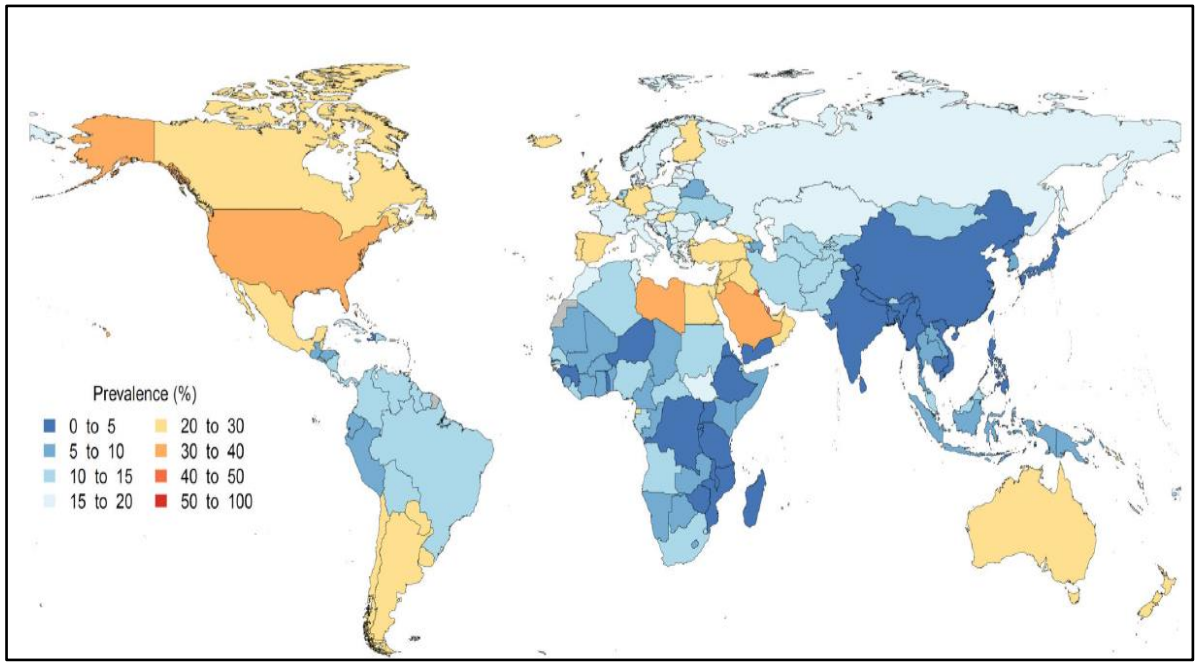


Figure 1.13 A global map showing the age–standardized prevalence of obesity (BMI ≥30) in 2013 amongst males aged ≥20 years (Reproduced with permission from Elsevier [188]).

Of particular concern was the finding that in 2013 adult South African women had the highest rate of obesity in the whole of sub-Saharan Africa. An alarming 42.0% (40.6-43.3) of them were classified as being obese (Figure 1.14). In addition, 69.3% (68.1-70.4) of South African women in the same age group were overweight [188]. Overweight and obesity in childbearing years brings about many concerns and risks for pregnancies and future offspring. Amongst a group of pregnant South African women in Johannesburg, 44% were reportedly obese or morbidly obese and there was a significantly higher prevalence of complications, one being GDM, amongst the obese/morbidly obese group compared to women non-obese women [79].

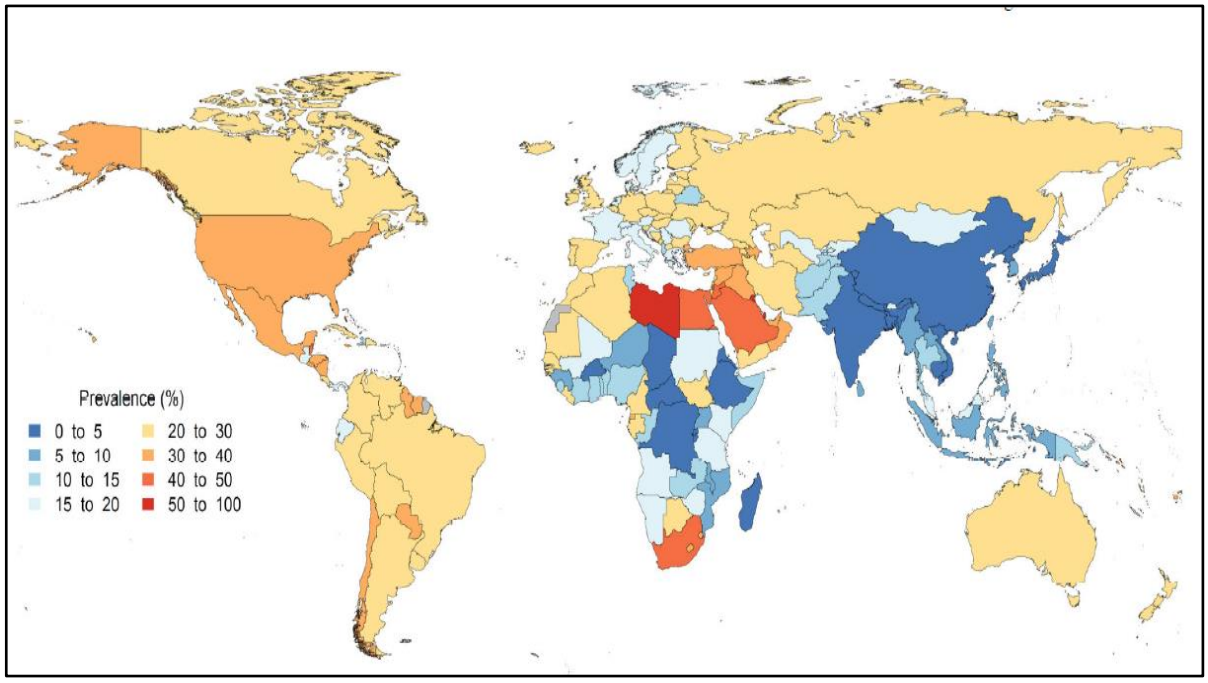


Figure 1.14 A global map showing the age–standardized prevalence of obesity (BMI ≥30) in 2013 amongst females aged ≥20 years (Reproduced with permission from Elsevier [188]).

More recent statistics on South Africa obtained through surveys conducted in 2016 by the Department of Health show that 68% of women and 31% of men aged 15 years or older are overweight or obese. In addition, one in five women of the same age group is morbidly obese. In terms of ethnicity, Coloured women are the most overweight/obese followed by Indian/Asian women, black women and then white women. The mean BMI amongst women living in non-urban areas of South Africa is 28.6 kg/m^2 compared to their urban counterparts who have a mean BMI of 29.6 kg/m^2 [192]. Such results illustrate the growing epidemic of obesity, particularly amongst women, in South Africa. Whilst urbanisation does seem to bring with it a higher prevalence of obesity, non-urban areas are becoming affected too. A decline in physical activity, an increase in sedentary behaviour and a shift towards more Westernised diets are key drivers behind the obesity epidemic [187].

1.5.2 The Burden of Diabetes in South Africa

Like obesity, a rapid increase in diabetes has been observed across Africa. From 1980 to 2014 the prevalence of diabetes in African men increased from 3.4% (1.5–6.3) to 8.5% (6.5–10.8) and from 4.1% (2.0–7.5) to 8.9% (6.9–11.2) in African women. Overall, northern and southern African regions have higher rates of diabetes than the global average [189]. South Africa has contributed considerably to the global increase in diabetes with 2.3 million people affected in the country (the majority of which have T2DM) [193]. In 2014 the leading causes of death in South Africa were tuberculosis, diabetes mellitus and cerebrovascular diseases. From 2014 to 2015 diabetes moved from being the third most common cause of death to being the second. Along with diabetes an increase in hypertensive disorders and heart disease has been observed [186].

Diabetes adds a significant amount of strain on a country's healthcare system and economy. Heath facilities become burdened, productivity declines and cases of disability increase [193]. Bertram et al. [194] estimated that in South Africa in 2009 T2DM alone caused 42 919 Years Lost due to Disability (YLD) which is a measure of loss of life due to ill health or disability. In addition, the authors calculated YLD for diabetes-related complications; a further 13 458 YLD were calculated due to do retinopathy, 4 527 from amputations, 7 233 from stroke and 5 577 from ischaemic heart disease bringing the total YLD for diabetes to 73 714. Being a developing country already burdened by HIV and poverty, South Africa cannot afford to be further burdened by diabetes.

Recently, the South African government proposed a sugar tax on sugar-sweetened beverages in an attempt to curb obesity and T2DM [195]. This has come about as a result of studies that have estimated the potential positive effects of such a tax on morbidity, mortality and economy. Manyema et al. [196] estimated that over 20 years a 20% tax on sugar-sweetened beverages in South Africa would reduce deaths caused by T2DM by 21 000 (95% uncertainty interval (UI) 14 000, 29 000), Disability-Adjusted Life Years (DALYs) (number of lost years of healthy life) attributed to T2DM by 374 000 (95% UI 299 000, 463 000) and healthcare costs by approximately ZAR10.0 billion (95% UI ZAR6.8, 14.0 billion). A significant reduction in the consumption of sweetened beverages is likely to occur with the implementation of the sugar tax [197]. As of yet the sugar tax has not been imposed in South Africa. Government claim to implement it sometime during 2018. Until then, further interventions to avert the obesity and diabetes crises are desperately needed.

1.5.3 Maternity Care in South Africa

Antenatal care, and healthcare to children under six years of age, are free services in the public healthcare sector of South Africa [198]. The public healthcare facilities of the country consist of clinics, community healthcare centres, and district, regional, tertiary and central hospitals. The different facilities offer variable levels of care. Clinics and community healthcare centres are structured and staffed to manage more common, low-risk medical problems whereas the hospitals are set to manage more complicated cases. High-risk patients are referred to higher level facilities through a structured referral system [198].

Antenatal care is offered at all types of healthcare facilities. However, the level of care and services offered differ between facilities. Some community health centres are standalone maternity services run by midwives and are referred to as midwife obstetric units (MOUs). Dating and monitoring of pregnancies by ultrasonography are generally not offered at these facilities. Gestational age is therefore calculated by using a woman's last menstrual period (LMP) which is only valid if she is certain of her dates. If the LMP dates are unknown or appear to be incorrect the symphysis-fundal height (SFH) measurement is used after 24 weeks gestation for singleton pregnancies. Under 20 weeks gestation bimanual and abdominal palpation can be used to estimate gestational age but the healthcare professional needs to be skilled and experienced at the technique in order for an accurate age assessment to be obtained [198].

South Africa has for many years adopted the Basic Antenatal Care (BANC) approach in the public healthcare sector. The BANC approach was implemented in order to improve the quality of care to pregnant women and reduce the rates of maternal and child mortality. The BANC guidelines stipulate five antenatal visits for a pregnant woman at < 20, 20, 26, 32 and 38 weeks gestation [199, 200]. The guidelines were designed based on the Focussed Antenatal Care (FANC) model adopted by the WHO. More recently the WHO have revised their antenatal guidelines and have now proposed the 2016 WHO Antenatal Care (ANC) model which involves a pregnant woman being followed up at an antenatal clinic for a minimum of eight visits; once in the first trimester (up to 12 weeks of gestation), twice in the second trimester (at 20 and 26 weeks of gestation) and five times in the third trimester (at 30, 34, 36, 38 and 40 weeks). The more frequent visits and contact with healthcare professionals is predicted to reduce perinatal deaths and improve antenatal care [201].

In South Africa nurses and midwives are largely responsible for antenatal care at clinics and community healthcare centres. Therefore, all deliveries that occur at clinics with labour wards and MOUs are vaginal deliveries. District, regional and tertiary hospitals manage high-risk antenatal cases and offer 24-hour labour and delivery services including Caesarean sections. Generally, district hospitals have visiting specialist obstetricians whereas regional and tertiary hospitals employ specialist obstetricians on a permanent basis. Prenatal ultrasound services should be present at all levels of hospitals but are generally not available at antenatal clinics and community healthcare centres. Regional and tertiary hospitals serve as academic teaching hospitals. Regional hospitals offer services at a general specialist level whereas tertiary hospitals offer both specialist and sub-specialist care and management [198]. Central hospitals offer extremely specialised tertiary and quaternary services on a national level and are involved in the training of healthcare professionals. There are ten central hospitals in South Africa, four of which are based in the Gauteng Province [202].

Whilst the majority (96%) of pregnant South African women deliver their babies in a health facility and 97% of the deliveries are assisted by a skilled healthcare professional [192], maternal deaths during pregnancy, childbirth and the puerperium period (delivery to about six weeks post-delivery) remain a concern. The South African Department of Health reported that in 2014 the institutional Maternal Mortality Ratio (iMMR) in South Africa was 140.81 per 100 000 live births [203]. This figure declined in 2016 with the latest reported iMMR ratio being 134.33 per 100 000 live births [204]. In April 2017, South Africa proposed the adoption of the 2016 WHO ANC model [205, 206] in an attempt to improve overall care and management of pregnant women and reduce mortality rates.

1.5.3.1 Screening for Gestational Diabetes Mellitus in South Africa

The Guidelines for Maternity Care in South Africa recommends screening women for GDM based on the presence of risk factors at their first antenatal visit and then again at 28 weeks gestation if the first screen was negative [198]. These guidelines list certain risk factors for GDM that are considered important in South Africa (Table 1.3). However, several different GDM screening protocols and guidelines exist across the nine provinces of the country. The criteria used to identify women at risk for GDM may differ between and even within provinces. Women with GDM are usually referred to specialist diabetes units, generally within tertiary/central hospitals [198].

Table 1.3 Risk factors for gestational diabetes mellitus according to the National Guidelines for Maternity Care in South Africa [198]

Underlying patient factors	Patient from an ethnic group with high prevalence of diabetes (e.g. Indian) BMI ≥35 kg/m^2 Age ≥40 years
Previous history	Previous history of gestational diabetes First degree relative with diabetes Previous unexplained intrauterine fetal death Previous macrosomic baby (birth weight ≥4 kg)
Current pregnancy	Polyhydramnios Large for gestational age fetus Glycosuria (glucose 1+ or more on urine dipstick)

More recently, in 2017, the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) endorsed the above risk factors for GDM but excluded the maternal age cut-off and decreased the BMI cut-off to ≥30 kg/m^2. In addition, they added a history of polycystic ovarian syndrome, polyhydramnios, stillbirths, unexplained perinatal deaths, and previous babies with congenital abnormalities as being reasons to screen a woman for GDM [131].

1.6 Summary of the Literature Review

The literature review has highlighted some important aspects around GDM. The condition usually arises as a result of pre-existing chronic insulin resistance and decreased β-cell reserves, coupled with the natural insulin resistance induced by late pregnancy. Uncontrolled GDM has detrimental consequences, both short- and long-term, to the mother and her offspring. Exposure to hyperglycaemia *in utero* appears to increase and accelerate fetal growth and is associated with macrosomia and LGA neonates which often require delivery interventions, specifically Caesarean sections. Of note is the effect of GDM on fetal programming which increases risks of obesity and T2DM later in life. In addition, despite most mothers reverting to normal glucose metabolism after delivery, they are at increased risk of developing T2DM in later years.

There are several different screening protocols for GDM and a global consensus as to which protocol to use has not yet been reached. Many countries adopt a selective screening approach as opposed to a universal one which means some women with GDM are missed. The OGTT is the gold standard test for diagnosing GDM and is usually performed between 24 and 28 weeks gestation; the gestational period when the natural insulin-resistant state of pregnancy peaks.

Certain risk factors increase one's chance of developing GDM, the most influential is a high BMI. Treatment through dietary advice and exercise manages the majority of GDM cases and in instances where other interventions are required, insulin and metformin appear to be drugs of choice to effectively control women's glucose levels. Treatment for GDM seems to successfully avert the adverse consequence of *in utero* exposure to hyperglycaemia but the long-term effects on fetal programming are unknown. With the global rise in T2DM and obesity is assumed that GDM prevalence is on the rise too.

1.7 Gaps in the Literature and the Motivation for the Study

It is known that the prevalence of T2DM in South Africa is on the rise. Given that GDM and T2DM are aetiologically indistinct one would assume that the prevalence of GDM is increasing too. With South African women being the most obese amongst women in sub-Saharan Africa it is possible that, as with the Pima Indian population, T2DM is increasing in South Africa because of an increase in overweight and obesity as well as *in utero* exposure to GDM. In addition, African ethnicity is a known risk factor for GDM.

Given the fact that the South African public healthcare sector utilises a selective screening approach for GDM with various GDM diagnostic criteria being utilised across the country, the true extent of GDM amongst South African women is unknown. Understanding the degree of the problem could indicate whether alternative GDM screening protocols are necessary. In addition, there is no literature describing the effects of GDM on fetal growth in African babies. In general, global data on *in utero* growth in relation to GDM is limited. Understanding if/how GDM affects fetal growth can pinpoint areas where obstetricians can intervene to not only improve maternal care but also to improve the care of the unborn child.

Considering the large majority of pregnancies in a population are of low- intermediate risk, most South African women in the public healthcare sector are managed through local antenatal clinics and healthcare centres. Therefore, the majority of women do not have access to prenatal ultrasound services. Gestational age assessment in clinics and healthcare centres is generally made based on LMP dates and palpation. Accurate gestational age dating is important when performing an OGTT; it should ideally be performed at 24-28 weeks gestation although the wide range allows some room for error. However, determining whether a baby is LGA at birth is dependent on knowing the gestational age at delivery. If routine GDM screening is to be introduced into local antenatal clinics, gestational age dating methods other than ultrasound will most likely be relied upon and therefore determining their accuracy is important.

Understanding the extent of GDM in the black South African population will assist in identifying whether current screening regimes are sufficient and where intervention programmes, such as nutritional advice/awareness/counselling and blood glucose monitoring, should be introduced. Research into the effects of *in utero* exposure to GDM is needed amongst South African babies to understand whether the reported adverse outcomes, such as

macrosomia, found in other population groups are in fact applicable to the local setting. Such research will add to the mandate of improving maternal and child health in the country.

1.8 Aims and Objectives

The overarching aim of this study was to investigate GDM amongst black South African women living in Soweto, Johannesburg, South Africa.

The specific objectives were:

1. To determine what GDM prevalence figures exist for Africa.
2. To estimate the prevalence of GDM amongst black South African women living in urban Soweto and assess their clinical management.
3. To assess the effects of GDM exposure on fetal growth and neonatal birth measures.
4. To compare gestational age estimation using last menstrual period to that using fetal ultrasound.

1.9 Study Hypotheses

With reference to the objectives above the hypotheses were:

1. Very little data on the prevalence of GDM exist in Africa.
2. The prevalence of GDM amongst women in urban Soweto is higher than that reported in rural Limpopo (8.8%) [207] using the same diagnostic criteria.
3. Exposure to GDM *in utero* causes increased fetal growth and birth measurements.
4. Gestational age dating using last menstrual period is inaccurate amongst South African women.

1.10 The Study in Relation to the Gestational Diabetes Mellitus Conceptual Framework

As discussed in this chapter, there are several factors described in the literature that influence the development of GDM. Some of these are modifiable, such as weight, lifestyle and diet, and others are not, such as genetic make-up and ethnicity. Together with these modifiable and non-modifiable factors a woman's obstetric history also influences her risk of developing GDM. Figure 1.15 illustrates the conceptual framework for GDM and highlights the areas that this PhD study addresses (as indicated by the red dashed line boxes). Within these areas of interest this PhD will assess certain factors that influence GDM development (indicted in red text) and the effects of GDM on the fetal growth and neonatal birth size.

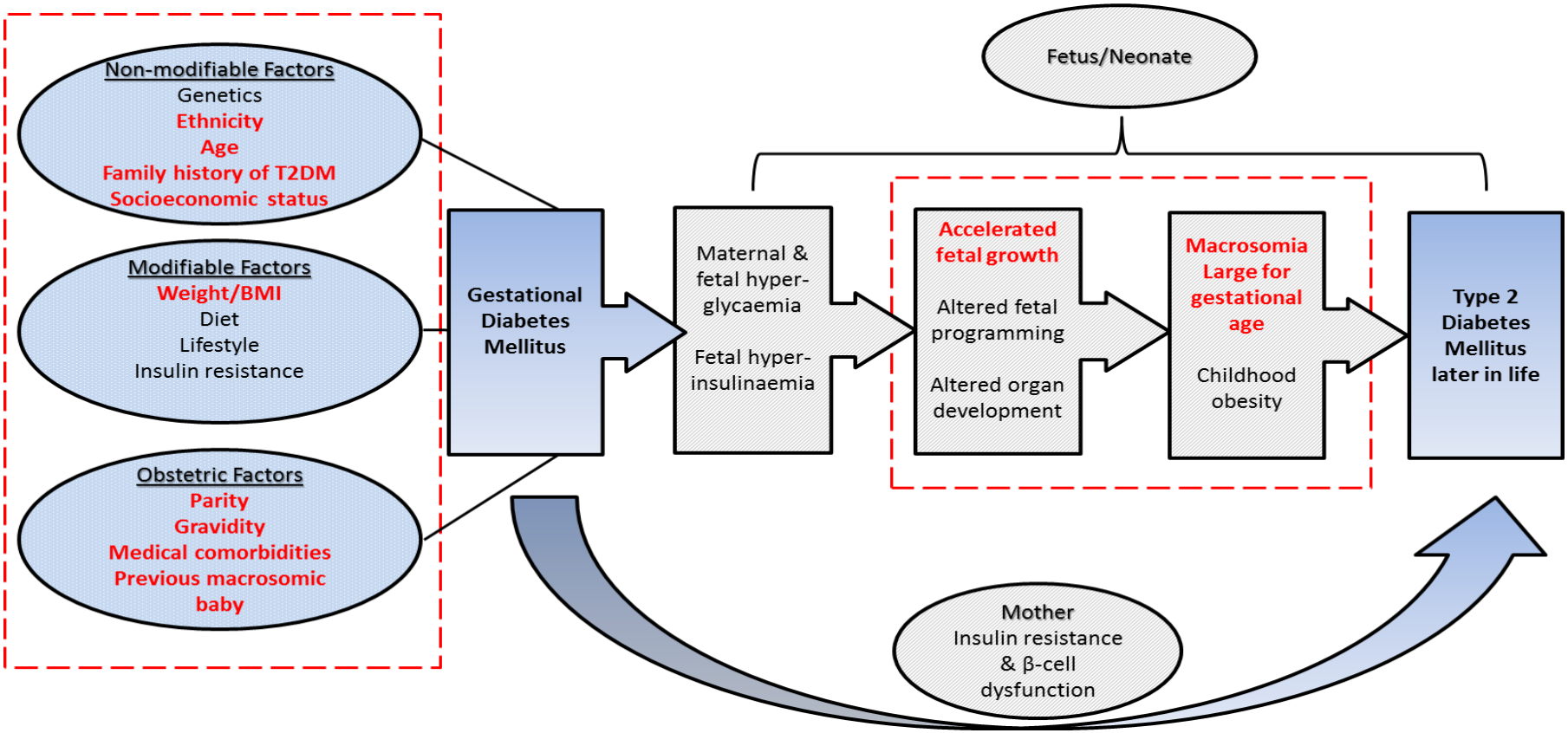


Figure 1.15 The conceptual framework for gestational diabetes mellitus (GDM) in relation to the PhD study [Informed by 72]. The areas that this PhD study focuses on are indicated by the red dashed-line boxes and red text.

CHAPTER 2

STUDY CONTEXT AND METHODS

This chapter describes the study setting, study sample and the methodological approaches used in order to achieve each of the objectives. The study was based in Soweto in Johannesburg, South Africa, and data collection spanned four years. More detail on specific methodology and statistical analyses used is described in each of the empirical papers (Section Two).

2.1 Soweto, Johannesburg, South Africa

Soweto is a large urban settlement situated within the city of Johannesburg in the Gauteng province of South Africa (Figure 2.1a). Its name is derived from the term ‘South-Western Townships' (So-We-To) [208]. The township was formed during the apartheid era when people of different races were divided and relocated to specific areas. Soweto was formally established in 1963 [209] and is now one of the largest townships in South Africa [210].

Soweto is a densely populated township consisting of around 1.3 million people, 98.5% of which are of black African ethnicity. Soweto is comprised of approximately 200 km^2 of land divided into several suburbs (Figure 2.1b). In total there are around 355 331 Sowetan households and 6 357 persons per km^2. Regarding living conditions, 84.2% of the dwellings are formal with 55% of them having piped water inside the dwelling. Electricity and flushing toilets connected to a sewerage system are available to 90% of the dwellings. Most households have a refrigerator, stove, television, radio and cellular telephone. From an economic perspective, there are an increasing number of residents in Soweto whose household incomes would group them as being of middle socioeconomic status (SES) but the majority of individuals come from low SES with 18.7% of households having no income [211]. Poverty is a serious problem in Soweto with a large number of individuals being unemployed and having low education levels [210]; only 38.3% of individuals in Soweto successfully completed high school and 9.3% have gone on to tertiary education [211].

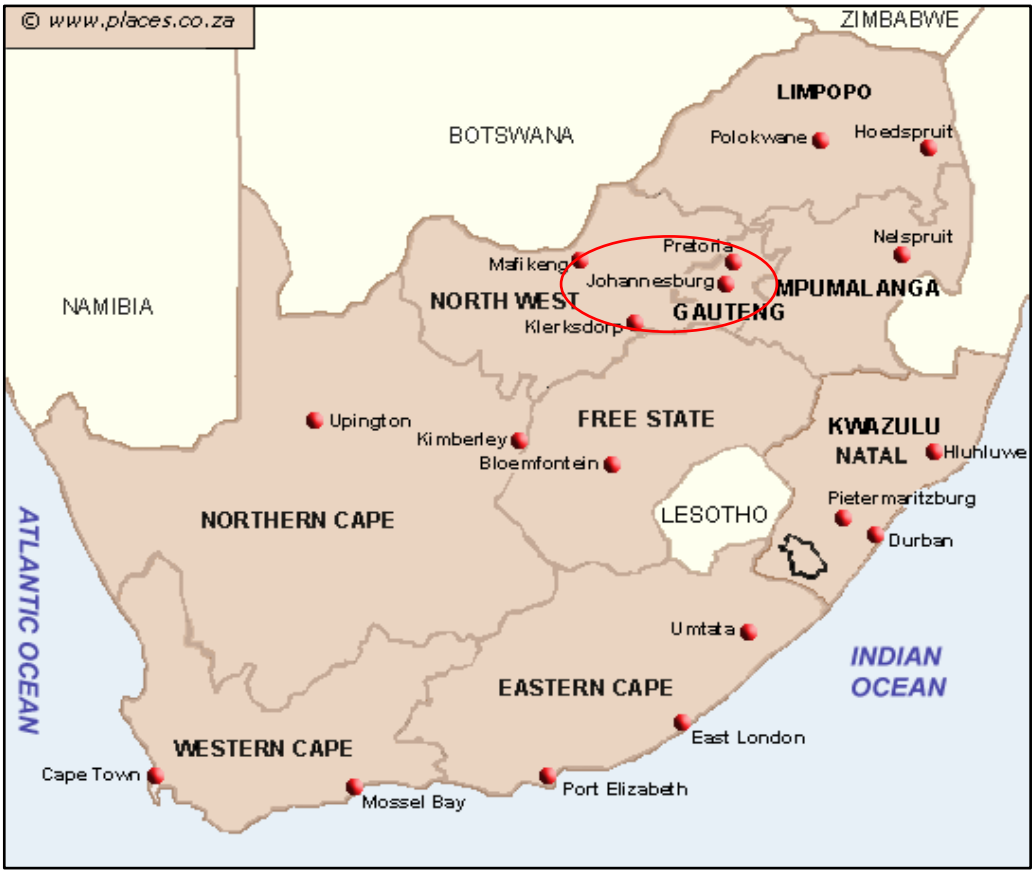


Figure 2.1a Map of South Africa showing Johannesburg in the Gauteng province [212]



Figure 2.1b A map of Soweto spanning 200 km^2 South-West of Johannesburg [213]

Whilst there are 11 official languages in South Africa, the most commonly spoken home languages in Soweto are IsiZulu (37.1%), Sesotho (15.5%), Setswana (12.9%), Xitsonga (8.9%) and IsiXhosa (8.7%). Approximately 2.3% of the Sowetan population speak English [211].

There are around 46 primary healthcare clinics and community centres scattered around Soweto, one district hospital, Zola-Jabulani District Hospital, which only opened in 2014, and one central provincial hospital, Chris Hani Baragwanath Academic Hospital [210]. Chris Hani Baragwanath Academic Hospital takes on a vast number of referrals from the other health facilities in the region.

2.2 Chris Hani Baragwanath Academic Hospital

Chris Hani Baragwanath Academic Hospital (CHBAH) is one of the ten central public hospitals in South Africa that provide specialised tertiary and quaternary services and are involved in research and the training of healthcare professionals [202]. It is one of the third largest hospitals in the world and was originally built in 1941 as a military hospital. The hospital has approximately 3 200 beds and over 6 000 staff members. It serves as a teaching hospital for the University of the Witwatersrand and a referral centre for other areas of South Africa as well as neighbouring countries. The Obstetrics Unit has 300 beds and facilitates approximately 17 000 deliveries per year [214].

2.2.1 Current Screening and Management of Gestational Diabetes Mellitus in Soweto

At CHBAH a selective screening approach is used to identify women at risk of developing GDM [215]. Chris Hani Baragwanath Academic Hospital is the only health facility in the Soweto region that performs OGTTs. Pregnant women receiving their antenatal care at the surrounding antenatal clinics, who are identified as having risk factors for GDM, would be referred to CHBAH for further investigation. The hospital follows the latest Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) guidelines for the diagnosis and management of GDM; a two hour 75 g OGTT using the WHO 2013 diagnostic criteria [131].

Risk factors used at CHBAH for the identification of women at risk for GDM are those listed in Table 1.3. However, in addition to those the CHABH also considers a random blood glucose ≥ 8 mmol/l but < 11 mmol/l and recurrent infections e.g. urinary tract, vaginal thrush, as risk factors [215]

If diagnosed with GDM, women are referred to the specialist Obstetric Diabetes Clinic at CHBAH that manages diabetes in pregnancy. It is the only clinic in Soweto that manages such women. The Obstetric Diabetes Clinic at CHBAH was started in 1983 by Professor Kenneth Huddle [216]. The Clinic's current GDM management protocol stipulates that women should be closely monitored every two weeks until 32 weeks gestation after which they are monitored on a weekly basis until delivery. A dietician consults with all patients with GDM and prescribes a diet comprising of 40% carbohydrate, 40% fat and 20% protein. All women with GDM are sent home with a glucometer to regularly monitor their glucose levels. They are encouraged to do capillary glucose readings pre-meal, one or two hours postprandial, and late at night. Target blood glucose levels should be fasting <5.3 mmol/l, one hour postprandial <7.8 mmol/l, and two hours postprandial <6.7 mmol/l. If there is no improvement in glucose levels after a couple of weeks of diet therapy women are prescribed metformin. If glucose levels are not well controlled on metformin, or if the drug is not well tolerated, treatment with insulin is initiated. Insulin, as opposed to metformin, may be started immediately in situations where initial glucose readings are extremely highly [131]. Induction of labour or Caesarean section is planned for around 38 weeks' gestation. Postpartum follow-up for women diagnosed with GDM involves an OGTT six weeks after delivery to assess glucose profiles and the need for further management [131, 215].

2.3 The MRC/Wits Developmental Pathways for Health Research Unit

The Medical Research Council (MRC) and University of the Witwatersrand's (Wits) Developmental Pathways for Health Research Unit (DPHRU) is a research unit, directed by Professor Shane Norris, nested within the Department of Paediatrics. The Unit is renowned for its Birth to Twenty Study (Bt20); a longitudinal birth cohort study consisting predominantly of black males and females born between 23 April and 8 June 1990 in the Soweto-Johannesburg region [217]. The mandate of DPRHU is "to investigate genetic, physiological, psychosocial and lifestyle determinants of growth and development, and risk of disease across the lifespan." Figure 2.2 describes the life course framework on which DPHRU bases its research.

The DPHRU is based at CHBAH in Soweto. The Unit has its own laboratory where samples can be run in real-time, several interview rooms, a large treatment/procedure room, a research hub for students and several offices. All interviews, data collection and data capturing pertaining to studies are performed on site.

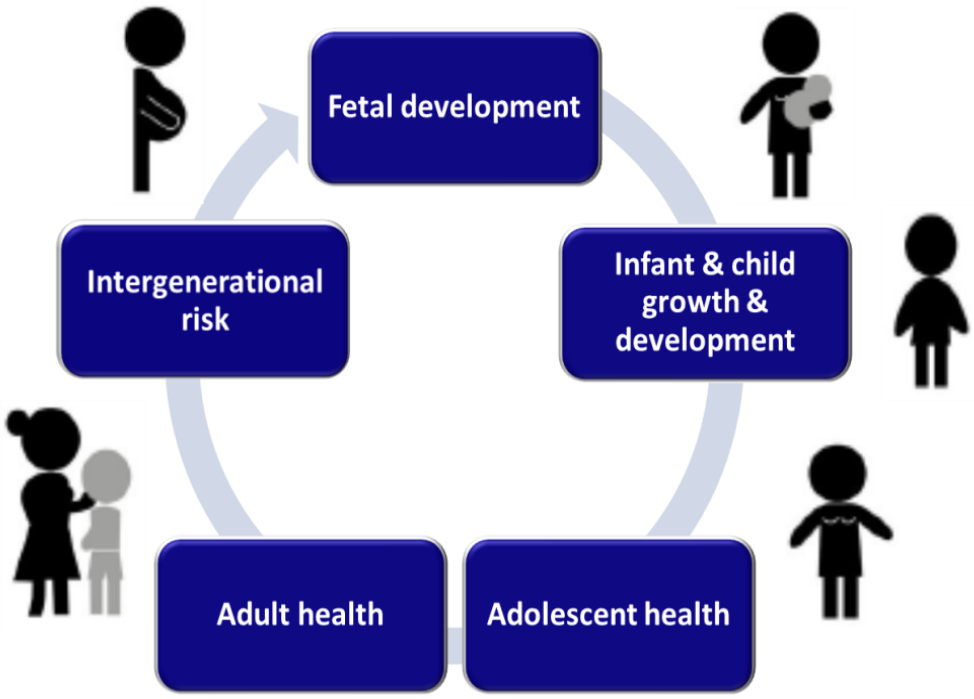


Figure 2.2 The life course framework at the MRC/Wits Developmental Pathways for Health Research Unit.

2.4 Participant Recruitment

Participants for this study were recruited from the Antenatal Clinic and Fetal Medicine Unit of CHBAH. The inclusion and exclusion criteria are described in Table 2.1. A total of 3 656 women who met the inclusion criteria were briefed on GDM and the study and were invited to access free screening for GDM when they were 24-28 weeks pregnant. Women in the GDM screening part of the study were seen at one time point (24-28 weeks gestation) where an OGTT was performed and questionnaires were administered.

A subset of the 3 656 women (n=1 017) recruited at the beginning of the study, formed the longitudinal Soweto First 1000 Days study (S1000). The S1000 study aimed to include approximately 1 000 women who would be followed up throughout their pregnancies until delivery. The 1 017 women enrolled into S1000 were amongst the first 1 050 women recruited. The S1000 name refers to the first 1000 days of life; from conception up until two years of age. The overarching aim of the S1000 study was to investigate the effects of a multitude of maternal factors, of which GDM was one, on fetal development and infant outcomes with the long-term goal of identifying areas for intervention to optimise both maternal and child health. These women were followed up at several time points during their pregnancies and at delivery and one of their follow-up visits included an OGTT.

Details around participants, sample size and methodology used are described in each of the empirical papers presented in Section Two

Table 2.1 Inclusion and exclusion criteria of study participants

Inclusion Criteria	**Exclusion Criteria**
Black South African females	Women diagnosed with diabetes
≥18 years of age	Women diagnosed with epilepsy
Residing in Soweto	Fetal abnormalities detected
≤20 weeks pregnant confirmed by ultrasound	
Singleton pregnancies	
Able to give informed consent	

2.5 Data Collection

Data collection occurred from 1 June 2013 until 30 April 2017. Research assistants fluent in English and several of the common African languages were involved in explaining the objectives of the studies (GDM screening study and S1000 study) to potential participants and obtaining informed, written consent. Participants also received information sheets (Appendix B) describing what each study would involve. Once informed consent (Appendix B) had been obtained from study participants data collection took place at the DPHRU research site. Study data were collected and managed using Research Electronic Data Capture (REDCap) hosted at The University of the Witwatersrand. REDCap is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources [218]. The data collection sheets related to the PhD study can be found in Appendix C.

2.5.1 Soweto First 1000 Days Study

The S1000 study was a longitudinal study that involved the participants being followed up at several time points during their pregnancies:

- Visit 1: <14 weeks gestation
- Visit 2: 14-18 weeks gestation
- Visit 3: 19-23 weeks gestation
- Visit 4: 24-28 weeks gestation
- Visit 5: 29-33 weeks gestation
- Visit 6: 34-38 weeks gestation
- Delivery

If women were between 14 and 20 weeks pregnant at the time of recruitment (inclusion criteria included participants being ≤20 weeks pregnant) they would be allocated to the appropriate "visit" determined by their gestational age. Data collection at each time point involved an ultrasound scan by a qualified and fully trained research sonographer. In addition

to the ultrasound scan, anthropometric measures were taken and a series of questionnaires were administered by trained research nurses (fully qualified registered nurses) and research assistants. The questionnaires included questions pertaining to demographics, obstetric history, socioeconomic status, family history of diabetes and personal health. All questionnaires were administered in English but all research nurses and assistants were able to speak several of the African languages and so any queries could be clarified in the participant's home language.

At 24-28 weeks gestation participants underwent a two hour 75 g OGTT (Appendix D contains the OGTT protocol). Regarding delivery, the majority of women in the study were scheduled to deliver at CHBAH. The DPHRU research nurses and assistants had permission to collect birth data from CHBAH. There was a research nurse and assistant on call 24 hours a day for participants to call when they went into labour or as soon as they had delivered their babies. Once the research nurse and assistant were notified of the pending delivery or newborn's arrival they would go to the respective ward at CHBAH to collect neonatal anthropometric measurements (weight, length and head circumference) within 24 hours of delivery. In cases where the research nurse and assistant were unable to collect the data, for example when the mother delivered at a local clinic, anthropometric measurements obtained by the hospital/clinic staff were obtained.

On a very small subset of neonates, body composition measures were taken using the PeaPod®. The machine was housed at the DPHRU which meant the newborn baby could only be brought to the Unit once he/she and his/her mother had been discharged. PeaPod® data was collected within three weeks post-delivery.

2.6 Methodology

Several methods were employed in this study; anthropometry (maternal and neonatal), fetal ultrasonography, body composition analysis, the OGTT, blood pressure measurements, haemoglobin measurements and questionnaires. The specific and detailed methodologies used to achieve each objective of the study are described in detail in the empirical papers in Section Two. The methodological approaches taken and data required to achieve each objective of the PhD are outlined below:

- **Objective 1: To determine what GDM prevalence figures exist for Africa**

A systematic review was performed. The methodology used was in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [219]. A stringent search of the literature was performed and the studies included in the systematic review were critiqued. This study is described in Chapter 3.

- **Objective 2: To estimate the prevalence of GDM amongst black South African women living in urban Soweto and assess their clinical management**

This was a cross-sectional study employing data at one time point (24-28 weeks gestation) on a large sample of women. Oral glucose tolerance test data were required. In addition, demographic data, maternal anthropometry, obstetric and family history data, blood pressure and haemoglobin readings were needed in order to assess possible GDM risk factors. Details around the management and treatment of GDM were also required. This study is described in Chapter 4.

- **Objectives 3: To assess the effects of GDM exposure on fetal growth and neonatal birth measures**

This was a longitudinal cohort study whereby the women enrolled into the S1000 study were followed up throughout their pregnancies and at delivery. Data from fetal ultrasound scans performed over the course of gestation, maternal anthropometry, neonatal birth anthropometry, PeaPod® data and OGTT data were required. This study is described in Chapter 5.

- **Objective 4: To compare gestational age estimation using last menstrual period to that using fetal ultrasound**

Data from participants of the S1000 study were used for this component of the PhD study. Fetal ultrasound dating scans and reported dates of participants' last menstrual periods were required as well as the dates of birth of their babies. Hospital-recorded ages at birth were also obtained. This study is described in Chapter 6.

2.7 Sample Size

Prior to the commencement of the study 2013, sample size calculations were performed to determine the ideal sample for 1) establishing what the prevalence of GDM is amongst women in Soweto and 2) detecting a difference in fetal growth measures between fetuses exposed to GDM compared to unexposed fetuses. These calculations were made using the findings from two previous studies; a GDM prevalence study amongst black South African women living in Limpopo [207] and an Italian study that investigated fetal growth in relation to GDM [220].

- **Sample size required to estimate the prevalence of GDM amongst black South African women living in urban Soweto**

Mamabolo et al. [207] reported an 8.8% prevalence of GDM using the IADPSG criteria amongst black women living in Limpopo, South Africa. Based on this a minimum sample size of 769 (power of 80%, CI of 95% and a width of 2% (between the ranges of 7% and 11%)) was calculated in order to determine the prevalence of GDM amongst women living in Soweto. Epi Info (Centre for Disease Control, Version 3.5.1) was used to calculate this. A final sample size of 1 906 was included in the GDM prevalence study (Chapter 4)

- **Sample size required to assess the effects of GDM exposure on fetal growth**

Giampietro et al. [220] reported a mean (± standard deviation) abdominal circumference of 27.0 ± 1.7 cm in the second trimester amongst fetuses exposed to GDM whereas the mean (± standard deviation) abdominal circumference amongst fetuses of normal pregnancies was 26.3

± 2.9 cm. This data (two independent means) was used to perform a sample size calculation using a two-sided test with a distribution ratio of 0.2 (N2/N1) in Stata (StataCorp, Version 12). A minimum sample size of 441 fetuses (74 GDM-exposed and 367 unexposed fetuses) (power of 80% and α significance level of 0.05) was calculated in order to detect a difference in fetal growth between GDM-exposed and unexposed fetuses. A final sample size of 741 fetuses, including 82 GDM-exposed and 659 unexposed fetuses, was included in the fetal growth component of the study (Chapter 5).

2.8 Data Analysis

All statistical analyses were run using Stata (StataCorp, version 12). Details pertaining to the statistical tests used are provided in each of the empirical papers.

2.9 Ethical Approval

Ethics clearance for the PhD study was granted by the University of the Witwatersrand's Human Research Ethics Committee (HREC) (Medical); ethics clearance certificate M130309: "Glucose metabolism and pregnancy in South African women". In addition, ethics clearance certificates M120524 and M150461 were issued for the S1000 study and GDM screening study respectively. Copies of all three ethics clearance certificates can be found in Appendix E.

SECTION 2
EMPIRICAL PAPERS

CHAPTER 3
GESTATIONAL DIABETES MELLITUS IN AFRICA: A SYSTEMATIC REVIEW

This chapter describes a systematic review that was performed in order to gain an understanding of what literature exists on gestational diabetes mellitus in Africa and what the average prevalence of the condition on the African continent is. Understanding the extent of a problem is a crucial step before one can ascertain whether interventions, further research and potential policy changes are warranted. The work described in this chapter was published in the peer-reviewed journal, PLoS One (Appendix J):

Macaulay, S., Dunger, D.B. & Norris, S.A. (2014) Gestational diabetes mellitus in Africa: A systematic review. *PLoS One*, 9 (6): e97871. doi:10.1371/journal.pone.

3.1 Introduction

Diabetes mellitus (DM) is a group of conditions that contribute significantly to the increasing health and financial burden in many countries around the world [37]. The prevalence of and screening methods for the clinical subgroups, Type 1 diabetes mellitus and Type 2 diabetes mellitus (T2DM), are relatively well researched and understood in most countries. However, those pertaining to the subgroup known as gestational diabetes mellitus (GDM) are less established [221]. Gestational diabetes mellitus is defined by the World Health Organization as being "any degree of glucose intolerance with onset or first recognition during pregnancy" and should therefore include glucose readings that fall within the impaired glucose tolerance (IGT) diagnostic range, as well as those within the diagnostic range for diabetes [47, 222]. More recently, the American Diabetes Association defines GDM as "diabetes diagnosed during pregnancy that is not clearly overt diabetes" [151].

Pregnancy itself induces changes in maternal glucose metabolism and insulin sensitivity. As pregnancy progresses the demand for insulin production on the mother's pancreas increases. In most instances, pregnant women are able to meet the increased insulin demand but in some cases these needs are not met resulting in poor glycaemic control and consequently GDM. Certain factors including having a family history of diabetes, being over 25 years of age, being obese, belonging to a particular ethnic group (African American, Hispanic, Indian) and having previously given birth to a baby weighing 4 kg or more (macrosomia), put women at greater risk of developing GDM [73, 223].

Pregnancies affected by GDM pose a risk for adversities such as the need for Caesarean sections due to fetal macrosomia. Macrosomia occurs as a result of accelerated fetal growth fuelled by maternal hyperglycaemia [58]. In approximately 95% of GDM cases maternal glucose metabolism returns to normal after delivery of the baby [71], however, an association between GDM and the development of T2DM in the mother later in life exists [13, 224]. In addition, research into the long term effects of poor maternal glucose metabolism on the fetus has revealed that offspring born to mothers with GDM are susceptible to IGT and obesity [225, 226]. With these associations in mind it would be important to identify pregnant women at risk for GDM so that prevention management such as lifestyle modifications can be implemented [227].

Consensus regarding screening for and classification of GDM is yet to be achieved globally [221]. The most recognised diagnostic test for GDM is the oral glucose tolerance test (OGTT) usually performed between 24-28 weeks gestation [228]. Different screening regimes for GDM exist and as a result studies investigating prevalence of GDM are often diverse in terms of methods employed, cut-off values used and consequently, results obtained [140].

Not only do different testing methods exist but the availability of GDM screening differs from country to country and even within countries. Although it would be ideal to screen every pregnant woman for GDM it is not always feasible from a cost perspective, particularly in low- or middle-income countries (LMICs). In many LMICs, and some high income countries, women tend to be selected for screening only if they fulfil certain GDM risk-associated criteria [229]. Due to this selective screening process one may expect the true extent of GDM in such countries to remain relatively unknown. Furthermore, prevalence rates may be dependent upon the specificity and sensitivity of the selective screening process in identifying at- risk women.

The effects of urbanisation have not only had a profound impact on developing countries' economies but also on public health. The transition from rural to urban ways of life is often associated with changes in eating habits, body mass and composition, and reduction in physical activity. The movement towards more Westernised diets involves increased consumption of fats, sugars and refined carbohydrates. As a result, LMICs are experiencing a rapid increase in overweight and obesity as well as NCDs diseases, such as diabetes, that accompany such conditions [37, 38]. Considering this, the prevalence of GDM should be increasing too. Reported prevalence figures for GDM in two high income countries, the United Kingdom and the United States of America, are 2-3% and 2-10% respectively [229]. A study that assessed GDM in the south of India, a LMIC, reported a far greater prevalence of 13.9% [230]. Gestational diabetes mellitus prevalence estimates for another LMIC, Brazil, are thought to be 7.0-7.6% [229].

Diabetes was essentially unknown in Africa in 1901, yet in 2013 19.8 million people were reportedly living with the condition and this number is predicted to increase to 41.5 million in 2035 equating to a 109% increase [231]. In Africa, the movement from a rural lifestyle to a more industrial urbanised way of life is largely responsible for the evolving problem of chronic diseases, of which diabetes is a major contributor [232].

The explosion in the prevalence of diabetes undoubtedly represents a serious public health burden. In addition, it is more than likely to bring along with it a considerable increase in GDM. However, with regard to GDM in Africa, the situation appears relatively unknown. From a cost perspective, many African countries employ a selective screening approach for GDM and the estimated percentage of pregnant women screened is unclear [229]. In order to suggest policy changes regarding screening for GDM, which will ultimately prevent the effects of GDM on the mother and her offspring, and in turn reduce the financial and health burden to a country, it is essential that the extent of the condition is well understood. Therefore, we performed a systematic search to identify research into diagnostic strategies, screening approaches and reported GDM prevalence figures on the African continent.

3.2 Methods

3.2.1 Protocol and Registration

This project was not prospectively registered. A protocol was developed during the planning process.

3.2.2 Information Sources and Search Strategy

The PRISMA guidelines (Appendix F) for the reporting of systematic reviews were followed [219]. Two authors (SM and SAN) independently performed a literature search using three electronic databases; PubMed, Scopus and the Cochrane Library. The following search terms and combinations were used: “gestational diabetes” and Africa; “impaired fasting glucose” and pregnancy and Africa; diabetes and pregnancy and Africa; “impaired glucose tolerance” and pregnancy and Africa; “gestational diabetes” and “African countries.” In addition, the search terms “gestational diabetes,” together with the names of each individual country in Africa were used. For example, “gestational diabetes” and Egypt; “gestational diabetes” and Namibia; “gestational diabetes” and “South Africa” were entered into the search. The list of all 54 recognised African countries included in the search can be found in Appendix G. Finally, “gestational diabetes” and “Sub-Saharan Africa” were searched for. Where possible, filters were set for studies pertaining to humans but articles written in all languages were included. The search was performed in September 2013. No time limits were set in an attempt

to gather all articles published up until the end of September 2013. Once duplicate references were removed the titles and abstracts of the references were screened.

Studies pertaining to African countries that included the following were considered relevant:

1) Screening methods for GDM
2) Criteria used to diagnose GDM
3) Prevalence of GDM

If an article failed to mention any of the above three points it was excluded. In addition, studies were excluded if they were:

1) On Type 1 and/or Type 2 diabetes only
2) Overviews of GDM
3) Editorials
4) Molecular studies
5) Solely on the outcomes and/or problems associated with macrosomic infants with no reference to GDM prevalence and screening
6) Focussed on perinatal mortality and congenital abnormality rates in babies born to mothers with diabetes
7) Solely comparisons of GDM testing regimes

3.2.3 Data Extraction

Full text articles were obtained and reviewed. Data were then extracted regarding country, region (rural/urban), population group, sample size, age of pregnant women in the cohort, gestational age, how the investigators defined GDM, how they tested for GDM and what GDM prevalence was reported. In addition, data were also extracted from abstracts that included how GDM was screened for, what criteria were used and what prevalence figures were obtained in the study but for which full text articles could not be obtained.

3.2.4 Assessment of Reporting Quality and Risk of Bias

The reporting quality of each study was assessed using the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist [233] guided by the published detailed explanation on how to use the checklist [234]. The combined checklist designed for cohort, case-control and cross-sectional studies was utilised (Appendix H). A quality assessment score out of 22 was determined for each study by assigning a point per STROBE item addressed. Good/fair quality papers were categorised as having a score of ≥14/22 and poor quality papers were classified as having a score of <14/22. All studies, regardless of their STROBE score, were retained in the systematic review.

Bias was assessed using the Risk of Bias Tool for Prevalence Studies developed by Hoy et al. [235] adapted specifically for this systematic review (Appendix I). The tool consists of ten items which address four areas of bias and an eleventh item includes a summary risk of bias assessment. The items assess both external and internal validity. Each study was rated as having a low, moderate or high risk of bias. Studies were classified as having a low risk of bias when eight or more of the ten questions were answered as "yes (low risk)", a moderate risk of bias when six to seven of the questions were answered as "yes (low risk)" and a high risk of bias when five or fewer questions were answered as "yes (low risk)".

3.3 Results

3.3.1 Study Selection

The three databases searched identified a total of 568 records. A total of 102 duplicates were removed resulting in 466 unique records after which 362 records were excluded based on their titles being considered irrelevant to the search topic. Of the 104 abstracts screened, 67 abstracts were considered to be relevant. Due to lack of access to the particular journals, despite several attempts, including trying to obtain the articles via the interlibrary loans service and contacting the authors directly, seven full text articles could not be obtained. After reviewing the full text articles of 60 of the records, 14 met all the criteria for the systematic review. In addition, one abstract, for which the full text article could not be obtained, was also considered relevant to the systematic review. A French-speaking colleague read, translated and extracted data from the one article written in French. Articles that were excluded were

those in which information regarding classification of, diagnostic criteria for and screening methods for GDM was missing, where methodology was unclear and where investigations were performed on immigrant women as opposed to women representative of the local pregnant population (Figure 3.1).

3.3.2 Reporting Quality and Risk of Bias

The STROBE scores per study and the risk of bias results are listed in Table 3.1. Quality and risk of bias assessments were not performed on the study for which only an abstract could be obtained [236] and for the systematic review that provided details on that one particular study [237].

With regard to reporting quality and referring to the STROBE checklist (Appendix H), describing the study design, sources of bias, statistical methods used and study limitations were areas where a number of the studies fell short.

Out of the 13 studies that underwent a risk of bias assessment, four (31%) were considered to have a high risk of bias; five were classified as having a moderate risk of bias (38%) and four (31%) were considered to have a low risk of bias.

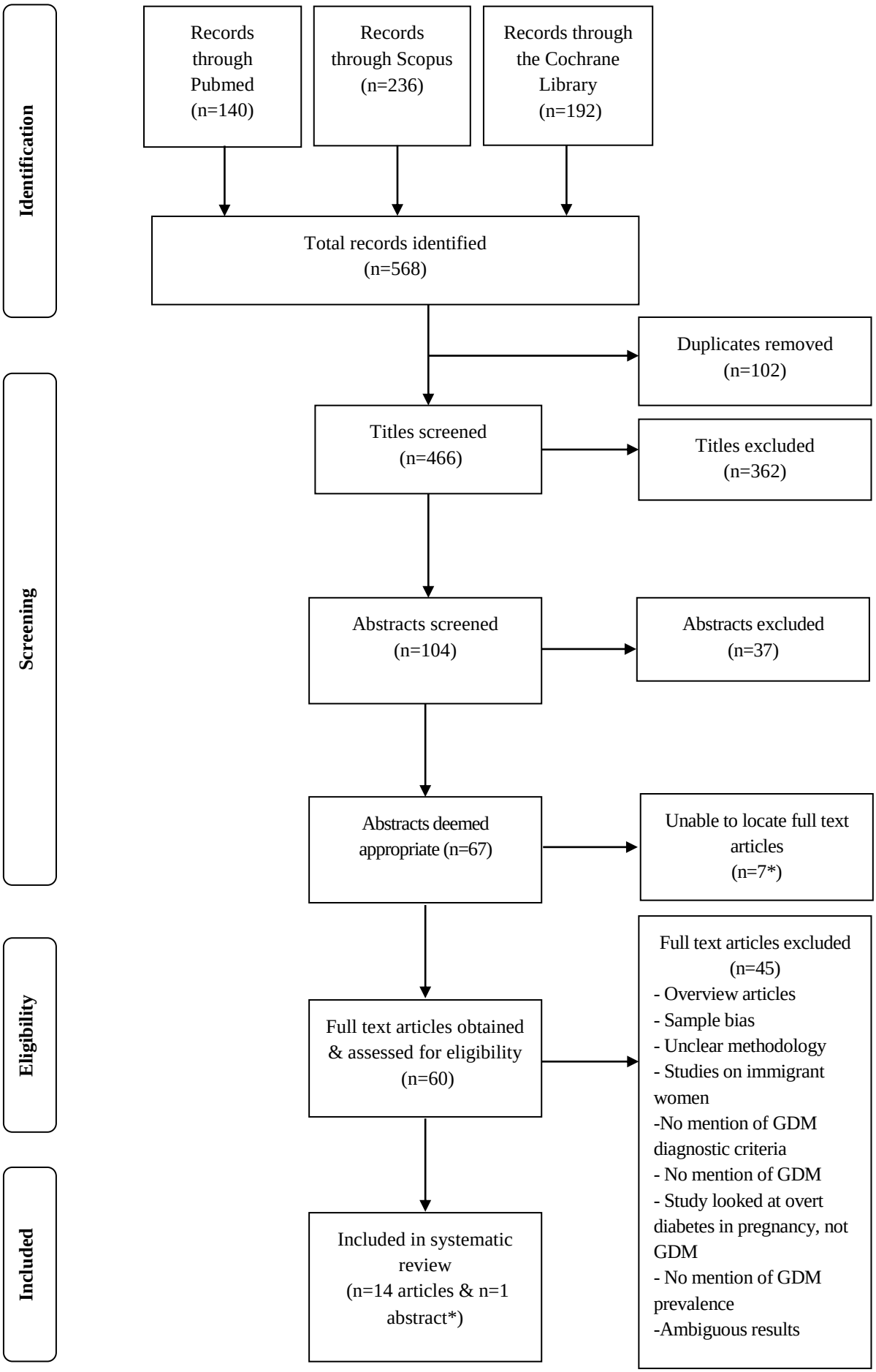


Figure 3.1 Flow diagram illustrating the number of included and excluded studies in the systematic review on gestational diabetes mellitus in Africa

Table 3.1 Reporting quality and risk of bias assessments

Author	STROBE reporting quality score*	Overall risk of bias**
Seyoum et al., 1999	18/22	Low
Bouhsain et al., 2009	16/22	High
Challis et al., 2002	11/22	Moderate
Olarinoye et al., 2004	18/22	Low
Adegbola & Ajayi, 2008	17/22	Moderate
Kamanu et al., 2009	19/22	High
Kuti et al., 2012	19/22	Moderate
Anzaku & Musa, 2013	17/22	Low
Ozumba et al., 2004	12/22	High
Jackson & Coetzee, 1979	15/22	Moderate
Ranchod et al., 1991	16/22	Low
Mamabolo et al., 2006	18/22	Moderate
Basu et al., 2010	19/22	High
Swai, 1991[‡] (mentioned in: Hall et al., 2011)	Not assessed	Not assessed

*Good/fair quality papers were categorised as having a score of ≥14/22, poor quality papers were classified as having a score of <14/22; [‡]As only the abstract was available an assessment of the reporting quality and risk of bias could not be performed

3.3.3 Study Characteristics

Thirteen original research articles, one systematic review article and one abstract pertaining to an original research study were finally included in the systematic review thus totalling 14 African research studies (Figure 3.1). The systematic review article [238] discussed studies in Sub-Saharan Africa and contained suitable information concerning the study for which only an abstract was available. The earliest study was published in 1979 and the latest in 2013, therefore the original individual studies included in the review involved research spanning 35 years. Overall, information regarding GDM classification, screening methods and prevalence was obtained for six African countries; Ethiopia, Morocco, Mozambique, Nigeria, South Africa and Tanzania. Two of the 14 studies looked at GDM prevalence amongst women with risk factors (selective screening), another three studies were case control studies assessing GDM prevalence amongst women at increased risk for the condition versus women without

risk factors, and the remaining nine studies involved universal GDM screening of pregnant women. With reference to Table 3.2:

Ethiopia

Only one study on GDM in rural Ethiopia, performed over a decade ago, was included. This was a well reported study with a low risk of bias. The OGTT was utilised as the diagnostic test based on the WHO 1985 criteria and a GDM prevalence of 3.7% was reported [239].

Morocco

The one article pertaining to research performed in urban Morocco was published in 2009 and was written in French. The authors reported a relatively high prevalence of GDM; 7.7% using the Carpenter and Coustan's criteria. However, the authors stated that all women who tested positive on a glucose challenge screening test should have then been referred for an OGTT yet only 40% of these women received an OGTT. This suggests that the GDM prevalence could actually have been higher if all women requiring an OGTT were in fact tested. The authors did report that the GDM prevalence was similar to the prevalence of Type 2 diabetes in that population. Unfortunately no reference was made to the ethnicity of the study participants and considering Morocco has several ethnic groups it is difficult to say who this prevalence figure applies to [240]. In addition, the risk of potential bias within this study was high.

Mozambique

Only one case control study, of relatively poor reporting quality and moderate risk of bias, was analysed from Mozambique. The study was conducted in 2002 in an urban/ suburban setting and the population group was not stated. Considering the majority of the Mozambican population is black, it is assumed that the cohort consisted of black females. Authors of the study reported a GDM prevalence of 11% amongst women who had late fetal deaths (cases) and 7.3% amongst women who had delivered live newborns (controls). The investigators diagnosed GDM using their own diagnostic criteria which classified glucose readings for diabetes mellitus and IGT as GDM [241].

Nigeria

Six Nigerian studies, all on urban populations, were evaluated. These studies were conducted between the years 2004-2013 [89, 237, 242-245]. Five of the six studies were classified as having good/fair reporting quality and one was classified as poor. The risk of bias across the six studies ranged between low, moderate and high. All the studies used the OGTT as the

method to detect GDM but different glucose loads were employed (50 g, 75 g and 100 g) over a time period of one to three hours.

One study focussed solely on determining the prevalence of GDM amongst women with risk factors which included (i) history of fetal macrosomia; (ii) maternal obesity; (iii) previous intrauterine death; (iv) first degree relative with diabetes; (v) glycosuria and (vi) history of GDM in a previous pregnancy [237]. Another two studies were case control studies whereby women with risk factors for GDM [244] or women who had delivered macrosomic babies [245] were classified as cases, and women without risk factors [244] or women who had delivered normal weight babies [245] served as the controls. Prevalence of GDM was higher amongst the cases in both studies; 6.2% versus 4.6% (utilising the Carpenter and Coustan's criteria) [244] and 2.5% versus 1.5% (utilising the investigators own diagnostic criteria) [245]. However, Kamanu et al. [245], who used their own diagnostic criteria as mentioned above, diagnosed GDM based on a 1 hour 50 g OGTT (>7.8 mmol/l) and only followed up borderline results with a 75 g 2 hour OGTT. Usually the 50 g glucose load is referred to as a glucose challenge test and women who test positive on the challenge test are followed up with a further OGTT. This is referred to as the two step approach [151]. It is unconventional for a 50 g OGTT to be performed independently as a diagnostic test and so the results of this study could be questionable.

Excluding the two case-control studies discussed above, the other four Nigerian studies utilised the WHO diagnostic criteria (two used the WHO 1985 criteria and two used the WHO 1999 criteria). One of these four studies compared the detection rate of the three hour 75 g OGTT using the WHO 1985 criteria to the three hour 100 g OGTT using the NDDG criteria. The 75 g OGTT with WHO 1985 diagnostic criteria yielded a higher GDM prevalence (11.6% versus 4.5%). Conversely, this study found that the incidence of fetal macrosomia was higher (66.7%) amongst women diagnosed with GDM by the 100 g OGTT using the NDDG criteria than amongst women diagnosed with GDM by the 75 g OGTT using the WHO 1985 criteria (23.1%) [242].

South Africa

Four South African studies, conducted between 1979 and 2010, were included in the systematic review [79, 207, 246, 247]. One study focused predominantly on Indian women [247], two on black women [79, 207] and the other did not state the ethnicity of the women [246]. The study by Jackson et al. [246] tested women for GDM because they had one or

more risk factors. These risk factors included (i) a parent or sibling with diabetes; (ii) repeated miscarriages; (iii) obesity; (iv) previous macrosomic infant; (v) glycosuria; (vi) previous hyperglycaemia; (vii) previous infant with a severe congenital anomaly; (viii) previous perinatal death; (ix) polyhydramnios and (x) Indian ethnicity. In addition, this particular study utilised a 2 hour 50 g OGTT and the investigators' own diagnostic criteria. A 50 g glucose load is usually used for the glucose challenge test and an OGTT generally utilises either 75 g or 100 g of glucose [151]. The glucose load chosen for an OGTT by the investigators is unusual. However, this study was performed in 1979 and can therefore be considered outdated. Optimisation of the OGTT for the diagnosis of GDM has developed and improved greatly since then.

All but one study employed a two hour OGTT for the diagnosis of GDM. The one study that did not employ an OGTT was interestingly the most recent study in South Africa, conducted in 2010, which tested fasting or random blood glucose levels and referred to an institutional protocol for diagnostic criteria [79]. Ranchod et al. [247] compared the WHO 1999 criteria and DSPG of EASD criteria; WHO criteria produced a higher GDM prevalence (3.8% versus 1.6%). Overall, the four South African studies produced GDM prevalence figures ranging from 1.6% to 8.8%.

Tanzania

One study, published in 1991, was included on GDM prevalence in rural Tanzania [236]. Unfortunately, the full text article could not be obtained but data was extracted from the abstract and the review article [238]. This study involved an OGTT on a small sample of women (n=189) using the WHO 1985 diagnostic criteria. A prevalence of 0% was determined. Unfortunately, as the full text article could not be obtain, reporting quality and risk of bias for this study could not be assessed.

Table 3.2 Prevalence of gestational diabetes mellitus (GDM) in Africa

Author	Country	Region (rural/ urban)	Population group	Sample size	Age of women	Gestational age when tested for GDM	GDM diagnostic criteria used	Diagnostic test used to determine GDM	GDM prevalence
Seyoum et al., 1999	Ethiopia	Tigray (rural)	Black	890	27.4 ± 7.1 yrs (15-50 yrs)	24+ weeks	WHO criteria (1985)*	2hr 75g OGTT	3.7% (33/890)
Bouhsain et al., 2009	Morocco	De Rabat (urban)	Not stated	426	28.8 ± 6.1 yrs	24-28 weeks	Carpenter and Coustan's criteria*	3hr 100g OGTT	7.7% (8/426)
Challis et al., 2002	Mozambique	Maputo (urban/ suburban)	Not stated (assumed Black)	Cases: 109 women with late fetal deaths	Mean of 25 yrs	>27 weeks	Fasting blood glucose of ≥6.7 mmol/l and/or OGTT 2 hr blood glucose of ≥9.0 mmol/l	2hr 75g OGTT	11% (12/109 cases)
				Controls: 110 women with live births		Post delivery			7.3% (8/110 controls)
Olarinoye et al., 2004	Nigeria	Lagos (urban)	Black	248 (138; 75g OGTT, 110; 100g OGTT)	30.7 ± 4.2 yrs (18-41 yrs)	≥28 weeks	WHO criteria (1985)* - 75g OGTT	3hr 75g OGTT	11.6% (16/138) -75g OGTT
							NDDG criteria (1979)*- 100g OGTT	3hr 100g OGTT	4.5% (5/110)- 100g OGTT
Adegbola & Ajayi, 2008	Nigeria	Lagos (urban)	Black	Cases: 113 women with risk factors	19-45 yrs	24-28 weeks and repeated at 30-32 weeks	Carpenter and Coustan's criteria*	3hr 100g OGTT	6.2% (7/113 cases)
				Controls: 109 women without risk factors					4.6% (5/109 controls)
Kamanu et al., 2009	Nigeria	Aba (urban)	Black	Cases: 240 women with macrosomic babies	19-45 yrs	24-28 weeks	1hr 50g OGTT >7.8 mmol/l	1hr 50g OGTT	2.5% (6/240 cases)
				Controls: 8800 women with normal weight babies			Borderline results: 2hr 75g OGTT plasma glucose level >10 mmol/l at 1 hr and >8.6 mmol/l at 2 hr	Borderline cases followed up with 2hr 75g OGTT	1.5% (134/8800 controls)
Kuti et al., 2011	Nigeria	Ibadan (urban)	Black	765	19-45 yrs	4-40 weeks	WHO criteria (1999)*	2hr 75g OGTT	13.9% (106/765) (amongst women with risk factors)

Table 3.2 (Continued)

Author	Country	Region (rural/ urban)	Population group	Sample size	Age of women	Gestational age when tested for GDM	GDM diagnostic criteria used	Diagnostic test used to determine GDM	GDM prevalence
Anzaku & Musa, 2013	Nigeria	Jos (urban)	Black	253	19-42 yrs	24-28 weeks	WHO criteria (1985)*	2hr 75g OGTT	8.3% (21/253)
Ozumba et al., 2004	Nigeria	Enugu (urban)	Black	12030	15-54 yrs	≥28 weeks	WHO criteria (1999)*	2 hr 75g OGTT	1% (122/12030)
Jackson & Coetzee, 1979	South Africa	Cape Town (urban)	Not stated	558	Not stated	All gestations (test repeated in 3rd trimester)	When 2 of the following 3 criteria were exceeded on 2 separate GTT: 1) Fasting level: 5.5 mmol/l 2) Maximum level: 10.0 mmol/l (excluding the 30 min figure) 3) 2 hr level: 6.7 mmol/l	2hr 50g OGTT	3% (17/558) (amongst women with risk factors)
Ranchod et al., 1991	South Africa	Pietermartizburg (urban)	Indian (majority) and Coloured (minority)	1717	Not stated	28-32 weeks	WHO criteria (1985)* and DSPG of EASD criteria (1988)*	2hr 75g OGTT	3.8% (65/1717): WHO 1.6% (27/1717): DPSG of EASD
Mamabolo et al., 2007	South Africa	Limpopo (rural)	Black	262	25.5 ± 6.9 yrs	28-36 weeks	WHO criteria (1999)*	2hr 75g OGTT	8.8% (23/262)
Basu et al., 2010	South Africa	Johannesburg (urban)	Black (94%), White (4%), Mixed (1.7%), Asian (0.5%)	767	13-31 yrs	23-32 weeks	Institutional protocol: Fasting blood glucose: >8.0 mmol/l or random blood glucose: 11.0 mmol/l	Fasting or random blood glucose levels	1.8% (14/767)
Swai et al.,1991**; Hall et al., 2011	Tanzania	Unknown (rural)	Black	189	Unavailable	Unavailable	WHO criteria (1985)*	2hr 75g OGTT	0% (0/189)

*Refer to Table 1.2; **Could not obtain full text article

3.4 Discussion

As far as the authors are aware, no other systematic review has assessed the prevalence of GDM across the African continent. This systematic review therefore focussed on studies in African countries that provided details on the GDM screening methods employed, the diagnostic criteria used and the prevalence figures obtained.

Africa consists of 54 countries [248] yet only six African countries, equating to a mere 11%, were represented in this systematic review. The percentage of countries for which prevalence figures were found in a systematic review that assessed GDM in Asia was 26% [249]. Although still low, this regional representation is better than the one found in the current review. This highlights the fact that little seems to be known about the prevalence and potential burden of GDM in African countries. Before health care policies and guidelines can successfully be drawn up and implemented, it is important for one to establish the extent of a particular problem. It is evident that the extent of GDM in Africa as a whole is not well investigated. Africa has been plagued with undernutrition and GDM may not be considered a public health concern. However, as African countries shift economically a double burden of under- and overnutrition emerges. With the increase in overnutrition, particularly in females, GDM may be naively overlooked.

The results of the systematic review illustrate that the majority of the studies tested for GDM at around 24-28 weeks gestation, the recommended gestational age for when an OGTT should be performed [66]. In addition, the most commonly employed method for GDM screening in Africa is the two hour 75 g OGTT with glucose reference ranges as stipulated by the WHO 1985 or 1999 diagnostic criteria (Table 3.2). Two of the reported studies made comparisons between different diagnostic criteria and screening methods. One of the Nigerian studies showed that the two hour 75 g OGTT using the WHO 1985 criteria diagnosed more than double the amount of women that the 100 g OGTT using the NDDG criteria [242]. In addition, one of the South African studies also illustrated a two-fold detection rate using the 1985 WHO criteria versus the DSPG of EASD criteria [247]. Based on these findings, whether the 75 g OGTT over-diagnoses GDM in women is debatable and warrants further investigation. This statement is supported by the authors of the systematic review on GDM Asia who commented that the choice of diagnostic criteria greatly affects GDM prevalence [249].

Many lessons have been learnt from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study which showed that there is a continuous association between maternal blood glucose levels below those diagnostic of diabetes, and adverse outcomes, such as increased neonatal birth weight [100]. As a result of these findings various groups have reconsidered the diagnostic criteria for GDM. The IADSPG diagnostic criteria and WHO 2013 diagnostic criteria are not as stringent as some of the other/previous criteria mainly because only one abnormal value, as opposed to two, is sufficient to make a diagnosis of GDM (Table 1.1). As a result of using the newer criteria it is very likely that the prevalence of GDM will increase. This has both positive and negative consequences. For example, more women will be diagnosed with GDM and receive treatment and management which in turn will decrease the effects of maternal hyperglycaemia on the mother and developing fetus. On the other hand, the health system in a country could become overburdened with GDM pregnancies, which could impact heavily on a country's economy. However, considering the potential adverse pregnancy outcomes and the long term effects of GDM on mother and baby, it may be beneficial to the individuals, as well as a country's health system and economy, to diagnose and manage more women than less. None of the studies reported in this systematic review used the WHO 2013 or IADPSG criteria.

The percentage of women affected with GDM in this review was as low as 0% in rural Tanzania [236] and as high as 13.9% amongst urban Nigerian women with risk factors [237]. This disparity in prevalence is possibly due to the different methodology and study designs employed across the 14 studies. Without the availability of a standardised universal screening protocol the question is raised as to whether or not the prevalence figures that were obtained through the various studies are in fact true reflections of the African situation. In addition, with respect to the discussion above regarding the newer IADSPG and WHO 2013 diagnostic criteria, should the 14 studies reported in this systematic review have utilised either of the said criteria the GDM prevalence figures obtained would most likely have been greater.

Two of the studies, one performed in Nigeria and the other in South Africa, only tested women with risk factors for GDM and therefore employed the selective screening approach within their methodology [237, 246]. Certain risk factors have indeed been proven to be very useful in identifying women at risk for GDM; when BMI is >30 versus <20 kg/m^2 a woman has a three times greater risk of developing GDM. Ethnicity is also another key factor for assessing the risk of developing GDM; Asian women are five times more likely to develop GDM than Caucasian women, and African-American women are two times more likely to

develop GDM than Caucasian women [221]. The study by Kuti et al. [237] in Nigeria reported a high GDM prevalence (13.9%) amongst these women and the authors found the strongest associations between the following risk factors and a diagnosis of GDM: being over 30 years of age (although this was not used as a risk factor in the sample selection process), having a family history of diabetes and having previously been diagnosed with GDM.

The South African study that tested women with risk factors produced a much lower prevalence of GDM (3%) but did report a strong association between glycosuria, previous hyperglycaemia and having two or more of the listed risk factors with a diagnosis of GDM [246]. These studies support that certain maternal risk factors have a high specificity in identifying women at risk of developing GDM. This selective screening approach may certainly have an important role in resource-limited settings.

The countries with the most studies pertaining to GDM were South Africa and Nigeria, which had four and six studies reported respectively. With particular reference to South Africa, considering there are 22 million black females living in the country, representing approximately 80% of the entire female population [250], two studies on GDM in black women, one in a rural setting [207] and one in an urban setting [79], involving a total cohort of approximately 983 women, cannot be considered representative of the South African GDM scenario. In addition, out of the six African countries for which GDM prevalence figures were obtained, only Nigeria and South Africa have reported relatively recent figures on macrosomia rates. In Nigeria it is thought that macrosomia accounts for 7.5% [251] to 8.1% [252, 253] of births which ties in with the high GDM prevalence figures of 8.3% [89] and 13.9% [237] as reported by the two Nigerian studies in this review. This suggests macrosomia may be a marker for GDM prevalence. With respect to South Africa, one study conducted on black patients in urban Soweto reported a 2.3% macrosomia prevalence [254] but recent unpublished data from the South African Department of Health indicates a surprisingly low macrosomia rate of 1.7% (Buchmann, E.J. 2013, personal communication). If macrosomia rates are indicative of GDM rates then it is imperative that research on GDM is conducted in other African countries. Algeria and Uganda's macrosomia prevalence figures are reported as 14.9% and 8.4% respectively [251], this raises concern regarding their possible GDM figures.

It is alarming that very little appears to be known about GDM in African countries. Research studies, such as those listed in this systematic review, and particularly those that screen all women in the study cohort for GDM, are exceptionally useful in assessing the prevalence of

the problem. Based on the 14 reported studies included in the systematic review, if one ignores the prevalence figures obtained from the two studies that focussed on higher risk women [237, 246] and takes the prevalence of GDM amongst the control group in the case control studies [241, 244, 245], and selects the prevalence figures obtained by the WHO diagnostic criteria as opposed to those obtained by the NDDG criteria in one study [242] and the DSPG of EASD criteria in another study [247], the overall prevalence of GDM in Africa is estimated to be approximately 5% (60.1/12); approximately two and a half to seventeen times greater than some high income countries (Denmark (2-3%), the UK (2-3%) and Germany (0.3-0.8%)) [229].

Interestingly, few studies were performed on rural populations. As a direct consequence of urbanisation it would be expected that the prevalence of GDM would be higher amongst urban populations as opposed to rural populations. Out of the four South African studies (three urban and one rural) the study in rural Limpopo produced the highest GDM prevalence (8.8%) amongst a representative sample of local pregnant women [207]. However, one of the limitations in making comparisons between the rural and urban studies in this review is the different GDM screening methods employed and diagnostic criteria used. In addition, some studies looked at women already at high risk for GDM. Other limitations to this review include only published studies, as opposed to grey literature, being searched and roughly one third of the studies included in the review having a high risk of bias and another third having a moderate risk of bias.

This systematic review has illustrated a gap in the knowledge of GDM in Africa with only 11% of the African continent being represented. More epidemiological based studies on GDM in African countries need to be performed in order to provide reliable information and thus clarity on the extent of GDM. An ideal scenario would be if one set of diagnostic criteria and one testing method was employed across the continent in order to produce comparable data. In addition, comparisons between GDM prevalence amongst rural and urban populations within a country should be carried out in order to assess the extent of the effects of urbanisation on public health.

Understanding and subsequently attempting to curb the prevalence of GDM in developing countries is imperative for maternal and child health. As GDM often results in macrosomic infants, birth trauma and the need for Caesarean sections at delivery are expected. This is precarious as it impacts both maternal and child survival during delivery, and places a

significant economic burden on the health system, which in many African countries is already struggling with limited resources.

Furthermore, for most countries macrosomia appears to have been overlooked with the justified focus on low birth weight and small for gestational age statistics. The Developmental Origins of Health and Disease research describes how the developing fetus is susceptible to its environment and that certain *in utero* events can in fact alter fetal programming and produce different phenotypes. Low birth weight is representative of poor fetal nutrition and growth, and has been shown to be associated with a range of chronic conditions, including T2DM [3]. However, high birth weight requires as much consideration as there is evidence to support that fetal overnutrition also poses risk for T2DM and other chronic conditions later in life [120]. With the emerging increase in T2DM and obesity, macrosomia will become an important factor in maternal and child health and should be reported on and monitored by the health care system as a marker for GDM sooner than later.

As Africa continues along its economic and concomitant urbanisation and lifestyle transitions, the double burden of both under- and overnutrition is a cause for concern. Therefore, epidemiologists, public health specialists, health professionals, and policy leaders need to place GDM and macrosomia as key elements in their maternal and child health framework, thus enabling policies and practice to minimise the risk of maternal impaired glucose metabolism during pregnancy.

CHAPTER 4

THE PREVALENCE OF GESTATIONAL DIABETES MELLITUS AMONGST BLACK SOUTH AFRICAN WOMEN IS A PUBLIC HEALTH CONCERN

This chapter presents the research study that investigated the prevalence of GDM amongst South African women living in Soweto. It describes the GDM prevalence and risk factors amongst the study population and as well as the efficacy of the OGTT using the WHO 2013 diagnostic criteria. This work has been published in the peer-reviewed journal, Diabetes Research and Clinical Practice (Appendix J):

Macaulay, S., Ngobeni, M., Dunger, D.B. & Norris, S.A. (2018) The prevalence of gestational diabetes mellitus amongst black South African women is a public health concern. *Diabetes Research and Clinical Practice*, 139: 278-287. doi:10.1016/j.diabres.2018.03.012.

4.1 Introduction

Many low- and middle-income countries are experiencing demographic, nutritional and epidemiological transitions which have resulted in a surge of non-communicable diseases. Shifts in dietary patterns, urbanisation and a decrease in physical activity are key elements in these transitions [38]. South Africa is witnessing this at a rapid rate with approximately 2.3 million individuals affected by diabetes [193] ranking it the second most common cause of death in 2015 [186]. In addition, overweight and obesity are on the rise, particularly amongst South African women with 68% of them aged 15 years and above being classified as overweight or obese [192].

Gestational diabetes mellitus (GDM), defined as any degree of glucose intolerance diagnosed for the first time in a woman during pregnancy that is not clearly overt diabetes [66, 67, 255], is considered a precursor for Type 2 diabetes mellitus (T2DM) for both the affected mother and her unborn child [120]. Several risk factors have been shown to increase a woman's chance of developing GDM; increasing age, a family history of T2DM, high parity, belonging to a particular ethnic group (Hispanic, African, African American, Asian), having previously delivered a baby weighing 4 kg or more, and being overweight or obese [73]. Given the large number of individuals in South Africa affected by diabetes, together with the alarming prevalence of overweight/obesity amongst women, it is hypothesised that GDM is of significant concern too. Very few studies reporting on the prevalence of GDM exist in Africa overall [150] and only two have been conducted on a small number of black South African women; one in rural Limpopo (n=262) [207] and the other in urban Johannesburg (n=545) [256].

The gold standard test for diagnosing GDM is the oral glucose tolerance test (OGTT) performed at 24-28 weeks gestation [146]. Ideally, all pregnant women should be tested for GDM (universal screening) [66, 67]. However, South Africa's public healthcare system employs a selective screening approach for GDM whereby only women with certain risk factors are tested [198]. This approach is viewed as the most cost-effective in resource-poor settings. The downfall to selective screening is the lack of data on the actual prevalence of the condition, and also the risk of affected women being missed; selective screening is thought to miss up to 50% of women with GDM [160]. There has been some debate around whether a

fasting plasma glucose reading alone is sufficient for diagnosing GDM [257, 258] and predicting adverse neonatal outcomes [259]. In resource-poor settings where OGTTs are not feasible, a fasting plasma glucose screen may be an alternative option for detecting women with GDM.

Furthermore, the existence of several OGTT diagnostic criteria has complicated estimating GDM prevalence both between and even within countries. The International Association for Diabetes and Pregnancy Study Group (IADPSG) proposed a new set of diagnostic criteria in 2010 [66] which was adopted by the World Health Organization (WHO) in 2013 with the addition of a two-hour plasma glucose cut-off value of ≥11.1 mmol/l as diagnostic of overt diabetes [67]. Recently, the Society for Endocrinology, Metabolism and Diabetes of South Africa recommended the use of the WHO 2013 diagnostic criteria [260].

The overarching aim of this study was to determine the prevalence of GDM using the WHO 2013 diagnostic criteria amongst a large cohort of black South African women living in an urban township in Johannesburg. In addition, given South Africa's current propensity to utilise a selective screening approach for GDM, the study also aimed to describe GDM-associated risk factors amongst the study population, assess the efficacy of the fasting plasma glucose reading alone in detecting GDM, and report on the clinical management of women with GDM.

4.2 Participants, Materials and Methods

4.2.1 Participants

The study was conducted at the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU) situated in Soweto in Johannesburg, South Africa. Approximately 1.3 million people reside in Soweto, the majority of which are black South Africans [261].

Chris Hani Baragwanath Academic Hospital (CHBAH) is the one central academic hospital servicing the Soweto region. Pregnant patients at CHBAH represent of a mix of high-, moderate- and low-risk pregnancies. Several women are referred solely due to having had a previous Caesarean section or for fetal ultrasounds which are generally not available at the

local antenatal clinics. Pregnant women attending the Antenatal Clinic and Fetal Medicine Unit at CHBAH were screened for eligibility for the study. The inclusion criteria included black South African women (ethnicity was self-reported), ≥18 years of age, living in Soweto and ≤20 weeks pregnant with singleton pregnancies. In addition, participants could not have any known type of diabetes at the time of recruitment nor could they have epilepsy (due to concern of antiepileptic medication affecting glucose metabolism). All participants had to have had a fetal ultrasound from which their gestational age was calculated. Screened women who fulfilled the inclusion criteria were informed about the study, educated on GDM and invited to participate in the study. Those who expressed an interest in participating in the study were given an appointment at the Research Unit for when they were 24-28 weeks pregnant. The study took place from 1 June 2013 to the 30 April 2017.

4.2.2 Anthropometry

As this was a screening study all data were collected at one time point; a participant's enrolment appointment at 24-28 weeks gestation. Height (cm) was determined using the SECA stadiometer (Hamburg, Germany) and weight (kg) was determined using the SECA digital weighing scale (Hamburg, Germany). These measurements were used to calculate body mass index (BMI; kg/m^2). Furthermore, BMI was classified into the WHO categories for underweight (<18.5 kg/m^2), normal weight (≥18.5-24.9 kg/m^2), overweight (≥25-29.9 kg/m^2) and obese (≥30 kg/m^2) [262].

Blood pressure was measured using the Microlife Blood Pressure Monitor for Pregnant Women (Microlife AG Swiss Corporation, Widnau, Switzerland) whilst women were seated. An appropriate sized cuff was placed on the right arm of a participant. Three sets of systolic and diastolic blood pressure readings were taken with two minutes of rest between each set. The first set of readings was discarded and the average of the second and third set was used for the analyses. A diagnosis of hypertension was made when the systolic blood pressure (SBP) reading was ≥140 mmHg and/or the diastolic blood pressure (DBP) reading was ≥90 mmHg [263].

Haemoglobin (Hb) levels were measured using the HemoScan HB Meter (Alterna Biotech, Inc, California, USA). A diagnosis of anaemia was made when Hb levels were <110 g/l [264].

All measurements were taken by trained research nurses. Senior scientists and nurses qualified in anthropometry conducted regular training sessions (every six months) for research nurses. They also performed quality control checks that ensured the coefficient of variation of all anthropometric measures between research nurses was less than 1%.

4.2.3 The Oral Glucose Tolerance Test

Participants had been asked to attend their appointment after an overnight fast (a minimum of ten hours of fasting). A finger-prick blood sample was taken to perform a fasting capillary blood glucose reading using a hand-held glucometer; ACCU-CHEK® (Roche, Indianapolis, USA). If the capillary blood glucose reading was <7 mmol/l and the participant was sure she had fasted the research nurse proceeded with a venous blood sample to test fasting plasma glucose and then administered a two-hour 75 g OGTT. Participants with capillary glucose readings of ≥7 mmol/l were immediately referred to CHBAH for further investigation into possible overt diabetes. In view of potential hyperglycaemia-related complications, an OGTT in the research setting was not performed on these women and they were therefore excluded from the study. Whilst it is not recommended to use capillary glucose readings in place of venous glucose readings [265] the use of capillary glucose readings is considered acceptable if used as an initial screen [266].

The OGTT involved participants drinking 75 g of glucose powder dissolved in approximately 250 ml of water. The drink was consumed within five minutes and the participants remained seated throughout the process. Venous blood samples were drawn at one hour and two hours post-glucose load. Blood was collected in vacutainers containing fluoride and oxalate. Blood samples were immediately sent to the laboratory on site and processed in real-time so as to reduce further glycolysis in the blood collection tubes.

Venous (plasma) glucose samples were tested using the Randox RX Daytona Chemistry Analyzer. A diagnosis of GDM was made according to the WHO 2013 diagnostic criteria (fasting plasma glucose of 5.1-6.9 mmol/l, or, one-hour plasma glucose of ≥10.0 mmol/l or two-hour plasma glucose of 8.5-11.0 mmol/l). Overt diabetes was diagnosed as a fasting plasma glucose level of ≥7.0 mmol/l or a two-hour plasma glucose reading of ≥11.1 mmol/l [66, 67]. The Clinical and Laboratory Standards Institute document EP15 was used for the verification of performance for precision and trueness of the Randox RX Daytona Chemistry

Analyzer. A random selection of 150 samples was run in duplicate to ascertain the coefficient of variation of the laboratory technician, which was determined to be 2.3%.

Different sets of GDM diagnostic criteria produce varying results which makes the comparison of results across studies difficult. Therefore, in order to compare our findings to those of other fairly recent African studies we also determined the prevalence of GDM amongst our cohort using the WHO 1999 diagnostic criteria (fasting plasma glucose of ≥7.0 mmol/l or a two-hour plasma glucose of ≥7.8 mmol/l) [47] and the IADPSG diagnostic criteria (same as the WHO 2013 criteria except the two-hour plasma glucose reading is ≥8.5 mmol/l, there is no cut-off value for overt diabetes) [66].

4.2.4 Questionnaires

Participants were asked questions pertaining to their demographics and obstetric and family histories. The demographic questions included age, marital status, level of education and a household asset score. The household asset score is the sum of the number of eleven assets (electricity, radio, television, refrigerator, cellular telephone, personal computer, farm animals, agricultural land, bicycle, motorcycle, motor vehicle) a participant has. This score is used as an indicator of household socioeconomic status and has been used in other studies based in Soweto [267].

Obstetric-related questions included how many previous pregnancies (gravidity) and births (parity) a woman had and if she had previously delivered a macrosomic (≥4 kg at birth) neonate. Whilst the cut-off value for macrosomia is debatable, South Africa tends to define macrosomia as a birth weight of 4 kg or more [198]. The family history questionnaire involved asking participants if they specifically had a sibling, parent or grandparent with diabetes.

4.2.5 Management and Follow-up

Women diagnosed with overt diabetes and GDM were referred to the specialist Obstetric Diabetes Clinic at CHBAH for management. The management protocol at the Clinic involves following women up every two weeks until 32 weeks gestation and then once a week until

delivery. Induction of labour or Caesarean section is planned for around 38 weeks' gestation. All women are seen by a dietician who prescribes a diabetic diet and advises on necessary dietary and lifestyle modifications. Women may also be prescribed medication. All women are given a glucometer for self-monitoring of blood glucose [215]. Research assistants telephoned these women to follow-up on their referrals.

4.2.6 Statistical Analyses

Stata Version 12 **(**StataCorp, College Station, Texas**)** was used for statistical analyses. The Shapiro-Wilk and Skewness and Kurtosis tests were used to assess the distribution of continuous data. Normally distributed continuous variables were presented as means ± standard deviations (SD) and those that were not normally distributed were presented as medians (interquartile range (IQR)). Categorical data were presented as frequencies and percentages. Differences between categorical variables were determined using the Chi-square test. The Student's t-test was used to analyse differences between normally distributed variables and the Mann-Whitney test was used to analyse differences between non-normally distributed variables. The Kruskal-Wallis H test, with the Conover-Iman test of multiple comparisons, was used to analyse the glucose readings according to BMI category. Significance was assumed at a two-tailed p value of $p<0.05$. A multiple logistic regression analysis was performed to investigate whether the well-described risk factors (age, BMI, parity, family history of diabetes and previous delivery of a macrosomic baby) were associated with GDM in this cohort. An evaluation of the fasting glucose reading alone, in terms of sensitivity, specificity and predictive values to diagnose or rule out GDM, was performed using the MedCalc Diagnostic Test Evaluation Calculator (MedCalc Software, Ostend, Belgium).

4.2.7 Ethical Approval

The University of the Witwatersrand's Human Research Ethics Committee (Medical) granted clearance for the study (Certificate references: M120524, M130309 and M150461) (Appendix E). Study participants gave informed, written consent.

4.3 Results

A total of 3 656 eligible women were invited to participate in the study. Of those invited to participate, 2009 (55%) underwent an OGTT. There were 1647 (45%) women who did not undergo an OGTT for various reasons (Figure 4.1). As the women who did not undergo and OGTT had met the inclusion criteria at recruitment, they did not differ from the group of women who did undergo an OGTT with regard to ethnicity and geographical area of residence. In addition, we compared the ages of the women who did not undergo an OGTT to those who did; there was no significant difference between the groups (p=0.242).

A final sample of 1 906 women was included in the study (Figure 4.1). Of these women, six (0.3%) were diagnosed with overt diabetes; one had a fasting plasma glucose ≥7.0 mmol/l and the other five had two-hour plasma glucose readings of ≥11.1 mmol/l. A total of 174 women were diagnosed as having GDM according to the WHO 2013 criteria which resulted in a GDM prevalence of 9.1% (95% confidence interval (CI) 7.9, 10.5). As shown in Figure 4.1, five women had capillary fasting glucose readings of > 7 mmol/l and were therefore excluded from the OGTT. In addition, two women had apparently been diagnosed through the antenatal clinic as having GDM and were therefore also excluded from having an OGTT. Assuming the five women with elevated capillary glucose levels did have GDM, the addition of them plus the two diagnosed at the antenatal clinic would total 181 women with GDM. This would slightly increase the GDM prevalence to 9.5% (95% CI 8.2, 10.9) using the WHO 2013 criteria. However, as a diagnosis of GDM was not confirmed or refuted in these seven women through a research-based OGTT, analyses were only performed on the 174 women confirmed to have GDM.

For comparative purposes we also calculated the prevalence of GDM amongst our study cohort using the WHO 1999 and the IADPSG criteria. Using the WHO 1999 criteria 107/1906 women were classified as having GDM producing a prevalence of 5.6% (95% CI 4.6, 6.7), and using the IADPSG criteria 179/1906 women had GDM producing a prevalence of 9.4% (95% CI 8.2, 10.8).

The 174 women with GDM according to the WHO 2013 criteria were significantly older, had more household assets and were therefore of a higher household socioeconomic status, and were more likely to report a positive family history of diabetes than the women with normal glucose profiles. In addition, they had significantly higher BMIs at the time of the OGTT than

the women with normal glucose profiles with 59.2% of them falling into the obese category (Table 4.1).

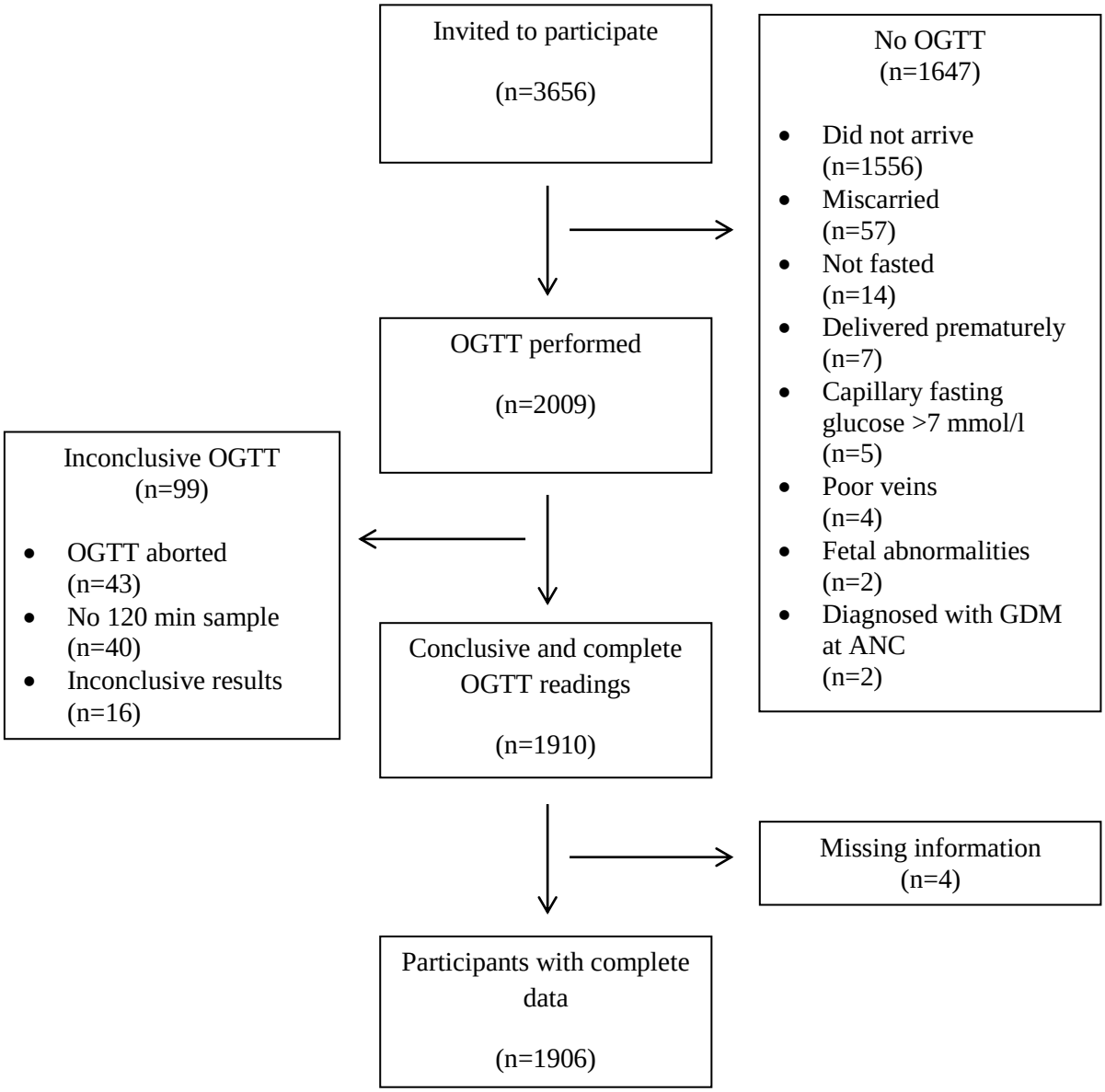


Figure 4.1 Study participation in the GDM prevalence study. OGTT denotes oral glucose tolerance test; ANC, antenatal clinic.

Women with GDM were slightly more advanced in terms of gestational age at the time of the OGTT than those with normal glucose profiles but there was no difference in parity or gravidity between the two groups. The overall rate of having delivered a previous baby with macrosomia was low for the entire group (4.7%) and did not differ significantly between those with and those without GDM. Interestingly, women with GDM were more likely to be diagnosed with anaemia compared to those with normal glucose profiles (Table 4.1).

Table 4.1 Characteristics of the study participants

	Median (IQR) or n (%) or mean ± SD			
Characteristics	**Total participants (n=1906)**	**Participants with normal glucose profiles (n=1726)**	**Participants with GDM (n=174)**	***P* value**
Age (years)	30 (25-35)	30 (25.0-34.5)	31 (27-36)	0.001*
Household socioeconomic status †	5 (5-6) 5.4 ± 1.2	5 (5-6) 5.4 ± 1.2	5 (5-6) 5.7 ± 1.2	0.014*
Education				
No schooling/primary school	47 (2.5%)	42 (2.4%)	5 (2.9%)	0.936
Secondary school	1480 (77.7%)	1341 (77.7%)	135 (77.6%)	
Tertiary education	379 (19.9%)	343 (19.9%)	34 (19.5%)	
Marital Status				
Single	1340 (70.3%)	1217 (70.5%)	119 (68.4%)	0.560
Married/cohabiting	566 (29.7%)	509 (29.5%)	55 (31.6%)	
Family history of diabetes (n=1904)	476 (25.0%)	419 (24.3%)	56 (32.2%)	0.022*
Weight (kg) §	74.7 (64.7-86.1)	74.4 (64.2-85.3)	78.6 (67.9-91.0)	0.001*
Height (cm)	159.1 (155.1-163.1)	159.2 (155.1-163.2)	158.9 (156.1-162.2)	0.621
BMI (kg/m^2) §	29.5 (25.7-33.7)	29.3 (25.5-33.5)	31.5 (27.4-35.6)	<0.001*
BMI categories §				
Normal	395 (20.7%)	369 (21.4%)	26 (14.9%)	0.004*
Overweight	606 (31.8%)	561 (32.5%)	45 (25.9%)	
Obese	905 (47.5%)	796 (46.1%)	103 (59.2%)	
Haemoglobin (g/l) (n=1804)	131 (113-157)	131 (114-157)	126 (106-150)	0.007*
Anaemia (n=1804)	375 (20.8%)	325 (19.9%)	50 (30.9%)	0.001*
Systolic blood pressure (mmHg) (n=1898)	108 (100.5-116.5)	108 (100.5-116.5)	108.5 (102.0-116.0)	0.589
Diastolic blood pressure (mmHg) (n=1898)	68.5 (63-75)	68.5 (62.5-72.5)	69.8 (64.5-76.5)	0.026*
Hypertensive (n=1898)	64 (3.4%)	56 (3.3%)	8 (4.6%)	0.352
Gestational age †	26 (25-27) 25.8 ± 1.5	26 (25-27) 25.7 ± 1.5	26 (25-27) 26.1 ± 1.4	0.001*
Previous pregnancies				
None	230 (12.1%)	215 (12.5%)	15 (8.6%)	0.078
One to two	1191 (62.5%)	1084 (62.8%)	104 (59.8%)	
Three or more	485 (25.5%)	427 (24.7%)	55 (31.6%)	
Previous births				
None	494 (25.9%)	453 (26.3%)	40 (23.0%)	0.183
One to two	1237 (64.9%)	1109 (64.3%)	123 (70.7%)	
Three or more	175 (9.2%)	164 (9.5%)	11 (6.3%)	
Previous macrosomic neonate (n=1904)	90 (4.7%)	81 (4.7%)	9 (5.2%)	0.779
HIV status				
Negative	1309 (68.7%)	1190 (69.0%)	115 (66.1%)	0.439
Positive	597 (31.3%)	536 (31.1%)	59 (33.9%)	

Gestational diabetes mellitus (GDM) diagnosed according to World Health Organization's 2013 criteria; Sample size indicated if less than 1906; Total of 1906 women includes six women diagnosed with overt diabetes; *P<0.05 indicates a statistically significant difference; †Mean (± standard deviation (SD)) indicated where p<0.05 but median (interquartile range (IQR)) does not indicate the difference; §At the time of the oral glucose tolerance test (OGTT) (24-28 weeks gestation)

Blood glucose readings were first analysed across the entire study population (n=1 906) stratified according to BMI category. Glucose readings and prevalence of GDM differed significantly across the three BMI categories; the highest prevalence was amongst the women in the obese category. Of note, all six women with overt diabetes were classified as obese (Table 4.2).

Table 4.2 Glucose readings and gestational diabetes mellitus prevalence according to body mass index category

	Body Mass Index Category (at 24-28 weeks gestation)			***P* value**
	Normal weight (n=395)	**Overweight (n=606)**	**Obese (n=905)**	
Fasting glucose (mmol/l)				
Median (IQR)	4.0 (3.6-4.4)	4.0 (3.6-4.4)	4.0 (3.7-4.6)	<0.001[*‡¶]
Mean ± SD	4.0 ± 0.6	4.1 ± 0.6	4.2 ± 0.7	
1-hour glucose (mmol/l)				
Median (IQR)	5.3 (4.4-6.1)	5.5 (4.6-6.7)	6.0 (5.0-7.2)	<0.001[*§‡¶]
Mean ± SD	5.4 ± 1.4	5.7 ± 1.5	6.2 ± 1.6	
2-hour glucose (mmol/l)				
Median (IQR)	5.1 (4.3-5.9)	5.3 (4.5-6.2)	5.6 (4.7-6.6)	<0.001[*§‡¶]
Mean ± SD	5.1 ± 1.2	5.4 ± 1.2	5.7 ± 1.4	
Women with GDM (%)	26/395 (6.7%)	45/606 (7.4%)	103/899[†] (11.5%)	0.004[*‡¶]

Gestational diabetes mellitus (GDM) diagnosed according to the World Health Organization's 2013 criteria; Interquartile range (IQR); Standard deviation (SD); [*]*P*<0.05 indicates a statistically significant difference; [†]Denominator less six women with overt diabetes; [§]Significant difference between normal and overweight; [‡]Significant difference between normal and obese; [¶]Significant difference between overweight and obese

As expected, plasma glucose levels taken at the three time points of the OGTT differed significantly ($p<0.05$) between the women with GDM and those with normal glucose profiles. The mean ± SD for the fasting, one-hour and two-hour glucose readings were 5.4 ± 0.5 mmol/l, 8.0 ± 1.7 mmol/l and 7.4 ± 1.5 mmol/l respectively amongst the women with GDM. In comparison, the fasting, one-hour and two-hour glucose readings amongst the women with normal glucose profiles were 4.0 ± 0.5 mmol/l, 5.6 ± 1.3 mmol/l and 5.3 ± 1.1 mmol/l respectively. The majority of women with GDM (116/174; 66.7%) were diagnosed on a fasting plasma glucose reading alone, whilst a further 29/174 (16.7%) had an abnormal fasting glucose plus one or two additional abnormal readings (Table 4.3). Therefore, a total of

145/174 (83.3%) women had an abnormal fasting plasma glucose reading which alone is diagnostic of GDM. Based on this, the fasting plasma glucose test had an 83.3% sensitivity (95% CI 77.0, 88.5), 100% specificity (95% CI 99.8, 100), 0.17 negative likelihood ratio (95% CI 0.12, 0.23) and a 98.4% negative predictive value (95% CI 97.7, 98.8) of diagnosing GDM.

Table 4.3 Analysis of the World Health Organization's 2013 criteria in diagnosing gestational diabetes mellitus

Abnormal plasma glucose reading(s)	**Women diagnosed N (%)**
Fasting glucose alone (5.1-6.9 mmol/l)	116 (66.7)
1-hour glucose alone (≥10 mmol/l)	3 (1.7)
2-hour glucose alone (8.5-11.0 mmol/l)	18 (10.3)
Fasting + 1-hour glucose + 2-hour glucose	7 (4.0)
Fasting glucose + 1-hour glucose	8 (4.6)
Fasting glucose + 2-hour glucose	14 (8.0)
1-hour glucose + 2-hour glucose	8 (4.6)
Total	174 (100)

A multiple logistic regression analysis was performed to see which of the well-described GDM risk factors were significantly associated with a diagnosis of GDM amongst our cohort of women. Table 4 shows that being ≥35 years of age, having a BMI of ≥30 kg/m^2 at the time of the OGTT and having a positive family history of diabetes were significant risk factors. High parity and a previous macrosomic baby were not significant risk factors amongst the women in our study. When fasting glucose was added to the logistic regression model as a continuous variable the only significant risk factor was age ≥35 years (p=0.039).

Table 4.4 Multiple logistic regression analysis for risk factors associated with gestational diabetes mellitus

Risk factor	Odds Ratio	95% Confidence interval	*P* Value
Age			
18-24 years (reference)			
25-34 years	1.6	1.0, 2.6	0.066
≥ 35 years	2.5	1.5, 4.4	0.001*
Family history of diabetes			
No (reference)			
Yes	1.4	1.0, 2.0	0.038*
BMI †			
Normal (reference)			
Overweight	1.1	0.7, 1.9	0.598
Obese	1.7	1.1, 2.7	0.021*
Previous delivery of a macrosomic baby			
No (reference)			
Yes	1.0	0.5, 2.0	0.994
Parity			
Low (<2 births) (reference)			
High (≥2 births)	0.7	0.52, 1.1	0.094

†Body Mass Index (BMI) at the time of the oral glucose tolerance test (24-28) weeks gestation; *$P<0.05$ indicates a statistically significant difference

Of the 174 women diagnosed with GDM, 123 (70.7%) followed through with their referral to the Obstetric Diabetes Clinic whereas 38 (21.8%) chose not to attend the Clinic and 13 (7.5%) could not be contacted. All the women who attended the Clinic received diet therapy. A total of 70/123 (56.9%) women were managed through diet therapy alone and 59/123 (48%) required medication; 58 were prescribed metformin and one was prescribed insulin. Of the six women diagnosed with overt diabetes, 5/6 (83.3%) followed through with their referral to the Obstetric Diabetes Clinic and 4/5 (80%) were managed by medication (two on metformin and two on insulin) and one was managed through diet therapy alone.

4.4 Discussion

Our study reports a GDM prevalence of 9.1% (95% CI 7.9, 10.5) using the WHO 2013 diagnostic criteria amongst black South African women living in urban Soweto. Our results also illustrate that being classified as obese at 24-28 weeks gestation, being ≥35 years of age and having a family history of diabetes are positively associated with GDM development. Most women with GDM in our study were controlled through diet/lifestyle modification. Furthermore, the fasting plasma glucose reading alone appears to have a high sensitivity in detecting GDM.

A concerning finding was the large number of eligible women (1 556/3 656; 42.6%) invited to participate in the study who did not follow through with their invitation (Figure 4.1). This highlights potential issues around the awareness of GDM, its effects, and the importance of screening for it. A qualitative study assessing the reasons why these women chose not to participate would be helpful; we speculate that a general lack of awareness as well as logistically issues such as transport costs and time off work may be some reasons. The same can be said for the 21.8% of women with GDM who did not follow through with their referrals to the Obstetric Diabetes Clinic. A follow-up study to understand the reasons behind these women's decisions may identify areas for intervention including further education on GDM. In addition, a limitation of this study was that demographic data, other than age, was not collected on the 42.6% of women who chose not to participate. It would have been helpful to have collected data including socioeconomic status and BMI on the non-participants to determine if they differed significantly from the study group or not which. Such findings may have potentially influenced the estimated GDM prevalence.

Regarding the BMI results amongst the study participants, it is possible that they may be overestimations as a woman's BMI at 24-28 weeks gestation is not an accurate reflection of her non-pregnant BMI. However, the highest prevalence of obesity in the whole of sub-Saharan Africa is amongst South African women; an alarming 42% (40.6-43.4) of women over 20 years of age in South Africa are obese [188]. Therefore, the high rate of obesity found in our study (47.5% amongst all participants) is very similar to that of the general South African adult female population.

In terms of GDM prevalence, two recent African studies reported a GDM prevalence of 8.1% in Nigeria using the WHO 2013 criteria [159] and a 2.9% (95% CI, 1.6, 4.2) prevalence using

the IADPSG criteria in Kenya [268]. Using the same diagnostic criteria, our prevalence results are higher than those reported in these who African countries. Regarding South Africa, only two previous studies have assessed GDM in black women. A study conducted in 2007 in rural Limpopo reported a GDM prevalence of 8.8% (95% CI 5.6, 12.9) using the WHO 1999 criteria [207]. Interestingly, using the same diagnostic criteria the GDM prevalence is lower amongst our urban cohort (5.6% (95% CI 4.6, 6.7)) than the rural cohort in Limpopo. Given the changes in dietary habits and physical activity that are associated with urbanisation one might have expected our study to have produced a higher prevalence. The study set in Limpopo had a much smaller sample size (262 women of which 23 had GDM) and wider 95% CI for the prevalence rate than ours; it is possible that had a larger sample size been used the GDM prevalence in the Limpopo study may have been lower.

The second South African study, set in Johannesburg, reported an extremely high prevalence of GDM amongst 554 black women using the IADPSG criteria; 25.8% (CI not stated) [256]. This prevalence figure is more than twice that obtained from our study using the same diagnostic criteria (9.4% (95% CI 8.2, 10.8)). The authors do not mention the prevalence of overt diabetes amongst their study participants although the median (IQR) fasting plasma glucose levels amongst the GDM group is reported as 5.8 (3.9-13.4) mmol/l. The IQR suggests some women in the GDM group had fasting glucose readings of ≥7.0 mmol/l which according to the IADPSG criteria is indicative of overt diabetes.

As with all single centred studies, selection bias and the generalisability of the results become important questions. Given the rigor of our study and removal of women with overt diabetes we are confident that 9.1% (95% CI 8.2, 10.8) is an accurate reflection of the prevalence of GDM (using the WHO 2013 criteria) amongst black South African women living in urban Soweto. Ideally, a multicentre study should be conducted across South Africa to determine the true burden of GDM. Although comparing GDM prevalence rates within and between countries is difficult due to the different diagnostic criteria, a rough estimation of GDM prevalence in Africa based on a limited number of published studies was reported to be around 5% [150]. From a worldwide perspective, GDM is thought to complicate approximately 7% (range 1-14%) of all pregnancies [269]. Therefore, a prevalence of 9.1% is of concern.

As mentioned previously, South Africa currently employs a selective screening approach based on risk factors for GDM detection. Being ≥35 years, obese and having a family history

of diabetes were shown to be significant risk factors associated with GDM development. However, only age ≥35 years remained significant when fasting glucose was added to the model. This suggests that fasting glucose is capturing the impact of adiposity on, and the genetic contribution (family history) to, glucose metabolism. Regarding fasting glucose, our results give evidence that a fasting plasma glucose screen is very effective in detecting GDM. Performing an OGTT is timely and costly and South Africa's healthcare system is already heavily burdened making universal screening for GDM an unlikely possibility. Based on our results we would recommend that at least a fasting plasma glucose test be considered as a GDM screening tool for all pregnant women in South Africa.

An interesting finding was that women with GDM had significantly lower levels of haemoglobin and a higher rate of anaemia than those with normal glucose profiles. Other investigators have reported the opposite with high levels of haemoglobin being associated with GDM [270] and iron deficient anaemia being protective against the condition [271]. It is possible that whilst the majority of women with GDM in our study were classified as obese, their diets were likely to have been excessive in refined carbohydrates, fats and sugars but deficient in micronutrients, including iron. Another possibility may be that the anaemia is a result of the pathophysiology of diabetes, such as renal insufficiency or periodontal disease [272-274].

In terms of clinical management, 56.9% of women with GDM in our study were managed solely through diet therapy. The American Diabetes Association (ADA) suggests that lifestyle modification (diet therapy, physical activity and weight management) can control 70-85% of women with GDM [116]. Whilst the percentage of women managed through dietary modification alone in our study was less than what the ADA suggests it is still very encouraging that more than half the women with GDM did not require medication. Such a finding is promising as it suggests that from a healthcare system's perspective managing GDM might not be that costly, and from a patient's perspective medication-burden might not be of concern to most.

4.5 Conclusion

Our study is the largest GDM prevalence study in South Africa to date. A 9.1% prevalence of GDM according to the WHO 2013 criteria is concerning and warrants further discussion around screening approaches for GDM in the South African public healthcare sector. Whilst diagnosing more women with GDM may add burden to a country's health system, the possible benefits of diagnosing and managing such women, and in turn preventing the cycle of diabetes, should be considered. Optimising the health and nutrition of women should be prioritised to minimise excessive weight gain and the current GDM selective screening protocol needs to be reconsidered; the long term effects of such will be instrumental in reducing the prevalence of diabetes.

CHAPTER 5

THE EFFECTS OF GESTATIONAL DIABETES MELLITUS ON FETAL GROWTH AND NEONATAL BIRTH MEASURES

The previous chapter illustrated that there is a high prevalence of GDM amongst pregnant women living in Soweto. The 9.1% GDM prevalence raises concern about the number of fetuses exposed to hyperglycaemia and how GDM exposure affects fetal growth and neonatal size at birth. This next chapter describes the study that investigated longitudinal fetal growth and size at birth amongst GDM-exposed and unexposed babies. This chapter contains supplementary tables that are alluded to in the text but are presented independently at the end of the chapter. This work has been accepted for publication by the peer-reviewed journal, Diabetic Medicine. The article is currently available as an advanced online publication (Appendix J):

Macaulay, S., Munthali, R.J., Dunger, D.B. & Norris, S.A. (2018) The effects of gestational diabetes mellitus on fetal growth and neonatal birth measures in an African cohort. *Diabetic Medicine.* Advanced online publication. doi: 10.1111/dme.13668.

5.1 Introduction

An optimal *in utero* environment is essential for healthy fetal growth and both maternal under- and overnutrition can increase the offspring's risk for non-communicable diseases [275].

During pregnancy maternal tissues become insensitive to insulin thus reducing glucose absorption in order to shunt glucose to the developing fetus. As a result, the maternal pancreas is required to increase insulin secretion [53]. Insulin resistance peaks at around 20 weeks' gestation. Some women are unable to meet this increased insulin demand resulting in hyperglycaemia and consequently, gestational diabetes mellitus (GDM) [68].

Uncontrolled maternal hyperglycaemia causes fetal overnutrition which can affect fetal growth [106]. Macrosomia (birth weight independent of gestational age at delivery of ≥4.0 kg) and being large for gestational age (birth weight >90th centile for gestational age) [87], are well-described complications of GDM. An increase in fat is responsible for GDM-exposed babies being larger than unexposed babies [100]. For these children, GDM exposure puts them at increased risk of obesity and Type 2 diabetes later in life [276].

The oral glucose tolerance test (OGTT), performed at 24-28 weeks gestation, is the gold standard for diagnosing GDM [146]. A diagnosis of GDM is therefore usually made late into the second trimester, however, the effects of fetal exposure to hyperglycaemia may present earlier. Understanding if and when an increase in fetal growth is observed in GDM-affected pregnancies could help clinicians identify at-risk women and their unborn babies.

The aim of this study was to evaluate longitudinal fetal growth and birth outcomes amongst pregnant African women with and without GDM.

5.2 Participants and Methods

5.2.1 Study Design

The MRC/Wits Developmental Pathways for Health Research Unit of the University of the Witwatersrand, Johannesburg, South Africa, conducted a prospective longitudinal pregnancy cohort study from June 2013 until July 2016. During this time 1 017 women were enrolled into the Soweto First 1000 Days study.

5.2.2 Study Participants

Women were recruited from the Antenatal Clinic and Fetal Medicine Unit at Chris Hani Baragwanath Academic Hospital; an academic central hospital located in urban Soweto, Johannesburg. Women eligible for enrolment into the study were black South African females (ethnicity was self-reported), ≥18 years of age, residing in Soweto, and pregnant with singleton pregnancies which were preferably less than 14 weeks gestation but no more than 20 weeks gestation. Women could not have been diagnosed with any known type of diabetes or epilepsy (due to the concern of certain antiepileptic drugs interfering with glucose metabolism) at the time of recruitment. Fetal abnormalities excluded women from the study.

5.2.3 Ethical Approval

The University of the Witwatersrand's Human Research Ethics Committee (Medical) granted clearance for the study (Certificate references: M120524 and M130309). Study participants gave informed, written consent to participate.

5.2.4 Data Collection

A participant's first visit to the research unit involved a dating scan, anthropometric measures, and the completion of pregnancy-related and sociodemographic questionnaires. A household socioeconomic status (SES) score was calculated as the total number of specified household assets that a woman had. This scoring system has been used in South African research studies as a proxy for household SES [267].

5.2.4.1 Ultrasonography

A pregnancy dating scan involved measuring the fetal crown–rump-length at <14 weeks + 0 days, or the biparietal diameter, head circumference and femur length in more advanced pregnancies (>14 weeks but <20 weeks). Thereafter, participants were invited for follow-up scans every five weeks. Follow-up visits were at; 14-18 weeks, 19-23 weeks, 24-28 weeks, 29-33 weeks and 34-38 weeks gestation. Gestational age at each visit and at delivery was calculated from the gestational age determined by the dating scan. A Philips HD-9 (Philips Ultrasound, Bothell, Washington, USA) ultrasound machine was used. The majority of the scans underwent external quality assessment by colleagues at Oxford University (UK).

5.2.4.2 The Oral Glucose Tolerance Test

At 24-28 weeks gestation participants underwent a two-hour 75 g OGTT after an overnight fast. Prior to administering the OGTT research nurses performed a finger prick capillary fasting glucose test using a glucometer on the participants. Women who had fasted but whose capillary glucose levels were ≥7.0 mmol/l were immediately referred to the hospital due to concern of them having overt diabetes. An OGTT under research conditions was therefore not conducted on these women. The World Health Organization's (WHO) 2013 criteria for diagnosing GDM were used (fasting plasma glucose of 5.1-6.9 mmol/l, or, one-hour plasma glucose of ≥10.0 mmol/l or two-hour plasma glucose of 8.5-11.0 mmol/l). A fasting plasma glucose of ≥7.0 mmol/l or a two-hour plasma glucose of ≥11.1 mmol/l is diagnostic of overt diabetes [67]. Venous blood samples were run on site using the RX Daytona Chemistry Analyzer (Randox, London, UK). A random selection of 150 samples was run in duplicate to ascertain the coefficient of variation of the laboratory technician, which was determined to be 2.3%.

Women diagnosed with GDM were referred to the Obstetric Diabetes Clinic at the hospital. The Clinic's protocol involves regular monitoring of women with GDM; they are seen every two weeks until 32 weeks gestation and then once a week until delivery. Induction of labour or Caesarean section is planned for around 38 weeks' gestation. A dietician consults with the women and a diet comprising of 40% carbohydrate, 40% fat and 20% protein is recommended. All women are provided with a glucometer for at-home capillary glucose monitoring which they are encouraged to do pre-meal, one or two hours postprandial, and late

at night. Target blood glucose levels should be fasting <5.3 mmol/l, one hour postprandial <7.8 mmol/l, and two hours postprandial <6.7 mmol/l. Women who do not meet the glucose targets following one to two weeks of dietary modification are prescribed metformin. If glucose levels are not well-controlled on metformin, or if metformin is not well-tolerated, women are moved onto insulin therapy. In instances where initial glucose readings are exceedingly highly, metformin may be bypassed and insulin therapy initiated immediately [131].

5.2.4.3 Anthropometry

Maternal weight (kg) and height (cm) were taken at enrolment using the SECA stadiometer and SECA digital weighing scale (SECA, Hamburg, Germany) respectively.

Neonatal birth weight (g), length (cm) and head circumference (cm) were measured using the calibrated SECA Baby Scale 376 (SECA, Hamburg, Germany), Harpenden Infantometer (Holtain, London, UK) and a metal head circumference tape measure (CMS ref.3105) (Chasmors Ltd, London, UK) respectively. Measurements were taken within 24 hours of delivery. The coefficient of variation of all anthropometric measures between research nurses and assistants was less than 1%.

Ponderal Index ((birth weight (g)/ birth length (cm)^3) x 100) measured relative weight-for-length at birth and was used as an indicator of neonatal body composition (<2.0 g/cm^3: small; 2.0-2.49 g/cm^3 marginal; 2.5-2.99 g/cm^3 normal; ≥3.0 g/cm^3 overweight) [277].

The International Newborn Size at Birth Standards Application tool [278] calculated centiles and z-scores for birth weight. This tool takes into account the gestational age at delivery (total days) and the sex of the newborn. The classification of neonates based on birth weight was as follows: <10th centile: small for gestational age; 10th - 90th centile: appropriate for gestational age; and >90th centile: large for gestational age [84]. Macrosomia was defined as a birth weight irrespective of gestational age at delivery of ≥4.0 kg [198]. Birth weight of <2.5 kg irrespective of gestational age at delivery was considered low birth weight [279].

A subset of neonates underwent a fat mass (g) assessment using the PeaPod® (Cosmed, Concord, California, USA). The PeaPod® determines infant body composition through Air

Displacement Plethysmography. Body density is calculated (mass/volume) and thereafter fat percentage and fat-free mass is determined.

5.2.5 Statistical Analyses

Stata **(**StataCorp, version 12.0, College Station, Texas, USA**)** was used for data analyses. The Shapiro-Wilk and Skewness and Kurtosis tests assessed the distribution of continuous data. Descriptive continuous variables that were normally distributed were presented as means ± standard deviations (SD) and those that were not normally distributed were presented as medians (interquartile range (IQR)). Categorical data were presented as frequencies and percentages. Differences between categorical variables were determined using the Chi-square test. The Student's t-test and one-way ANOVA (with the Bonferroni adjustment for multiple comparisons) were used to analyse differences between normally distributed variables. Differences between variables that were not normally distributed were determined using the Mann-Whitney test and the Kruskal-Wallis H test (with the Conover-Iman test of multiple comparisons). Multiple linear regression analysis was applied to study associations between certain variables and Ponderal Index and fat mass. Significance was assumed at a two-tailed *p* value of $p<0.05$.

Linear mixed effects modelling (LMM) [280, 281] was used to examine the differences in fetal growth, assessed separately for biparietal diameter, head circumference, abdominal circumference and femur length, over the course of pregnancy. Linear mixed effects modelling is a flexible modelling statistical technique that deals with the correlation of repeated measurements. Time varying and time-invariant fixed effects were also analysed using LMM. All models were run with a random intercept and a random slope with unstructured residual correlation. In the final model, fixed effects were the covariates, GDM status and time (gestational age in days), whereas random effects were the intercept and visit. Different interactions were explored and included in the model if significant. Potential confounders were fetal sex, baseline BMI, weekly change in weight gain and baseline maternal age. The GDM and non-GDM groups stratified by fetal sex were analysed, and then the pooled dataset was analysed. A likelihood ratio test was used to select the best fitting model; with or without random effects.

5.3 Results

Of the 1017 women enrolled in the study, 807 (79.4%) underwent an OGTT and of those all three glucose readings were available on 741 (91.8%) women. Figure 5.1 describes how the sample sizes for the various aspects of this study were derived.

Most participants were enrolled at 12.0 (13.0-14.0) weeks gestation. Eighty three (11.2%) of the 741 women were diagnosed with GDM and none had overt diabetes. Women with GDM were significantly older, heavier from a weight and BMI perspective, had a higher gravidity and parity and were more likely to have had a family history of diabetes than women without GDM (Table 5.1).

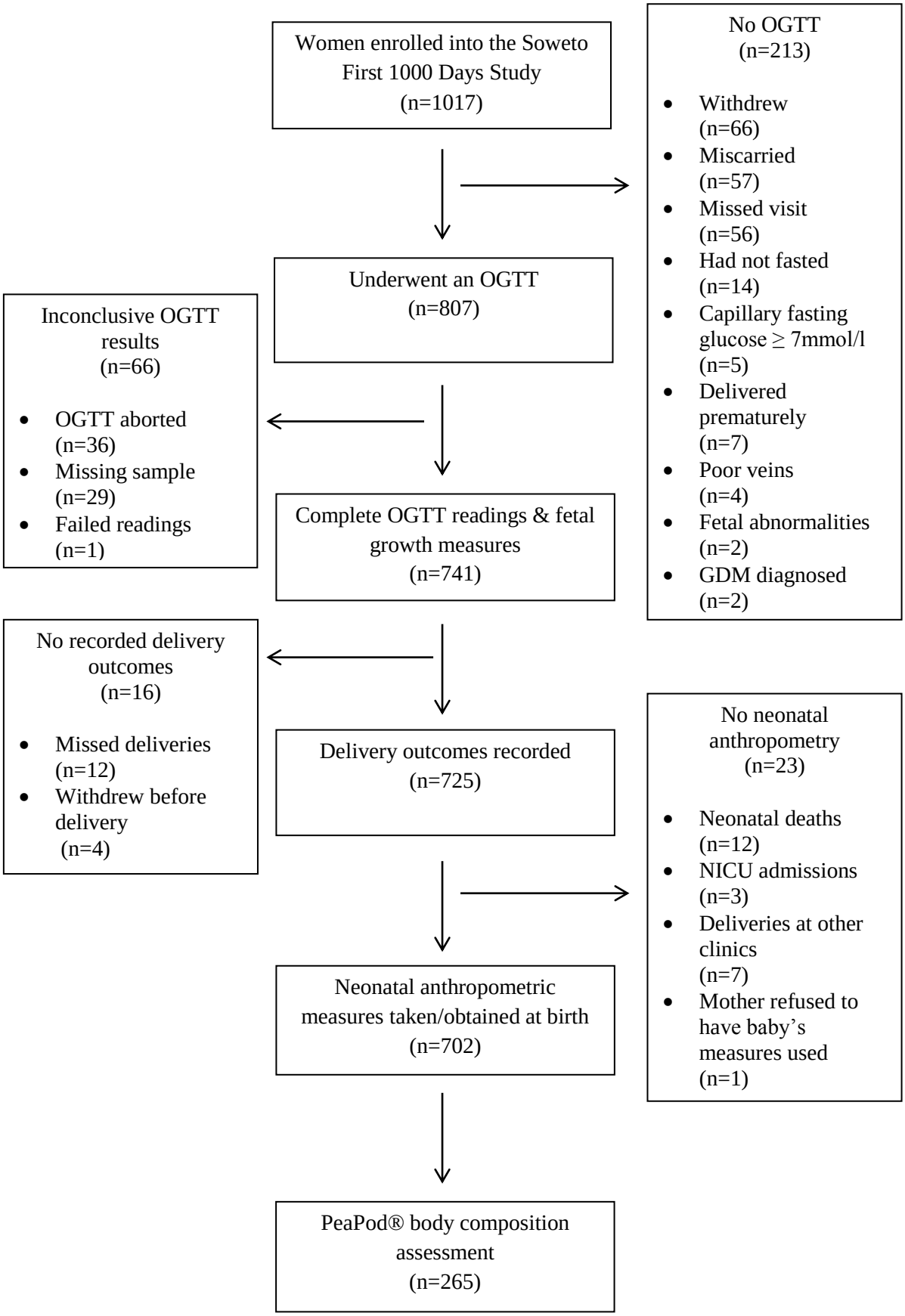


Figure 5.1 Sample size for the study investigating the effects of gestational diabetes mellitus (GDM) on fetal growth and neonatal birth size. OGTT denotes oral glucose tolerance test; NICU, neonatal intensive care unit.

Table 5.1 Characteristics of the women participating in the S1000 Study

Characteristic	Total women	Women without GDM	Women with GDM	*P* value
Total	741 (100.0%)	658 (88.8%)	83 (11.2%)	
Age (years)	29 (25-34)	29 (24-34)	31 (27-36)	<0.001*
Household socioeconomic status (asset score/11)	6 (5-6)	6 (5-6)	6 (5-6)	0.569
Level of education				
No schooling/primary school	16 (2.2%)	13 (2.0%)	3 (3.6%)	0.356
Secondary school	535 (72.2%)	472.0 (71.7%)	63 (75.9%)	
Tertiary education	190 (25.6%)	173 (26.3%)	17 (20.5%)	
Marital status				
Single	462 (62.4%)	415 (63.1%)	47 (56.6%)	0.254
Married/cohabiting	279 (37.7%)	243 (36.9%)	36 (43.4%)	
Family history of diabetes (n=739)	19 (25.9%)	161 (24.5%)	30 (36.1%)	0.023*
Weight (kg)	69.1 (59.4-80.2)	68.6 (59.0-79.3)	73.0 (63.9-86.8)	0.002*
BMI	27.6 (23.7-31.4)	27.4 (23.3-31.1)	29.2 (25.2-34.8)	0.001*
Weight gain (kg) (enrolment to OGTT)	5.0 (2.9-7.0)	5.0 (2.9-7.0)	4.4 (3.0-6.7)	0.326
Total gestational weight gain (kg)	9.1 (6.2-12.1)	9.2 (6.2-12.2)	7.3 (5.9-11.4)	0.127
Gestational age at enrolment (weeks)	12 (11-13)	12 (11-13)	13 (11-13)	0.627
Gravidity †	2 (1-3)	2 (1-3)	2 (1-3)	0.010*
	1.9 ± 1.3	1.8 ± 1.3	2.2 ± 1.3	
Parity	1 (0-2)	1 (0-2)	1 (1-2)	0.016*
Previous macrosomic infant (n=739)	40 (5.4%)	33 (5.0%)	7 (8.4%)	0.197
HIV positive at enrolment	234 (31.6%)	204 (31.0%)	30 (36.1%)	0.342
Fasting glucose (mmol/l)	4.2 (3.8-4.6)	4.1 (3.7-4.4)	5.2 (5.1-5.6)	<0.001*
60 mins post-glucose load (mmol/l)	5.9 (5.0-7.1)	5.7 (4.9-6.8)	8.3 (7.1-9.7)	<0.001*
120 mins post-glucose load (mmol/l)	5.5 (4.7-6.4)	5.4 (4.6-6.2)	7.5 (6.5-8.6)	<0.001*

Values given as median (interquartile range) or n (%); Sample size (n) indicated if less than 741; Gestational diabetes mellitus (GDM) diagnosed using the World Health Organization's 2013 criteria; *P<0.05 indicates a statistically significant difference; BMI, Body mass index; †Mean ± standard deviation given as well when median (interquartile range) does not illustrate the difference between the groups

5.3.1 Fetal Growth Measures

A total of 4040 fetal ultrasounds were performed with each participant receiving a median of 6 (5-6) scans. The median gestational weeks for each of the follow-up visits were: Visit 2: 17 (16-18) weeks; Visit 3: 22 (21-22) weeks; Visit 4: 27 (26-27) weeks; Visit 5: 32 (31-32) weeks; and Visit 6: 37 (36-37) weeks. Compared to female fetuses, males had significantly larger biparietal diameters, head circumferences and abdominal circumferences but femur length did not differ significantly between the sexes (Supplementary Table S5.1).

The GDM-exposed fetuses had significantly larger biparietal diameters, head circumferences and abdominal circumferences than the unexposed group, particularly at Visit 4: 27 (26-27) weeks when a diagnosis of GDM was made. Abdominal circumference was significantly larger across all five visits in GDM-exposed versus unexposed fetuses. Femur length did not differ between the two groups (Figure 5.2 and Supplementary Table S5.2).

The effect of maternal BMI on fetal growth in the whole group was compared. Maternal BMI had a statistically significant effect on femur length throughout pregnancy with larger mothers having fetuses with longer femurs. There was a significant difference in head circumference at 32 (31-32) weeks gestation and in abdominal circumference at 27 (26-27) and 32 (31-32) weeks gestation ($p<0.001$) between fetuses of women in the different BMI categories (Table S5.3). When stratified by GDM status, maternal BMI did not seem to affect fetal size in the group with GDM. In the group without GDM, abdominal circumference and head circumference differed significantly at specific gestational time points, and femur length differed significantly throughout pregnancy (Supplementary Table S5.4).

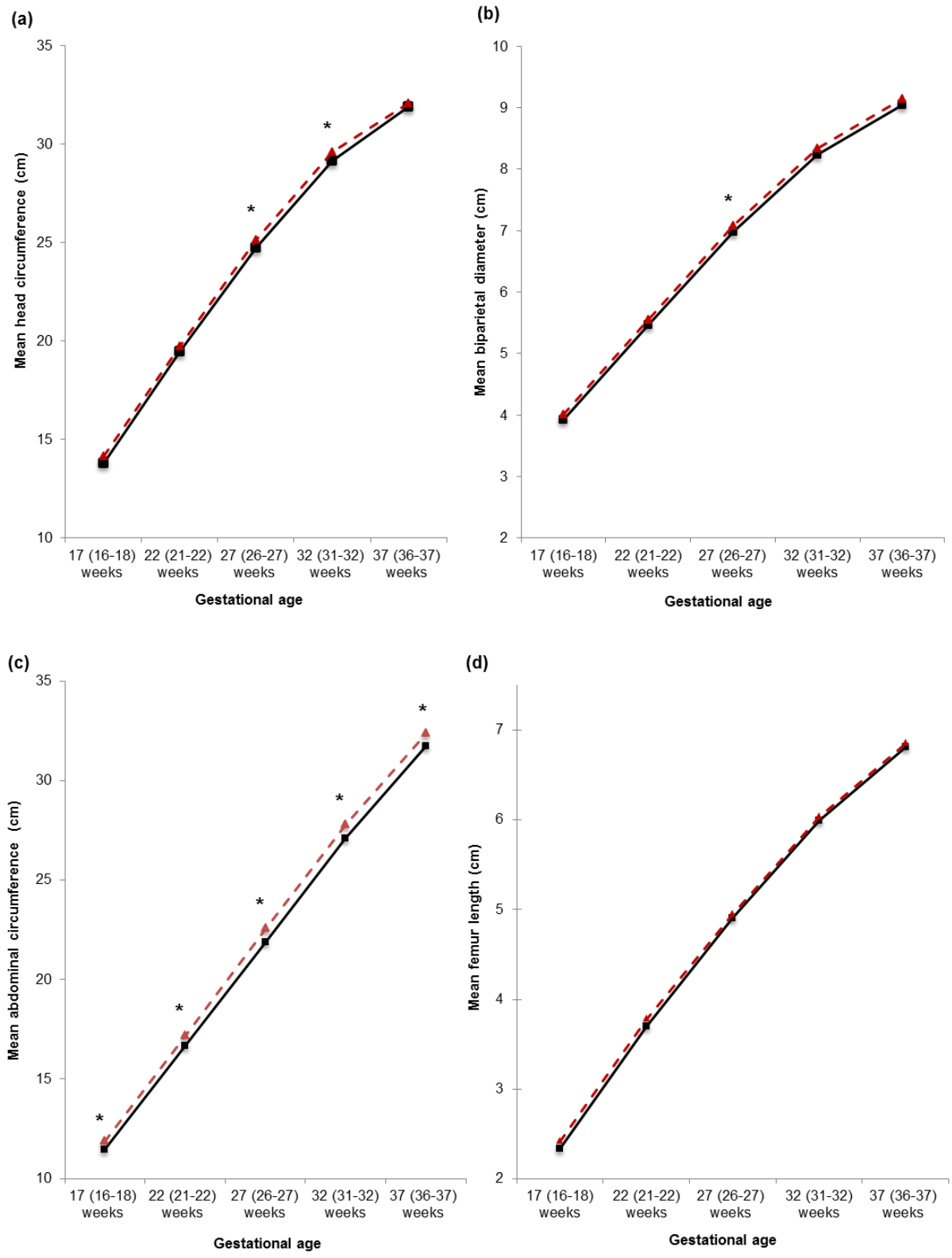


Figure 5.2 Head circumference (a), biparietal diameter (b), abdominal circumference (c) and femur length (d) of fetuses exposed to gestational diabetes mellitus (GDM) (n=83; red dashed line) versus unexposed fetuses (n=658; solid black line) against gestational age in weeks. The asterisk (*) denotes a statistically significant difference (p<0.05) with GDM-exposed fetuses being larger than unexposed fetuses.

The results from the LMM revealed that biparietal diameter, head circumference and femur length were not associated with GDM status (results not shown). However, the pooled model for abdominal circumference showed a positive association between GDM status and abdominal circumference (p=0.007) (Table 5.2). When stratified by sex, the same results were found for male fetuses (p=0.009) but not for female fetuses (p=0.286). The LMM was rerun using the interaction term 'sex*GDM'. The interaction term was significant for male fetuses (p=0.009) but not female fetuses (p=0.388) therefore substantiating the results above.

When maternal BMI and weight change (kg) per week were included in the pooled model, GDM still had an effect on abdominal circumference but BMI and weight change appeared to have a direct effect on abdominal circumference independent of GDM. There were no interactions between BMI and GDM, and weight change and GDM. Over time, the head circumference/abdominal circumference ratio became smaller amongst the GDM-exposed group with head circumference remaining unchanged but abdominal circumference increasing in size.

Table 5.2 Linear mixed modelling results showing fixed effects on abdominal circumference size (cm) *in utero*

	Coefficient	***P* value**	**95% Confidence interval**
Gestational age (days)	0.15	<0.001	0.14, 0.15
Maternal age (years)	0.00	0.725	-0.02, 0.01
Positive GDM status	0.24	0.007	-0.11, 0.39
Visit number (n)	-0.01	0.959	-0.40, 0.19
BMI at enrolment (kg/m^2)	0.02	<0.001	0.00, 0.02
Average weight change per week (kg)	0.31	0.034	-0.12, 0.67

GDM, gestational diabetes mellitus; BMI, body mass index

5.3.2 Management of Women with Gestational Diabetes Mellitus

All 83 women with GDM were referred to the Obstetric Diabetes Clinic, most followed through with their referrals (55/83 (66.3%)). All received diet therapy and 18 (32.7%) were treated with medication; 17 (94.4%) with metformin and one (5.6%) with insulin.

5.3.3 Delivery Outcomes

Of the whole group (n=725), women who delivered early (<39 weeks gestation encompassing the preterm and early-term categories [282]; n=342) were of higher parity than those who delivered later (≥39 weeks gestation; n=383); 1 (1-2) versus 1 (0-1) (p=0.007) but did not differ otherwise (results not shown). Women with GDM delivered early than women without GDM; 38 (37-39) weeks versus 39 (38-40) weeks (p=0.001), and the Caesarean section rate was higher amongst the group with GDM; 73.2% versus 56.4% (p=0.004) (Table 5.3). Male neonates had significantly larger birth weights, lengths and head circumferences than females (Supplementary Table S5.5).

A multiple linear regression analysis, adjusting for gestational age at delivery and neonatal sex, was run to determine if GDM was associated with birth weight. There was no significant association between GDM exposure and birth weight (p=0.134) but gestational age at delivery was significantly associated with birth weight (p<0.001).

There was no significant difference in birth size between the GDM-exposed and unexposed neonates. The Ponderal Index did not differ between the two groups (p=0.245) (Table 5.3). Similarly, when stratified by sex, there were no differences in birth measures between GDM-exposed and unexposed neonates (Table 5.4). Multiple linear regression analysis was run to predict Ponderal Index from sex, gestational age at delivery and GDM exposure. None of these variables were significantly associated with Ponderal Index (R^2=0.004).

Fat mass (g) was determined on 265 of the neonates (25 GDM-exposed and 240 unexposed neonates) by the PeaPod®. The median fat mass for the whole group was 419.1 g (296.9-565.1). The median fat mass for the GDM-exposed neonates was 439.8 g (340.0-549.9) and for the unexposed neonates was 415.5 g (292.9-566.3) (p=0.530). A multiple linear regression analysis was run to predict fat mass based on sex, gestational age at delivery, birth length and

GDM exposure. A positive regression equation was found (R^2 =0.091). Sex (β=57.09, p=0.018), gestational age at delivery (β=19.26, p=0.016) and birth length (β=14.13, p=0.002) were statistically significantly associated with fat mass but GDM exposure was not (β=38.24, p=0.349).

Table 5.3 Delivery outcomes and birth anthropometry for neonates exposed to gestational diabetes mellitus (GDM) and unexposed neonates

Variable	Total neonates	Unexposed neonates	GDM-exposed neonates	*P* value
Delivery outcomes	**n=725**	**n=643**	**n=82**	
Sex of neonate				
Male	374 (51.6%)	329 (51.2%)	45 (54.9%)	0.526
Female	351 (48.4%)	314 (48.8%)	37 (45.1%)	
Gestational age at delivery (weeks)	39 (38-40)	39 (38-40)	38 (37-39)	0.001*
Preterm delivery	102 (14.1%)	86 (13.4%)	16 (19.5%)	0.132
Mode of delivery				
Vaginal delivery	303 (41.8%)	281 (43.7%)	22 (26.8%)	0.004*
Caesarean section	422 (58.2%)	362 (56.3%)	60 (73.2%)	
Birth anthropometry	**n=702**	**n=620**	**n=82**	
Birth weight (g)	3030.0 (2685.0-3300.0)	3030.0 (2695.0-3304.5)	3067.5 (2600.0-3250.0)	0.562
Birth weight category				
Small for gestational age	125 (17.8%)	113 (18.2%)	12 (14.6%)	0.709
Appropriate for gestational age	532 (75.8%)	467 (75.3%)	65 (79.3%)	
Large for gestational age	45 (6.4%)	40 (6.5%)	5 (6.1%)	
Macrosomic	17 (2.4%)	16 (2.6%)	1 (1.2%)	0.451
Low birth weight	113 (16.1%)	97 (15.7%)	16 (19.5%)	0.371
Birth length (cm) (n =701)	48.6 (46.7-50.2)	48.7 (46.8-50.3)	48.2 (46.5-49.2)	0.080
Weight for length ratio (kg/m)	6.2 (5.6-6.7)	6.2 (5.6-6.7)	6.2 (5.6-6.6)	0.706
Birth head circumference (cm) (n =701)	34.0 (33.1-35.0)	34.1 (33.2-35.0)	34.0 (33.0-35.0)	0.237
Ponderal Index (g/cm^3)	2.6 (2.4-2.9)	2.6 (2.4-2.8)	2.7 (2.4-2.9)	0.245

Values given as median (interquartile range) or n (%); *P<0.05 indicates a statistically significant difference.

Table 5.4 Birth outcomes of male and female neonates exposed to gestational diabetes mellitus compared to unexposed male and female neonates

	Male Neonates			Female Neonates		
	GDM-exposed	**Unexposed**	***P* value**	**GDM-exposed**	**Unexposed**	***P* value**
Weight (g)	3080 (2540-3250)	3055 (2740-3345)	0.421	3035 (2675-3230)	3000 (2635-3240)	0.923
Length (cm)	48.2 (46.3-49.7)	49.0 (47.0-51.0)	0.072	48.1 (46.5-49.0)	48.1 (46.3-50.0)	0.486
Head circumference (cm)	34.2 (33.0-35.8)	34.3 (33.2-35.2)	0.953	33.6 (33.0-34.5)	34.0 (33.1-35.0)	0.083
Ponderal Index	2.7 (2.4-2.9)	2.6 (2.4 -2.8)	0.375	2.7 (2.5-2.9)	2.6 (2.4-2.9)	0.448

Values given as median (interquartile range)

5.4 Discussion

Our study has shown that GDM influences fetal growth; fetuses exposed to hyperglycaemia were larger than unexposed fetuses. The difference in abdominal circumference size was seen as early as 16-18 weeks gestation. Sovio et al. [283] showed that excessive growth of the abdominal circumference in GDM-exposed fetuses occurs between 20-28 weeks gestation thus preceding a diagnosis of GDM [283] but our results show that this increased growth occurs even earlier.

The high Caesarean section rate observed for the entire study population (58.2%) is not surprising given the fact that Chris Hani Baragwanath Academic Hospital is a highly specialised central hospital. The national Caesarean section rate target set by the South African Department of Health for central hospitals is 50% [284]. A study that investigated reasons for Caesarean sections at Chris Hani Baragwanath Academic Hospital found fetal distress to be the most common indication, followed by previous Caesarean section and labour dystocia respectively [285]. The higher parity found amongst women who delivered early (<39 weeks) in the current study suggests those women may have had previous Caesarean sections therefore necessitating a further Caesarean section which resulted in earlier delivery. The earlier deliveries and higher Caesarean section rate amongst the women with GDM align with the hospital's GDM management protocol. The induction rate amongst women with GDM at the hospital is thought to be around 60-65% (Nicolaou, V, 2018 personal communication).

The LMM results showed a positive association over the course of gestation between GDM and abdominal circumference for the whole sample but when stratified by sex this was only observed amongst male fetuses. This is likely to be a finding of sexual dimorphism. Male sex is an independent risk factor for adverse pregnancy outcomes with females having an advantage over males [286]. Male fetuses are thought to be more susceptible to the *in utero* environment as they capitalise on ways of increasing their growth and are therefore more responsive to the current maternal diet than their female counterparts [287]. A recent study found that GDM-exposed male offspring had higher BMIs and an increased risk of obesity across late childhood, adolescence and early adulthood, compared to unexposed offspring, but, this difference was not observed amongst female offspring [288].

Abdominal circumference is thought to be a good indicator for fetopathy [102]. An abdominal circumference of ≥35 cm in the third trimester is apparently predictive of 93% of macrosomic births [104]. The mean abdominal circumference measurement at 37 weeks gestation amongst the GDM-exposed fetuses in the current study was 32.36 ± 1.79 cm (Supplementary Table S5.2). Whilst the GDM-exposed fetuses had larger abdominal circumferences than the unexposed fetuses, the threshold was still below that predictive of macrosomia.

The absence of a difference in birth size between the GDM-exposed and unexposed neonates could possibly be related to the management of the women with GDM. Most women with GDM received clinical intervention (dietary advice at the least). Management of GDM by nutritional counselling and diet therapy has been shown to significantly reduce the mean birth weight and neonatal fat mass, as well as frequency of LGA and macrosomic neonates [289]. However, another possibility could be that the *in utero* effects of hyperglycaemia are in fact small.

The lack of difference in birth size between GDM-exposed and unexposed neonates has been reported by others [105]. However, those investigators found that Ponderal Indexes and body fat were higher amongst the GDM-exposed neonates [105]. Our study found no difference in Ponderal Index between the two groups (p=0.245). From the PeaPod® results on the subset of neonates the GDM-exposed group appeared to have 5.8% (24.3 g) more fat mass than the unexposed group (439.8 g versus 415.5 g) but the difference was not statistically significant. This 5.8% difference may be clinically relevant and given the larger abdominal circumference seen *in utero* amongst GDM-exposed fetuses it is possible that at birth they have more adiposity around the abdomen than anywhere else. Long-term follow up of these babies is needed as there is no African GDM-exposed cohort data.

The small sample size for the PeaPod® assessment (n=265) may be a limiting factor in detecting any significant difference in fat mass between the two groups. One might also question whether the study was underpowered to detect a difference in birth measures between the two groups. However, a sample size calculation using mean birth weights from a previous South African GDM study [207] determined that a minimum sample size of 144 neonates (72 GDM-exposed and 72 unexposed neonates) (power of 90% and α significance level of 0.05) was required in order to detect a significant difference in birth weight. Our study was sufficiently powered with 82 GDM-exposed and 620 unexposed neonates.

This unique study has demonstrated a significant association between GDM-exposure and fetal growth in African babies. Second trimester abdominal circumference measures indicated fetal overgrowth with male fetuses being more susceptible to the hyperglycaemic environment than females. Despite there being no difference in birth size between GDM-exposed and unexposed neonates, the increased fetal abdominal circumference may still confer metabolic risk later on in life, particularly amongst male offspring [290].

5.5 Supplementary Tables

Table S5.1 Fetal measurements by fetal sex at five time points during pregnancy

	Total Group (n=725)	Males (n=374)	Females (n=351)	*P* value
Biparietal diameter				
17 (16-18) weeks	3.94 ± 0.44	4.00 ± 0.43	3.87 ± 0.45	0.001*
22 (21-22) weeks	5.48 ± 0.41	5.56 ± 0.41	5.38 ± 0.40	<0.001*
27 (26-27) weeks	6.99 ± 0.44	7.07 ± 0.44	6.90 ± 0.43	<0.001*
32 (31-32) weeks	8.25 ± 0.41	8.33 ± 0.42	8.17 ± 0.39	<0.001*
37 (36-37) weeks	9.05 ± 0.43	9.11 ± 0.43	8.98 ± 0.42	<0.001*
Head circumference				
17 (16-18) weeks	13.86 ± 1.55	14.04 ± 1.53	13.65 ± 1.54	0.001*
22 (21-22) weeks	19.50 ± 1.39	19.74 ± 1.35	19.20 ± 1.37	<0.001*
27 (26-27) weeks	24.81 ± 1.45	25.11 ± 1.44	24.46 ± 1.38	<0.001*
32 (31-32) weeks	29.19 ± 1.38	29.45 ± 1.43	28.91 ± 1.27	<0.001*
37 (36-37) weeks	31.91 ± 1.34	32.16 ± 1.29	31.64 ± 1.35	<0.001*
Abdominal circumference				
17 (16-18) weeks	11.50 ± 1.48	11.68 ± 1.47	11.30 ± 1.48	0.001*
22 (21-22) weeks	16.73 ± 1.45	16.92 ± 1.42	16.47 ± 1.44	<0.001*
27 (26-27) weeks	21.96 ± 1.83	22.19 ± 1.87	21.70 ± 1.76	<0.001*
32 (31-32) weeks	27.16 ± 1.81	27.28 ± 1.83	27.05 ± 1.77	0.103
37 (36-37) weeks	31.81 ± 2.05	31.90 ± 2.05	31.69 ± 2.02	0.246
Femur length				
17 (16-18) weeks	2.35 ± 0.40	2.36 ± 0.39	2.33 ± 0.40	0.471
22 (21-22) weeks	3.71 ± 0.36	3.71 ± 0.35	3.70 ± 0.36	0.619
27 (26-27) weeks	4.90 ± 0.36	4.91 ± 0.36	4.89 ± 0.36	0.294
32 (31-32) weeks	5.99 ± 0.34	5.98 ± 0.35	6.01 ± 0.33	0.280
37 (36-37) weeks	6.81 ± 0.38	6.80 ± 0.36	6.82 ± 0.41	0.471

Values given in cm as mean ± standard deviation; *$P<0.05$ indicates a statistically significant difference

Table S5.2 Fetal growth measurements in gestational diabetes mellitus (GDM)-exposed versus unexposed fetuses

	Total fetuses (n=741)	**Unexposed fetuses (n=658)**	**GDM-exposed fetuses (n=83)**	***P* value**
Biparietal diameter				
17 (16-18) weeks	3.94 ± 0.44	3.93 ± 0.45	4.01 ± 0.41	0.142
22 (21-22) weeks	5.48 ± 0.41	5.47 ± 0.41	5.56 ± 0.41	0.059
27 (26-27) weeks	6.99 ± 0.44	6.98 ± 0.43	7.08 ± 0.51	0.005*
32 (31-32) weeks	8.25 ± 0.41	8.24 ± 0.40	8.34 ± 0.49	0.103
37 (36-37) weeks	9.05 ± 0.43	9.04 ± 0.43	9.14 ± 0.44	0.132
Head circumference				
17 (16-18) weeks	13.86 ± 1.55	13.82 ± 1.53	14.18 ± 1.61	0.083
22 (21-22) weeks	19.50 ± 1.39	19.47 ± 1.39	19.75 ± 1.35	0.055
27 (26-27) weeks	24.81 ± 1.45	24.76 ± 1.40	25.16 ± 1.75	0.001*
32 (31-32) weeks	29.19 ± 1.38	29.14 ± 1.35	29.59 ± 1.56	0.019*
37 (36-37) weeks	31.91 ± 1.34	31.89 ± 1.35	32.10 ± 1.30	0.281
Abdominal circumference				
17 (16-18) weeks	11.50 ± 1.48	11.46 ± 1.46	11.87 ± 1.56	0.030*
22 (21-22) weeks	16.73 ± 1.45	16.67 ± 1.44	17.16 ± 1.47	0.002*
27 (26-27) weeks	21.96 ± 1.83	21.89 ± 1.76	22.53 ± 2.25	0.001*
32 (31-32) weeks	27.16 ± 1.81	27.09 ± 1.77	27.75 ± 2.08	0.011*
37 (36-37) weeks	31.81 ± 2.05	31.75 ± 2.07	32.36 ± 1.79	0.027*
Femur length				
17 (16-18) weeks	2.35 ± 0.40	2.34 ± 0.40	2.41 ± 0.40	0.267
22 (21-22) weeks	3.71 ± 0.36	3.70 ± 0.36	3.78 ± 0.33	0.095
27 (26-27) weeks	4.90 ± 0.36	4.90 ± 0.36	4.94 ± 0.37	0.206
32 (31-32) weeks	5.99 ± 0.34	5.99 ± 0.34	6.03 ± 0.34	0.296
37 (36-37) weeks	6.81 ± 0.38	6.81 ± 0.39	6.85 ± 0.37	0.788

Values given in cm as mean ± standard deviation; *$P<0.05$ indicates a statistically significant difference

Table S5.3 Fetal measurements by maternal body mass index (BMI) categories at five time points during pregnancy

	Maternal BMI			
	Normal weight (n=245)	**Overweight (n=248)**	**Obese (n=248)**	***P* value**
Biparietal diameter				
17 (16-18) weeks	3.97 ± 0.45	3.89 ± 0.44	3.96 ± 0.43	0.154
22 (21-22) weeks	5.47 ± 0.41	5.44 ± 0.42	5.52 ± 0.40	0.104
27 (26-27) weeks	6.95 ± 0.42	7.00 ± 0.42	7.03 ± 0.47	0.108
32 (31-32) weeks	8.21 ± 0.40	8.26 ± 0.43	8.29 ± 0.41	0.087
37 (36-37) weeks	9.01 ± 0.43	9.08 ± 0.46	9.06 ± 0.40	0.232
Head circumference				
17 (16-18) weeks	13.93 ± 1.61	13.69 ± 1.50	13.97 ± 1.52	0.101
22 (21-22) weeks	19.50 ± 1.40	19.37 ± 1.41	19.62 ± 1.34	0.168
27 (26-27) weeks	24.67 ± 1.36	24.82 ± 1.37	24.92 ± 1.59	0.113
32 (31-32) weeks	28.98 ± 1.37	29.19 ± 1.37	29.40 ± 1.36	0.005[§]
37 (36-37) weeks	31.77 ± 1.38	32.02 ± 1.36	31.95 ± 1.29	0.191
Abdominal circumference				
17 (16-18) weeks	11.55 ± 1.52	11.34 ± 1.40	11.62 ± 1.51	0.108
22 (21-22) weeks	16.68 ± 1.48	16.60 ± 1.41	16.90 ± 1.43	0.055
27 (26-27) weeks	21.67 ± 1.66	21.87 ± 1.83	22.33 ± 1.93	<0.001[§‡]
32 (31-32) weeks	26.80 ± 1.71	27.13 ± 1.96	27.58 ± 1.69	<0.001[§‡]
37 (36-37) weeks	31.56 ± 1.92	31.86 ± 2.19	32.02 ± 2.02	0.097
Femur length				
17 (16-18) weeks	2.34 ± 0.42	2.29 ± 0.38	2.41 ± 0.38	0.005[‡]
22 (21-22) weeks	3.69 ± 0.37	3.66 ± 0.35	3.78 ± 0.34	0.004[‡]
27 (26-27) weeks	4.87 ± 0.35	4.87 ± 0.34	4.97 ± 0.38	0.002[§‡]
32 (31-32) weeks	5.95 ± 0.34	5.96 ± 0.32	6.06 ± 0.36	<0.001[§‡]
37 (36-37) weeks	6.76 ± 0.36	6.79 ± 0.41	6.89 ± 0.38	0.010[§]

[§]Statistically significant difference (p<0.05) between normal and obese; [‡]Statistically significant difference (p<0.05) between overweight and obese

Table S5.4 Fetal measurements by maternal body mass index (BMI) and gestational diabetes mellitus (GDM) status at five time points during pregnancy

	Unexposed Fetuses (n=658)				GDM-Exposed Fetuses (n=83)			
	Maternal BMI				Maternal BMI			
	Normal weight (n=228)	Overweight (n=201)	Obese (n=229)	*P* value	Normal weight (n=17)	Overweight (n=27)	Obese (n=39)	*P* value
Biparietal diameter								
17 (16-18) weeks	3.97 ± 0.45	3.88 ± 0.45	3.95 ± 0.44	0.178	4.00 ± 0.54	3.97 ± 0.37	4.05 ± 0.38	0.713
22 (21-22) weeks	5.47 ± 0.42	5.42 ± 0.41	5.52 ± 0.40	0.070	5.53 ± 0.39	5.60 ± 0.40	5.55 ± 0.42	0.836
27 (26-27) weeks	6.95 ± 0.43	6.97 ± 0.42	7.03 ± 0.44	0.219	7.02 ± 0.36	7.19 ± 0.37	7.04 ± 0.62	0.328
32 (31-32) weeks	8.20 ± 0.38	8.24 ± 0.42	8.29 ± 0.40	0.082	8.30 ± 0.53	8.41 ± 0.49	8.31 ± 0.48	0.709
37 (36-37) weeks	8.99 ± 0.42	9.06 ± 0.45	9.07 ± 0.41	0.200	9.32 ± 0.50	9.25 ± 0.46	8.95 ± 0.32	0.034*
Head circumference								
17 (16-18) weeks	13.92 ± 1.57	13.66 ± 1.50	13.88 ± 1.53	0.155	13.95 ± 2.10	13.95 ± 1.50	14.46 ± 1.43	0.419
22 (21-22) weeks	19.49 ± 1.40	19.29 ± 1.43	19.63 ± 1.31	0.069	19.64 ± 1.37	20.02 ± 1.09	19.61 ± 1.51	0.689
27 (26-27) weeks	24.66 ± 1.38	24.73 ± 1.37	24.90 ± 1.44	0.245	24.83 ± 1.11	25.62 ± 1.10	25.00 ± 2.23	0.090
32 (31-32) weeks	28.97 ± 1.35	29.11 ± 1.35	29.36 ± 1.32	0.013§	29.14 ± 1.68	29.84 ± 1.44	29.62 ± 1.59	0.401
37 (36-37) weeks	31.75 ± 1.38	31.95 ± 1.37	31.99 ± 1.28	0.226	32.15 ± 1.23	32.57 ± 1.22	31.62 ± 1.28	0.055
Abdominal circumference								
17 (16-18) weeks	11.54 ± 1.48	11.30 ± 1.42	11.53 ± 1.49	0.173	11.75 ± 2.03	11.65 ± 1.23	12.09 ± 1.54	0.523
22 (21-22) weeks	16.67 ± 1.48	16.52 ± 1.44	16.84 ± 1.38	0.071	16.80 ± 1.59	17.26 ± 0.99	17.27 ± 1.70	0.718
27 (26-27) weeks	21.65 ± 1.67	21.77 ± 1.85	22.27 ± 1.70	0.001§,‡	21.98 ± 1.58	22.69 ± 1.38	22.66 ± 2.88	0.321
32 (31-32) weeks	26.79 ± 1.68	27.09 ± 1.94	27.44 ± 1.60	0.001§	26.87 ± 2.07	27.49 ± 2.07	28.29 ± 1.99	0.062
37 (36-37) weeks	31.52 ± 1.93	31.81 ± 2.23	31.96 ± 2.04	0.136	32.49 ± 1.61	32.24 ± 1.87	32.44 ± 1.85	0.920

Table S5.4 (Continued)

	Unexposed Fetuses (n=658)				GDM-Exposed Fetuses (n=83)			
	Maternal BMI				Maternal BMI			
	Normal weight (n=228)	Overweight (n=201)	Obese (n=229)	*P* value	Normal weight (n=17)	Overweight (n=27)	Obese (n=39)	*P* value
Femur length								
17 (16-18) weeks	2.34 ± 0.42	2.28 ± 0.38	2.40 ± 0.38	0.014[‡]	2.32 ± 0.52	2.35 ± 0.35	2.49 ± 0.36	0.294
22 (21-22) weeks	3.70 ± 0.38	3.64 ± 0.35	3.78 ± 0.35	0.002[‡]	3.66 ± 0.29	3.86 ± 0.34	3.77 ± 0.32	0.243
27 (26-27) weeks	4.87 ± 0.36	4.86 ± 0.35	4.97 ± 0.36	0.001[§,‡]	4.86 ± 0.24	5.01 ± 0.24	4.93 ± 0.47	0.229
32 (31-32) weeks	5.95 ± 0.34	5.96 ± 0.32	6.06 ± 0.35	0.001[§,‡]	5.97 ± 0.25	6.02 ± 0.29	6.06 ± 0.40	0.669
37 (36-37) weeks	6.77 ± 0.36	6.78 ± 0.41	6.89 ± 0.38	0.012[§,‡]	6.70 ± 0.31	6.85 ± 0.34	6.90 ± 0.42	0.685

Values given in cm as mean ± standard deviation; *No statistically significant difference between the BMI groups after the Bonferroni adjustment for multiple comparisons was applied; [§]Statistically significant difference ($p<0.05$) between normal and obese; [‡]Statistically significant difference ($p<0.05$) between overweight and obese

Table S5.5 Delivery outcomes and birth anthropometry of male versus female neonates

Variable	Total	Males	Females	*P* value
Delivery outcomes	**n=725**	**n=374**	**n=351**	
Gestational age at delivery (weeks)	39 (38-40)	39 (37-40)	39 (38-40)	0.481
Preterm delivery (<37 weeks)	102 (14.1%)	54 (14.4%)	48 (13.7%)	0.768
Mode of delivery (n = 724)				
Vaginal delivery	303 (41.8%)	151 (40.4%)	152 (43.3%)	0.424
Caesarean section	422 (58.2%)	223 (59.6%)	199 (56.7%)	
Birth anthropometry	**n=702**	**n=363**	**n=339**	
Birth weight (g)	3030 (2685-3300)	3060 (2730-3330)	3000 (2640-3240)	0.032*
Birth weight category				
Small for gestational age	125 (17.8%)	63 (17.4%)	62 (18.3%)	0.942
Appropriate for gestational age	532 (75.8%)	277 (76.3%)	225 (75.2%)	
Large for gestational age	45 (6.4%)	23 (6.3%)	52 (6.5%)	
Macrosomic	17 (2.4%)	12 (3.3%)	5 (22.4%)	0.115
Low birth weight	113 (16.1%)	51 (14.1%)	62 (18.3%)	0.127
Birth length (cm) (n=701)	48.6 (46.7-50.2)	49.0 (47.0-50.9)	48.1 (46.3-50.0)	0.001*
Weight for length ratio (kg/m)	6.2 (5.6-6.7)	6.2 (5.7-6.7)	6.1 (5.6-6.6)	0.138
Birth head circumference (cm) (n=701)	34.0 (33.1-35.0)	34.3 (33.20-35.3)	34.0 (33.0-35.0)	0.005*
Ponderal Index (g/cm^3)	2.6 (2.4-2.9)	2.6 (2.4-2.8)	2.6 (2.4-2.9)	0.459
Ponderal Index categories				
Small	41 (5.9%)	25 (6.9%)	16 (4.7%)	0.347
Marginal	196 (28.0%)	99 (27.4%)	97 (28.6%)	
Normal	371 (52.9%)	196 (54.1%)	175 (51.6%)	
Overweight	93 (13.3%)	42 (11.6%)	51 (15.0%)	

Values given as median (interquartile range) or n (%); Sample size (n) indicated if less than 725; *$P<0.05$ indicates a statistically significant difference

CHAPTER 6

THE RELIABILITY AND VALIDITY OF GESTATIONAL AGE DATING BY LAST MENSTRUAL PERIOD IN A LOW-MIDDLE-INCOME SETTING

Chapter 5 highlighted that *in utero* exposure to GDM resulted in increased fetal growth but no difference in birth size was observed between GDM-exposed versus unexposed neonates. Furthermore, the number of large for gestational age neonates at delivery amongst the GDM-exposed group was surprisingly very low. Building on from this is the question around accurate gestational age dating. In South Africa, the majority of the antenatal care facilities do not have prenatal ultrasound services and gestational age dating is therefore reliant upon the estimation from a woman's last menstrual period (LMP) date. This chapter describes research that was conducted to determine the reliability, sensitivity and specificity of gestational age dating using LMP. This work has been submitted to the peer-reviewed journal, the Journal of Obstetrics and Gynaecology Research, where it has been reviewed and recommendations by the reviewers have been made. It has since been revised and resubmitted to the journal:

Macaulay, S., Buchmann, E.J., Dunger, D.B. & Norris, S.A. The reliability and validity of last menstrual period for gestational age estimation in a low-middle-income setting. *The Journal of Obstetrics and Gynaecology Research*. Reviewed, revised and resubmitted for review.

6.1 Introduction

Accurate gestational age dating is important during pregnancy and after delivery. Precise timing of deliveries by Caesarean section or induction of labour for high-risk, complicated or post-date pregnancies is essential. At birth, from an obstetric point of view, classifying a newborn as large for gestational age might point towards the mother having had gestational diabetes during her pregnancy which has risks for subsequent pregnancies [107]. From a paediatric perspective, a large for gestational age neonate flags long term cardiometabolic risks [291-293]. Gestational age determination influences clinical decisions and management and it is therefore important that accurate estimations are made.

When performed with precision, prenatal ultrasound is the gold standard for gestational age dating. Ideally, gestational age dating by fetal ultrasound should be performed at <14 weeks but up to 20 weeks gestation is acceptable [294]. As prenatal ultrasonography requires costly equipment and skilled medical professionals many pregnant women in resource-poor settings have limited or no access to the service [295].

The majority of primary level antenatal clinics in the South African public healthcare sector do not offer routine prenatal ultrasonography. Other gestational age dating methods including the last menstrual period (LMP), fetal symphysis-fundal height (SFH) measurement and palpation are utilised. Symphysis-fundal height is not appropriate for dating early pregnancies and palpation requires skill and expertise [198]. For these reasons, many antenatal clinics rely on the reported LMP. However, LMP has its own limitations; it is self-reported and dependent upon accurate recall, as well as the assumption of a regular 28 day cycle with ovulation occurring on day 14 [296]. Therefore, the validity and reliability of LMP to assess gestational age is often questioned.

Four South African studies [297-300] have investigated LMP as a gestational age assessment method over the past ten years. Whilst these studies assessed the reliability of LMP, none investigated its validity (sensitivity and specificity). Furthermore, three of the four studies assessed gestational age dating in the context of termination of pregnancy [297, 299, 300] and termination of pregnancy study populations tend to underestimate their gestational age compared to women who take their pregnancies to term [297, 301, 302]. The remaining study assessed gestational age dating amongst pregnant women in Cape Town in the Western Cape Province [298].

The current study was set in Johannesburg in the Gauteng Province of South Africa. Johannesburg and Cape Town differ in various ways other than geographical location. Johannesburg, spanning 1 645 km^2, is the most populated city in South Africa followed by Cape Town which spans 2 461 km^2. The two cities differ in terms of population structure with the majority of Johannesburg inhabitants being black African and the majority of Cape Town inhabitants being mixed ancestry. The two cities also have different provincial healthcare policies [303]. The aim of the study was to assess the reliability and validity of LMP as a gestational age dating method amongst pregnant women in Johannesburg, and, to compare gestational age estimates by LMP and ultrasound to those recorded at delivery by the hospital/clinic staff.

6.2 Methods

6.2.1 Participants

This study involved 741 participants enrolled into a longitudinal pregnancy cohort study called the Soweto First 1000 Days (S1000) study. The study took place during June 2013 to July 2016. The overarching aim of the S1000 study was to investigate the effects of maternal factors on fetal development and infant outcomes. Participants were recruited from the Antenatal Clinic and Fetal Medicine Unit of Chris Hani Baragwanath Academic Hospital. Eligibility criteria included women being 18 years of age or older, of South African black ethnicity (self-reported), residing in Soweto, and pregnant with naturally conceived singleton pregnancies of preferably less than 14 weeks gestation but no more than 20 weeks gestation (according to ultrasound assessment). Participants were followed up over the course of their pregnancies.

6.2.2 Questionnaires

At enrolment all participants were interviewed by research nurses and asked a series of demographic and obstetric-related questions. The demographic questions included age, marital status, level of education and a household asset score (the sum of eleven assets; electricity, radio, television, refrigerator, cellular telephone, personal computer, farm animals,

agricultural land, bicycle, motorcycle, motor vehicle) which was used as a proxy for household socioeconomic status.

6.2.3 Gestational Age Dating Methods

6.2.3.1 Last Menstrual Period

Participants were prompted using a calendar to assist them with recall of their LMP dates. The gestational age of a pregnancy with a reported LMP was determined using the Obdisk Pregnancy Calculator; a pregnancy wheel that assumes a 28 day menstrual cycle and a gestational age of 280 days (40 weeks) at birth.

6.2.3.2 Ultrasonography

All women enrolled into the study received a dating ultrasound. The dating scan involved dating the pregnancy using the fetal crown-rump-length at <14 weeks + 0 days gestation, or the biparietal diameter, head circumference, abdominal circumference and femur length in more advanced pregnancies. All ultrasounds were conducted by two board-certified sonographers using the Philips HD-9 (Philips Ultrasound, Bothell, Washington, USA) machine. The ultrasound machine used the Robinson and Fleming formula to calculate the gestational age based on the crown-rump length [16] and the formulae used to calculate gestational age using the other fetal biometric measurements in more advanced pregnancies were as per the Intergrowth-21st study described in detail by Papageorghiou et al. [304]. More than half of the crown-rump-length and fetal biometry measures underwent external quality assessment by colleagues at Oxford University (UK) as per the Intergrowth-21st ultrasound quality control protocol [305]. All scans analysed were considered to be within the acceptable limits.

6.2.3.3 Discrepancies in Gestational Age Dating Methods

A difference of more than five days at ≤8 weeks and 6 days was considered discrepant, a difference of more than seven days at 9 weeks–15 weeks and 6 days was considered

discrepant and finally a difference of more than ten days at 16 weeks–21 weeks and 6 days was considered discrepant [296].

The hospital-recorded gestational age was captured and gestational age at delivery was also estimated according to both the LMP and dating scan. As per the American College of Obstetrician and Gynecologists' (ACOG) guidelines, at birth neonates were classified as being 'preterm', 'early-term', 'full-term', 'late-term' or 'post-term' according to their gestational age at delivery (Table 6.1) [282].

Table 6.1 The American College of Obstetrician and Gynecologists' recommendations for defining deliveries according to gestational age

Birth category	Gestational age in weeks	Total gestational age in days
Preterm	<37 weeks	<259
Early-term	37 weeks 0 days - 38 weeks 6 days	259 – 272
Full-term	39 weeks 0 days - 40 weeks 6 days	273 - 286
Late-term	41 weeks 0 days - 41 weeks 6 days	287 – 293
Post-term	≥42 weeks 0 days	≥294

The ability of LMP and ultrasound to identify critical gestational age cut-offs used regularly in obstetric practice was also considered. For example, ≥37 weeks gestation versus ≤36 weeks and 6 days to identify women needing elective delivery for hypertensive disorders [306]; ≥39 weeks gestation versus ≤38 weeks and 6 days for women needing elective Caesarean section or induction of labour for a clinical indication or by their choice [307, 308] and ≥41 weeks gestation versus ≤40 weeks and 6 days for elective delivery for prolonged pregnancy [309].

6.2.4 Anthropometry

Anthropometric measures including, weight (kg), height (cm) and body mass index (BMI) were taken on each of the study participants at enrolment. The World Health Organization's BMI categories for underweight (<18.5 kg/m^2), normal weight (≥18.5-24.9 kg/m^2), overweight (≥25-29.9 kg/m^2) and obese (≥30 kg/m^2) were used [262].

6.2.5 Statistical Analyses

Stata Version 12 **(**StataCorp, College Station, Texas**)** was used for statistical analyses. The Shapiro-Wilk and the Skewness and Kurtosis tests were used to assess the distribution of the continuous data. Continuous variables that were normally distributed were presented as means (standard deviations (SD)) and those that were not normally distributed were presented as medians (interquartile range (IQR)). Categorical data were presented as frequencies and percentages. The Student's t-test was used to analyse differences between normally distributed variables and the Mann-Whitney test was used to analyse differences between non-normally distributed variables. Differences between categorical variables were determined using the Chi-square test. A multiple logistic regression analysis was performed to determine the predictors of having a discrepancy between LMP-based and ultrasound-based gestational age estimates. The Kruskal-Wallis H test, with the Conover-Iman test of multiple comparisons, was used to analyse the gestational age estimates at delivery according to ultrasound (at enrolment), LMP and hospital records. Significance was assumed at a two-tailed *p* value of $p<0.05$.

Using ultrasound as the gold standard method, a Bland-Altman plot [310] was performed. This involved plotting the difference in gestational age (days) at enrolment as determined by ultrasound and LMP on the vertical axis against the mean gestational age (days) of ultrasound and LMP on the horizontal axis. The standard deviation, bias and 95% lower and upper limits of agreement were calculated.

A Lin's concordance correlation coefficient was also calculated to assess the concordance between LMP-based gestational age dating and ultrasound-based dating (gold standard). The Lin's coefficient measures the goodness of fit of the line. A Lin's coefficient of 1 indicates perfect agreement between two methods whereas a value of 0 indicates no agreement [311].

Cohen's Kappa (*k*) statistic was used to evaluate the degree of agreement between gestational age estimation by ultrasound and LMP in categorising neonates as preterm, early-term, full-term, late-term and post-term. The following levels of agreement were considered: 'poor', $k<0.00$; 'slight', $k=0.00$-0.20; 'fair', $k=0.21$-0.40; 'moderate', $k=0.41$-0.60; 'substantial', $k=0.61$-0.80; 'almost perfect', $k=0.81$-1.00 [312].

Finally, using ultrasound as the gold standard, the sensitivity, specificity, positive and negative predictive values of LMP in classifying neonates into birth categories, and in determining critical gestational age cut-offs for induction of labour or elective Caesarean sections, was assessed.

6.2.6 Ethical Approval

The study was approved by the University of the Witwatersrand's Human Research Ethics Committee (Medical) (certificate references: M120524 and M130309). All study participants gave written, informed consent.

6.3 Results

6.3.1 Participants' Characteristics

Of the 741 women in the study, 498 (67.2%) reported LMP dates for which they felt certain about, 51 (6.9%) reported LMP dates but felt uncertain about them and 192 (25.9%) could not report their LMP dates at all. Women who were unable to report LMP dates were significantly younger and more advanced in their pregnancies than the women who did report LMP dates (Table 6.2). Multiple logistic regression analysis revealed no significant predictors for reporting an LMP (results not shown).

Most women (593/741; 80%) were enrolled at <14 weeks + 0 days gestation and therefore had a dating ultrasound based on crown-rump-length measurement. The remaining 148/741 women (20%) were ≥14 weeks gestation and had a dating scan that assessed the biparietal diameter, head circumference, abdominal circumference and femur length.

Table 6.2 Demographic characteristics of women with known versus unknown last menstrual period (LMP) dates

	Median (IQR) or n (%)			
	Total participants (n=741)	**Participants with LMP dates (n=549)**	**Participants without LMP dates (n=192)**	***P* value**
Age (years)	29.0(25.0-34.0)	30.0 (25.0-34.0)	28.0 (24.0-33.5)	0.026*
Household socioeconomic status ‡	6 (5-6)	6 (5-6)	6 (5-6)	0.692
Education				0.455
No schooling/primary school	16 (2.2%)	13 (2.4%)	3 (1.6%)	
Secondary school	535 (72.2%)	390 (71.0%)	145 (75.5%)	
Tertiary education	190 (25.6%)	136 (26.6%)	44 (22.9%)	
Marital status				0.151
Single	472.0 (62.4%)	334 (60.8%)	128 (66.7%)	
Married/cohabiting	279.0 (37.7%)	215 (39.2%)	64 (33.3%)	
Weight (kg)	69.1 (59.4-80.2)	69.0 (59.2-80.3)	69.3 (60.1-79.9)	0.883
Height (cm)	158.7 (154.8-162.6)	158.6 (154.6-162.7)	158.8 (155.1-162.3)	0.784
BMI (kg/m^2)	27.6 (23.7-31.4)	27.6 (23.6-31.3)	27.5 (24.3-31.4)	0.843
BMI categories				0.902
Normal	245 (33.1%)	184 (33.5%)	61 (31.8%)	
Overweight	248 (33.5%)	182 (33.2%)	66 (34.4%)	
Obese	248 (33.5%)	183 (33.3%)	65 (33.9%)	
US-based GA (days)§	89 (79-97)	89 (79-96)	91.5 (83-100.)	0.006*
Gravidity	2 (1-3)	2 (1-3)	2 (1-3)	0.113
Parity	1 (0-2)	1 (0-2)	1 (0-2)	0.251

Interquartile range (IQR); Last menstrual period (LMP); Ultrasound (US); Gestational age (GA); Body Mass Index (BMI); *Statistically significant, $p<0.05$; ‡Household asset score out of 11; §At the time of enrolment (<20 weeks gestation)

Of the total number of women who reported an LMP (n=549; 498 who felt certain and 51 who felt uncertain about their LMP dates), 210/549 (38.3%) of them had discrepancies between their ultrasound-based and LMP-based gestational age estimates (Table 6.3). A multiple logistic regression analysis revealed that weight and household SES were significantly associated with these discrepancies (Table 6.4).

Table 6.3 Characteristics of the study participants with reported last menstrual period dates and ultrasound assessments

	Median (IQR) or n (%)			
	Total participants	**Discrepant[†] participants**	**Non-discrepant participants**	***P* value**
Participants	**n=549[¶]**	**n=210**	**n=339**	
Age (years)	30 (25-34)	30 (25-34)	29 (25-35)	0.782
Household socioeconomic status [‡]	6 (5-6)	5 (5-6)	6 (5-7)	0.017[*]
Education				0.145
No schooling/primary school	13 (2.4%)	5 (2.4%)	8 (2.4%)	
Secondary school	390 (71.0%)	159 (75.7%)	231 (68.1%)	
Tertiary education	146 (26.6%)	46 (21.9%)	100 (29.5%)	
Marital status				0.823
Single	334 (60.8%)	129 (61.4%)	205 (60.5%)	
Married/cohabiting	215 (39.1%)	81 (38.6%)	134 (39.5%)	
Weight (kg)	69.0 (59.2-80.3)	68.4 (56.3-79.2)	69.1 (60.3-81.1)	0.047[*]
Height (cm)	158.6 (154.6-162.7)	157.8 (154.0-162.7)	159.0 (154.8-162.8)	0.214
BMI (kg/m^2)	27.6 (23.6-31.3)	27.1 (23.1-31.2)	27.8 (24.0-31.4)	0.102
BMI categories				0.095
Normal	184 (33.5%)	82 (39.1%)	102 (30.1%)	
Overweight	182 (33.2%)	63 (30.0%)	119 (35.1%)	
Obese	183 (33.3%)	65 (31.0%)	118 (34.8%)	
Certain about LMP	498 (90.7%)	186 (88.6%)	312 (92.0%)	0.174
LMP-based GA (days)[§]	88 (75-99)	85 (72-107)	88 (76-96)	0.941
US-based GA (days)[§]	89 (78-96)	88 (77-95)	90 (78-96)	0.384
Gravidity	2 (1-3)	2 (1-3)	2 (1-3)	0.972
Parity	1 (0-2)	1 (0-2)	1 (0-2)	0.803
Participants	**n=536[¶]**	**n=205**	**n=331**	
Mode of delivery				0.780
NVD	213 (39.7%)	83 (40.5%)	130 (39.3%)	
Caesarean Section	323 (60.3%)	122 (59.5%)	201 (60.7%)	
Newborn sex				0.091
Male	268 (50.0%)	112 (54.6%)	156 (47.1%)	
Female	268 (50.0%)	93 (45.4%)	175 (52.9%)	

Interquartile range (IQR); Last menstrual period (LMP); Body Mass Index (BMI); Ultrasound (US); Gestational age (GA); Normal vaginal delivery (NVD); [*]Statistically significant, $p<0.05$; [†]Discrepant according to the American College of Obstetricians & Gynecologists' guidelines [296]; [‡]Household asset score out of 11; [§]At the time of enrolment (< 20 weeks gestation); [¶] A total of 549/741 study participants had both LMP and ultrasound gestational age assessments. Of the 549 women, only 536 had delivery data recorded.

Table 6.4 Multiple logistic regression analysis for risk factors associated with having discrepancies[†] between gestational age dating by last menstrual period versus ultrasound

Risk factor	**Odds ratio**	**95% Confidence interval**	***P* value**
Age (years)	0.99	0.96, 1.03	0.778
Weight (kg)	0.99	0.98, 1.00	0.049[*]
Level of education (categories)			
No schooling/primary school (reference)			
Secondary school	1.17	0.37, 3.70	0.790
Tertiary education	0.84	0.25, 2.80	0.772
Household asset score (n)	0.85	0.74, 1.00	0.039[*]
Gravidity (n)	0.98	0.79, 1.22	0.878
Certainty of reported LMP	0.59	0.33, 1.08	0.086

[†]Discrepancies according to the American College of Obstetricians & Gynecologists' guidelines [296]: >5 days at ≤8 weeks & 6 days, >7 days at 9 weeks–15 weeks & 6 days, >10 days at 16 weeks–21 weeks & 6 days between the two methods; LMP, last menstrual period; [*]statistically significant $p<0.05$

6.3.2 Gestational Age Distribution

The distribution of gestational age at enrolment for the 549 women who had both reported LMP dates and dating scans is presented in Figure 6.1. According to their LMP-based estimates 300 (54.6%) women had underestimated how far pregnant they were, 219 (39.9%) women had overestimated how far pregnant they were and 30 women (5.5%) had LMP-based gestational age estimates that were exactly the same as the ultrasound-based estimates.

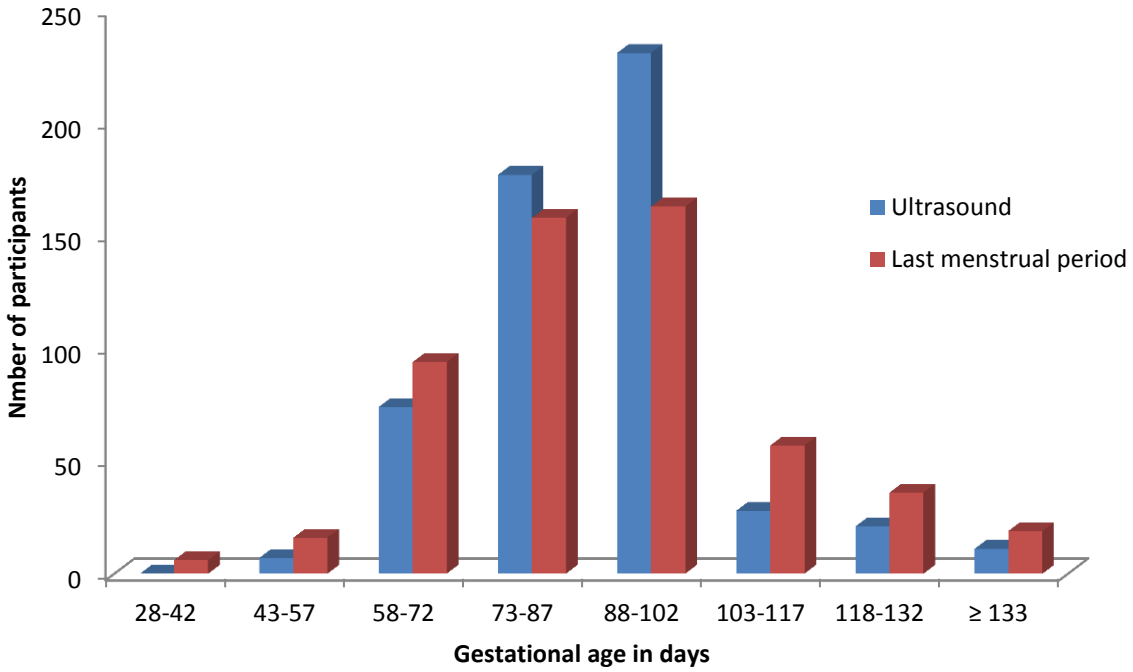


Figure 6.1 The distribution of gestational age at enrolment determined by prenatal ultrasound and last menstrual period.

6.3.3 Level of Agreement of Gestational Age Dating by Last Menstrual Period and Ultrasonography

A Bland-Altman plot illustrating LMP-based gestational age estimates versus ultrasound-based gestational age estimates (from the dating scan) is represented in Figure 6.2. The standard deviation of the differences was 13.5 days and the 95% limits of agreement were calculated as -26.7 and 26.3. A bias (mean difference) of -0.2 (Confidence Interval (CI): 1.4, 0.9) days between the two methods was determined.

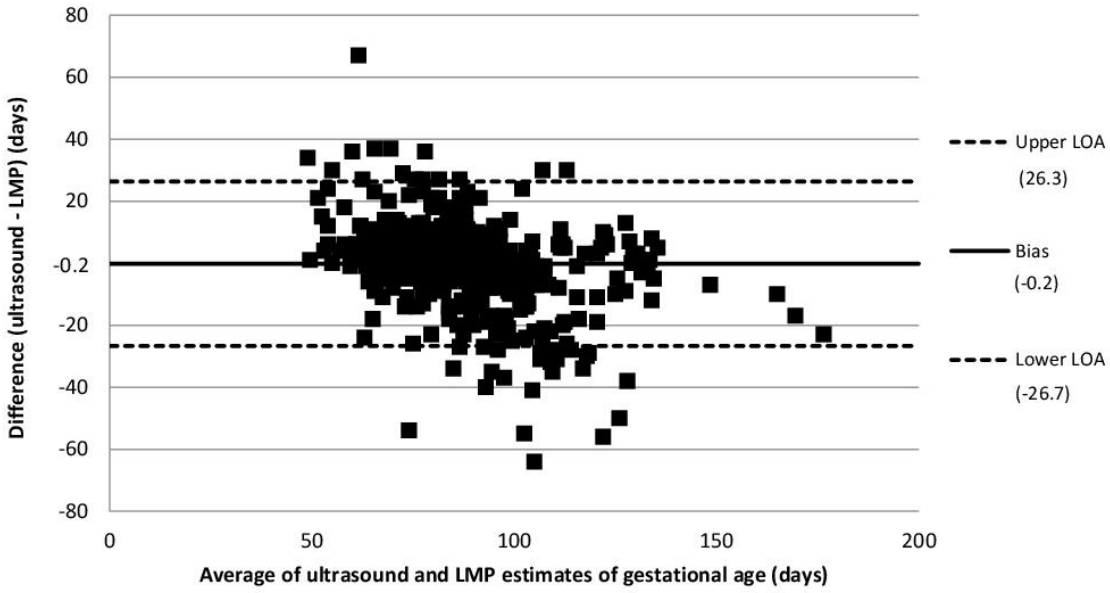


Figure 6.2 A Bland-Altman plot comparing gestational age estimation by ultrasound and last menstrual period (LOA; limits of agreement).

A Lin's concordance correlation coefficient of 0.75 (Standard Error (SE): 0.02; 95% CI 0.71, 0.78) was calculated for LMP-based gestational age dating using ultrasound-based dating as the gold standard.

6.3.4 Reliability of Gestational Age Dating by Last Menstrual Period

Compared to LMP-based and ultrasound-based estimates, hospital recorded gestational ages at birth were significantly shorter. The majority of neonates were classified as full-term according to all three estimates. Hospital recorded gestational age estimates classified a larger number of preterm and early-term neonates whereas LMP classified far more neonates as being post-term (Table 6.5). There was a significant difference (p<0.05) in the classification of neonates across all the birth categories by the three estimations.

Table 6.5 Birth categories according to gestational age estimates by ultrasound, last menstrual period, and hospital records

	Ultrasound	**Hospital Records**	**LMP**	***P* value**
GA at delivery (median (IQR) days)	273.0 (266.0-280.0)	268.0 (259.5-277.0)	272.0 (261.5-281.0)	<0.001[†§]
Birth categories (n (%))				<0.050[‡]
Preterm	72 (13.4%)	108 (20.1%)	104 (19.4%)	
Early-term	187 (34.9%)	193 (36.0%)	172 (32.1%)	
Full-term	240 (44.8%)	203 (37.9%)	177 (33.0%)	
Late-term	31 (5.8%)	23 (4.3%)	33 (6.2%)	
Post-term	6 (1.1%)	9 (1.7%)	50 (9.3%)	

IQR, interquartile range; Birth categories according to the American College of Obstetricians and Gynecologists' definitions (Table 6.1);[†]*P* value determined by the Kruskal-Wallis H test with the Conover-Iman posthoc test; [§]Significant difference between: ultrasound and hospital recorded gestational age (GA), and, last menstrual period (LMP) and hospital recorded GA; [‡]*P* value determined by the Chi-square test. Significant difference between birth categories determined by ultrasound and hospital records, ultrasound and LMP, and LMP and hospital records.

An overall Kappa statistic of 0.64 (95% CI: 0.54, 0.71), p<0.001, was calculated when all birth categories were grouped, indicating a 'substantial' agreement between LMP-based and ultrasound-based gestational age dating. When analysed by each birth category, the agreement between the two methods in classifying neonates as preterm was 'substantial' but 'slight' in classifying post-term neonates. There was 'fair' agreement between the two methods in classifying term pregnancies (Table 6.6).

Table 6.6 Preterm, term, and post-term deliveries according to gestational age estimates by early prenatal ultrasound (gold standard) and last menstrual period (n=536)

Birth Category[†]	Ultrasound	LMP	Kappa	95% CI
Preterm	72 (13.4%)	104 (19.4%)	0.64	0.54, 0.71
Early-term	187 (34.9%)	172 (32.1%)	0.30	0.22, 0.38
Full-term	240 (44.8%)	177 (33.0%)	0.34	0.25, 0.41
Late-term	31 (5.8%)	33 (6.2%)	0.24	0.11, 0.38
Post-term	6 (1.1%)	50 (9.3%)	0.05	-0.03, 0.17

LMP, Last menstrual period; CI, Confidence interval; [†]According to the American College of Obstetricians and Gynecologists' definitions (Table 6.1); Kappa levels of agreement: 'Poor', k<0.00; 'slight', k=0.00-0.20; 'fair', k=0.21-0.40; 'moderate', k=0.41-0.60; 'substantial', k=0.61-0.80; 'almost perfect', k=0.81-1.00.

6.3.5 Validity of Gestational Age Dating by Last Menstrual Period

Using the ultrasound-based gestational age estimates as the gold standard, LMP had a fairly high specificity across the five birth categories but its sensitivity and positive predictive value varied (Table 6.7). Furthermore, the sensitivity and specificity of LMP decreased as the gestational age increased which was evident from the results indicating LMP's ability in determining the critical gestational age cut-offs used in obstetric practice (Table 6.8).

Table 6.7 Validity of gestational age estimates according to last menstrual period[†] in classifying birth categories

Birth Category[‡]	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
Preterm	84.7 (74.3, 92.1)	90.5 (87.5, 93.0)	58.1 (48.1, 67.7)	97.4 (95.5, 98.7)
Early-term	51.3 (43.9, 58.7)	78.2 (73.5, 82.4)	55.8 (48.1, 63.4)	75.0 (70.2, 79.4)
Full-term	51.2 (44.7, 57.7)	81.8 (76.9, 86.0)	69.5 (62.1, 76.2)	67.4 (62.3, 72.2)
Late-term	29.0 (14.2, 48.0)	95.2 (93.0, 96.9)	27.3 (13.3, 45.5)	95.6 (93.5, 97.2)
Post-term	33.3 (4.33, 77.7)	91.1 (88.3, 93.4)	4.08 (0.5, 14.0)	99.2 (97.9, 99.8)

[†]Compared to ultrasound estimates as the gold standard; CI, Confidence interval; [‡]According to the American College of Obstetricians and Gynecologists' definitions (Table 6.1)

Table 6.8 Validity of gestational age estimates according to last menstrual period[†] in determining critical gestational age cut-offs

Gestational age cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
≥37 weeks versus ≤36 weeks + 6 days	90.7 (87.7, 93.2)	84.7 (74.3, 92.1)	97.5 (95.5, 98.7)	58.7 (48.6, 68.2)
≥39 weeks versus ≤38 weeks + 6 days	71.8 (66.1, 77.1)	76.4 (70.8, 81.5)	76.5 (70.9, 81.6)	71.7 (66.0, 77.0)
≥41 weeks versus ≤40 weeks + 6 days	54.1 (36.9, 70.5)	87.4 (84.1, 90.2)	24.1 (15.4, 34.7)	96.2 (94.1, 97.8)

[†]Compared to ultrasound estimates as the gold standard; CI, Confidence interval

6.4 Discussion

Our study assessed LMP and prenatal ultrasound as gestational age dating methods amongst a group of pregnant South African women. Our results have shown that 74% of the women were able to report an LMP. The 25.9% of women who could not recall an LMP is substantially greater than the 8.3% reported amongst Nigerian women [313]. The women who could not report an LMP were significantly younger and more advanced in their pregnancies than women who did report an LMP. These findings speak to the issue of recall bias; the further along one is in one's pregnancy the less likely one is to recall the LMP date. Despite being younger, the women who could not report an LMP date were not less educated than those who could.

Of the women in our study who did report an LMP (n=549), 67.2% felt certain about their dates. This is higher than the 56.0% of women in the Cape Town based study who felt certain about their LMP dates [298]. Lighter weight women and those of lower household SES were more likely to have discrepancies between the LMP-based and ultrasound-based gestational age estimates. Other studies have reported similar findings regarding socioeconomic status and having discrepancies between LMP-based and ultrasound-based gestational age estimates, but have also found that women with discrepancies were more likely to be less educated, single and younger than women without gestational age estimate discrepancies [314-316].

The hospital records appeared to underestimate gestational age at birth. This suggests that hospital staff members are not scrutinising the antenatal records at delivery for information on gestational age and that rough estimates are being recorded. This highlights a gap in the healthcare system where strengthening is required. If antenatal records were electronically captured and therefore easily accessible and linked to delivery records, errors and discrepancies may be reduced. The current system, however, still utilises and relies on manually recorded details which may not necessarily be available at the delivery thus allowing room for error in gestational age estimation.

Compared to ultrasound, dating by LMP overestimated gestational age by 0.2 days. The overestimation of gestational age by LMP has been reported by other investigators; another South African study reported LMP overestimating gestational age by 0.5 days [299], a Vietnamese study reported an overestimation of 1.4 days [317] and two American studies reported an overestimation of gestational age by LMP by 0.8 days [315] and 2.8 days [318] respectively. Delayed ovulation is thought to be one of the reasons why gestation is overstated by LMP [314]. The 0.2 day overestimation found in our study is not considered clinically significant. Therefore, together with the Lin's correlation coefficient of 0.75 (close to 1.00), our results indicate that LMP and ultrasound appear to be fairly well correlated in early pregnancy.

The 'substantial' agreement (k=0.64) between LMP and ultrasound in classifying neonates into the birth categories is better than that reported by Deputy et al. (2017) who found a 'moderate' agreement between the two methods in categorising neonates at birth (k=0.41) in Vietnam [317]. Hoffman et al. [315] reported exactly the same Kappa coefficient ('slight', k=0.05 (95% CI -0.02, 0.13)) as ours for the level of agreement between LMP and ultrasound in classifying post-term births (defined in their study as >41 weeks gestation) in an American study. Gernand et al. [316] also showed that the level of agreement between the two methods in classifying preterm births (defined by them as <259 days gestation) was better (k=0.74) than that for post-term births (k=0.24) (defined by them as <293 days gestation). Despite the cut-off values for post-term being marginally different across the three above-mentioned studies, they highlight that LMP is poor in identifying pregnancies/neonates of >41 weeks gestation in both high- and low-to-middle-income settings. The over-classification of neonates as post-term by LMP has been reported in several countries and populations [315, 317, 319-321].

Whilst the specificity of LMP in classifying neonates into the birth categories was generally high, the sensitivity and positive predictive values differed per birth category. The sensitivity and positive predictive value of LMP in identifying preterm births were high but low for late-term and post-term births. Dietz et al. [314] reported similar findings in a high-income setting with LMP having a sensitivity and positive predictive value of 64.3% and 58.7% respectively for preterm births, and 33.6% and 3.7% respectively for post-term births. Our results are comparable to those of Dietz et al. [314]. The low sensitivity and positive predictive value of LMP for classifying post-term births seems to be a recognised problem [316, 320]. Furthermore, whilst LMP can be relied upon to inform clinical decisions around delivery for pregnancies affected by hypertension (≥37 weeks), its sensitivity in informing timing of elective Caesarean sections or inductions of labour for other clinical indications or by maternal choice (≥39 weeks) or prolonged pregnancies (≥41 weeks) is poor. Using LMP to estimate due date and identify pregnancies over 39 weeks is very likely to result in several unnecessary inductions of labour and Caesarean sections which carry their own risks and add burden to the hospital system.

Taking into account South Africa's under-resourced and heavily burdened healthcare system, the results from our study are useful in understanding how and when LMP can be utilised as an alternative to ultrasound for dating pregnancies. In the absence of prenatal ultrasonography, LMP appears to be a reliable, inexpensive substitute for estimating gestational age during early pregnancy amongst women in Soweto, Johannesburg. However, due to its poor sensitivity, LMP should not be used to inform clinical decisions around delivery beyond 39 weeks gestation. Relying on LMP is likely to result in unnecessary inductions of labour and Caesarean sections. Gestational age dating by ultrasound has been shown to reduce the need for post-term induction [322]. Therefore, whilst ultrasound equipment is expensive and requires trained operators, the long-term benefits of using ultrasound to date pregnancies and inform clinical decisions may in fact turn out to be the more cost-effective option.

SECTION 3
INTEGRATED DISCUSSION

CHAPTER 7
DISCUSSION AND CONCLUSION

This chapter provides a summary of the key findings from the empirical papers presented in Chapters 3 to 6. It also discusses two main themes that have emerged from this research and how the findings add to the body of knowledge on GDM from a global and national perspective. The strengths and limitations of the PhD study are highlighted and recommendations in the context of South African policies governing maternal and child 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.

7.1 Consolidated Findings

This PhD study aimed to answer four specific objectives with the intention of providing a better understanding of the extent and impact of GDM, and how screening for the condition in the South African context can be improved. Each objective was achieved through an empirical study presented as an individual chapter within this thesis. Table 7.1 provides a summary of the key findings from each chapter.

Table 7.1 Consolidated findings from the four empirical studies

Objective	Chapter	Main Findings
To determine what GDM prevalence figures exist for Africa.	3	• Little data on GDM in Africa exists. Only 6/54 (11%) of the countries on the African continent had reported GDM prevalence figures. • A multitude of GDM diagnostic criteria are used across the African continent making the comparison of existing prevalence figures difficult. • At the time the systematic review was published, only four studies on GDM in South Africa had been undertaken, of which only two involved black women. • Prevalence figures for GDM ranged from 0% to 13.9% and an average GDM prevalence across Africa was estimated to be around 5%.
To estimate the prevalence of GDM amongst black South African women living in urban Soweto and assess their clinical management.	4	• Using the WHO 2013 diagnostic criteria a GDM prevalence of 9.1% (95% CI 7.9, 10.5) was determined amongst black South African women living in Soweto. • Women with GDM had significantly higher weights and BMIs, were significantly older, of higher household socioeconomic status, more likely to report a family history of diabetes, and more likely to be diagnosed with anaemia than women without GDM. • An age of ≥35 years, BMI ≥30 kg/m^2, and a family history of diabetes were significant risk factors for the development of GDM. • The fasting plasma glucose reading had a high sensitivity (83.3% (95% CI 77.0, 88.5)) in diagnosing GDM. • Just over half the women with GDM (56.9%) were managed by diet therapy alone.
To assess the effects of GDM exposure on fetal growth and neonatal birth measures.	5	• GDM exposure was associated with an increase in fetal growth, especially abdominal circumference, which was already seen at 16-18 weeks gestation. • Male fetuses in particular showed a significant association between GDM exposure and increased abdominal circumference. • There was no difference in birth measurements between the GDM-exposed and unexposed neonates.
To compare gestational age estimation using last menstrual period to that using fetal ultrasound	6	• Using fetal ultrasound as the gold standard, last menstrual period (LMP) overestimated gestational age by 0.2 days. • Gestational age estimates by ultrasound and LMP were fairly well correlated in early pregnancy. • LMP has a low sensitivity in identifying late-term and post-term pregnancies/neonates.

7.2 Hypotheses Revisited

The study's hypotheses are listed below with an explanation regarding whether the research findings support or reject them.

1. **Very little data on the prevalence of GDM exist in Africa.**
   The systematic review that assessed GDM prevalence in Africa supports this hypothesis. Very little data exist on GDM in Africa with only 11% of African countries having reported on its prevalence.

2. **The prevalence of GDM amongst women in urban Soweto is higher than that reported in rural Limpopo (8.8%) using the same diagnostic criteria.**
   When the same diagnostic criteria were applied the GDM prevalence amongst the urban cohort of women in the current study was in fact less (5.6% (95% CI 4.6, 6.7) than that reported in rural Limpopo. Therefore the above hypothesis regarding prevalence of GDM is rejected.

3. **Exposure to GDM *in utero* results in increased fetal growth and birth measurements.**
   The above hypothesis is partly supported and partly refuted by the study outcomes. Whilst an increase in fetal growth was observed amongst the GDM-exposed fetuses (particularly abdominal circumference and specifically amongst male fetuses), there was no significant difference in any of the birth measures between GDM-exposed and unexposed neonates.

4. **Gestational age estimation by LMP has poor reliability and validity in dating South African pregnancies.**
   As gestational age dating by LMP and ultrasound were found to be well correlated in early pregnancy, LMP can be viewed as being a reliable alternative to ultrasound dating in early pregnancy. However, LMP is not sensitive enough in identifying late-term and post-term pregnancies.

7.3 Emerging Research Themes

The specific results from each of the four empirical studies have been discussed in detail and compared to findings from similar studies in the respective chapters. Through the consolidation of the findings two key thematic areas have emerged. Firstly, the consequences of prenatal exposure to GDM, and secondly, health systems strengthening to optimise maternal health. These are discussed below.

7.3.1 Consequences of Prenatal Exposure to Gestational Diabetes Mellitus

The results obtained through this study have illustrated that *in utero* exposure to GDM does affect fetal growth, particularly that of the abdominal circumference, and interestingly, amongst male fetuses only. Overall, there was a lack of difference in birth measures at delivery between GDM-exposed neonates compared to unexposed neonates, and when stratified by sex, no difference in birth measures between the GDM-exposed versus unexposed groups were seen either. Furthermore, on the subset of infants that underwent a PeaPod® assessment, no significant difference in fat mass was seen amongst the GDM-exposed versus unexposed groups. This raises the question of whether the adverse effects of GDM exposure were entirely mitigated at birth, or, despite no difference in birth size being observed, did the exposure to GDM alter fetal programming thus predisposing these infants to obesity and diabetes in their later years? Whilst Chapter 5 touched on the topic of sexual dimorphism the following section will discuss this topic in further detail and also discuss the potential postnatal consequences of GDM exposure to the neonates in the current study.

7.3.1.1 Sexual Dimorphism

It is well described in the literature that males are at higher risk for several health issues and adverse pregnancy outcomes compared to their female counterparts. For example, higher incidences of preterm births [286] and birth defects [323] have been noted amongst male neonates compared to females. In addition, men have been shown to have higher blood pressure and shorter lifespans than women [324]. This has been referred to as “the male disadvantage” [325]. These findings point towards sex differences in developmental and physiological processes which fit into the framework of DOHaD.

It is speculated that male and female fetuses have different growth strategies. Males are generally larger in size at birth than females and this was supported through the findings of the study presented in Chapter 5. The male fetus' mandate is to grow as fast and as large as possible. The placentas of male fetuses tend to be very efficient at transporting nutrients but have less reserve capacity than females as nutrients are utilised for fetal growth rather than placental growth. This growth strategy means male fetuses are highly dependent on the current maternal diet in maximising food supply. This is problematic in situations of undernutrition as there is little placental reserve capacity to compensate for the lack of nutrients. Likewise, in situations of maternal overnutrition, such as GDM involving an excessive amount of nutrients, male fetuses are thought to overcapitalise on the surplus of nutrients to fuel their growth. Conversely, female fetuses invest in the development of the placenta and with more placental reserve capacity they are better protected against nutrient shortages as they are less dependent on the current maternal diet. This also makes female fetuses more adaptable to changes in maternal diet [287, 326]. Epigenetic studies have found an increase in the expression of genes involved in placental development and the preservation of pregnancy amongst female placentas compared to males [327].

Considering the placenta is the interface between the fetus and mother, its ability to function and respond in an adverse situation would determine how well or poorly the fetus adapts. Female placentas appear more responsive to stress signals than male placentas which makes female fetuses better at adapting to adversities than males [328]. Several studies have supported this. Maternal psychosocial stress has been linked to an increased risk of behavioural deficits and schizophrenia amongst male offspring [329, 330]. In addition, animal studies have shown that pregnant mice on high fat diets produce significantly smaller male embryos than female embryos, and, maternal obesity results in deregulation of significantly more genes in male brains compared to those of females [331].

Regarding GDM, certain studies have illustrated sex differences with respect to prevalence as well as response to treatment. Whilst there were slightly more male fetuses compared to female fetuses exposed to GDM in the current study, the difference was not statistically significantly (45 versus 37, p=0.530). However, others have shown that pregnant women carrying male fetuses are at increased risk of developing GDM than those carrying female fetuses [332]. The mechanisms behind such findings remain unclear but it is suggested that differential placental gene expression plays a role. Bahado-Singh et al. [333] showed that treatment by means of diet therapy and insulin (where needed) for exposure to mild GDM was

more effective in male fetuses than females. Treatment-exposed male neonates had significantly lower birth weights and fat mass than female neonates. This could be explained by the hypothesis that males are more susceptible to the *in utero* environment and are therefore more likely to be responsive to treatment. In later years (age five to ten years), however, only female offspring of women treated for mild GDM were shown to have significantly lowered fasting glucose levels compared to the offspring of the untreated group [290]. Another example of sexual dimorphism related to GDM exposure was reported in a Spanish study that assessed the prevalence of macrosomia, LGA and SGA amongst GDM-exposed and unexposed neonates. The investigators reported a significant difference in the prevalence of the three birth weight categories between male and female fetuses, but of most interest was the finding that GDM was a predictor of macrosomia in male fetuses only (odds ratio 1.67, 95% CI 1.12, 2.49). They concluded that close observation of fetal growth may be warranted in women with GDM who are pregnant with male fetuses [334].

7.3.1.2 Postnatal Risks

Despite no difference in birth size being observed between the GDM-exposed versus unexposed neonates, and most mothers having received some form of treatment (diet therapy at the least), it is still very possible that the exposure to GDM caused physiological changes during fetal development which will have long-term consequences. It has been shown that even in the case of treated GDM phospholipid metabolism is disturbed and levels of a certain placental transporter protein (MFSD2a; major facilitator super family domain containing 2a protein) is decreased which affects the transfer of docosahexaenoic acid (DHA); an omega-3 fatty acid that is essential for neurodevelopment. In addition, high fetal fat mass, as determined by larger abdominal circumference size, is associated with lower DHA levels [335]. This disrupted physiological process may very well have long-term consequences for the child. Furthermore, considering the positive association found in the current study between abdominal circumferences in male fetuses with GDM exposure, it would be reasonable to speculate that despite having normal birth measures they are at increased metabolic risk.

In terms of the female neonates in the current study, it is hypothesised that as female fetuses are less sensitive to unfavourable *in utero* events and GDM treatment has been shown to have positive long-term benefits amongst female offspring [290], the adverse effects of GDM

exposure may in fact have been attenuated thus reducing future risk for obesity and T2DM. This would, however, need to be confirmed through longitudinal observation of the female offspring. The HAPO Follow-up Study (HAPO FUS) [126] will hopefully lend some insight into the future metabolic risks of children exposed the GDM *in utero*.

Based on the findings from the current study it can be said that whilst all pregnancies affected by GDM should be managed as high-risk, those particularly involving male fetuses should be flagged and monitored very closely both pre- and postnatally and mothers should be informed to carefully monitor male infant weight gain.

7.3.2 Health Systems Strengthening

A major theme that has presented itself through this study is the need to strengthen the current South African healthcare system in order to offer an optimal service to pregnant women. This theme encompasses enhancing screening for GDM, as well as widening the availability of ultrasound services and developing a system that connects antenatal data with birth data. Together these will be value-added services that will have long-term benefits to patients, the healthcare system and economy of the country through the reduction of morbidities.

The need to optimise physical maternal health, such as good nutrition and weight control, has also been identified. In view of the DOHaD hypothesis there are intergenerational benefits to ensuring mothers have optimal diets, healthy BMIs and good prenatal care, and so investing in the health of pregnant women is invaluable.

7.3.2.1 Prenatal Ultrasound Services

The results presented in Chapter 6 illustrate that whilst gestational age estimation by LMP and ultrasound are well correlated in early pregnancy, gestational age dating by ultrasound is required in order to make accurate clinical decisions regarding timing of delivery, particularly in cases of late-term and post-term pregnancies. Increasing the availability of prenatal ultrasound services to pregnant women in antenatal clinics has major benefits. In the context of a GDM pregnancy, ultrasound would offer accurate gestational age dating, fetal growth assessment and fetal sexing; three essential components for the management of a GDM-affected pregnancy. The current study has identified that fetal abdominal circumference is

affected by the hyperglycaemic *in utero* environment, therefore, ultrasound monitoring of abdominal circumference size would be helpful in identifying excessive fetal growth and potential macrosomic or large for gestational age neonates (especially male neonates). Ultrasound monitoring of fetal abdominal circumference has been shown to effectively guide the management of GDM-affected pregnancies, even amongst women with significant obesity [336].

7.3.2.2 Hospital Record Keeping

The observed discrepancies in the hospital-recorded gestational ages versus ultrasound-determined gestational ages at birth as presented in Chapter 6 highlights disconnect between antenatal and delivery records. This could be mitigated if records were more accessible (and not dependent on the patient personally bringing in her antenatal file at the time of delivery) and an electronic system was in place.

A patient database to link patient antenatal records including ultrasound scans (if performed), management and treatment of maternal morbidities, such as GDM, and other important medical information should be set up. This will improve access to patient information which may reduce the number of gestational age dating errors made at delivery and improve the overall care and management of pregnant women. Implementation of such a system will, however, require technical infrastructure, skilled personnel, funding, ethical consideration, organisation and sustainability [337]. However, there are long-term benefits to establishing an electronic medical record system in low-resource settings. The implementation of an electronic medical record management system has proven to be successful and effective in a resource-poor antenatal clinic in Kenya [338]. Patient satisfaction with service provision, improved documentation of patient medical histories, and reduced consultation time were some of the reported benefits [339]. A study performed in Malawi demonstrated that from a financial perspective, the benefits of an electronic medical system outweigh the costs [340].

7.3.2.3 Screening for Gestational Diabetes Mellitus

Considering the costs and time involved in OGTTs, it is understandable that universal screening for GDM using the traditional OGTT is not employed in the resource-strapped South African public healthcare system. However, the high prevalence (9.1%) of GDM found amongst Sowetan women supports the need for improved GDM screening to be implemented in the antenatal clinics within the Soweto region. Other South African researchers have investigated the use of a patient-provided breakfast meal in place of the 75 g OGTT to screening for GDM, however, the amount of sugar and starch varied greatly amongst the meals provided by the patients that this non-standardised testing option was considered ineffective [341]. In the current study, the use of a fasting plasma glucose reading alone was proven to be effective in identifying the majority of women with GDM. Others have supported this finding [342]. The current study also showed that of those women who chose to take up the offer of free screening for GDM by arriving for their OGTT appointment, most had complied with the instruction to fast overnight, very few could not proceed with an OGTT due to not having fasted (Figure 4.1). This implies that if healthcare workers take the time to explain the importance of testing one's fasting blood glucose adherence to fasting beforehand is good. With the mandate of improving maternal health in mind, implementing GDM screening in order to identify and treat women with the condition is essential with both short- and long-term health benefits. Instead of relying on risk factors to identify women at risk of GDM, and performing a full OGTT, a universal screen using a fasting blood glucose test may be feasible.

7.3.2.4 Nutritional Counselling and Health Education

As discussed in Chapter 5, the majority (47%) of women with GDM were classified as 'obese' in the first trimester of pregnancy. In addition, a high prevalence of anaemia was noted amongst the women with GDM (30.9%), possibly due to poor nutrition at the micronutrient level. Therefore, a significant number of South African pregnancies involve obesity coupled with malnutrition and hyperglycaemia which presents a suboptimal situation for fetal growth and development and, consequently, transgenerational risks for NCDs. In addition, the large number of women in the GDM prevalence study (Chapter 4) who did not follow through with their invitation to participate in the study could very well be due to a lack of awareness around the condition and the importance of screening for it. Amongst pregnant

women in Samoa, another LMIC, only 58% were aware that diabetes could occur for the first time during pregnancy and very few women were aware of the common risk factors [343]. The need to educate women about the risk factors for and consequences of GDM is essential.

Educational strategies should be developed in order to educate South African pregnant women about GDM and the importance of maintaining a healthy BMI and eating food that provides a nutritional balance. Until now, the prevalence of GDM amongst black women living in Soweto remained unknown and was therefore not considered a public health concern. The WHO recommends nutritional counselling for all pregnant women. In addition, they also advocate counselling regarding physical activity in pregnancy in order to prevent excessive weight gain [201]. The current study did not assess physical activity but other researchers have reported low levels of physical activity which decreased as pregnancy progressed amongst women in Soweto [344]. A lack of education around physical activity was shown to be one of the barriers in getting pregnant women to be more active [345].

Not only does obesity increase risk for comorbidities and pregnancy complications it also impacts on the quality of life of a woman. A South African study reported low self-esteem and decreased physical function amongst obese pregnant women [346]. This adds a mental health component to the optimisation of maternal health and well-being.

Food insecurity in South Africa is reality, with many poor people unable to afford nutritious and balanced diets. Fast food is cheap and easily accessible, even in rural areas [347]. This increases the risk of overweight and obesity as well as malnutrition. It has been suggested that a solution to this problem is to introduce community based gardens of vegetable and grain crops to improve access to a variety of healthy food options [348]. Understanding why so many pregnant women are overweight or obese and what exactly they are eating will help facilitate possible dietary and behavioural interventions.

Nutritional counselling by registered dieticians does not form part of routine antenatal care in the South African public healthcare system. Likewise, informative advice around physical activity in pregnancy is not routinely offered. If both nutritional counselling and physical activity advice by trained specialists formed part of routine antenatal care, the prevalence of overweight and obesity amongst pregnant women might decrease which in turn might reduce the prevalence of GDM.

7.4 Contextual Relevance

The findings from this study are relevant from an international and national perspective and have contributed to the global body of literature on the topic of GDM. The need for universally applicable GDM screening guidelines has been identified with emphasis placed on the applicability and feasibility of such guidelines in low-resource settings [140]. Being a LMIC the results from this study may have relevance to other LMICs who are not only observing an increase in overweight and obesity and therefore possible GDM, but are also faced with similar dilemmas in terms of under-resourced public healthcare systems. The current study has illustrated how investigating the prevalence of GDM in a country is a necessary first step in understanding the extent of the problem followed by the assessment of whether current GDM screening practices and protocols are sufficient or should be reviewed.

7.4.1 The African and South African Context

As highlighted in Chapter 3, very little data exist on GDM in Africa. The current study was unique in that it was the first to assess the effects of GDM on fetal growth in Africa.

Regarding South Africa, the National Development Plan of the Department of Health lists nine health goals that it wishes to have achieved by the year 2030. Some of these goals focus on improving the overall health and well-being of the South African population and others focus on strengthening the healthcare system [349]. The nine 2030 health goals are listed below:

1. "Raise the life expectancy of South Africans to at least 70 years
2. Progressively improve tuberculosis prevention and cure
3. Reduce maternal, infant and child mortality
4. Significantly reduce prevalence of non-communicable disease
5. Reduce injury, accidents and violence by 50% from 2010 levels
6. Complete health system reforms
7. Primary healthcare teams provide healthcare to families and communities
8. Universal healthcare coverage
9. Fill posts with skilled committed and competent individuals" [349]

Goals “significantly reduce prevalence of non-communicable disease” and “complete health system reforms” resonate with the results of the current study. Furthermore, the Department of Health has identified a set of priorities that represent the interventions required for achieving the 2030 vision [349]. Amongst these priorities are five that the findings of this study speak to:

1. “Address the social determinants that affect health and disease
2. Strengthen the health system
3. Improve health information systems
4. Improve quality by using evidence
5. Prevent and reduce the disease burden and promote health” [349]

The following sections will address each of the five priorities listed above with GDM in mind.

Regarding the **“social determinants that affect health and disease,”** [349] the WHO list ‘food’, ‘the social gradient’ and ‘early life’ amongst the important determinants of health [350]. These three factors have been flagged by this study as being important in the development of GDM and in turn, T2DM. Obesity and diet inadequacies on the micronutrient level amongst pregnant females were identified. Whilst this study did not investigate dietary behaviour it is possible that food choices amongst the study population are influenced by cost with less nutritious meals being more economical and, therefore, more often consumed.

In order to **“strengthen the health system”** [349], the 9.1% prevalence of GDM amongst black South African women in Soweto suggests current GDM screening policies should be reconsidered. In addition, the study has shown that prenatal ultrasound services are essential in all antenatal clinics. Strengthening the health system by providing ultrasound services and screening and treatment for GDM to all pregnant women will contribute towards optimising maternal and child health.

Consideration needs to be given towards how to “**improve health information systems”** [349] in the South African public healthcare sector. The development of an efficient and reliable patient management system that allows the linking of patient records that can be accessed with ease by treating medical specialists should improve the quality of patient care.

The findings and suggestions from the current study can inform the National Department of Health and other stakeholders on the current issues surrounding GDM screening and management. This would help them "**improve quality by using evidence"**[349]. Lastly, by addressing and implementing all of the above and introducing patient education strategies the ability to **"prevent and reduce disease burden and promote health"** [349] may become a reality.

7.4.1.1 Recommendations Arising From This Research

With the focus of strengthening the health system and improving the care of pregnant women and their unborn babies in Soweto, South Africa, certain recommendations arising from the results of this research can be made.

- Firstly, given the high prevalence of GDM amongst women living in Soweto, it is recommended that routine screening for GDM be implemented in antenatal clinics. As LMP provides an accurate estimation of gestational age in early pregnancy, it could be used to book a woman for a glucose screening appointment when she is 24-28 weeks pregnant. A fasting plasma glucose reading, not a full OGTT, need only be performed.

- Secondly, ultrasound services should be established in all antenatal clinics in order to accurately date pregnancies which will aid clinical decisions around delivery and also identify fetal sex which has been shown to be an important factor in GDM-affected pregnancies.

- Thirdly, the growth of fetuses exposed to GDM should be carefully monitored. Attention should be paid to the fetal abdominal circumference with male fetuses, in particular, being observed closely.

- Finally, now that the prevalence of GDM has been established to be worthy of concern and consideration, education strategies for healthcare professionals as well as pregnant women should be implemented in order to increase awareness about GDM.

7.5 Strengths and Limitations

As with all studies, this PhD study had its strengths as well as some limitations. The main strength of this study is its novelty. It is the first study ever in Africa to investigate and report on how GDM affects fetal growth. In addition, it is the largest GDM prevalence study (n=1 906) conducted in South Africa to date. Methodologically speaking, the steps taken to ensure a well-described sample and accurate measurements from maternal anthropometry, fetal growth measures to glucose readings, ensured this study was robust and had rigour.

Whilst limitations to each of the empirical studies have been discussed in the separate chapters, a further four will be discussed here. Firstly, with regard to the empirical study on the effects of GDM on fetal growth and neonatal birth measures (Chapter 5) it would have been ideal to have obtained an abdominal circumference measurement on each neonate at birth in order to ascertain whether the increase in abdominal circumference amongst the GDM-exposed fetuses was also present at birth. Furthermore, other neonatal anthropometric measures, including skinfold thickness and upper arm circumference, could have been helpful and should be considered for future studies.

Secondly, a maternal postpartum fasting glucose reading (at the least), in the absence of any GDM treatment, may have been beneficial in determining how many women diagnosed with GDM reverted to normal glucose metabolism after delivery.

Thirdly, in terms of the GMD prevalence study (Chapter 4) it would have been helpful to have obtained more information on the women who chose not to take up the offer of free GDM screening (1 556/3 656; 42%). Their reasons for declining screening may have provided some insight into how study uptake could have been improved, such as the need for further information and education strategies around GDM and the purpose of the study. In addition, determining if the women who chose not to undergo GDM screening differed from the group of women who did (in terms of variables other than age, ethnicity and geographical location) would have been useful.

And lastly, the current study did not report on insulin levels. Understanding the fasting insulin profiles of women with GDM would give insight into the thresholds of insulin insensitivity and the association with hyperglycaemia. In other words, analysing insulin levels would give

insight into the degree of insulin insensitivity that is required for a diagnosis of GDM to be made.

7.6 Future Studies

This study has highlighted several research areas for subsequent studies to focus on. These further studies will help researchers gain a better understanding of the causes, consequences and impact of GDM in the South African context. Some possible future studies are listed below:

- **Assessing Women's Knowledge of Gestational Diabetes Mellitus**

The low uptake rate for participation in the GDM prevalence study (Chapter 4) makes one wonder whether the women understood the severity of GDM and its consequences. A study exploring women's knowledge on the subject would be helpful in identifying knowledge gaps and might lend insight into how to create awareness around the topic. Such a study could involve a mixed methods approach with women completing a questionnaire for quantitative analyses as well as being interviewed for qualitative analyses.

- **Cost Analysis for the Implementation of Glucose Screening in Antenatal Clinics**

The outcomes of the research presented in Chapter 4 showed that a fasting plasma glucose reading alone is highly sensitive in diagnosing GDM. This would be far less time consuming and most likely more cost effective, than a full OGTT. A formal cost analysis study looking at the possibility of implementing universal screening for GDM using a fasting plasma glucose reading in South African antenatal clinics would be extremely beneficial.

- **Long-Term Follow-Up of Babies Exposed to Gestational Diabetes Mellitus**

As per the fetal growth and neonatal outcomes component of this study (Chapter 5) the effects of GDM on fetal growth were seen *in utero* but appeared to be attenuated at birth. Despite this, the exposure to GDM *in utero* may have affected fetal metabolic programming and the increase in abdominal circumference observed prenatally may be associated with a

predisposition towards obesity later on. A longitudinal study assessing offspring exposed to GDM would provide researchers with novel insight into the long-term consequences of fetal exposure to GDM amongst black South African children. Of particular interest would be the assessment of fat mass in these individuals through methods including dual-energy x-ray absorptiometry (DEXA) and air displacement plethysmography (PeaPod® and BodPod®).

- **Assessing Insulin Profiles during Pregnancy**

Whilst plasma glucose levels are used to confirm or rule out a diagnosis of GDM, insulin insensitivity is the driving force behind the hyperglycaemia. Therefore, it would be of interest to investigate maternal fasting insulin levels in conjunction with fasting glucose levels during pregnancy. A Homeostasis Model Assessment (HOMA) estimate could be performed to indicate β-cell function and insulin sensitivity [351] and provide a deeper understanding of glucose metabolism during pregnancy.

- **Investigating Factors Associated with Gestational Diabetes Mellitus**

Now that the prevalence of GDM amongst Sowetan women has been established, further work is needed in order to understand the factors and comorbidities associated with GDM amongst this cohort of women. For example, the high rate of anaemia found amongst the GDM cohort (Chapter 5) was an interesting and unique finding. Whilst some suggestions were made as to why the high rate of anaemia was present, further investigation into this is warranted. Having a better understanding of what predisposes a woman to develop GDM and the pathophysiology of the condition thereafter can assist in targeting areas of interventions and management. The interaction of HIV status, obesity and anaemia on the development of GDM would be interesting to investigate as would be the genetic contribution towards GDM development. Given the genetic uniqueness of the black South African population, a GDM genetic association study would be of great interest.

7.7 Conclusion

In conclusion, this study has demonstrated that the prevalence of GDM amongst black South African women living in urban Soweto is concerning. It has also highlighted that a hyperglycaemic *in utero* environment does affect fetal growth with sexual dimorphism influencing susceptibility. The re-evaluation of current GDM selective screening policies and the improvement of antenatal care services are required in order to optimise maternal health during pregnancy amongst South African women.

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APPENDICES

APPENDIX A: AUTHORS' AGREEMENT

Authors' Agreement

By signing this declaration, the co-authors listed below agree to the use of the specified article by the student, Shelley Macaulay, as part of her Thesis.

1. **Macaulay, S.**, Dunger, D.B. & Norris, S.A. (2014) Gestational diabetes mellitus in Africa: A systematic review. *PLoS One*, 9 (6): e97871. doi:10.1371/journal.pone.

Co-author Name	Signature	Date
Professor David B. Dunger		20/03/2018
Professor Shane A. Norris		20/03/2018

2. **Macaulay, S.**, Ngobeni, M., Dunger, D.B. & Norris, S.A. (2018) The prevalence of gestational diabetes mellitus amongst black South African women is a public health concern. *Diabetes Research and Clinical Practice*, 139: 278-287. doi:10.1016/j.diabres.2018.03.012

Co-author Name	Signature	Date
Ms Martha Ngobeni		20/03/2018
Professor David B. Dunger		20/03/2018
Professor Shane A. Norris		20/03/2018

3. **Macaulay, S.**, Munthali, R.J., Dunger, D.B. & Norris, S.A. (2018) The effects of gestational diabetes mellitus on fetal growth and neonatal birth measures in an African cohort. *Diabetic Medicine.* Advanced online publication. doi: 10.1111/dme.13668. .

Co-author Name	**Signature**	**Date**
Dr Richard J. Munthali		20/03/2018
Professor David B. Dunger		20/03/2018
Professor Shane A. Norris		20/03/2018

4. **Macaulay, S.**, Buchmann, E.J. Dunger, D.B. & Norris, S.A. The reliability and validity of last menstrual period for gestational age estimation in a low-middle-income setting. Submitted to the *Journal of Obstetrics and Gynaecology Research.* Reviewed, revised and resubmitted.

Co-author Name	**Signature**	**Date**
Professor Eckhart J. Buchmann		20/03/2018
Professor David B. Dunger		20/03/2018
Professor Shane A. Norris		20/03/2018

APPENDIX B: INFORMATION AND CONSENT SHEETS

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

GESTATIONAL DIABETES SCREENING STUDY
INFORMATION SHEET

Hello, we are Shelley Macaulay, Martha Ngobeni and Professor Shane Norris from the Developmental Pathways for Health Research Unit and the University of the Witwatersrand and we are conducting a research study to investigate the prevalence of gestational diabetes in Soweto. Before you decide to participate, we would like you to understand why the research is being done, and what it would involve for you and your baby.

What is involved in the sub-study?

This study will be conducted at the MRC/Wits Developmental Pathways for Health Research Unit based at Chris Hani Baragwanath Academic Hospital (CHBAH). There are certain investigations that will be performed at our Research Unit, but you still need to go for your regular antenatal clinic and doctor checkups. If you agree to participate in the study we will:

- Collect blood samples from you for glucose metabolism.
- Ask you a series of questions related to yourself and your pregnancy.

Below are detailed procedures of what the study involves:

Interviewer-completed questionnaire

At your visit we will fill in a questionnaire with your help about your education, household circumstances, employment, your family and your health during your pregnancy. If you are uncomfortable about answering any of the questions you need not answer them. If you refuse to answer a question, you will not be penalised or lose any benefits to which you are entitled to in the study.

Measurements

We would like to measure your weight, height and blood pressure the visit.

The Oral Glucose Tolerance Test

When we eat certain foods our bodies break the food down into sugar (glucose) which provides us with energy. When our blood sugar levels rise our bodies produce a hormone called insulin to control the sugar levels. However, sometimes there can be too much sugar in our blood stream which can cause problems. One of the most common problems resulting from too much sugar is a condition called diabetes. Some women can develop diabetes during

pregnancy without ever having had it before (this is called gestational diabetes). Developing diabetes during pregnancy can impact the mother's and baby's health. Therefore, we want to determine your glucose and insulin levels.

When you are around 24-28 weeks (6-7 months) pregnant an oral glucose tolerance test (OGTT) will be performed on you. You will have to have fasted from 10pm the night before you come for this visit. You may drink water but must not eat any food or drink anything else. The test will involve you drinking approximately 1 cup of a sweet sugary drink. Before you drink the liquid a nurse will prick your finger to check your fasting blood sugar level and she will take a blood sample from a vein in your arm. You will then be given the sugary liquid to drink. This has to be drunk within 5 minutes. Thereafter, a blood sample will be taken from you 1 hour and 2 hours after you have swallowed the drink. As soon as this test is completed we will offer you a sandwich and a drink.

If your OGGT results suggest that you may have gestational diabetes, you will be telephonically contacted and asked to come in to our Unit to get a referral letter for the Obstetric Diabetes Clinic at CHBAH.

Possible risks

Sample of blood: You may experience discomfort, bleeding, and/or bruising. You may feel dizzy or faint.

What to do if you have problems: If it is discovered that you may have a health problem when your blood results or other results are received, you will be notified and the right health care practitioner to help you with your problem and treatment will be recommended.

Possible benefits:

If we find any problems during your visit we will refer you to CHBAH for management.

Costs to you:

Participation in the research will involve no cost to you. You will be given a sandwich and fruit juice once your measurements and assessments have been completed. You will also be given R100 for transport costs.

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.

Records of your participation in this research:

You have the right to privacy. The principal investigators will keep information about your participation in locked files. Your data collected will be labelled with a code to ensure privacy.

- Ethical approval: The Gestational Diabetes study, will submit a protocol to the University of Witwatersrand's, Human Research Ethics Committee (HREC)

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

Your questions:
The investigators listed on the first page of this form are available to answer your questions about this research. You may contact the investigators at any time on the following number (011) 933-1122. If you require any further information or have any questions/complaints about the study please contact the Human Research Ethics Committee of the University of the Witwatersrand: Chairperson Prof P Cleaton-Jones, Chairperson Tel 011 717 2301. Secretariat: Zanele Ndlovu and Langutani Masingi 011 717 1252/1234 zanele.ndlovu@wits.ac.za or langutani.masingi@wits.ac.za

YOU WILL HAVE A COPY OF THIS INFORMATION SHEET TO KEEP

If you are happy to take part in the study please read and sign the attached consent form and contact us to confirm your participation.

Your signature on the consent form certifies the following:
· You have read the information provided in this consent form.
· You have received answers to all of your questions.
· You have freely decided to participate in this research.
· 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

GESTATIONAL DIABETES SCREENING STUDY
CONSENT SHEET

I agree to myself being a participant in the study. The goals and methods of the study are clear to me. I understand that the study will involve a visit which will include an interview, a glucose tolerance test and blood taking. All the details and purposes of this study have been explained to me. I understand that I have the right to refuse to participate in the study. I agree to participation in the study on the condition that:

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

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

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

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

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

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

7. I will receive a referral note to a health service if any result is out of the normal range or a problem is detected during the course of the study.

PARTICIPANT

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

RESEARCH ASSISTANT

Printed Name	**Signature**	**Date and Time**

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

SOWETO FIRST 1000 DAYS STUDY (S1000)
(FETAL GROWTH STUDY)
INFORMATION SHEET

Hello, my name is Professor Shane Norris, and I am a Researcher for the Department of Paediatrics at the University of Witwatersrand. Together with other colleagues from the University we will be conducting a research study to investigate the effects of nutrition on fetal growth and development. Before you decide to participate, we would like you to understand why the research is being done, and what it would involve for you and your baby.

What is involved in the study?

The study will be conducted at our offices in Chris Hani Baragwanath Hospital (MRC/Wits Developmental Pathways for Health Research Unit). For this study there are certain investigations that we will be performing at our offices, but you will still get your routine antenatal management and care at your local antenatal clinic.

If you agree to take part in the study, we will collect the following research data:

- 6 ultrasound scans at 4-6 weekly intervals. At each scan, we will measure your baby, and the blood flow to the placenta and the baby. Each ultrasound scan session will take approximately 30-60 minutes.
- Blood samples for micronutrients and glucose metabolism.
- Ask you a series of questions related to yourself and your pregnancy.

When you enroll in our study we will assign a Research Nurse to you so that you can contact her if you require any further advice or assistance during your pregnancy. You will also be required to contact the nurse when you go into labour so that she can arrange to be present at the delivery of your baby. At the delivery, the research nurse will take a cord blood sample, a faecal sample and your placenta for investigations. When your baby is born, we would like to weigh your baby and measure his/her length and head circumference. This is to help us better understand how babies grow.

Procedures

Interviewer-completed questionnaires

At each visit at our offices, we will fill in some questionnaires with your help about your education, household circumstances and employment, your physical activity and dietary intake, events that have recently happened in your life and your health. We will also ask you questions around your pregnancy planning and menstrual and obstetric histories as well as

some questions about your family. If you are uncomfortable about answering any of the questions you need not answer them.

Blood taking

A nurse will collect blood from you (from a vein in your arm) at these visits:

1. Visit 1: (±15 ml total; 3 teaspoons of blood) for biochemical analysis (e.g. Vitamin D, folate) and DNA analysis (see separate information and consent sheet).
2. Visit 4: (±25 ml total; 5 teaspoons) for glucose, insulin, and biochemical analysis (e.g. lipids and Vitamin D analysis).
3. Visit 6: (±10 ml total; 2 teaspoons) for DNA and RNA analysis (see separate consent sheet).

Sometimes when blood is taken you may feel a prick at the place where the needle enters your body. Afterwards there may be some slight bruising. Sterile, disposable syringes will be used once only so there is no chance of infection. This procedure is safe and there is only a slight prick as the needle is placed through the skin. There will be no charge for these blood tests. The results from the blood tests will be absolutely confidential; this means a code will be used instead of your name. We will tell you the results of your blood tests and explain them in detail so that you can understand what they mean. If the results indicate there is any health concern we will assist in referring you to the appropriate doctors.

Haemoglobin

At each visit a nurse will perform a finger prick test on you to check your haemoglobin levels. Haemoglobin is a protein that carries oxygen in your blood.

Ultrasound

We would like to perform 5 ultrasounds (sonar examinations) on your baby at various stages during your pregnancy. Ultrasound measurements are safe and carry no risk to you or your baby. If any problems are detected on ultrasound we will refer you to the appropriate doctors at Chris Hani Baragwanath Hospital. It is important to realise that ultrasound is a screening technique, which means that it is not 100% effective in detecting fetal abnormalities and so sometimes problems with the baby can be missed.

The Oral Glucose Tolerance Test

When we eat certain foods our bodies break the food down into sugar (glucose) which provides us with energy. When our blood sugar levels rise our bodies produce a hormone called insulin to control the sugar levels. However, sometimes there can be too much sugar in our blood stream which can cause problems. One of the most common problems resulting from too much sugar is a condition called diabetes. Some women can develop diabetes during pregnancy without ever having had it before (this is called gestational diabetes). Developing diabetes during pregnancy can impact the mother's and baby's health. Therefore, we want to determine your glucose and insulin levels.

When you are around 24-28 weeks (6-7 months) pregnant an oral glucose tolerance test (OGTT) will be performed on you. You will have to have fasted from 10pm the night before you come for this visit. You may drink water but must not eat any food or drink anything else.

The test will involve you drinking approximately 1 cup of a sweet sugary drink. Before you drink the liquid a nurse will prick your finger to check your fasting blood sugar level and she will take two blood samples from a vein in your arm (one for glucose and one for insulin testing). You will then be given the sugary liquid to drink. This has to be drunk within 5 minutes. Thereafter, two blood samples will be taken from you at 30 minutes, 1 hour and 2 hours after you have swallowed the drink.

As soon as this test is completed we will offer you a sandwich and a drink.

Delivery Process

You need to be booked into your local antenatal clinic for regular antenatal care and management. Usually, you will deliver your baby at the clinic at which you received your antenatal care. You will have a research nurse available to you throughout your pregnancy. Once you are ready to be admitted to hospital/clinic, you will need to contact the research nurse. If you deliver at CHBAH the research nurse will be present at your delivery. Once you have delivered your baby we will collect some biological samples; cord blood, a faecal sample and the placenta, and we would also like to measure your baby's weight, length and head circumference.

PeaPod

The PeaPod is a machine that determines infant body composition. The machine is safe and non-invasive. The process entails the baby being placed into a warmed test chamber where body mass and body volume is measured. The baby will lie inside the chamber for a few minutes and you will be able to see him/her through a glass lid. PeaPod assessments will be done as soon after delivery as possible.

Possible risks

Sample of blood: You may experience discomfort, bleeding, and/or bruising. You may feel dizzy or faint.

What to do if you have problems: If it is discovered that you may have a health problem when your blood results or other results are received, you will be notified and the right health care practitioner to help you with your problem and treatment will be recommended.

Possible benefits:

You will have 6 ultrasound scans which monitor the growth of your baby. If we find any problems during follow-up visits we will refer you to back to your antenatal clinic for further management and referral.

Costs to you:

Collecting a sample of blood and testing it in a research laboratory will not cost you anything. You will be given a sandwich and fruit juice once your measurements and assessments have been completed. You will also be given R50 for transport costs.

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.

Records of your participation in this research:
You have the right to privacy. The principal investigator will keep information about your participation in locked files. Your samples will be labelled with a code to ensure your privacy.

- Ethical approval: This study protocol has been submitted to the University of the Witwatersrand's, Human Research Ethics Committee (HREC), and written approval has been granted by that committee. Ethics Clearance number: M120524

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

Your questions:
The investigator listed on the first page of this form is available to answer your questions about this research. You may contact the investigator at any time on the following number (011) 933-1122. If you require any further information or have any questions/complaints about the study please contact the Human Research Ethics Committee of the University of the Witwatersrand on (011) 717-1234 or anisa.keshav@wits.ac.za

YOU WILL HAVE A COPY OF THIS INFORMATION SHEET TO KEEP

If you are happy to take part in the study please read and sign the attached consent form and contact us to confirm your participation.

Your signature on the consent form certifies the following:
· You have read the information provided in this consent form
· You have received answers to all of your questions.
· You have freely decided to participate in this research.
· 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

SOWETO FIRST 1000 DAYS STUDY (S1000)
CONSENT SHEET

I agree to myself being a participant in the study. The goals and methods of the study are clear to me. I understand that the study will involve interviews, measurements, collection of biological samples, blood taking, an oral glucose tolerance test and ultrasound examinations. All the details and purposes of this study have been explained to me. I understand that I have the right to refuse to participate in the study. I agree to participation in the study on the condition that:

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

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

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

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

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

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

7. I will receive a referral note to a health service if any result is out of the normal range or a problem is detected during the course of the study.

PARTICIPANT

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

RESEARCH ASSISTANT

Printed Name	**Signature**	**Date and Time**

APPENDIX C: PHD DATA COLLECTION SHEETS

GLUCOSE METABOLISM AND PREGNANCY

IN SOUTH AFRICAN WOMEN STUDY

DPHRU
MRC/Wits Developmental Pathways for Health Research Unit

GESTATIONAL DIABETES SCREENING STUDY

Visit date: DD/MM/YYYY

Subject code: _____

DEMOGRAPHICS

1) Age of participant (years): ☐☐

2) Marital status:

Single	☐	Widowed	☐
Married/Cohabiting	☐	Separated/Divorced	☐

3) Total number of years of formal education: ☐☐

4) Highest level of education attended:

No school attended	☐	Professional/technical training	☐
Primary	☐	University	☐
Secondary	☐		

HOUSEHOLD SOCIOECONOMIC STATUS

5) Does the woman's household have or own any of the following: (cross the boxes that apply)

Electricity	☐	Cell phone	☐	Bicycle	☐
Radio	☐	Personal computer	☐	Motorcycle/scooter	☐
Television	☐	Farm animals	☐	Car/Truck/Tractor	☐
Refrigerator	☐	Agricultural land	☐		

MATERNAL HEALTH

6) Height: ☐☐☐ . ☐ cm

7) Weight (at this visit): ☐☐☐ . ☐ kg

8) Blood pressure (reading 1): Systolic ☐☐☐ mmHg

Diastolic ☐☐☐ mmHg

Blood pressure (reading 2): Systolic ☐☐☐ mmHg

Diastolic ☐☐☐ mmHg

Blood pressure (reading 3): Systolic ☐☐☐ mmHg

Diastolic ☐☐☐ mmHg

9) HIV status: Positive ☐ Negative ☐

10) Haemoglobin level: ☐☐ . ☐ g/l

FAMILY HISTORY OF DIABETES

11) Does anyone in your family have diabetes? (Blood relatives only) If no, skip to 12

Yes ☐ No ☐

11.1) If yes, which of the following family members have/had diabetes?

Mother ☐

Father ☐

Sister (s) ☐

Brother (s) ☐

Maternal grandmother ☐

Maternal grandfather ☐

Paternal grandmother ☐

Paternal grandfather ☐

OBSTETRIC HISTORY

12) Gestational age according to ultrasound: ☐☐ weeks ☐ days

13) Number of previous pregnancies excluding this pregnancy (if 0, ignore): ☐☐

14) Date of last delivery, miscarriage or termination: _____DD/MM/YYYY_____

15) Number of previous miscarriages: ☐☐

16) Number of previous births: ☐☐

17) Did any of her previous babies weigh 4 kg or more at birth Yes ☐ No ☐

17.1) If YES, record the weights and sex of the baby/ies:

Weight:_____ Male ☐ Female ☐

Weight:_____ Male ☐ Female ☐

Weight:_____ Male ☐ Female ☐

Weight:_____ Male ☐ Female ☐

----------END OF GDM SCREENING STUDY DATA COLLECTION SHEET----------

GLUCOSE METABOLISM AND PREGNANCY IN SOUTH AFRICAN WOMEN STUDY

S1000 STUDY: FETAL GROWTH & NEONATAL OUTCOMES

Visit date: DD/MM/YYYY

Subject code: _____

DEMOGRAPHICS

1) Age of participant (years): ☐☐

2) Marital status:

Single	☐	Widowed	☐
Married/Cohabiting	☐	Separated/Divorced	☐

3) Total number of years of formal education ☐☐

4) Highest level of education attended:

No school attended	☐	Professional/technical training	☐
Primary	☐	University	☐
Secondary	☐		

HOUSEHOLD SOCIOECONOMIC STATUS

5) Does the woman's household have or own any of the following: (cross the boxes that apply)

Electricity	☐	Cell phone	☐	Bicycle	☐
Radio	☐	Personal computer	☐	Motorcycle/scooter	☐
Television	☐	Farm animals	☐	Car/Truck/Tractor	☐
Refrigerator	☐	Agricultural land	☐		

MATERNAL HEALTH

6) Height: ☐☐☐ ☐ . cm

7) Weight (at this visit): ☐☐☐ . ☐ kg

8) Blood pressure (reading 1): Systolic ☐☐☐ mmHg

Diastolic ☐☐☐ mmHg

Blood pressure (reading 2): Systolic ☐☐☐ mmHg

Diastolic ☐☐☐ mmHg

Blood pressure (reading 3): Systolic ☐☐☐ mmHg

Diastolic ☐☐☐ mmHg

9) HIV status : Positive ☐ Negative ☐

FAMILY HISTORYOF DIABETES

10) Does anyone in your family have diabetes? (Blood relatives only) If no, skip to 11

Yes ☐ No ☐

10.1) If yes, which of the following family members have/had diabetes?

Mother ☐

Father ☐

Sister (s) ☐

Brother (s) ☐

Maternal grandmother ☐

Maternal grandfather ☐

Paternal grandmother ☐

Paternal grandfather ☐

OBSTETRIC HISTORY

11) Number of previous pregnancies excluding this pregnancy ☐☐

12) Date of last delivery, miscarriage or termination: _____DD/MM/YYYY_____

13) Number of previous miscarriages: ☐☐

14) Number of previous births: ☐☐

15) Did any of her previous babies weigh 4 kg or more at birth? Yes ☐ No ☐

15.1) If YES, record the weights and sex of the baby/ies:

Weight:_____ Male ☐ Female ☐

Weight:_____ Male ☐ Female ☐

Weight:_____ Male ☐ Female ☐

Weight:_____ Male ☐ Female ☐

Weight:_____ Male ☐ Female ☐

Weight:_____ Male ☐ Female ☐

CURRENT PREGNANCY DATING

Last Menstrual Period

16) Is the first day of the last menstrual period known? Yes ☐ No ☐

17) If yes, date of LMP: _____DD/MM/YYYY_____

18) Was she certain of the date of her LMP? Yes ☐ No ☐

19) Estimated gestational age by LMP : ☐☐ weeks ☐ days

Fetal Ultrasound Dating Scan

20) Crown-rump Length (CRL) measurement: ☐☐☐ . ☐ mm

21) Estimated gestational age by CRL: ☐☐ weeks ☐ days

22) Biparietal diameter (BPD): ☐☐ . ☐ cm

23) Head circumference (HC): ☐☐ . ☐ cm

24) Abdominal circumference (AC): ☐☐ . ☐ cm

25) Femur length (FL): ☐☐ . ☐ cm

26) Gestational age based on ultrasound: ☐☐ weeks ☐ days

----------END OF STUDY ENTRY (VISIT 1) DATA COLLECTION SHEET----------

GLUCOSE METABOLISM AND PREGNANCY IN SOUTH AFRICAN WOMEN STUDY

PREGNANCY FOLLOW-UP & ULTRASOUND: VISITS 2, 3, 4, 5 & 6

Visit number: _____

Visit date: _____DD/MM/YYYY_____

Subject code: _____DD/MM/YYYY_____

PREGNANCY FOLLOW-UP

1) Weight (at this visit): ☐☐☐ . ☐ kg

ULTRASOUND EXAMINATION

Evaluate & record the following:

2) Biparietal diameter (BPD): ☐☐ . ☐ cm

3) Head circumference (HC): ☐☐ . ☐ cm

4) Abdominal circumference (AC): ☐☐ . ☐ cm

5) Femur length (FL): ☐☐ . ☐ cm

6) Gestational age based on ultrasound: ☐☐ weeks ☐ days

----------END OF PREGNANCY FOLLOW-UP & ULTRASOUND DATA COLLECTION SHEET--------

GLUCOSE METABOLISM AND PREGNANCY IN SOUTH AFRICAN WOMEN STUDY

ORAL GLUCOSE TOLERANCE TEST (VISIT 4)

Visit date: _____DD/MM/YYYY_____

Subject code: _____

ORAL GLUCOSE TOLERANCE TEST (OGTT)

1) Has she eaten or drunk anything, other than water, since 10pm last night?

   Yes ☐ No ☐

   If, NO, proceed with a finger prick glucose test

   If, YES, reschedule another appointment for an OGTT is she is < 28 weeks pregnant

2) Using the Accu-Chek® device and a new sterile needle, prick the woman's finger and test her fasting capillary glucose levels

   2.1) Record the reading ☐☐ . ☐ mmol/l and follow the instructions as stipulated by the OGTT Standard Operating Procedure

3) Once the glucose blood samples have been run in the laboratory, enter the readings below:

   3.1) Fasting plasma glucose reading: ☐☐. ☐ mmol/l

   3.2) One hour post-glucose load blood glucose reading: ☐☐. ☐ mmol/l

   3.3) Two hours post-glucose load blood glucose reading: ☐☐ . ☐ mmol/l

----------END OF OGTT DATA COLLECTION SHEET ----------

GLUCOSE METABOLISM AND PREGNANCY IN SOUTH AFRICAN WOMEN STUDY

DELIVERY

Date of assessment: _____

Mother's subject code: _____

BIRTH DETAILS

1) Mode of delivery:

   Vaginal spontaneous ☐ Vaginal assisted (e.g. forceps, vacuum) ☐

   Caesarean section ☐ Assisted breech or breech extraction ☐

2) Newborn sex: Male ☐ Female ☐

3) Date of delivery: _____DD/MM/YYYY_____

4) Gestational age based on the best obstetric estimate: ☐☐ weeks ☐ days

5) Newborn status at birth:

   Alive ☐ Intrapartum death ☐ Antepartum death ☐

ANTHROPOMETRIC MEASUREMENTS

(to be obtained within 24 hours of the birth)

First set		**Second set**	
Weight:	☐☐☐☐ g	Weight:	☐☐☐☐ g
Length:	☐☐.☐ cm	Length:	☐☐.☐ cm
Head circumference:	☐☐.☐ cm	Head circumference:	☐☐.☐ cm

----------END OF DELIVERY DATA COLLECTION SHEET----------

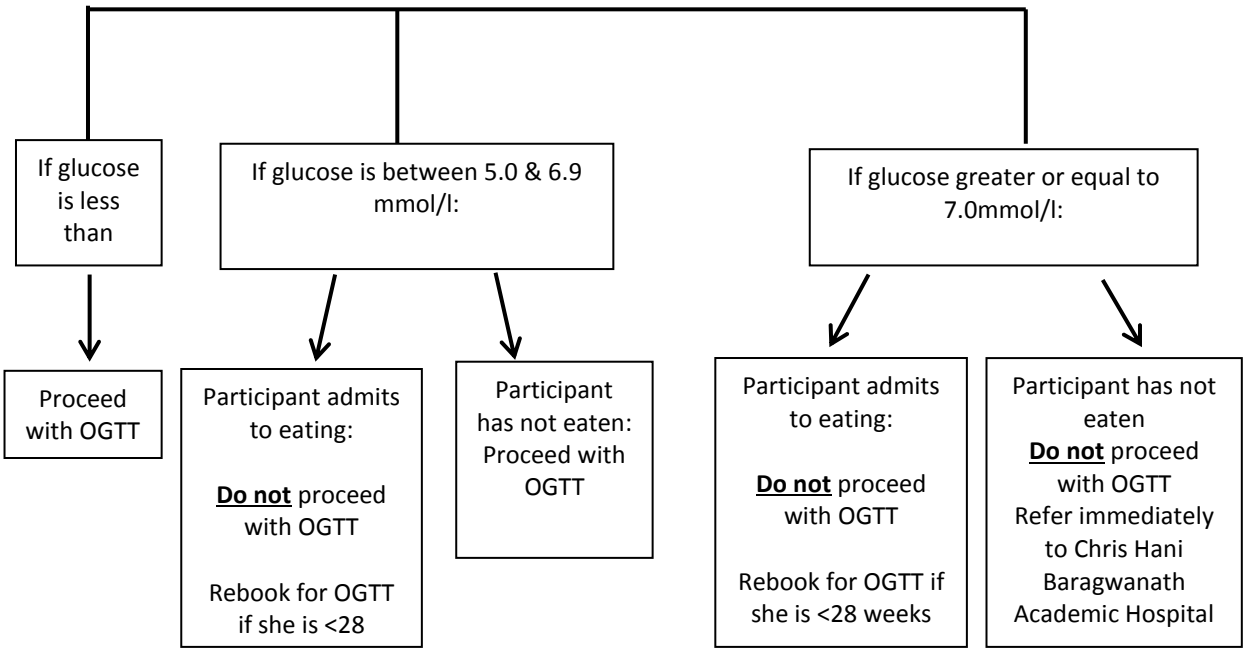
APPENDIX D: ORAL GLUCOSE TOLERANCE TEST PROCEDURE

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

STANDARD OPERATING PROCEDURE (SOP) FOR THE ORAL GLUCOSE TOLERANCE TEST (OGTT)

1. Make sure the pregnant woman is between 24-28 weeks gestation
2. Make sure she has fasted overnight (no food or drink (other than water) from 22h00 the night before)
3. Explain the process and purpose of the OGTT to the woman
4. Do a finger prick glucose test to check her fasting capillary glucose levels and refer to the flow chart below:



5. If finger prick results indicate that the OGTT can proceed insert a catheter into a vein of the woman and proceed with the following steps

6. Mix the glucose drink
    a. One box (75 g) of glucose powder mixed with 250 ml of luke warm water (luke warm water allows the glucose to dissolve better)
    b. Make sure you have a stop watch/clock
    c. Make sure the woman is comfortably seated
    d. Store the glucose drink on the side whilst you proceed with Step 7

7. Take a **fasting** blood glucose sample in a grey vacutainer (4 ml)
    a. Make sure the tube is full of blood
    b. Label the tube with the woman's study ID and **"O minutes glucose"**

8. Give the woman the glucose drink and make sure she drinks it within 5 minutes (watch the clock)

9. Once she has finished drinking the drink start your stop watch

10. Keep an eye on the clock. When it reaches **60 minutes** (one hour from when the woman finished drinking the glucose drink) take a glucose blood sample. Do not stop the stop watch, let it continue running.
    a. Glucose in a grey 4 ml vacutainer
    b. Label the tube with the woman's study ID and **"60 minutes glucose"**

11. Keep an eye on the clock. When it reaches **120 minutes** (two hours from when the woman finished drinking the glucose drink) take a glucose and insulin blood sample. Do not stop the stop watch, let it continue running.
    a. Glucose in a grey 4 ml vacutainer
    b. Label the tubes with the woman's study ID and **"120 minutes glucose"**

12. Remove the catheter

13. Ensure the woman is then given something to drink and eat

----------END OF OGTT SOP----------

APPENDIX E: ETHICS CLEARANCE CERTIFICATES

PhD Study's Ethics Clearance Certificate

R14/49 Miss Shelley Macaulay

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130309

NAME: (Principal Investigator)	Miss Shelley Macaulay
DEPARTMENT:	Developmental pathways Researc Unit CH Baragwanath Academic Hospital
PROJECT TITLE:	Glucose Metabolism and Pregnancy in South African Women
DATE CONSIDERED:	05/04/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shane Norris
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 17/05/2013

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

DECLARATION OF INVESTIGATORS

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

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

Principal Investigator Signature

Date 18/05/2013

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Soweto First 1000 Days Study Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Professor Shane Norris

CLEARANCE CERTIFICATE	**M120524**
PROJECT	Foetal Growth Study: Investigating Maternal Factors Associated with Foetal Growth and Delivery Outcomes
INVESTIGATORS	Professor Shane Norris.
DEPARTMENT	Developmental Pathways Research Unit
DATE CONSIDERED	25/05/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 14/07/2013 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

GDM Screening Study Ethics Clearance Certificate

R14/49 Martha Ngobeni

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150461

NAME: (Principal Investigator)	Martha Ngobeni
DEPARTMENT:	Paediatrics MRC/Wits Development Pathways for Health Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Growth and Body Composition of Infants Born to Mothers with, and those without, Gestatinal Diabetes Mellitus
DATE CONSIDERED:	24/04/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shane Norris and Shelley Macaulay
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/06/2015

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

DECLARATION OF INVESTIGATORS

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

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

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX F: PRIMSA GUIDELINES FOR SYSTEMATIC REVIEWS

Section/topic	#	Checklist item	Reported in section
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract (in published paper)
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Methods
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Methods
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Methods & Fig. 3.1

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Methods
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Methods
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Methods
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	N/A

APPENDIX G: LIST OF AFRICAN COUNTRIES

(Taken from: http://www.worldatlas.com)

1. Algeria
2. Angola
3. Benin
4. Botswana
5. Burkina
6. Burundi
7. Cameroon
8. Cape Verde
9. Central African Republic
10. Chad
11. Comoros
12. Congo
13. Congo, Democratic Republic of
14. Djibouti
15. Egypt
16. Equatorial Guinea
17. Eritrea
18. Ethiopia
19. Gabon
20. Gambia
21. Ghana
22. Guinea
23. Guinea-Bissau
24. Ivory Coast
25. Kenya
26. Lesotho
27. Liberia
28. Libya
29. Madagascar
30. Malawi
31. Mali
32. Mauritania
33. Mauritius
34. Morocco
35. Mozambique
36. Namibia
37. Niger
38. Nigeria
39. Rwanda
40. Sao Tome and Principe
41. Senegal
42. Seychelles
43. Sierra Leone
44. Somalia
45. South Africa
46. South Sudan
47. Sudan
48. Swaziland
49. Tanzania
50. Togo
51. Tunisia
52. Uganda
53. Zambia
54. Zimbabwe

APPENDIX H: STROBE CHECKLIST

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

APPENDIX I: RISK OF BIAS TOOL FOR PREVALENCE STUDIES

Adapted from the Risk of Bias Tool for Prevalence Studies developed by Hoy et al. [229]

Risk of Bias Item	**Answer:** **Yes (Low Risk) or No (High risk)**
External Validity	
1. Was the study target population a close representation of the national pregnant population in relation to relevant variables?	
2. Was the sampling frame a true or close representation of the target population?	
3. Was some form of random selection used to select the sample, OR, was a census undertaken?	
4. Was the likelihood of non-participation bias minimal?	
Internal Validity	
5. Were data collected directly from the subjects? (as opposed to medical records)	
6. Were acceptable diagnostic criteria for GDM used?	
7. Was a reliable and accepted method of testing for GDM utilised?	
8. Was the same mode of data collection used for all subjects?	
9. Was GDM tested for within the advised gestational period of 24-28 weeks?	
10. Were the numerator(s) and denominator(s) for the calculation of the prevalence of GDM appropriate?	
11. Summary item on the overall risk of study bias LOW RISK OF BIAS: 8 or more "yes" answers. Further research is very unlikely to change our confidence in the estimate. MODERATE RISK OF BIAS: 6 to 7 "yes" answers. Further research is likely to have an important impact on our confidence in the estimate and may change the estimate. HIGH RISK OF BIAS: 5 or fewer "yes" answers. Further research is very likely to have an important impact on our confidence in the estimate and is likely to change the estimate.	

APPENDIX J: PUBLISHED PAPERS

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PLOS ONE

Gestational Diabetes Mellitus in Africa: A Systematic Review

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Abstract

Background: Gestational diabetes mellitus (GDM) is any degree of impaired glucose tolerance first recognised during pregnancy. Most women with GDM revert to normal glucose metabolism after delivery of their babies; however, they are at risk of developing type 2 diabetes later in life as are their offspring. Determining a country's GDM prevalence can assist with policy guidelines regarding GDM screening and management, and can highlight areas requiring research. This systematic review assesses GDM prevalence in Africa.

Methods and Findings: Three electronic databases were searched without language restrictions; PubMed, Scopus and the Cochrane Library. Thirty-one search terms were searched. Eligible articles defined GDM, stated what GDM screening approaches were employed and reported GDM prevalence. The reporting quality and risk of bias within each study was assessed. The PRISMA guidelines for systematic reviews were followed. The literature search identified 466 unique records. Sixty full text articles were reviewed of which 14 were included in the systematic review. One abstract, for which the full text article could not be obtained, was also included. Information regarding GDM classification, screening methods and prevalence was obtained for six African countries; Ethiopia (n = 1), Morocco (n = 1), Mozambique (n = 1), Nigeria (n = 6), South Africa (n = 4) and Tanzania (n = 1). Prevalence figures ranged from 0% (Tanzania) to 13.9% (Nigeria) with some studies focussing on women with GDM risk factors. Most studies utilised the two hour 75 g oral glucose tolerance test and applied the World Health Organization's diagnostic criteria.

Conclusions: Six countries, equating to 11% of the African continent, were represented in this systematic review. This indicates how little is known about GDM in Africa and highlights the need for further research. Considering the increasing public health burden of obesity and type 2 diabetes, it is essential that the extent of GDM is understood in Africa to allow for effective intervention programmes.

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Introduction

Diabetes mellitus (DM) is a group of conditions that contribute significantly to the increasing health and financial burden in many countries around the world [1]. The prevalence of and screening methods for the clinical subgroups, type 1 diabetes mellitus and type 2 diabetes mellitus, are relatively well researched and understood in most countries. However, those pertaining to the subgroup known as gestational diabetes mellitus (GDM) are less established [2]. Gestational diabetes mellitus is defined by the World Health Organization as being "any degree of glucose intolerance with onset or first recognition during pregnancy" and should therefore include glucose readings that fall within the impaired glucose tolerance (IGT) diagnostic range, as well as those within the diagnostic range for diabetes [3,4]. More recently, the American Diabetes Association defines GDM as "diabetes diagnosed during pregnancy that is not clearly overt diabetes" [5].

Pregnancy itself induces changes in maternal glucose metabolism and insulin sensitivity. As pregnancy progresses the demand for insulin production on the mother's pancreas increases. In most instances, pregnant women are able to meet the increased insulin demand but in some cases these needs are not met resulting in poor glycaemic control and consequently GDM. Certain factors including having a family history of diabetes, being over 25 years of age, being obese, belonging to a particular ethnic group (African American, Hispanic, Indian) and having previously given birth to a baby weighing 4 kg or more (macrosomia), put women at greater risk of developing GDM [6,7].

Pregnancies affected by GDM pose a risk for adversities such as the need for Caesarean sections due to fetal macrosomia. Macrosomia occurs as a result of accelerated fetal growth fuelled by maternal hyperglycaemia [8]. In approximately 95% of GDM cases maternal glucose metabolism returns to normal after delivery of the baby [9], however, an association between GDM and the development of type 2 diabetes mellitus in the mother later in life exists [10,11]. In addition, research into the long term effects of poor maternal glucose metabolism on the fetus has revealed that offspring born to mothers with GDM are susceptible to IGT and

obesity [12,13]. With these associations in mind it would be important to identify pregnant women at risk for GDM so that prevention management such as lifestyle modifications can be implemented [14].

Consensus regarding screening for and classification of GDM is yet to be achieved globally [2]. The most recognised diagnostic test for GDM is the oral glucose tolerance test (OGTT) usually performed between 24–28 weeks gestation [15]. Different screening regimes for GDM exist and as a result studies investigating prevalence of GDM are often diverse in terms of methods employed, cut-off values used and consequently, results obtained [16]. Table 1 summarises some of the different screening regimes and respective glucose cut-off values used to diagnose GDM.

Not only do different testing methods exist but the availability of GDM screening differs from country to country and even within countries. Although it would be ideal to screen every pregnant woman for GDM it is not always feasible from a cost perspective, particularly in low- or middle-income countries (LMICs). In many LMICs, and some high income countries, women tend to be selected for screening only if they fulfil certain GDM risk-associated criteria [17]. Due to this selective screening process one may expect the true extent of GDM in such countries to remain relatively unknown. Furthermore, prevalence rates may be dependent upon the specificity and sensitivity of the selective screening process in identifying at- risk women.

The effects of urbanisation have not only had a profound impact on developing countries' economies but also on public health. The transition from rural to urban ways of life is often associated with changes in eating habits, body mass and composition, and reduction in physical activity. The movement towards more Westernised diets involves increased consumption of fats, sugars and refined carbohydrates. As a result, LMICs are experiencing a rapid increase in overweight and obesity as well as non-communicable diseases, such as diabetes, that accompany such conditions [1,18]. Considering this, the prevalence of GDM should be increasing too. Reported prevalence figures for GDM in two high income countries, the United Kingdom and the United States of America, are 2–3% and 2–10% respectively [17]. A study that assessed GDM in the south of India, a LMIC, reported a far greater prevalence of 13.9% [19]. Gestational diabetes mellitus prevalence estimates for another LMIC, Brazil, are thought to be 7.0–7.6% [17].

Diabetes was essentially unknown in Africa in 1901, yet in 2013 19.8 million people were reportedly living with the condition and this number is predicted to increase to 41.5 million in 2035 equating to a 109% increase [20]. In Africa, the movement from a rural lifestyle to a more industrial urbanised way of life is largely responsible for the evolving problem of chronic diseases, of which diabetes is a major contributor [21].

The explosion in the prevalence of diabetes undoubtedly represents a serious public health burden. In addition, it is more than likely to bring along with it a considerable increase in GDM. However, with regards to GDM in Africa, the situation appears relatively unknown. From a cost perspective, many African countries employ a selective screening approach for GDM and the estimated percentage of pregnant women screened is unclear [17]. In order to suggest policy changes regarding screening for GDM, which will ultimately prevent the effects of GDM on the mother and her offspring and in turn reduce the financial and health burden to a country, it is essential that the extent of the condition is well understood. Therefore, we performed a systematic search to identify research into diagnostic strategies, screening approaches and reported GDM prevalence figures on the African continent.

Methods

Protocol and Registration

This project was not prospectively registered. A protocol was developed during the planning process.

Information Sources and Search Strategy

The PRISMA guidelines (Checklist S1) for the reporting of systematic reviews were followed [22]. Two authors (SM and SAN) independently performed a literature search using three electronic databases; PubMed, Scopus and the Cochrane Library. The following search terms and combinations were used: "gestational diabetes" and Africa; "impaired fasting glucose" and pregnancy and Africa; diabetes and pregnancy and Africa; "impaired glucose tolerance" and pregnancy and Africa; "gestational diabetes" and "African countries." In addition, the search terms "gestational diabetes," together with the names of each individual country in Africa were used. For example, "gestational diabetes" and Egypt; "gestational diabetes" and Namibia; "gestational diabetes" and "South Africa" were entered into the search. The list of all 54 recognised African countries included in the search can be found in Appendix S1. Finally, "gestational diabetes" and "Sub-Saharan Africa" were searched for. Where possible, filters were set for studies pertaining to humans but articles written in all languages were included. The search was performed in September 2013. No time limits were set in an attempt to gather all articles published up until the end of September 2013. Once duplicate references were removed the titles and abstracts of the references were screened.

Studies pertaining to African countries that included the following were considered relevant:

1) Screening methods for GDM
2) Criteria used to diagnose GDM
3) Prevalence of GDM

If an article failed to mention any of the above three points it was excluded. In addition, studies were excluded if they were:

1) On type 1 and/or type 2 diabetes only
2) Overviews of GDM
3) Editorials
4) Molecular studies
5) Solely on the outcomes and/or problems associated with macrosomic infants with no reference to GDM prevalence and screening
6) Focussed on perinatal mortality and congenital abnormality rates in babies born to mothers with diabetes
7) Solely comparisons of GDM testing regimes

Data Extraction

Full text articles were obtained and reviewed. Data were then extracted regarding country, region (rural/urban), population group, sample size, age of pregnant women in the cohort, gestational age, how the investigators defined GDM, how they tested for GDM and what GDM prevalence was reported. In addition, data were also extracted from abstracts that included how GDM was screened for, what criteria were used and what prevalence figures were obtained in the study but for which full text articles could not be obtained.

Table 1. The different diagnostic criteria available for the diagnosis of gestational diabetes mellitus.

Group/Organisation	Screening test	Diagnostic criteria: blood glucose level thresholds
American Diabetes Association [5,52]	One step: 2 hr 75 g OGTT	At least one of the following must be met:
		Fasting: ≥5.1 mmol/l (92 mg/dl)
		1 hr: ≥10.0 mmol/l (180 mg/dl)
		2 hr: ≥8.5 mmol/l (153 mg/dl)
	OR Two step:	OR
	1) 1 hr 50 g (non-fasting) screen	If 1 hr: ≥10.0 mmol/l (180 mg/dl) proceed with step 2
	2) 3 hr 100 g OGTT	3 hr: ≥7.8 mmol/l (140 mg/dl)
Carpenter and Coustan [53]	3 hr 100 g OGTT	At least two of the following must be met:
		Fasting: ≥5.3 mmol/l (95.4 mg/dl)
		1 hr: ≥10.0 mmol/l (180 mg/dl)
		2 hr: ≥8.6 mmol/l (154.8 mg/dl)
		3 hr: ≥7.8 mmol/l (140 mg/dl)
Diabetes Pregnancy Study Group (DPSG) of the European Association for the Study of Diabetes (EASD) [54]	2 hr 75 g OGTT	Fasting: >5.2 mmol/l (93.6 mg/dl)
		OR
		2 hr: >9.0 mmol/l (162 mg/dl)
International Association of Diabetes and Pregnancy Study Groups (IADPSG) [42]	2 hr 75 g OGTT	At least one of the following must be met:
		Fasting: ≥5.1 mmol/l (92 mg/dl)
		1 hr: ≥10.0 mmol/l (180 mg/dl)
		2 hr: ≥8.5 mmol/l (153 mg/dl)
National Diabetes Data Group (NDDG) (1979) [55]	3 hr 100 g OGTT	At least two of the following must be met:
		Fasting: ≥5.8 mmol/l (105 mg/dl)
		1 hr: ≥10.6 mmol/l (190 mg/dl)
		2 hr: ≥9.2 mmol/l (165 mg/dl)
		3 hr: ≥8.0 mmol/l (145 mg/dl)
World Health Organization (1985) [56]	2 hr 75 g OGTT	Fasting: ≥7.8 mmol/l (140 mg/dl)
		OR
		2 hr: ≥7.8 mmol/l (140 mg/dl)
World Health Organization (1999) [4]	2 hr 75 g OGTT	Fasting: ≥7.0 mmol/l (126 mg/dl)
		OR
		2 hr: ≥7.8 mmol/l (140 mg/dl)
World Health Organization (2013) [20]	2 hr 75 g OGTT	At least one of the following must be met:
		Fasting: 5.1–6.9 mmol/l (92–125 mg/dl)
		1 hr: ≥10.0 mmol/l (180 mg/dl)
		2 hr: 8.5–11.0 mmol/l (153–199 mg/dl)

doi:10.1371/journal.pone.0097871.t001

Assessment of Reporting Quality and Risk of Bias

The reporting quality of each study was assessed using the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist [23] guided by the published detailed explanation on how to use the checklist [24]. The combined checklist designed for cohort, case-control and cross-sectional studies was utilised (Appendix S2). A quality assessment score out of 22 was determined for each study by assigning a point per STROBE item addressed. Good/fair quality papers were categorised as having a score of ≥14/22 and poor quality papers were classified as having a score of <14/22. All studies, regardless of their STROBE score, were retained in the systematic review.

Bias was assessed using the Risk of Bias Tool for Prevalence Studies developed by Hoy, Brooks, Woolfe et al., (2012) [25], adapted specifically for this systematic review (Appendix S3). The tool consists of ten items which address four areas of bias and an eleventh item includes a summary risk of bias assessment. The items assess both external and internal validity. Each study was rated as having a low, moderate or high risk of bias. Studies were classified as having a low risk of bias when eight or more of the ten questions were answered as "yes (low risk)", a moderate risk of bias when six to seven of the questions were answered as "yes (low risk)" and a high risk of bias when five or fewer questions were answered as "yes (low risk)".

Results

Study Selection

The three databases searched identified a total of 568 records. A total of 102 duplicates were removed resulting in 466 unique records after which 362 records were excluded based on their titles being considered irrelevant to the search topic. Of the 104 abstracts screened, 67 abstracts were considered to be relevant. Due to lack of access to the particular journals, despite several attempts, seven full text articles could not be obtained. After reviewing the full text articles of 60 of the records, 14 met all the criteria for the systematic review. In addition, one abstract, for which the full text article could not be obtained, was also considered relevant to the systematic review. A French-speaking colleague read, translated and extracted data from the one article written in French. Articles that were excluded were those in which information regarding classification of, diagnostic criteria for and screening methods for GDM was missing, where methodology was unclear and where investigations were performed on immigrant women as opposed to women representative of the local pregnant population (Figure 1).

Reporting Quality and Risk of Bias

The STROBE scores per study and the risk of bias results are listed in Table 2. Quality and risk of bias assessments were not performed on the study for which only an abstract could be obtained [26] and for the systematic review that provided details on that one particular study [27]. With regards to reporting quality and referring to the STROBE checklist (Appendix S2), describing the study design, sources of bias, statistical methods used and study limitations were areas where a number of the studies fell short.

Out of the 13 studies that underwent a risk of bias assessment, four (31%) were considered to have a high risk of bias; five were classified as having a moderate risk of bias (38%) and four (31%) were considered to have a low risk of bias.

Study Characteristics

Thirteen original research articles, one systematic review article and one abstract pertaining to an original research study were finally included in the systematic review thus totalling 14 African research studies (Figure 1). The systematic review article [28] discussed studies in Sub-Saharan Africa and contained suitable information concerning the study for which only an abstract was available. The earliest study was published in 1979 and the latest in 2013, therefore the original individual studies included in the review involved research spanning 35 years. Overall, information regarding GDM classification, screening methods and prevalence was obtained for six African countries; Ethiopia, Morocco, Mozambique, Nigeria, South Africa and Tanzania. Two of the 14 studies looked at GDM prevalence amongst women with risk factors (selective screening), another three studies were case control studies assessing GDM prevalence amongst women at increased risk for the condition versus women without risk factors, and the remaining nine studies involved universal GDM screening of pregnant women. With reference to Table 3:

Ethiopia. Only one study on GDM in rural Ethiopia, performed over a decade ago, was included. This was a well reported study with a low risk of bias. The OGTT was utilised as the diagnostic test based on the WHO 1985 criteria and a GDM prevalence of 3.7% was reported [29].

Morocco. The one article pertaining to research performed in urban Morocco was published in 2009 and was written in French. The authors reported a relatively high prevalence of GDM; 7.7% using the Carpenter and Coustan's criteria. However, the authors stated that all women who tested positive on a glucose challenge screening test should have then been referred for an OGTT yet only 40% of these women received an OGTT. This suggests that the GDM prevalence could actually have been higher if all women requiring an OGTT were in fact tested. The authors did report that the GDM prevalence was similar to the prevalence of type 2 diabetes in that population. Unfortunately no reference was made to the ethnicity of the study participants and considering Morocco has several ethnic groups it is difficult to say who this prevalence figure applies to [30]. In addition, the risk of potential bias within this study was high.

Mozambique. Only one case control study, of relatively poor reporting quality and moderate risk of bias, was analysed from Mozambique. The study was conducted in 2002 in an urban/suburban setting and the population group was not stated. Considering the majority of the Mozambican population is black, it is assumed that the cohort consisted of black females. Authors of the study reported a GDM prevalence of 11% amongst women who had late fetal deaths (cases) and 7.3% amongst women who had delivered live new-borns (controls). The investigators diagnosed GDM using their own diagnostic criteria which classified glucose readings for diabetes mellitus and IGT as GDM [31].

Nigeria. Six Nigerian studies, all on urban populations, were evaluated. These studies were conducted between the years 2004–2013 [27,32–36]. Five of the six studies were classified as having good/fair reporting quality and one was classified as poor. The risk of bias across the six studies ranged between low, moderate and high. All the studies used the OGTT as the method to detect GDM but different glucose concentrations were employed (50 g, 75 g and 100 g) over a time period of one to three hours.

One study focussed solely on determining the prevalence of GDM amongst women with risk factors which included (i) history of fetal macrosomia; (ii) maternal obesity; (iii) previous intrauterine death; (iv) first degree relative with diabetes; (v) glycosuria and (vi) history of GDM in a previous pregnancy [27]. Another two studies were case control studies whereby women with risk factors for GDM [33] or women who had delivered macrosomic babies [34] were classified as cases, and women without risk factors [33] or women who had delivered normal weight babies [34] served as the controls. Prevalence of GDM was higher amongst the cases in both studies; 6.2% versus 4.6% (utilising the Carpenter and Coustan's criteria) [33] and 2.5% versus 1.5% (utilising the investigators own diagnostic criteria) [34]. However, Kamanu et al., (2009), who used their own diagnostic criteria as mentioned above, diagnosed GDM based on a 1 hour 50 g OGTT (> 7.8 mmol/l/140 mg/dl) and only followed up borderline results with a 75 g 2 hour OGTT [34]. Usually the 50 g glucose load is referred to as a glucose challenge test and women who test positive on the challenge test are followed up with a further OGTT. This is referred to as the two step approach [5]. It is unconventional for a 50 g OGTT to be performed independently as a diagnostic test and so the results of this study could be questionable.

Excluding the two case-control studies discussed above, the other four Nigerian studies utilised the WHO diagnostic criteria (two used the WHO 1985 criteria and two used the WHO 1999 criteria). One of these four studies compared the detection rate of the three hour 75 g OGTT using the WHO 1985 criteria to the three hour 100 g OGTT using the NDDG criteria. The 75 g OGTT with WHO 1985 diagnostic criteria yielded a higher GDM prevalence (11.6% versus 4.5%). Conversely, this study found that the incidence of fetal macrosomia was higher (66.7%) amongst women diagnosed with GDM by the 100 g OGTT using

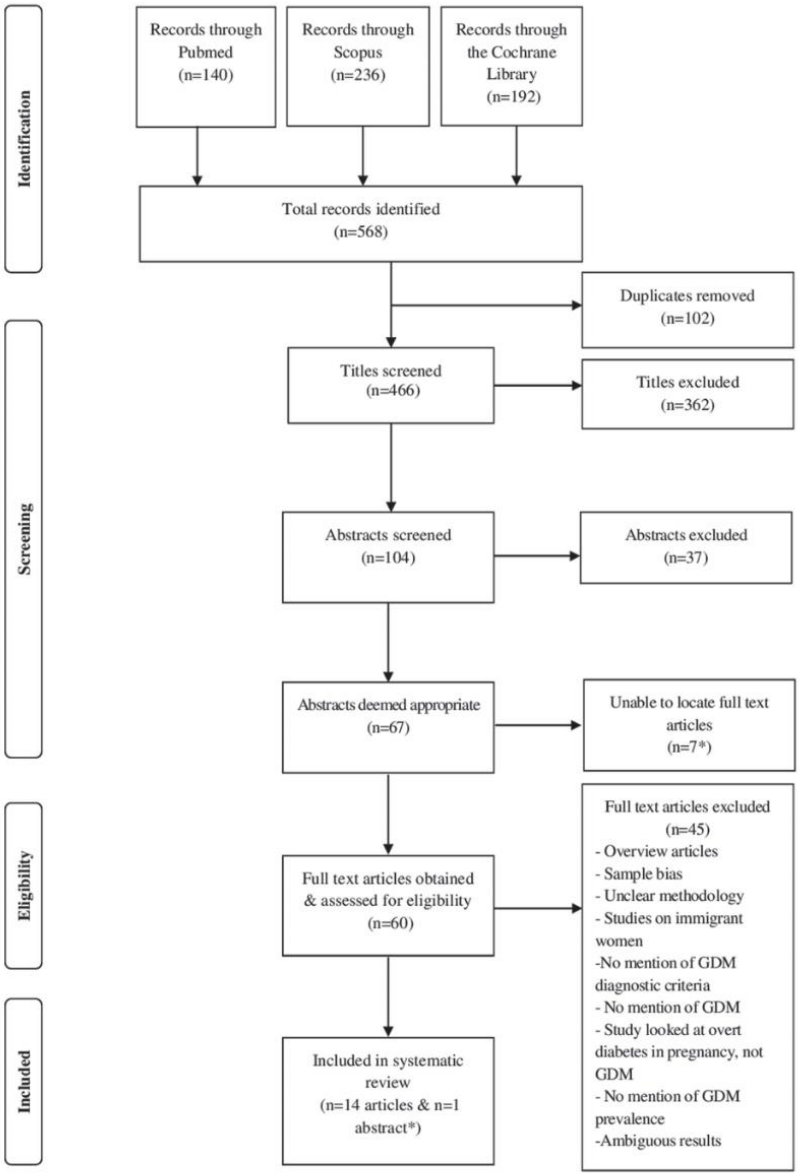


Figure 1. Flow diagram illustrating the number of included and excluded studies in the systematic review on gestational diabetes mellitus in Africa.
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Table 2. Reporting quality and risk of bias assessments.

Author	STROBE reporting quality score*	Overall risk of bias
Seyoum et al., 1999 [29]	18/22	Low
Bouhsain et al., 2009 [30]	16/22	High
Challis et al., 2002 [31]	11/22	Moderate
Olarinoye et al., 2004 [32]	18/22	Low
Adegbola & Ajayi, 2008 [33]	17/22	Moderate
Kamanu et al., 2009 [34]	19/22	High
Kuti et al., 2012 [27]	19/22	Moderate
Anzaku & Musa, 2013 [35]	17/22	Low
Ozumba et al., 2004 [36]	12/22	High
Jackson & Coetzee, 1979 [37]	15/22	Moderate
Ranchod et al., 1991 [38]	16/22	Low
Mamabolo et al., 2006 [39]	18/22	Moderate
Basu et al., 2010 [40]	19/22	High
Swai et al., 1991[#] [26]	Not assessed	Not assessed

*Good/fair quality papers were categorised as having a score of ≥14/22, poor quality papers were classified as having a score of <14/22.
[#]As only the abstract was available an assessment of the reporting quality and risk of bias could not be performed.
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the NDDG criteria than amongst women diagnosed with GDM by the 75 g OGTT using the WHO 1985 criteria (23.1%) [32].

South africa. Four South African studies, conducted between 1979 and 2010, were included in the systematic review [37–40]. One study focused predominantly on Indian women [38], two on black women [39,40] and the other did not state the ethnicity of the women [37]. The study by Jackson and Coetzee (1979) tested women for GDM because they had one or more risk factors. These risk factors included (i) a parent or sibling with diabetes; (ii) repeated miscarriages; (iii) obesity; (iv) previous macrosomic infant; (v) glycosuria; (vi) previous hyperglycaemia; (vii) previous infant with a severe congenital anomaly; (viii) previous perinatal death; (ix) polyhydramnios and (x) Indian ethnicity. In addition, this particular study utilised a 2 hour 50 g OGTT and the investigators' own diagnostic criteria [37]. A 50 g glucose load is usually used for the glucose challenge test and an OGTT generally utilises either 75 g or 100 g of glucose [5]. The glucose load chosen for an OGTT by the investigators is unusual. However, this study was performed in 1979 and can therefore be considered outdated. Optimisation of the OGTT for the diagnosis of GDM has developed and improved greatly since then.

All but one study employed a two hour OGTT for the diagnosis of GDM. The one study that did not employ an OGTT was interestingly the most recent study in South Africa, conducted in 2010, which tested fasting or random blood glucose levels and referred to an institutional protocol for diagnostic criteria [40]. Ranchod et al., (1991) compared the WHO 1999 criteria and DSPG of EASD criteria; WHO criteria produced a higher GDM prevalence (3.8% versus 1.6%) [38]. Overall, the four South African studies produced GDM prevalence figures ranging from 1.6% to 8.8%.

Tanzania. One study, published in 1991, was included on GDM prevalence in rural Tanzania [26]. Unfortunately, the full text article could not be obtained but data was extracted from the abstract and the review article [28]. This study involved an OGTT on a small sample of women (n = 189) using the WHO 1985 diagnostic criteria. A prevalence of 0% was determined. Unfortunately, as the full text article could not be obtain, reporting quality and risk of bias for this study could not be assessed.

Discussion

As far as the authors are aware, no other systematic review has assessed the prevalence of GDM across the African continent. This systematic review therefore focussed on studies in African countries that provided details on the GDM screening methods employed, the diagnostic criteria used and the prevalence figures obtained.

Africa consists of 54 countries [41] yet only six African countries, equating to a mere 11%, were represented in this systematic review. The percentage of countries for which prevalence figures were found in a systematic review that assessed GDM in Asia was 26% [42]. Although still low, this regional representation is better than the one found in the current review. This highlights the fact that little seems to be known about the prevalence and potential burden of GDM in African countries. Before health care policies and guidelines can successfully be drawn up and implemented, it is important for one to establish the extent of a particular problem. It is evident that the extent of GDM in Africa as a whole is not well investigated. Africa has been plagued with under-nutrition and GDM may not be considered a public health concern. However, as African countries shift economically a double burden of under- and over-nutrition emerges. With the increase in over-nutrition, particularly in females, GDM may be naively overlooked.

The results of the systematic review illustrate that the majority of the studies tested for GDM at around 24–28 weeks gestation, the recommended gestational age for when an OGTT should be performed [42]. In addition, the most commonly employed method for GDM screening in Africa is the two hour 75 g OGTT with glucose reference ranges as stipulated by the WHO 1985 or 1999 diagnostic criteria (Table 3). Two of the reported studies made comparisons between different diagnostic criteria and screening methods. One of the Nigerian studies showed that the two hour 75 g OGTT using the WHO 1985 criteria diagnosed more than double the amount of women that the 100 g OGTT

Table 3. Prevalence of Gestational Diabetes Mellitus (GDM) in Africa.

Author	Country	Region (rural/urban)	Population group	Sample size	Age of women	Gestational age when tested for GDM	GDM diagnostic criteria used	Diagnostic test used to determine GDM	GDM prevalence
Seyoum et al., 1999 [29]	Ethiopia	Tigray (rural)	Black	890	27.4±7.1 yrs (15–50 yrs)	24+ weeks	WHO criteria (1985)*	2 hr 75 g OGTT	3.7% (33/890)
Bouhsain et al., 2009 [30]	Morocco	De Rabat (urban)	Not stated	426	28.8±6.1 yrs	24–28 weeks	Carpenter and Coustan's criteria*	3 hr 100 g OGTT	7.7% (8/426)
Challis et al., 2002 [31]	Mozam-bique	Maputo (urban/suburban)	Not stated (assumed Black)	Cases: 109 (women with late fetal deaths)	Mean of 25 yrs	>27 weeks	Fasting blood glucose of ≥6.7 mmol/l (120.6 mg/dl) and/or OGTT 2 hr blood glucose of ≥9.0 mmol/l (162 mg/dl)	2 hr 75 g OGTT	11% (12/109 cases)
				Controls: 110 (women with live births)		Post delivery			7.3% (8/110 controls)
Olarinoye et al., 2004 [32]	Nigeria	Lagos (urban)	Black	248 (138; 75 g OGTT, 110; 100 g OGTT)	30.7±4.2 yrs (18–41 yrs)	≥28 weeks	WHO criteria (1985)* –75 g OGTT	3 hr 75 g OGTT	11.6% (16/138) –75 g OGTT
							NDDG criteria (1979)* - 100 g OGTT	3 hr 100 g OGTT	4.5% (5/110)- 100 g OGTT
Adegbola & Ajayi, 2008 [33]	Nigeria	Lagos (urban)	Black	Cases: 113 women with risk factors	19–45 yrs	24–28 weeks and repeated at 30–32 weeks	Carpenter and Coustan's criteria*	3 hr 100 g OGTT	6.2% (7/113 cases)
				Controls: 109 women without risk factors					4.6% (5/109 controls)
Kamanu et al., 2009 [34]	Nigeria	Aba (urban)	Black	Cases: 240 women with macrosomic babies	19–45 yrs	24–28 weeks	1 hr 50 g OGTT >7.8 mmol/l (140 mg/dl).	1 hr 50 g OGTT	2.5% (6/240 cases)
							Borderline results: 2 hr 75 g OGTT plasma glucose level >10 mmol/l (180 mg/dl) at 1 hr and >8.6 mmol/l (154.8 mg/dl) at 2 hr	Borderline cases followed up with a 2 hr 75 g OGTT	
				Controls: 8800 women with normal weight babies					1.5% (134/8800 controls)
Kuti et al., 2012 [27]	Nigeria	Ibadan (urban)	Black	765	19–45 yrs	4–40 weeks	WHO criteria (1999)*	2 hr 75 g OGTT	13.9% (106/765) (amongst women with risk factors)
Anzaku & Musa, 2013 [35]	Nigeria	Jos (urban)	Black	253	19–42 yrs	24–28 weeks	WHO criteria (1985)*	2 hr 75 g OGTT	8.3% (21/253)
Ozumba et al., 2004 [36]	Nigeria	Enugu (urban)	Black	12030	15–54 yrs	≥28 weeks	WHO criteria (1999)*	2 hr 75 g OGTT	1% (122/12030)

Table 3. Cont.

Author	Country	Region (rural/urban)	Population group	Sample size	Age of women	Gestational age when tested for GDM	GDM diagnostic criteria used	Diagnostic test used to determine GDM	GDM prevalence
Jackson & Coetzee, 1979 [37]	South Africa	Cape Town (urban)	Not stated	558	Not stated	All gestations (test repeated in 3rd trimester)	When 2 of the following 3 criteria were exceeded on 2 separate GTT:	2 hr 50 g OGT	3% (17/558) (amongst women with risk factors)
							2) Maximum level: 10.0 mmol/l (180 mg/dl) (excluding the 30 min figure)		
							3)2 hr level: 6.7 mmol/l (120.6 mg/dl)		
Ranchod et al., 1991 [38]	South Africa	Pieter-martizburg (urban)	Indian (majority) and Coloured (minority)	1717	Not stated	28–32 weeks	WHO criteria (1985)* and DSPG of EASD criteria (1988)*	2 hr 75 g OGT	3.8% (65/1717): WHO
									1.6% (27/1717): DPSG of EASD
Mamabolo et al., 2006 [39]	South Africa	Limpopo (rural)	Black	262	25.5±6.9 yrs	28–36 weeks	WHO criteria (1999)*	2 hr 75 g OGT	8.8% (23/262)
Basu et al., 2010 [40]	South Africa	Johannesburg (urban)	Black (94%), White (4%), Mixed (1.7%) and Asian (0.5%)	767	13–31 yrs	23–32 weeks	Institutional protocol: Fasting blood glucose: >8.0 mmol/l (180 mg/dl) or random blood glucose: 11.0 mmol/l (198 mg/dl)	Fasting or random blood glucose levels	1.8% (14/767)
Swai et al., 1991** [26]; Hall et al., 2011[28]	Tanzania	Unknown (rural)	Black	189	Unavailable	Unavailable	WHO criteria (1985)*	2 hr 75 g OGT	0% (0/189)

*Refer to Table 1;
**Could not obtain full text article.
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using the NDDG criteria [32]. In addition, one of the South African studies also illustrated a two-fold detection rate using the 1985 WHO criteria versus the DSPG of EASD criteria [38]. Based on these findings, whether the 75 g OGTT over-diagnoses GDM in women is debatable and warrants further investigation. This statement is supported by the authors of the systematic review on GDM Asia who commented that the choice of diagnostic criteria greatly affects GDM prevalence [43].

Many lessons have been learnt from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study which showed that there is a continuous association between maternal blood glucose levels below those diagnostic of diabetes, and adverse outcomes, such as increased neonatal birth weight [44]. As a result of these findings various groups have reconsidered the diagnostic criteria for GDM. The IADSPG diagnostic criteria and WHO 2013 diagnostic criteria are not as stringent as some of the other/previous criteria mainly because only one abnormal value, as opposed to two, is sufficient to make a diagnosis of GDM (Table 1). As a result of using the newer criteria it is very likely that the prevalence of GDM will increase. This has both positive and negative consequences. For example, more women will be diagnosed with GDM and receive treatment and management which in turn will decrease the effects of maternal hyperglycaemia on the mother and developing fetus. On the other hand, the health system in a country could become overburdened with GDM pregnancies, which could impact heavily on a country's economy [45]. However, considering the potential adverse pregnancy outcomes and the long term effects of GDM on mother and baby, it may be beneficial to the individuals, as well as a country's health system and economy, to diagnose and manage more women than less. None of the studies reported in this systematic review used the WHO 2013 or IADPSG criteria.

The percentage of women affected with GDM in this review was as low as 0% in rural Tanzania [26] and as high as 13.9% amongst urban Nigerian women with risk factors [27]. This disparity in prevalence is possibly due to the different methodology and study designs employed across the 14 studies. Without the availability of a standardised universal screening protocol the question is raised as to whether or not the prevalence figures that were obtained through the various studies are in fact true reflections of the African situation. In addition, with respect to the discussion above regarding the newer IADSPG and WHO 2013 diagnostic criteria, should the 14 studies reported in this systematic review have utilised either of the said criteria the GDM prevalence figures obtained would most likely have been greater.

Two of the studies, one performed in Nigeria and the other in South Africa, only tested women with risk factors for GDM and therefore employed the selective screening approach within their methodology [27,37]. Certain risk factors have indeed been proven to be very useful in identifying women at risk for GDM; when BMI is >30 versus <20 kg/m^2 a woman has a three times greater risk of developing GDM. Ethnicity is also another key factor for assessing the risk of developing GDM; Asian women are five times more likely to develop GDM than Caucasian women, and African-American women are two times more likely to develop GDM than Caucasian women [2]. The study by Kuti et al., (2012) in Nigeria reported a high GDM prevalence (13.9%) amongst these women and the authors found the strongest associations between the following risk factors and a diagnosis of GDM: being over 30 years of age (although this was not used as a risk factor in the sample selection process), having a family history of diabetes and having previously been diagnosed with GDM [27].

The South African study that tested women with risk factors produced a much lower prevalence of GDM (3%) but did report a strong association between glycosuria, previous hyperglycaemia and having two or more of the listed risk factors with a diagnosis of GDM [37]. These studies support that certain maternal risk factors have a high specificity in identifying women at risk of developing GDM. This selective screening approach may certainly have an important role in resource-limited settings.

The countries with the most studies pertaining to GDM were South Africa and Nigeria, which had four and six studies reported respectively. With particular reference to South Africa, considering there are 22 million black females living in the country, representing approximately 80% of the entire female population [44], two studies on GDM in black women, one in a rural setting [39] and one in an urban setting [40], involving a total cohort of approximately 983 women, cannot be considered representative of the South African GDM scenario. In addition, out of the six African countries for which GDM prevalence figures were obtained, only Nigeria and South Africa have reported relatively recent figures on macrosomia rates. In Nigeria it is thought that macrosomia accounts for 7.5% [45] to 8.1% [46,47] of births which ties in with the high GDM prevalence figures of 8.3% [35] and 13.9% [27] as reported by the two Nigerian studies in this review. This suggests macrosomia may be a marker for GDM prevalence. With respect to South Africa, one study conducted on black patients in urban Soweto reported a 2.3% macrosomia prevalence [48] but recent unpublished data from the South African Department of Health indicates a surprisingly low macrosomia rate of 1.7% [49]. If macrosomia rates are indicative of GDM rates then it is imperative that research on GDM is conducted in other African countries. Algeria and Uganda's macrosomia prevalence figures are reported as 14.9% and 8.4% respectively [45], this raises concern regarding their possible GDM figures.

It is alarming that very little appears to be known about GDM in African countries. Research studies, such as those listed in this systematic review, and particularly those that screen all women in the study cohort for GDM, are exceptionally useful in assessing the prevalence of the problem. Based on the 14 reported studies included in the systematic review, if one ignores the prevalence figures obtained from the two studies that focussed on higher risk women [27,37] and takes the prevalence of GDM amongst the control group in the case control studies [31,33,34], and selects the prevalence figures obtained by the WHO diagnostic criteria as opposed to those obtained by the NDDG criteria in one study [32] and the DSPG of EASD criteria in another study [38], the overall prevalence of GDM in Africa is estimated to be approximately 5% (60.1/12); approximately two and a half to seventeen times greater than some high income countries (Denmark (2–3%), the UK (2–3%) Germany (0.3–0.8%)) [17].

Interestingly, few studies were performed on rural populations. As a direct consequence of urbanisation it would be expected that the prevalence of GDM would be higher amongst urban populations as opposed to rural populations. Out of the four South African studies (three urban and one rural) the study in rural Limpopo produced the highest GDM prevalence (8.8%) amongst a representative sample of local pregnant women [39]. However, one of the limitations in making comparisons between the rural and urban studies in this review is the different GDM screening methods employed and diagnostic criteria used. In addition, some studies looked at women already at high risk for GDM. Other limitations to this review include only published studies, as opposed to grey literature, being searched and roughly one third of the studies included in the review having a high risk of bias and another third having a moderate risk of bias.

This systematic review has illustrated a gap in the knowledge of GDM in Africa with only 11% of the African continent being represented. More epidemiological based studies on GDM in African countries need to be performed in order to provide reliable information and thus clarity on the extent of GDM. An ideal scenario would be if one set of diagnostic criteria and one testing method was employed across the continent in order to produce comparable data. In addition, comparisons between GDM prevalence amongst rural and urban populations within a country should be carried out in order to assess the extent of the effects of urbanisation on public health.

Understanding and subsequently attempting to curb the prevalence of GDM in developing countries is imperative for maternal and child health. As GDM often results in macrosomic infants, birth trauma and the need for Caesarean sections at delivery are expected. This is precarious as it impacts both maternal and child survival during delivery, and places a significant economic burden on the health system, which in many African countries is already struggling with limited resources.

Furthermore, for most countries macrosomia appears to have been overlooked with the justified focus on low birth weight and small for gestational age statistics. The Developmental Origins of Health and Disease research describes how the developing fetus is susceptible to its environment and that certain *in utero* events can in fact alter fetal programming and produce different phenotypes. Low birth weight is representative of poor fetal nutrition and growth, and has been shown to be associated with a range of chronic conditions, including type 2 diabetes [50]. However, high birth weight requires as much consideration as there is evidence to support that fetal over-nutrition also poses risk for type 2 diabetes and other chronic conditions later in life [51]. With the emerging increase in type 2 diabetes and obesity, macrosomia will become an important factor in maternal and child health and should be reported on and monitored by the health care system as a marker for GDM sooner than later.

As Africa continues along its economic and concomitant urbanisation and lifestyle transitions, the double burden of both under- and over-nutrition is a cause for concern. Therefore, epidemiologists, public health specialists, health professionals, and policy leaders need to place GDM and macrosomia as key elements in their maternal and child health framework, thus enabling policies and practice to minimise the risk of maternal impaired glucose metabolism during pregnancy.

Supporting Information

Checklist S1 PRISMA 2009 checklist.
(DOC)

Appendix S1 The 54 countries in Africa according to the United Nations.
(DOCX)

Appendix S2 STROBE Statement: checklist of items that should be included in reports of observational studies.
(DOC)

Appendix S3 Risk of bias assessment tool.
(DOCX)

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Author Contributions

Conceived and designed the experiments: SM SAN. Performed the experiments: SM SAN. Analyzed the data: SM SAN. Contributed reagents/materials/analysis tools: SM SAN. Wrote the paper: SM SAN. Performed and updated the search: SM. Provided methodological advice and critically revised the manuscript: SM SAN DD.

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The prevalence of gestational diabetes mellitus amongst black South African women is a public health concern

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ABSTRACT

Aims: This study aimed to determine the prevalence of gestational diabetes mellitus (GDM) amongst black South African women, describe GDM-associated risk factors and clinical management, and evaluate the efficacy of the fasting plasma glucose reading in diagnosing GDM.

Methods: A cross-sectional screening study was performed. Pregnant women were recruited from the Chris Hani Baragwanath Academic Hospital in Johannesburg. A total of 1906 women underwent a two-hour 75 g oral glucose tolerance test at 24–28 weeks gestation. The World Health Organization's 2013 criteria were used to diagnose GDM.

Results: A total of 174/1906 (9.1% (95% confidence interval (CI) 7.9, 10.5)) women were diagnosed with GDM. These women had significantly higher weights and body mass indexes (BMIs), were significantly older, of higher household socioeconomic status, more likely to report a family history of diabetes, and more likely to be diagnosed with anaemia than women without GDM. An age of $\geq$35 years, BMI $\geq$ 30 kg/m^2, and a family history of diabetes were significant risk factors. The fasting plasma glucose reading had a high sensitivity (83.3% (95% CI 77.0, 88.5)) in diagnosing GDM and 56.9% of the women with GDM were managed by diet therapy alone.

Conclusion: This is the largest GDM prevalence study in South Africa to date. A diagnosis of GDM increases the risk of both mother and child developing Type 2 diabetes which causes further health complications, decreases longevity, and burdens a country's healthcare system. Therefore, a GDM prevalence of 9.1% is concerning and warrants further discussion around current GDM screening policies.

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1. Introduction

Many low- and middle-income countries are experiencing demographic, nutritional and epidemiological transitions which have resulted in a surge of non-communicable diseases. Shifts in dietary patterns, urbanisation and a decrease in physical activity are key elements in these transitions [1]. South Africa is witnessing this at a rapid rate with approximately 2.3 million individuals affected by diabetes [2] ranking it the second most common cause of death in 2015 [3]. In addition, overweight and obesity are on the rise, particularly amongst South African women with 68% of them aged 15 years and above being classified as overweight or obese [4].

Gestational diabetes mellitus (GDM), defined as any degree of glucose intolerance diagnosed for the first time in a woman during pregnancy, that is not clearly overt diabetes [5–7], is considered a precursor for Type 2 diabetes mellitus for both the affected mother and her unborn child [8]. Several risk factors have been shown to increase a woman's chance of developing GDM; increasing age, a family history of Type 2 diabetes mellitus, high parity, belonging to a particular ethnic group (Hispanic, African, African American, Asian), having previously delivered a baby weighing 4 kg or more, and being overweight or obese [9]. Given the large number of individuals in South Africa affected by diabetes, together with the alarming prevalence of overweight/obesity amongst women, it is hypothesised that GDM is of significant concern too. Very few studies reporting on the prevalence of GDM exist in Africa overall [10] and only two have been conducted on a small number of black South African women; one in rural Limpopo (n = 262) [11] and the other in urban Johannesburg (n = 545) [12].

The gold standard test for diagnosing GDM is the oral glucose tolerance test (OGTT) performed at 24–28 weeks gestation [13]. Ideally, all pregnant women should be tested for GDM (universal screening) [5,6]. However, South Africa's public healthcare system employs a selective screening approach for GDM whereby only women with certain risk factors are tested [14]. This approach is viewed as the most cost-effective in resource-poor settings. The downfall to selective screening is the lack of data on the actual prevalence of the condition, and also the risk of affected women being missed; selective screening is thought to miss close to 50% of women with GDM [15]. There has been some debate around whether a fasting plasma glucose reading alone is sufficient for diagnosing GDM [16,17] and predicting adverse neonatal outcomes [18]. In resource-poor settings where OGTTs are not feasible, a fasting plasma glucose screen may be an alternative option for detecting women with GDM.

Furthermore, the existence of several OGTT diagnostic criteria has complicated estimating GDM prevalence both between and even within countries. The International Association for Diabetes and Pregnancy Study Group (IADPSG) proposed a new set of diagnostic criteria in 2010 [5] which was adopted by the World Health Organization (WHO) in 2013 with the addition of a two-hour plasma glucose cut-off value of ≥11.1 mmol/l as diagnostic of overt diabetes [6]. Recently, the Society for Endocrinology, Metabolism and Diabetes of South Africa recommended the use of the WHO 2013 diagnostic criteria [19].

The overarching aim of this study was to determine the prevalence of GDM using the WHO 2013 diagnostic criteria amongst a large cohort of black South African women living in an urban township in Johannesburg. In addition, given South Africa's current propensity to utilise a selective screening approach for GDM, the study also aimed to describe GDM-associated risk factors amongst the study population, assess the efficacy of the fasting plasma glucose reading alone in detecting GDM, and report on the clinical management of women with GDM.

2. Participants, materials and methods

2.1. Participants

The study was conducted at the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU) situated in Soweto in Johannesburg, South Africa. Approximately 1.3 million people reside in Soweto, the majority of which are black South Africans [20].

Chris Hani Baragwanath Academic Hospital (CHBAH) is the one tertiary academic hospital servicing the Soweto region. Pregnant patients at CHBAH represent a mix of high-, moderate- and low-risk pregnancies. Several women are referred solely due to having had a previous Caesarean section or for fetal ultrasounds which are generally not available at the local antenatal clinics. Pregnant women attending the Antenatal Clinic and Fetal Medicine Unit at CHBAH were screened for eligibility for the study. The inclusion criteria included black South African women (ethnicity was self-reported), ≥18 years of age, living in Soweto and ≤20 weeks pregnant with singleton pregnancies. In addition, participants could not have any known type of diabetes at the time of recruitment nor could they have epilepsy (due to concern of antiepileptic medication affecting glucose metabolism). All participants had to have had a fetal ultrasound from which their gestational age was calculated. Screened women who fulfilled the inclusion criteria were informed about the study, educated on GDM and invited to participate in the study. Those who expressed an interest in participating in the study were given an appointment at the Research Unit for when they were 24–28 weeks pregnant. The study took place from 1 June 2013 to the 30 April 2017.

2.2. Anthropometry

As this was a screening study all data were collected at one time point; a participant's enrolment appointment at 24–28 weeks gestation. Height (cm) was determined using the SECA stadiometer (Hamburg, Germany) and weight (kg) was determined using the SECA digital weighing scale (Hamburg, Germany). These measurements were used to calculate body mass index (BMI; kg/m^2). Furthermore, BMI was classified into the WHO categories for underweight (<18.5 kg/m^2), normal

weight (≥18.5–24.9 kg/m^2), overweight (≥25–29.9 kg/m^2) and obese (≥30 kg/m^2) [21].

Blood pressure was measured using the Microlife Blood Pressure Monitor for Pregnant Women (Microlife AG Swiss Corporation, Widnau, Switzerland) whilst women were seated. An appropriate sized cuff was placed on the right arm of a participant. Three sets of systolic and diastolic blood pressure readings were taken with two minutes of rest between each set. The first set of readings was discarded and the average of the second and third set was used for the analyses. A diagnosis of hypertension was made when the systolic blood pressure (SBP) reading was ≥140 mmHg and/or the diastolic blood pressure (DBP) reading was ≥90 mmHg [22].

Haemoglobin (Hb) levels were measured using the HemoScan HB Meter (Alterna Biotech, Inc, California, USA). A diagnosis of anaemia was made when Hb levels were <110 g/l [23].

All measurements were taken by trained research nurses. Senior scientists and nurses qualified in anthropometry conducted regular training sessions (every six months) for research nurses. They also performed quality control checks that ensured the coefficient of variation of all anthropometric measures between research nurses was <1%.

2.3. The oral glucose tolerance test

Participants had been asked to attend their appointment after an overnight fast (a minimum of ten hours of fasting). A finger-prick blood sample was taken to perform a fasting capillary blood glucose reading using a hand-held glucometer; ACCU-CHEK® (Roche, Indianapolis, USA). If the capillary blood glucose reading was <7 mmol/l and the participant was sure she had fasted the research nurse proceeded with a venous blood sample to test fasting plasma glucose and then administered a two-hour 75 g OGTT. Participants with capillary glucose readings of ≥7 mmol/l were immediately referred to CHBAH for further investigation into possible overt diabetes. In view of potential hyperglycaemia-related complications, an OGTT in the research setting was not performed on these women and they were therefore excluded from the study. Whilst it is not recommended to use capillary glucose readings in place of venous glucose readings [24] the use of capillary glucose readings is considered acceptable if used as an initial screen [25].

The OGTT involved participants drinking 75 g of glucose powder dissolved in approximately 250 ml of water. The drink was consumed within five minutes and the participants remained seated throughout the process. Venous blood samples were drawn at one hour and two hours post-glucose load. Blood was collected in vacutainers containing fluoride and oxalate. Blood samples were immediately sent to the laboratory on site and processed in real-time so as to reduce further glycolysis in the blood collection tubes.

Venous (plasma) glucose samples were tested using the Randox RX Daytona Chemistry Analyzer. A diagnosis of GDM was made according to the WHO 2013 diagnostic criteria (fasting plasma glucose of 5.1–6.9 mmol/l, or, one-hour plasma glucose of ≥10.0 mmol/l or two-hour plasma glucose of 8.5–11.0 mmol/l). Overt diabetes was diagnosed as a fasting plasma glucose level of ≥7.0 mmol/l or a two-hour plasma glucose reading of ≥11.1 mmol/l [5,6]. The Clinical and Laboratory Standards Institute document EP15 was used for the verification of performance for precision and trueness of the Randox RX Daytona Chemistry Analyzer. A random selection of 150 samples was run in duplicate to ascertain the coefficient of variation of the laboratory technician which was determined to be 2.3%.

Different sets of GDM diagnostic criteria produce varying results which makes the comparison of results across studies difficult. Therefore, in order to compare our findings to those of other fairly recent African studies we also determined the prevalence of GDM amongst our cohort using the WHO 1999 diagnostic criteria (fasting plasma glucose of ≥7.0 mmol/l or a two-hour plasma glucose of ≥7.8 mmol/l) [26] and the IADPSG diagnostic criteria (same as the WHO 2013 criteria except the two-hour plasma glucose reading is ≥8.5 mmol/l, there is no cut-off value for overt diabetes) [5].

2.4. Questionnaires

Participants were asked questions pertaining to their demographics and obstetric and family histories. The demographic questions included age, marital status, level of education and a household asset score. The household asset score is the sum of the number of eleven assets (electricity, radio, television, refrigerator, cellular telephone, personal computer, farm animals, agricultural land, bicycle, motorcycle, motor vehicle) a participant has. This score is used as an indicator of household socioeconomic status and has been used in other studies based in Soweto [27].

Obstetric-related questions included how many previous pregnancies (gravidity) and births (parity) a woman had and if she had previously delivered a macrosomic (≥4 kg at birth) neonate. Whilst the cut-off value for macrosomia is debatable, South Africa tends to define macrosomia as a birth weight of 4 kg or more [14]. The family history questionnaire involved asking participants if they specifically had a sibling, parent or grandparent with diabetes.

2.5. Management and follow-up

Women diagnosed with overt diabetes and GDM were referred to the specialist Obstetric Diabetes Clinic at CHBAH for management. The management protocol at the Clinic involves following women up every two weeks until 32 weeks gestation and then once a week until delivery. Induction of labour or Caesarean section is planned for around 38 weeks' gestation. All women are seen by a dietician who prescribes a diabetic diet and advises on necessary dietary and lifestyle modifications. Women may also be prescribed medication. All women are given a glucometer for self-monitoring of blood glucose [28]. Research assistants telephoned these women to follow-up on their referrals.

2.6. Statistical analyses

Stata Version 12 (StataCorp, College Station, Texas) was used for statistical analyses. The Shapiro-Wilk and Skewness and Kurtosis tests were used to assess the distribution of continuous data. Normally distributed continuous variables were

presented as means ± standard deviations (SD) and those that were not normally distributed were presented as medians (interquartile range (IQR)). Categorical data were presented as frequencies and percentages. Differences between categorical variables were determined using the Chi-square test. The Student's *t*-test was used to analyse differences between normally distributed variables and the Mann-Whitney test was used to analyse differences between non-normally distributed variables. The Kruskal-Wallis H test, with the Conover-Iman test of multiple comparisons, was used to analyse the glucose readings according to BMI category. Significance was assumed at a two-tailed *p* value of $p < 0.05$. A multinomial logistic regression analysis was performed to investigate whether the well-described risk factors (age, BMI, parity, family history of diabetes and previous delivery of a macrosomic baby) were associated with GDM in this cohort. An evaluation of the fasting glucose reading alone, in terms of sensitivity, specificity and predictive values to diagnose or rule out GDM, was performed using the MedCalc Diagnostic Test Evaluation Calculator (MedCalc Software, Ostend, Belgium).

2.7. *Ethical approval*

The University of the Witwatersrand's Human Research Ethics Committee (Medical) granted clearance for the study (Certificate references: M120524, M130309 and M150461). Study participants gave informed, written consent.

3. Results

A total of 3656 eligible women were invited to participate in the study. Of those invited to participate, 2009 (55%) underwent an OGTT. There were 1647 (45%) women who did not undergo an OGTT for various reasons (Fig. 1). As the women who did not undergo and OGTT had met the inclusion criteria at recruitment, they did not differ from the group of women who did undergo an OGTT with regard to ethnicity and geographical area of residence. In addition, we compared the ages of the women who did not undergo an OGTT to those who did; there was no significant difference between the groups ($p = 0.242$).

A final sample of 1906 women was included in the study (Fig. 1). Of these women, six (0.3%) were diagnosed with overt diabetes; one had a fasting plasma glucose ≥7.0 mmol/l and the other five had two-hour plasma glucose readings of ≥11.1 mmol/l. A total of 174 women were diagnosed as having GDM according to the WHO 2013 criteria which resulted in a GDM prevalence of 9.1% (95% confidence interval (CI) 7.9, 10.5). For comparative purposes we also calculated the prevalence of GDM amongst our cohort using the WHO 1999 and the IADPSG criteria. Using the WHO 1999 criteria 107/1906 women were classified as having GDM producing a prevalence of 5.6% (95% CI 4.6, 6.7), and using the IADPSG criteria 179/1906 women had GDM producing a prevalence of 9.4% (95% CI 8.2, 10.8).

The 174 women with GDM according to the WHO 2013 criteria were significantly older, had more household assets and were therefore of a higher household socioeconomic status, and were more likely to have reported a positive family history of diabetes than the women with normal glucose profiles. In addition, they had significantly higher BMIs at the time of the OGTT than the women with normal glucose profiles with 59.2% of them falling into the obese category (Table 1).

Women with GDM were slightly more advanced in terms of gestational age at the time of the OGTT than those with normal glucose profiles but there was no difference in parity or gravidity between the two groups. The overall rate of having delivered a previous baby with macrosomia was low for the entire group (4.7%) and did not differ significantly between those with and those without GDM. Interestingly, women with GDM were more likely to be diagnosed with anaemia compared to those with normal glucose profiles (Table 1).

Blood glucose readings were first analysed across the entire study population (n = 1906) stratified according to BMI category. Glucose readings and prevalence of GDM differed significantly across the three BMI categories; the highest prevalence was amongst the women in the obese category. Of note, all six women with overt diabetes were classified as obese (Table 2).

As expected, plasma glucose levels taken at the three time points of the OGTT differed significantly ($p < 0.05$) between the women with GDM and those with normal glucose profiles. The mean ± SD for the fasting, one-hour and two-hour glucose readings were 5.4 ± 0.5 mmol/l, 8.0 ± 1.7 mmol/l and 7.4 ± 1.5 mmol/l respectively amongst the women with GDM. In comparison, the fasting, one-hour and two-hour glucose readings amongst the women with normal glucose profiles were 4.0 ± 0.5 mmol/l, 5.6 ± 1.3 mmol/l and 5.3 ± 1.1 mmol/l respectively. The majority of women with GDM (116/174; 66.7%) were diagnosed on a fasting plasma glucose reading alone, whilst a further 29/174 (16.7%) had an abnormal fasting glucose plus one or two additional abnormal readings (Table 3). Therefore, a total of 145/174 (83.3%) women had an abnormal fasting plasma glucose reading which alone is diagnostic of GDM. Based on this, the fasting plasma glucose test had an 83.3% sensitivity (95% CI 77.0, 88.5), 100% specificity (95% CI 99.8, 100), 0.17 negative likelihood ratio (95% CI 0.12, 0.23) and a 98.4% negative predictive value (95% CI 97.7, 98.8) of diagnosing GDM.

A multinomial logistic regression analysis was performed to see which of the well-described GDM risk factors were significantly associated with a diagnosis of GDM amongst our cohort of women. Table 4 shows that being ≥35 years of age, having a BMI of ≥30 kg/m^2 at the time of the OGTT and having a positive family history of diabetes were significant risk factors. High parity and a previous macrosomic baby were not significant risk factors amongst the women in our study. When fasting glucose was added to the logistic regression model as a continuous variable the only significant risk factor was age ≥35 years ($p = 0.039$).

Of the 174 women diagnosed with GDM, 123 (70.7%) followed through with their referral to the Obstetric Diabetes Clinic whereas 38 (21.8%) chose not to attend the Clinic and 13 (7.5%) could not be contacted. All the women who attended the Clinic received diet therapy. A total of 70/123 (56.9%) women were managed through diet therapy alone and

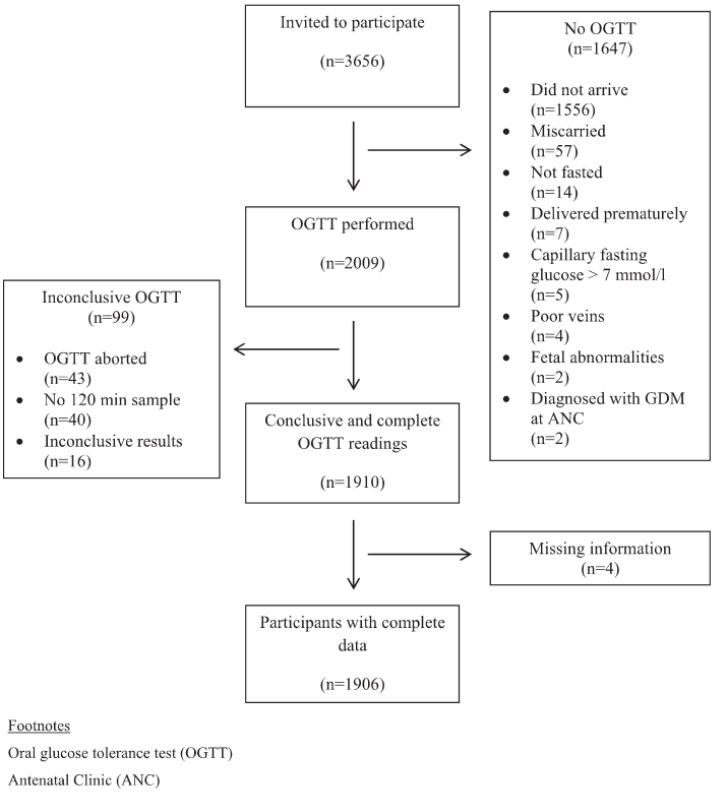


Fig. 1 – Study participation.

59/123 (48%) required medication; 58 were prescribed Metformin and one was prescribed insulin. Of the six women diagnosed with overt diabetes, 5/6 (83.3%) followed through with their referral to the Obstetric Diabetes Clinic and 4/5 (80%) were managed by medication (two on Metformin and two on insulin) and one was managed through diet therapy alone.

4. Discussion

Our study reports a GDM prevalence of 9.1% (95% CI 7.9, 10.5) using the WHO 2013 diagnostic criteria amongst black South African women living in urban Soweto. Our results also illustrate that being classified as obese at 24–28 weeks gestation, being ≥35 years of age and having a family history of diabetes are positively associated with GDM development. Most women with GDM in our study were controlled through diet/lifestyle modification. Furthermore, the fasting plasma glucose reading alone appears to have a high sensitivity in detecting GDM.

A concerning finding was the large number of eligible women (1556/3656; 42.6%) invited to participate in the study who did not follow through with their invitation (Fig. 1). This highlights potential issues around the awareness of GDM, its effects, and the importance of screening for it. A qualitative study assessing the reasons why these women chose not to participate would be helpful; we speculate that a general lack of awareness as well as logistically issues such as transport costs and time off work may be some reasons. The same can be said for the 21.8% of women with GDM who did not follow through with their referrals to the Obstetric Diabetes Clinic. A follow-up study to understand the reasons behind these women's decisions may identify areas for intervention including further education on GDM.

Regarding the BMI results amongst the study participants, it is possible that they may be overestimations as a woman's BMI at 24–28 weeks gestation is not an accurate reflection of her non-pregnant BMI. However, the highest prevalence of obesity in the whole of sub-Saharan Africa is amongst South African women; an alarming 42% (40.6–43.4) of women over

Table 1 – Characteristics of the study participants.

Characteristics	Median (IQR) or n (%) or mean ± SD			P value
	Total Participants (n = 1906)	Participants with normal glucose profiles (n = 1726)	Participants with GDM (n = 174)	
Age (years)	30 (25–35)	30 (25.0–34.5)	31 (27–36)	0.001
Household socioeconomic status[a]	5 (5–6) 5.4 ± 1.2	5 (5–6) 5.4 ± 1.2	5 (5–6) 5.7 ± 1.2	0.014
Education				
No schooling/primary school	47 (2.5%)	42 (2.4%)	5 (2.9%)	0.936
Secondary school	1480 (77.7%)	1341 (77.7%)	135 (77.6%)	
Tertiary education	379 (19.9%)	343 (19.9%)	34 (19.5%)	
Marital Status				
Single	1340 (70.3%)	1217 (70.5%)	119 (68.4%)	0.560
Married/cohabiting	566 (29.7%)	509 (29.5%)	55 (31.6%)	
Family history of diabetes (n = 1904)	476 (25.0%)	419 (24.3%)	56 (32.2%)	0.022
Weight (kg)[b]	74.7 (64.7–86.1)	74.4 (64.2–85.3)	78.6 (67.9–91.0)	0.001
Height (cm)	159.1 (155.1–163.1)	159.2 (155.1–163.2)	158.9 (156.1–162.2)	0.621
BMI (kg/m^2)[b]	29.5 (25.7–33.7)	29.3 (25.5–33.5)	31.5 (27.4–35.6)	<0.001
BMI categories[b]				
Normal	395 (20.7%)	369 (21.4%)	26 (14.9%)	0.004
Overweight	606 (31.8%)	561 (32.5%)	45 (25.9%)	
Obese	905 (47.5%)	796 (46.1%)	103 (59.2%)	
Haemoglobin (g/l) (n = 1804)	131 (113–157)	131 (114–157)	126 (106–150)	0.007
Anaemia (n = 1804)	375 (20.8%)	325 (19.9%)	50 (30.9%)	0.001
Systolic blood pressure (mmHg) (n = 1898)	108 (100.5–116.5)	108 (100.5–116.5)	108.5 (102.0–116.0)	0.589
Diastolic blood pressure (mmHg) (n = 1898)	68.5 (63–75)	68.5 (62.5–72.5)	69.8 (64.5–76.5)	0.026
Hypertensive (n = 1898)	64 (3.4%)	56 (3.3%)	8 (4.6%)	0.352
Gestational age[a]	26 (25–27) 25.8 ± 1.5	26 (25–27) 25.7 ± 1.5	26 (25–27) 26.1 ± 1.4	0.001
Previous pregnancies				
None	230 (12.1%)	215 (12.5%)	15 (8.6%)	0.078
One to two	1191 (62.5%)	1084 (62.8%)	104 (59.8%)	
Three or more	485 (25.5%)	427 (24.7%)	55 (31.6%)	
Previous births				
None	494 (25.9%)	453 (26.3%)	40 (23.0%)	0.183
One to two	1237 (64.9%)	1109 (64.3%)	123 (70.7%)	
Three or more	175 (9.2%)	164 (9.5%)	11 (6.3%)	
Previous macrosomic neonate (n = 1904)	90 (4.7%)	81 (4.7%)	9 (5.2%)	0.779
HIV status				
Negative	1309 (68.7%)	1190 (69.0%)	115 (66.1%)	0.439
Positive	597 (31.3%)	536 (31.1%)	59 (33.9%)	

Gestational diabetes mellitus (GDM) diagnosed according to World Health Organization's 2013 criteria; Sample size indicated if less than 1906; Total of 1906 women includes six women diagnosed with overt diabetes.

[a] Mean (±standard deviation (SD)) indicated where $p < 0.05$ but median (interquartile range (IQR)) does not indicate the difference.

[b] At the time of the oral glucose tolerance test (OGTT) (24–28 weeks gestation).

Table 2 – Glucose readings and gestational diabetes mellitus prevalence according to body mass index category.

	Body Mass Index Category (at 24–28 weeks gestation)			P value
	Normal weight (n = 395)	Overweight (n = 606)	Obese (n = 905)	
Fasting glucose (mmol/l)				
Median (IQR)	4.0 (3.6–4.4)	4.0 (3.6–4.4)	4.0 (3.7–4.6)	<0.001[c,d]
Mean ± SD	4.0 ± 0.6	4.1 ± 0.6	4.2 ± 0.7	
1-h glucose (mmol/l)				
Median (IQR)	5.3 (4.4–6.1)	5.5 (4.6–6.7)	6.0 (5.0–7.2)	<0.001[b,c,d]
Mean ± SD	5.4 ± 1.4	5.7 ± 1.5	6.2 ± 1.6	
2-h glucose (mmol/l)				
Median (IQR)	5.1 (4.3–5.9)	5.3 (4.5–6.2)	5.6 (4.7–6.6)	<0.001[b,c,d]
Mean ± SD	5.1 ± 1.2	5.4 ± 1.2	5.7 ± 1.4	
Women with GDM (%)	26/395 (6.7%)	45/606 (7.4%)	103/899[a] (11.5%)	0.004[c,d]

Gestational diabetes mellitus (GDM) diagnosed according to the World Health Organization's 2013 criteria; Interquartile range (IQR); Standard deviation (SD).

[a] Denominator less six women with overt diabetes.
[b] Significant difference between normal and overweight.
[c] Significant difference between normal and obese.
[d] Significant difference between overweight and obese.

Table 3 – Analysis of the World Health Organization's 2013 criteria in diagnosing gestational diabetes mellitus.

Abnormal plasma glucose reading(s)	Women diagnosed N (%)
Fasting glucose alone (5.1–6.9 mmol/l)	116 (66.7)
1-h glucose alone (≥10 mmol/l)	3 (1.7)
2-h glucose alone (8.5–11.0 mmol/l)	18 (10.3)
Fasting + 1-h glucose + 2-h glucose	7 (4.0)
Fasting glucose + 1-h glucose	8 (4.6)
Fasting glucose + 2-h glucose	14 (8.0)
1-h glucose + 2-h glucose	8 (4.6)
Total	174 (100)

Table 4 – Multivariate logistic regression analysis for risk factors associated with gestational diabetes mellitus.

Risk factor	Odds ratio	95% Confidence interval	P value
Age			
18–24 years (reference)			
25–34 years	1.6	1.0, 2.6	0.066
≥35 years	2.5	1.5, 4.4	0.001
Family history of diabetes			
No (reference)			
Yes	1.4	1.0, 2.0	0.038
BMI[a]			
Normal (reference)			
Overweight	1.1	0.7, 1.9	0.598
Obese	1.7	1.1, 2.7	0.021
Previous delivery of a macrosomic baby			
No (reference)			
Yes	1.0	0.5, 2.0	0.994
Parity			
Low (<2 births) (reference)			
High (≥2 births)	0.7	0.52, 1.1	0.094

[a] Body Mass Index (BMI) at the time of the oral glucose tolerance test (24–28 weeks gestation).

20 years of age in South Africa are obese [29]. Therefore, the high rate of obesity found in our study (47.5% amongst all participants) is very similar to that of the general South African adult female population.

In terms of GDM prevalence, two recent African studies reported a GDM prevalence of 8.1% in Nigeria using the WHO 2013 criteria [30] and a 2.9% (95% CI, 1.6, 4.2) prevalence using the IADPSG criteria in Kenya [31]. Using the same diagnostic criteria, our prevalence results are higher than those reported in these who African countries. Regarding South Africa, only two previous studies have assessed GDM in black women. A study conducted in 2007 in rural Limpopo reported a GDM prevalence of 8.8% (95% CI 5.6, 12.9) using the WHO 1999 criteria [11]. Interestingly, using the same diagnostic criteria the GDM prevalence is lower amongst our urban cohort (5.6% (95% CI 4.6, 6.7)) than the rural cohort in Limpopo. Given the changes in dietary habits and physical activity that are associated with urbanisation one might have expected our study to have produced a higher prevalence. The study set in Limpopo had a much smaller sample size (262 women of which 23 had GDM) and wider 95% CI for the prevalence rate than ours; it is possible that had a larger sample size been used the GDM prevalence in the Limpopo study may have been lower.

The second South African study, set in Johannesburg, reported an extremely high prevalence of GDM amongst 554 black women using the IADPSG criteria; 25.8% (CI not stated) [12]. This prevalence figure is more than twice that obtained from our study using the same diagnostic criteria (9.4% (95% CI 8.2, 10.8)). The authors do not mention the prevalence of overt diabetes amongst their study participants although the median (IQR) fasting plasma glucose levels amongst the GDM group is reported as 5.8 (3.9–13.4) mmol/l. The IQR suggests some women in the GDM group had fasting glucose readings of ≥7.0 mmol/l which according to the IADPSG criteria is indicative of overt diabetes.

As with all single centred studies, selection bias and the generalisability of the results become important questions. Given the rigor of our study and removal of women with overt diabetes we are confident that 9.1% (95% CI 8.2, 10.8) is an accurate reflection of the prevalence of GDM (using the WHO 2013 criteria) amongst black South African women living in urban Soweto. Ideally, a multicentre study should be conducted across South Africa to determine the true burden of GDM. Although comparing GDM prevalence rates within and between countries is difficult due to the different diagnostic criteria, a rough estimation of GDM prevalence in Africa based on a limited number of published studies was reported to be around 5% [10]. From a worldwide perspective, GDM is thought to complicate approximately 7% (range 1–14%) of all pregnancies [32]. Therefore, a prevalence of 9.1% is of concern.

As mentioned previously, South Africa currently employs a selective screening approach based on risk factors for GDM detection. Being ≥35 years, obese and having a family history of diabetes were shown to be significant risk factors associated with GDM development. However, only age ≥35 years remained significant when fasting glucose was added to the model. This suggests that fasting glucose is capturing the impact of adiposity on, and the genetic contribution (family history) to, glucose metabolism. Regarding fasting glucose, our results give evidence that a fasting plasma glucose screen is very effective in detecting GDM. Performing an OGTT is timely and costly and South Africa's healthcare system is already heavily burdened making universal screening for GDM an unlikely possibility. Based on our results we would recommend that at least a fasting plasma glucose test be considered as a GDM screening tool for all pregnant women in South Africa.

An interesting finding was that women with GDM had significantly lower levels of haemoglobin and a higher rate of anaemia than those with normal glucose profiles. Other investigators have reported the opposite with high levels of haemoglobin being associated with GDM [33] and iron deficient anaemia being protective against the condition [34]. It is possible that whilst the majority of women with GDM in our study were classified as obese, their diets were likely to have been excessive in refined carbohydrates, fats and sugars but deficient in micronutrients, including iron. Another possibility may be that the anaemia is a result of the pathophysiology of diabetes, such as renal insufficiency or periodontal disease [35–37].

In terms of clinical management, 56.9% of women with GDM in our study were managed solely through diet therapy. The American Diabetes Association (ADA) suggests that lifestyle modification (diet therapy, physical activity and weight management) can control 70–85% of women with GDM [38]. Whilst the percentage of women managed through dietary modification alone in our study was less than what the ADA suggests it is still very encouraging that more than half the women with GDM did not require medication. Such a finding is promising as it suggests that from a healthcare system's perspective managing GDM might not be that costly, and from a patient's perspective medication-burden might not be of concern to most.

5. Conclusion

Our study is the largest GDM prevalence study in South Africa to date. A 9.1% prevalence of GDM according to the WHO 2013 criteria is concerning and warrants further discussion around screening approaches for GDM in the South African public healthcare sector. Whilst diagnosing more women with GDM may add burden to a country's health system, the possible benefits of diagnosing and managing such women, and in turn preventing the cycle of diabetes, should be considered. Optimising the health and nutrition of women should be prioritised to minimise excessive weight gain and the current GDM selective screening protocol needs to be reconsidered; the long term effects of such will be instrumental in reducing the prevalence of diabetes.

Author contributions

SM conceived and designed the study, acquired the data, analysed and interpreted the data, drafted the original manuscript, critically reviewed and revised the manuscript. MN recruited participants, collected data and reviewed the manuscript. DBD provided methodological advice and critically

reviewed the manuscript. SAN conceived and designed the study, interpreted the data, provided methodological advice and critically reviewed the manuscript. All authors approved the final manuscript.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Research: Pregnancy

The effects of gestational diabetes mellitus on fetal growth and neonatal birth measures in an African cohort

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Abstract

Aim Fetal exposure to gestational diabetes mellitus (GDM) is said to alter fetal growth and increase the risk of macrosomia. However, little research on GDM exists in African populations. This study aimed to assess longitudinal fetal growth and neonatal birth measures among Black African babies exposed to GDM.

Methods Pregnant women (Soweto, South Africa) enrolled into a cohort study were followed up with repeated fetal ultrasounds. At 24–28 weeks' gestation a 2-h 75 g oral glucose tolerance test was performed and GDM was diagnosed using the World Health Organization's 2013 criteria. Neonatal birth measures were assessed.

Results The study involved 741 women; 83 (11.2%) with GDM and 658 (88.8%) without. A total of 4040 fetal ultrasounds were performed. GDM exposure was associated with an increase in fetal growth measures, especially abdominal circumference, which was already seen at 16–18 weeks' gestation. Male fetuses in particular, showed a significant association between GDM exposure and increased abdominal circumference (P = 0.009). Most women with GDM (66.3%) received management; all received diet therapy and 32.7% were prescribed medication. There was no difference in birth measures between the GDM-exposed and unexposed neonates.

Conclusion Repeated ultrasound measures identified the effects of GDM as early as 16–18 weeks' gestation, well before a diagnosis of GDM would usually be made. Sex differences in fetal growth were observed, with GDM-exposed male fetuses being more affected with larger abdominal circumferences than females. A low rate of macrosomia was observed compared with historical GDM populations.

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Introduction

An optimal *in utero* environment is essential for healthy fetal growth and both maternal under- and over-nutrition can increase the offspring's risk for non-communicable diseases [1].

During pregnancy maternal tissues become insensitive to insulin thus reducing glucose absorption to shunt glucose to the developing fetus. As a result, the maternal pancreas is required to increase insulin secretion [2]. Insulin resistance peaks at around 20 weeks' gestation. Some women are unable to meet this increased insulin demand resulting in hyperglycaemia and consequently, gestational diabetes mellitus (GDM) [3].

Uncontrolled maternal hyperglycaemia causes fetal over-nutrition which can affect fetal growth [4]. Macrosomia (birthweight independent of gestational age at delivery of ≥ 4.0 kg) and being large for gestational age (birthweight > 90th centile for gestational age) [5], are well-described complications of GDM. An increase in fat is responsible for GDM-exposed babies being larger than unexposed babies [6]. For these children, GDM exposure puts them at increased risk of obesity and Type 2 diabetes later in life [7].

The oral glucose tolerance test (OGTT), performed at 24–28 weeks' gestation, is the gold standard for diagnosing GDM [8]. A diagnosis of GDM is therefore usually made late into the second trimester, however, the effects of fetal exposure to hyperglycaemia may present earlier. Understanding if and when an increase in fetal growth is observed in GDM-affected pregnancies could help clinicians identify at-risk women and their unborn babies.

The aim of this study was to evaluate longitudinal fetal growth and birth outcomes among pregnant African women with and without GDM.

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What's new?

- This is the first study in Africa to describe gestational diabetes mellitus (GDM) exposure and its effects on fetal growth.
- Male fetuses in particular, were susceptible to the hyperglycaemic environment with abdominal circumference being an indicator of fetal overgrowth.
- A very low rate of macrosomia was present among GDM-exposed neonates compared with historical GDM populations.

Participants and methods

Study design

The MRC/Wits Developmental Pathways for Health Research Unit of the University of the Witwatersrand, Johannesburg, South Africa, conducted a prospective longitudinal pregnancy cohort study from June 2013 to July 2016. During this time, 1017 women were enrolled into the Soweto First 1000 Days Study.

Study participants

Women were recruited from the Antenatal Clinic and Fetal Medicine Unit at Chris Hani Baragwanath Academic Hospital; an academic central hospital located in urban Soweto, Johannesburg. Women eligible for enrolment into the study were Black South Africans (ethnicity was self-reported), ≥ 18 years of age, residing in Soweto, and pregnant with singleton pregnancies that were preferably < 14 weeks' gestation but no more than 20 weeks' gestation. Women could not have been diagnosed with any known type of diabetes or epilepsy (due to the concern of certain antiepileptic drugs interfering with glucose metabolism) at the time of recruitment. Fetal abnormalities excluded women from the study.

Ethics

The University of the Witwatersrand's Human Research Ethics Committee (Medical) granted clearance for the study (Certificate references: M120524 and M130309). Study participants gave informed, written consent to participate.

Data collection

A participant's first visit to the research unit involved a dating scan, anthropometric measures and the completion of pregnancy-related and sociodemographic questionnaires. A household socio-economic status (SES) score was calculated as the total number of specified household assets that a woman had. This scoring system has been used in South African research studies as a proxy for household SES [9].

Ultrasonography

A pregnancy dating scan involved measuring the fetal crown–rump length at < 14 weeks + 0 days, or the biparietal diameter, head circumference and femur length in more advanced pregnancies (> 14 weeks but < 20 weeks). Thereafter, participants were invited for follow-up scans every 5 weeks. Follow-up visits were at 14–18, 19–23, 24–28, 29–33 and 34–38 weeks' gestation. Gestational age at each visit and at delivery was calculated from the gestational age determined by the dating scan. A Philips HD-9 (Philips Ultrasound, Bothell, WA, USA) ultrasound machine was used. All scans underwent external quality assessment by colleagues at Oxford University (UK).

The oral glucose tolerance test

At 24–28 weeks' gestation participants underwent a 2-h 75-g OGTT after an overnight fast. Prior to administering the OGTT, research nurses performed a finger prick capillary fasting glucose test using a glucometer on the participants. Women who had fasted but whose capillary glucose levels were ≥ 7.0 mmol/l were immediately referred to the hospital due to concern of them having overt diabetes. An OGTT under research conditions was therefore not conducted on these women. The World Health Organization's (WHO) 2013 criteria for diagnosing GDM were used (fasting plasma glucose of 5.1–6.9 mmol/l or 1-h plasma glucose of ≥ 10.0 mmol/l or 2-h plasma glucose of 8.5–11.0 mmol/l). A fasting plasma glucose of ≥ 7.0 mmol/l or a 2-h plasma glucose of ≥ 11.1 mmol/l is diagnostic of overt diabetes [10]. Venous blood samples were run on site using the RX Daytona Chemistry Analyzer (Randox, London, UK). A random selection of 150 samples was run in duplicate to ascertain the coefficient of variation of the laboratory technician, which was determined to be 2.3%.

Women diagnosed with GDM were referred to the Obstetric Diabetes Clinic at the hospital. The clinic's protocol involves regular monitoring of women with GDM; they are seen every 2 weeks until 32 weeks' gestation and then once a week until delivery. Induction of labour or Caesarean section is planned for around 38 weeks' gestation. A dietitian consults with the women and a diet comprising of 40% carbohydrate, 40% fat and 20% protein is recommended. All women are provided with a glucometer for at-home capillary glucose monitoring which they are encouraged to do pre-meal, 1 or 2 h postprandial, and late at night. Target blood glucose levels should be: fasting < 5.3 mmol/l; 1 h postprandial < 7.8 mmol/l; and 2 h postprandial < 6.7 mmol/l. Women who do not meet the glucose targets following 1–2 weeks of dietary modification are prescribed metformin. If glucose levels are not well-controlled on metformin, or if metformin is not well-tolerated, women are moved onto insulin therapy. In instances where initial

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glucose readings are exceedingly highly, metformin may be bypassed and insulin therapy initiated immediately [11].

Anthropometry

Maternal weight (kg) and height (cm) were taken at enrolment using the SECA stadiometer and SECA digital weighing scale (SECA, Hamburg, Germany) respectively.

Neonatal birthweight (g), length (cm) and head circumference (cm) were measured using the calibrated SECA Baby Scale 376 (SECA), Harpenden Infantometer (Holtain, London, UK) and a metal head circumference tape measure (CMS ref.3105) (Chasmors Ltd, London, UK) respectively. Measurements were taken within 24 h of delivery. The coefficient of variation of all anthropometric measures between research nurses and assistants was < 1%.

Ponderal Index ([birthweight (g) birth length $(cm)^3$] × 100) measured relative weight-for-length at birth and was used as an indicator of neonatal body composition (< 2.0 g/cm^3, small; 2.0–2.49 g/cm^3, marginal; 2.5–2.99 g/cm^3, normal; ≥ 3.0 g/cm^3, overweight) [12].

The International Newborn Size at Birth Standards Application tool [13] calculated centiles and *z*-scores for birthweight. This tool takes into account gestational age at delivery (total days) and the sex of the newborn. The classification of neonates based on birthweight was as follows: < 10th centile, small for gestational age; 10th–90th centile, appropriate for gestational age; and > 90th centile, large for gestational age [14]. Macrosomia was defined as a birthweight irrespective of gestational age at delivery of ≥ 4.0 kg [15]. Birthweight of < 2.5 kg irrespective of gestational age at delivery was considered low birthweight [16].

A subset of neonates underwent a fat mass (g) assessment using the PeaPod® (Cosmed, Concord, CA, USA). The PeaPod® determines infant body composition through Air Displacement Plethysmography. Body density is calculated (mass/volume) and thereafter fat percentage and fat-free mass is determined.

Statistical analyses

Stata (version 12.0; StataCorp, College Station, TX, USA) was used for data analyses. The Shapiro–Wilk and skewness and kurtosis tests assessed the distribution of continuous data. Descriptive continuous variables that were normally distributed were presented as means ± SD and those that were not normally distributed were presented as medians [interquartile range (IQR)]. Categorical data were presented as frequencies and percentages. Differences between categorical variables were determined using the chi-squared test. The Student's *t*-test and one-way ANOVA (with the Bonferroni adjustment for multiple comparisons) were used to analyse differences between normally distributed variables. Differences between variables that were not normally distributed were determined using the Mann–Whitney test and the Kruskal–Wallis H test (with the Conover–Iman test of multiple comparisons). Multiple linear regression analysis was applied to study associations between certain variables and Ponderal Index and fat mass. Significance was assumed at a two-tailed *P*-value of < 0.05.

Linear mixed effects modelling (LMM) [17,18] was used to examine the differences in fetal growth, assessed separately for biparietal diameter, head circumference, abdominal circumference and femur length, over the course of pregnancy. LMM is a flexible modelling statistical technique that deals with the correlation of repeated measurements. Time-varying and time-invariant fixed effects were also analysed using LMM. All models were run with a random intercept and a random slope with unstructured residual correlation. In the final model, fixed effects were the covariates, GDM status and time (gestational age in days), whereas random effects were the intercept and visit. Different interactions were explored and included in the model if significant. Potential confounders were fetal sex, baseline BMI, weekly change in weight gain and baseline maternal age. The GDM and non-GDM groups stratified by fetal sex were analysed, and then the pooled dataset was analysed. A likelihood ratio test was used to select the best fitting model; with or without random effects.

Results

Of the 1017 women enrolled in the study, 807 (79.4%) underwent an OGTT and of those all three glucose readings were available on 741 (91.8%) women. Figure 1 describes how the sample sizes for the various aspects of this study were derived.

Most participants were enrolled at 12.0 (13.0–14.0) weeks' gestation. Eighty-three (11.2%) of the 741 women were diagnosed with GDM and none had overt diabetes. Women with GDM were significantly older, heavier from a weight and BMI perspective, had a higher gravidity and parity and were more likely to have had a family history of diabetes than women without GDM (Table 1).

Fetal growth measures

A total of 4040 fetal ultrasounds were performed with each participant receiving a median of 6 (5–6) scans. The median gestational weeks for each of the follow-up visits were: visit 2, 17 (16–18) weeks; visit 3, 22 (21–22) weeks; visit 4, 27 (26–27) weeks; visit 5, 32 (31–32) weeks; and visit 6, 37 (36–37) weeks. Compared with female fetuses, males had significantly larger biparietal diameters, head circumferences and abdominal circumferences but femur length did not differ significantly between the sexes (Table S1).

The GDM-exposed fetuses had significantly larger biparietal diameters, head circumferences and abdominal circumferences than the unexposed group, particularly at visit 4 [27

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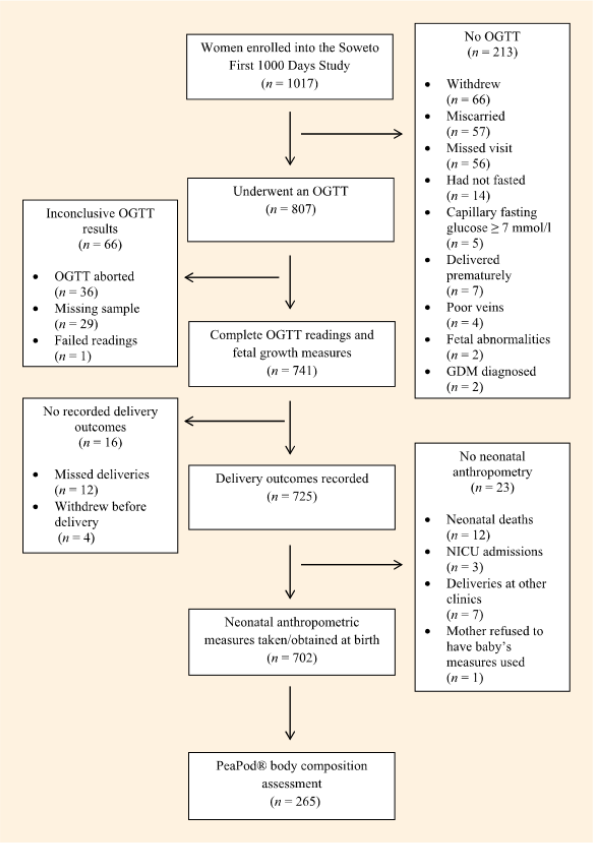


FIGURE 1 Flow diagram describing how the study sample was determined. OGTT, oral glucose tolerance test; GDM, gestational diabetes mellitus; NICU, neonatal intensive care unit.

(26–27) weeks] when a diagnosis of GDM was made. Abdominal circumference was significantly larger across all five visits in GDM-exposed vs. unexposed fetuses. Femur length did not differ between the two groups (Fig. 2; Table S2).

The effect of maternal BMI on fetal growth in the whole group was compared. Maternal BMI had a statistically significant effect on femur length throughout pregnancy with larger mothers having fetuses with longer femurs. There was a significant difference in head circumference at 32 (31–32) weeks' gestation and in abdominal circumference at 27 (26–27) and 32 (31–32) weeks' gestation ($P < 0.001$) between fetuses of women in the different BMI categories (Table S3). When stratified by GDM status, maternal BMI did not seem to affect fetal size in the group with GDM. In the group without GDM, abdominal circumference and head circumference differed significantly at specific gestational time points, and femur length differed significantly throughout pregnancy (Table S4).

The results from the LMM revealed that biparietal diameter, head circumference and femur length were not associated with GDM status. However, the pooled model for abdominal circumference showed a positive association with GDM status ($P = 0.007$) (Table 2). When stratified by sex, the same results were found for male fetuses ($P = 0.009$) but not for female fetuses ($P = 0.286$). When maternal BMI and weight change (kg) per week were included in the pooled model, GDM still had an effect on abdominal circumference

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Table 1 Characteristics of the women participating in the S1000 study

Characteristic	Total women	Women without GDM	Women with GDM	*P*-value
Total*	741 (100.0)	658 (88.8)	83 (11.2)	
Age (years)	29 (25–34)	29 (24–34)	31 (27–36)	< 0.001†
Household socioeconomic status (asset score/11)	6 (5–6)	6 (5–6)	6 (5–6)	0.569
Level of education*				
No schooling/primary school	16 (2.2)	13 (2.0)	3 (3.6)	0.356
Secondary school	535 (72.2)	472.0 (71.7)	63 (75.9)	
Tertiary education	190 (25.6)	173 (26.3)	17 (20.5)	
Marital status*				
Single	462 (62.4)	415 (63.1)	47 (56.6)	0.254
Married/cohabiting	279 (37.7)	243 (36.9)	36 (43.4)	
Family history of diabetes (n = 739)*	19 (25.9)	161 (24.5)	30 (36.1)	0.023†
Weight (kg)	69.1 (59.4–80.2)	68.6 (59.0–79.3)	73.0 (63.9–86.8)	0.002†
BMI	27.6 (23.7–31.4)	27.4 (23.3–31.1)	29.2 (25.2–34.8)	0.001†
Weight gain (kg) (enrolment to OGTT)	5.0 (2.9–7.0)	5.0 (2.9–7.0)	4.4 (3.0–6.7)	0.326
Total gestational weight gain (kg)	9.1 (6.2–12.1)	9.2 (6.2–12.2)	7.3 (5.9–11.4)	0.127
Gestational age at enrolment (weeks)	12 (11–13)	12 (11–13)	13 (11–13)	0.627
Gravidity‡	2 (1–3)	2 (1–3)	2 (1–3)	0.010†
	1.9 ± 1.3	1.8 ± 1.3	2.2 ± 1.3	
Parity	1 (0–2)	1 (0–2)	1 (1–2)	0.016†
Previous macrosomic infant (*n* = 739)*	40 (5.4)	33 (5.0)	7 (8.4)	0.197
HIV positive at enrolment*	234 (31.6)	204 (31.0)	30 (36.1)	0.342
Fasting glucose (mmol/l)	4.2 (3.8–4.6)	4.1 (3.7–4.4)	5.2 (5.1–5.6)	< 0.001†
60 min post-glucose load (mmol/l)	5.9 (5.0–7.1)	5.7 (4.9–6.8)	8.3 (7.1–9.7)	< 0.001†
120 min post-glucose load (mmol/l)	5.5 (4.7–6.4)	5.4 (4.6–6.2)	7.5 (6.5–8.6)	< 0.001†

OGTT, oral glucose tolerance test.
Values given as median (interquartile range) or *n (%). Sample size (n) indicated if less than 741. Gestational diabetes mellitus (GDM) diagnosed using the World Health Organization's 2013 criteria.
†$P < 0.05$ indicates a statistically significant difference.
‡Mean ± SD given as well when median (interquartile range) does not illustrate the difference between the groups.

but BMI and weight change appeared to have a direct effect on abdominal circumference independent of GDM. There were no interactions between BMI and GDM, and weight change and GDM. Over time, the head circumference/abdominal circumference ratio became smaller among the GDM-exposed group with head circumference remaining unchanged but abdominal circumference increasing in size.

Management of women with GDM

All 83 women with GDM were referred to the Obstetric Diabetes Clinic and most followed through with their referrals (55 of 83; 66.3%). All received diet therapy and 18 (32.7%) were treated with medication; 17 (94.4%) with metformin and one (5.6%) with insulin.

Delivery outcomes

Of the whole group ($n = 725$), women who delivered early (< 39 weeks' gestation encompassing the preterm and early term categories [19]; $n = 342$) were of higher parity than those who delivered later (≥ 39 weeks gestation; $n = 383$); 1 (1–2) vs. 1 (0–1) ($P = 0.007$) but did not differ otherwise (results not shown). Women with GDM delivered early than women without GDM; 38 (37–39) weeks vs. 39 (38–40) weeks ($P = 0.001$), and the Caesarean section rate was higher among the group with GDM; 73.2% vs. 56.3% ($P = 0.004$) (Table 3). Male neonates had significantly larger birthweights, lengths and head circumferences than females (Table S5).

There was no significant difference in birth size between the GDM-exposed and unexposed neonates. The Ponderal Index did not differ between the two groups ($P = 0.245$) (Table 3). Similarly, when stratified by sex, there were no differences in birth measures between GDM-exposed and unexposed neonates (Table 4). Multiple regression analysis was run to predict Ponderal Index from sex, gestational age at delivery and GDM exposure. None of these variables were significantly associated with Ponderal Index ($R^2 = 0.004$).

Fat mass (g) was determined on 265 of the neonates (25 GDM-exposed and 240 unexposed neonates) using the PeaPod®. The median fat mass for the whole group was 419.1 (296.9–565.1) g. The median fat mass for the GDM-exposed neonates was 439.8 (340.0–549.9) g and for the unexposed neonates was 415.5 (292.9–566.3) g ($P = 0.530$). A multiple regression analysis was run to predict fat mass based on sex, gestational age at delivery, birth length and GDM exposure. A positive regression equation was found ($R^2 = 0.091$). Sex ($\beta = 57.09$, $P = 0.018$), gestational age at delivery ($\beta = 19.26$, $P = 0.016$) and birth length ($\beta = 14.13$, $P = 0.002$) were statistically significantly associated with fat mass, but GDM exposure was not ($\beta = 38.24$, $P = 0.349$).

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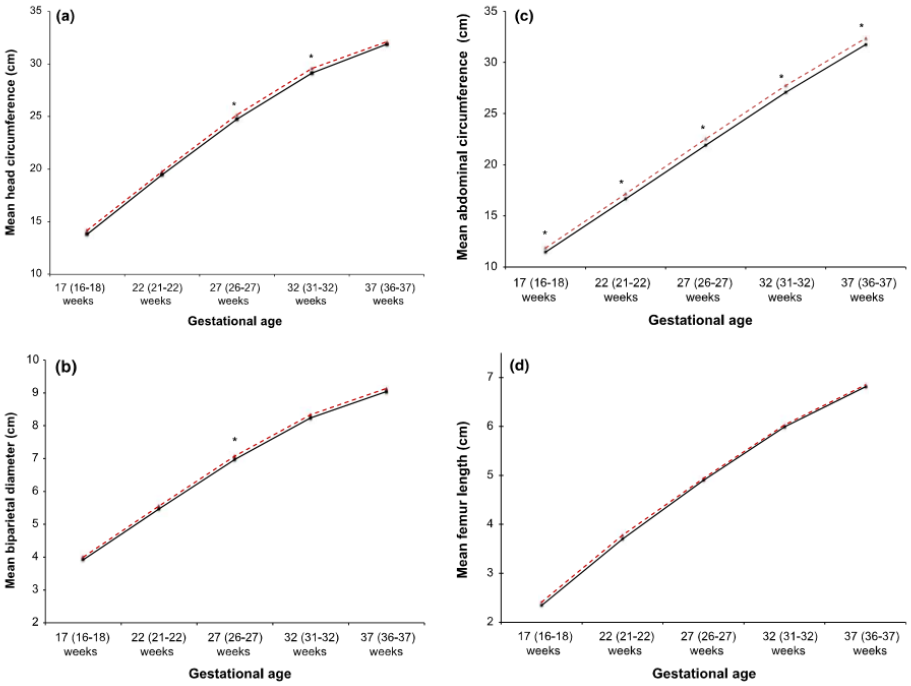


FIGURE 2 Head circumference (a), biparietal diameter (b), abdominal circumference (c) and femur length (d) of fetuses exposed to gestational diabetes mellitus (GDM) (n = 83; red dashed line) vs. unexposed fetuses (n = 658; solid black line) against gestational age in weeks. *Significant difference in size between the two groups ($P < 0.05$) with GDM-exposed fetuses being larger than unexposed fetuses.

Table 2 Linear mixed modelling results showing fixed effects on abdominal circumference size (cm) *in utero*

	Coefficient	*P*-value	95% CI
Gestational age	0.15	< 0.001	0.14, 0.15
Maternal age	0.00	0.725	−0.02, 0.01
Positive gestational diabetes mellitus status	0.24	0.007	−0.11, 0.39
Visit number	−0.01	0.959	−0.40, 0.19
BMI at enrolment	0.02	< 0.001	0.00, 0.02
Average weight change per week (kg)	0.31	0.034	−0.12, 0.67

Discussion

Our study has shown that GDM influences fetal growth; fetuses exposed to hyperglycaemia were larger than unexposed fetuses. The difference in abdominal circumference size was seen as early as 16–18 weeks' gestation. Sovio *et al.* [20] showed that excessive growth of the abdominal circumference in GDM-exposed fetuses occurs between 20 and 28 weeks' gestation thus preceding a diagnosis of GDM, but our results show that this increased growth occurs even earlier.

The high Caesarean section rate observed for the entire study population (58.2%) is not surprising given the fact that Chris Hani Baragwanath Academic Hospital is a highly specialized central hospital. The national Caesarean section rate target set by the South African Department of Health for central hospitals is 50% [21]. A study that investigated reasons for Caesarean sections at Chris Hani Baragwanath Academic Hospital found fetal distress to be the most common indication, followed by previous Caesarean section and labour dystocia, respectively [22]. The higher parity found among women who delivered early (< 39 weeks) in the current study suggests those women may have had previous Caesarean sections therefore necessitating a further Caesarean section which resulted in earlier delivery. The earlier deliveries and higher Caesarean section rate among the women with GDM align with the hospital's GDM management protocol. The induction rate among women with GDM

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Table 3 Delivery outcomes and birth anthropometry for neonates exposed to gestational diabetes mellitus and unexposed neonates

Variable	Total neonates	Unexposed neonates	GDM-exposed neonates	*P*-value
Delivery outcomes	*n* = 725	*n* = 643	*n* = 82	
Sex of neonate*				
Male	374 (51.6)	329 (51.2)	45 (54.9)	0.526
Female	351 (48.4)	314 (48.8)	37 (45.1)	
Gestational age at delivery (weeks)	39 (38–40)	39 (38–40)	38 (37–39)	0.001†
Preterm delivery*	102 (14.1)	86 (13.4)	16 (19.5)	0.132
Mode of delivery*				
Vaginal	303 (41.8)	281 (43.7)	22 (26.8)	0.004†
Caesarean section	422 (58.2)	362 (56.3)	60 (73.2)	
Birth anthropometry	*n* = 702	*n* = 620	*n* = 82	
Birthweight (g)	3030.0 (2685.0–3300.0)	3030.0 (2695.0–3304.5)	3067.5 (2600.0–3250.0)	0.562
Birthweight category*				
Small for gestational age	125 (17.8)	113 (18.2)	12 (14.6)	0.709
Appropriate for gestational age	532 (75.8)	467 (75.3)	65 (79.3)	
Large for gestational age	45 (6.4)	40 (6.5)	5 (6.1)	
Macrosomic*	17 (2.4)	16 (2.6)	1 (1.2)	0.451
Low birthweight*	113 (16.1)	97 (15.7)	16 (19.5)	0.371
Birth length (cm) (*n* =701)	48.6 (46.7–50.2)	48.7 (46.8–50.3)	48.2 (46.5–49.2)	0.080
Weight for length ratio (kg/m)	6.2 (5.6–6.7)	6.2 (5.6–6.7)	6.2 (5.6–6.6)	0.706
Birth head circumference (cm) (n =701)	34.0 (33.1–35.0)	34.1 (33.2–35.0)	34.0 (33.0–35.0)	0.237
Ponderal Index (g/cm^3)	2.6 (2.4–2.9)	2.6 (2.4–2.8)	2.7 (2.4–2.9)	0.245

Values given as median (interquartile range) or **n* (%). †$P < 0.05$ indicates a statistically significant difference.
GDM, gestational diabetes mellitus.

Table 4 Birth outcomes of male and female neonates exposed to gestational diabetes mellitus compared to unexposed male and female neonates

	Male neonates			Female neonates		
	GDM-exposed	Unexposed	*P*-value	GDM-exposed	Unexposed	*P*-value
Weight (g)	3080 (2540–3250)	3055 (2740–3345)	0.421	3035 (2675–3230)	3000 (2635–3240)	0.923
Length (cm)	48.2 (46.3–49.7)	49.0 (47.0–51.0)	0.072	48.1 (46.5–49.0)	48.1 (46.3–50.0)	0.486
Head circumference (cm)	34.2 (33.0–35.8)	34.3 (33.2–35.2)	0.953	33.6 (33.0–34.5)	34.0 (33.1–35.0)	0.083
Ponderal Index	2.7 (2.4–2.9)	2.6 (2.4–2.8)	0.375	2.7 (2.5–2.9)	2.6 (2.4–2.9)	0.448

Values given as median (interquartile range)
GDM, gestational diabetes mellitus.

at the hospital is thought to be around 60–65% (V. Nicolaou, pers. comm., 12 February 2018).

The LMM results showed a positive association over the course of gestation between GDM and abdominal circumference for the whole sample but when stratified by sex this was only observed among male fetuses. This is likely to be a finding of sexual dimorphism. Male sex is an independent risk factor for adverse pregnancy outcomes with females having an advantage over males [23]. Male fetuses are more susceptible to the *in utero* environment as they capitalize on ways of increasing their growth and are therefore more responsive to the current maternal diet than their female counterparts [24]. A recent study found that GDM-exposed male offspring had higher BMIs and an increased risk of obesity across late childhood, adolescence and early adulthood, compared with unexposed offspring, but, this difference was not observed among female offspring [25].

Abdominal circumference is thought to be a good indicator for fetopathy [26]. An abdominal circumference of ≥ 35 cm in the third trimester is apparently predictive of 93% of macrosomic births [27]. The mean abdominal circumference measurement at 37 weeks' gestation among the GDM-exposed fetuses in the current study was 32.36 ± 1.79 cm (Table S2). Although the GDM-exposed fetuses had larger abdominal circumferences than the unexposed fetuses, the threshold was still below that predictive of macrosomia.

The absence of a difference in birth size between the GDM-exposed and unexposed neonates could possibly be related to the management of the women with GDM. Most women with GDM received clinical intervention (dietary advice at the least). Management of GDM by nutritional counselling and diet therapy has been shown to significantly reduce the mean birthweight and neonatal fat mass, as well as the frequency of LGA and macrosomic neonates [28]. However, another possibility could be that the *in utero* effects of hyperglycaemia are in fact small.

The lack of difference in birth size between GDM-exposed and unexposed neonates has been reported by others [29].

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However, those investigators found that Ponderal Indexes and body fat were higher among the GDM-exposed neonates [29]. Our study found no difference in Ponderal Index between the two groups (P = 0.245). From the PeaPod® results on the subset of neonates, the GDM-exposed group appeared to have 5.8% (24.3 g) more fat mass than the unexposed group (439.8 vs. 415.5 g) but the difference was not statistically significant. This 5.8% difference may be clinically relevant and given the larger abdominal circumference seen *in utero* among GDM-exposed fetuses it is possible that at birth they have more adiposity around the abdomen than anywhere else. Long-term follow up of these babies is needed as there is no African GDM-exposed cohort data.

The small sample size for the PeaPod® assessment (n = 265) may be a limiting factor in detecting any significant difference in fat mass between the two groups. One might also question whether the study was underpowered to detect a difference in birth measures between the two groups. However, a sample size calculation using mean birthweights from a previous South African GDM study [30] determined that a minimum sample size of 144 neonates (72 GDM-exposed and 72 unexposed neonates) (power of 90% and α significance level of 0.05) was required in order to detect a significant difference in birthweight. Our study was sufficiently powered with 82 GDM-exposed and 620 unexposed neonates.

This unique study has demonstrated a significant association between GDM-exposure and fetal growth in African babies. Second trimester abdominal circumference measures indicated fetal overgrowth with male fetuses being more susceptible to the hyperglycaemic environment than females. Despite there being no difference in birth size between GDM-exposed and unexposed neonates, the increased fetal abdominal circumference may still confer metabolic risk later in life, particularly among male offspring [31].

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Competing interests

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Fetal measurements by fetal sex at five time points during pregnancy.
Table S2. Fetal growth measurements in gestational diabetes mellitus (GDM)-exposed vs. unexposed fetuses.
Table S3. Fetal measurements by maternal BMI categories at five time points during pregnancy.
Table S4. Fetal measurements by maternal BMI and gestational diabetes mellitus (GDM) status at five time points during pregnancy.
Table S5. Delivery outcomes and birth anthropometry of male vs. female neonates.

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APPENDIX K: PLAGARISM DECLARATION & TURNITIN REPORT

Plagiarism Declaration

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