THE IDENTIFICATION OF MICRORNA SIGNATURES ASSOCIATED WITH GLYCAEMIC CONTROL AND PREGNANCY OUTCOMES IN PREGNANCIES COMPLICATED BY TYPE 1, TYPE 2, AND GESTATIONAL DIABETES MELLITUS

By

Nompumelelo Lebogang Malaza

In fulfilment of the requirements for the

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DECLARATION

I, Nompumelelo Lebogang Malaza, declare that the dissertation/thesis, which I hereby submit for the degree Philosophiae Doctor at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

The author, whose name appears on the title page of this dissertation/thesis, has obtained, for the research described in this work, the applicable research ethics approval. The author declares that she has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

Signature: _____

Student name: Nompumelelo Lebogang Malaza (Student no. 12174506)

Date: 02 April 2024

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DEDICATION

I would like to dedicate my PhD to my beloved mother, Mazondo Malaza. I am deeply grateful for the countless sacrifices you have made for my education, and for supporting me every step of the way. Your unwavering faith and encouragement have been a source of strength throughout this journey. I would also like to pay tribute to my guardian angels, my late father, Reuben Mashego, and my late grandmother, Milile Malaza. Your guidance and support have been instrumental in helping me pursue my dreams. Though you are no longer with me, I know that you are watching over me with pride and love. Lastly, I want to give thanks to the Almighty Lord for being my constant companion and guiding me through every challenge in this journey.

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Malaza, N., Masete, M., Adam, S., Dias, S., Nyawo, T. and Pheiffer, C., 2022. A systematic review to compare adverse pregnancy outcomes in women with pregestational diabetes and gestational diabetes. *International Journal of Environmental Research and Public Health*, *19*(17), p.10846.

Masete, M., Dias, S., **Malaza, N.**, Adam, S. and Pheiffer, C., 2022. A big role for microRNAs in gestational diabetes mellitus. *Frontiers in Endocrinology*, *13*, p.892587.

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Malaza, N., Pheiffer, C., Masete, M., Dias, S. and Adam S., 25-26 October 2022. Comparison of obstetrics and perinatal outcomes in women with diabetes at Steve Biko Academic Hospital. *SAMRC Research and Capacity Development 16th Early Career Scientist Convention, Cape Town, South Africa.*

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LIST OF ABBREVIATIONS

ACE	Angiotensin-Converting Enzyme
ACOG	American College of Obstetrics and Gynaecology
AGA	Appropriate for gestational age
APOs	Adverse pregnancy outcomes
ARVs	Antiretrovirals
AUC	Area under the curve
BMI	Body mass index
CPRR	Competitive Programme for Rated Researchers
CS	Caesarean section
DGCR8	DiGeorge Syndrome Critical Region 8
DIP	Diabetes in pregnancy
DKA	Diabetic ketoacidosis
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FPG	Fasting plasma glucose
GA	Gestational age
GAD	Glutamic acid decarboxylase
GDM	Gestational diabetes
HAPO	Hyperglycaemia and Adverse Pregnancy Outcomes

HbA1c	Glycated haemoglobin
HIV	Human Immunodeficiency Virus
HMW	high molecular weight
IADPSG	International Association of Diabetes and Pregnancy Study Groups
IBM	International Business Machines
ICU	Intensive care unit
IOL	induction of labour
LGA	large for gestational age
MetDiet	Mediterranean diet
MiRNAs	MicroRNAs
NCDs	Non-communicable diseases
NICU	Neonatal intensive care unit
NRF	National Research Foundation
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
PTB	Preterm birth
qRT-PCR	Quantitative real-time PCR
RDS	Respiratory distress syndrome
RIA	Radioimmunoassay
RISC	RNA-induced silencing complex

RNA	Ribonucleic acid
ROC	Receiver Operating Characteristics
RT	Real-time
SA	South Africa
SAMRC	South African Medical Research Council
SBAH	Steve Biko Academic Hospital
SEM	Standard error of mean
SEMDSA	Society of Endocrinology, Metabolism and Diabetes in South Africa
SGA	Small for gestational age
SHBG	Sex hormone binding globulin
SNAT	System A sodium-dependent neutral amino acid transporter
T1DM	Type 1 diabetes
T2DM	Type 2 diabetes
USA	United States of America
UTR	Untranslated region
WHO	World Health Organization
ZnT8	Zinc transporter 8

ABSTRACT

Background. Diabetes in pregnancy (DIP) is associated with short- and long-term adverse pregnancy outcomes for both mother and child. However, pregestational type 1 (T1DM) and type 2 (T2DM) are associated with more common and severe pregnancy outcomes compared to gestational diabetes (GDM). Maternal biochemical and epigenetic markers and knowledge of diabetes have been associated with glycaemic control and adverse pregnancy outcomes in women with DIP, therefore they offer potential to serve as markers. This may aid in reducing adverse outcomes and improve mother and child health.

Aim. The aim of this study was to compare adverse pregnancy outcomes by DIP type and explore the candidacy of adiponectin, leptin, sex hormone binding globulin (SHBG), microRNAs (miRNAs) and diabetes knowledge to serve as markers of glycaemic control during pregnancy and perinatal outcomes in pregnancies complicated by T1DM, T2DM and GDM.

Methods. A prospective study was conducted at the high-risk antenatal clinic at Steve Biko Academic Hospital, Pretoria, South Africa between May 2017 and April 2023. The study population consisted of 232 pregnant women with pregestational T1DM (n=27) or T2DM (n=78), GDM (n=58), and normoglycaemia (n=69). Maternal serum adiponectin, leptin and SHBG levels were measured using enzyme linked immunosorbent assays (ELISAs). Maternal serum miRNAs were measured using quantitative real-time PCR. The diabetes knowledge and perceptions questionnaire was developed in three phases and content validity was tested in 20 women with GDM using researcher-administered interviews.

Results. Pregestational T1DM and T2DM were associated with an increased risk of preterm birth (p=0.002). Obesity was associated with a higher frequency of GDM (p=0.036), while body weight ≥ 80 kg was associated with caesarean section before the onset of labour (p<0.05). Lower maternal leptin levels were associated with large for gestational age (LGA; p=0.036), macrosomia

(birthweight more than 4 kg; p=0.060) and preterm birth (PTB; p=0.004). Lower levels of maternal SHBG were associated with macrosomia (p=0.025) and levels were negatively correlated with neonatal birthweight (r=-0.263, p=001). No association between maternal adiponectin levels and neonatal birth outcomes was observed. Four miRNAs (miR-124-3p, miR-128-3p, miR-20a-5p and miR-210-3p) were associated with small for gestational age (SGA) and were able to predict SGA, miR-124-3p (AUC=0.815), miR-128-3p (AUC=0.760), miR-20a-5p (AUC=0.841) and miR-210-3p (AUC=0.779). MiR-210-3p was associated with macrosomia and demonstrated good predictive ability (AUC=0.779). MiR-222-3p was increased in women with good glycaemic control compared to women with poor glycaemic control during pregnancy. A comprehensive questionnaire for evaluating diabetes knowledge in South African pregnant women with GDM was developed, which performed well in terms of content validity and was able to assess knowledge of diabetes in pregnant women with GDM.

Conclusion. To our knowledge, this is the first study to investigate the association between maternal adiponectin, leptin, SHBG and miRNAs with neonatal birth outcomes in South Africa. Our findings suggest that maternal leptin, SHBG and miRNAs may offer potential as biomarkers of neonatal birth outcomes and glycaemic control. Additionally, this is the first study to develop a questionnaire to evaluate diabetes knowledge in women with GDM in South Africa. The developed questionnaire may aid in identifying knowledge gaps in pregnant women with GDM, thereby enhancing education programs and developing interventions to improve glucose management and pregnancy outcomes in women with GDM. However, further studies in larger and multi-ethnic populations are warranted to explore the candidacy of biochemical and miRNA biomarkers for glycaemic control and neonatal birth outcomes. Additionally, the developed questionnaire requires a comprehensive validation process to evaluate construct validity, internal consistency, and test-retest reliability in future.

Word count: 565

Key words: diabetes in pregnancy (DIP), type 1 diabetes (T1DM), type 2 diabetes (T2DM), gestational diabetes (GDM), adiponectin, leptin, sex hormone-binding globulin (SHBG), microRNAs (miRNAs), diabetes knowledge, South Africa.

THESIS OUTLINE

CHAPTER 1 provides 1) the rationale, which gives context to the study, the research problem and highlights the importance and significance of the study, 2) aim, 3) objectives, and 4) thesis structure.

CHAPTER 2 provides a literature review on DIP, its prevalence, and describes the pathophysiology, adverse pregnancy outcomes associated with DIP, screening and diagnosis, and treatment options. This chapter also discusses biochemical markers and microRNAs (miRNAs) as potential biomarkers associated with adverse pregnancy outcomes. Lastly, this chapter discusses knowledge of diabetes as a tool to aid in glycaemic control and adverse outcomes.

CHAPTER 3 outlines the study designs used to address the different study aims and objectives, participant recruitment and the inclusion and exclusion criteria used in this study. This chapter also briefly outlines the selected outcomes and definitions, the experimental outline, and research methodologies used in the different chapters.

CHAPTER 4 presents a systematic review that compares adverse pregnancy outcomes between pregestational diabetes type 1 (T1DM) and type 2 (T2DM) diabetes and gestational diabetes (GDM). This review also discussed the pathophysiology of selected outcomes, the impact of maternal diabetes on maternal and neonatal health, and the need to improve care and diabetes in pregnancy education. This review was published in the *International Journal of Environmental Research and Public Health.* **Malaza N**, Masete M, Adam S, Dias S, Nyawo T, Pheiffer C. A

Systematic Review to Compare Adverse Pregnancy Outcomes in Women with Pregestational Diabetes and Gestational Diabetes. 2022; 19(17):10846.

CHAPTER 5 investigates the effects of maternal diabetes on obstetric and perinatal outcomes in a small sample (n= 183) of women attending the diabetic antenatal clinic at Steve Biko Academic Hospital in Pretoria, South Africa. This manuscript was published in *South African Journal of Obstetrics and Gynaecology.* **Malaza N**, Pheiffer C, Dias S, Adam S. Comparison of obstetric and perinatal outcomes in women with diabetes at Steve Biko Academic Hospital. 2023; 29(1).

CHAPTER 6 investigates the association between maternal adiponectin, leptin, and sex hormone-binding globulin (SHBG) with neonatal birth outcomes in pregnancies complicated by maternal diabetes. This chapter will be submitted as a research article to *The Journal of Diabetes & Metabolic Disorders:* **Malaza N**, Adam S, Masete M, Moloto P, Dias S, Pheiffer C. Evaluation of Serum Adiponectin, Leptin and Sex Hormone Binding Globulin levels in South African women with Diabetes in Pregnancy.

CHAPTER 7 investigates the association between maternal serum miRNAs and glycaemic control and pregnancy outcomes in pregnancies complicated by maternal diabetes. This chapter will be submitted as a research article to the *Journal of Molecular Medicine*: **Malaza N**, Masete M, Adam S, Dias S, Pheiffer C. Maternal circulating microRNAs in South African pregnant women are associated with foetal growth and birthweight.

CHAPTER 8 outlines the development process of a questionnaire to assess diabetes knowledge in women with GDM. This chapter also discusses the preliminary results from the testing of the developed questionnaire.

CHAPTER 9 summarises and discusses the findings from the individual chapters, followed by integration and synthesis of the overall findings of the thesis. The significance of the study findings, novelty, and how the results from this study contribute to existing research and advancing knowledge are discussed. The strengths and limitations of the study and recommendations for future research are stated. Lastly, the impact of the study on the public health system is discussed.

A combined list of REFERENCES can be found at the end of the thesis for easy referral.

The thesis ends with appendices including ethics approval and amendment letters, consent forms and supplementary data for chapters 4 and 8.

CHAPTER 1

INTRODUCTION

1.1. Study Overview

Globally, about 16.2% of pregnancies are complicated by diabetes (International Diabetes Federation, 2021). Diabetes in pregnancy (DIP) presents either as pregestational type 1 diabetes (T1DM) or type 2 diabetes (T2DM), T1DM or T2DM first diagnosed during pregnancy, or gestational diabetes mellitus (GDM) (International Diabetes Federation, 2021). DIP is associated with adverse short- and long-term outcomes for both mothers and their children, with the severity and frequency of the adverse effects related to the type of diabetes and degree of hyperglycaemia (Malaza et al., 2022; Schaefer-Graf et al., 2018). Adverse pregnancy outcomes (APOs) can affect up to 25% of newborns born to diabetic mothers (Eriksson, 2009). Short-term adverse outcomes include pre-eclampsia, caesarean section (C/S), congenital anomalies, small for gestational age (SGA), large for gestational age (LGA), macrosomia and preterm birth. In the long-term, women with pregestational diabetes are more likely to develop diabetic complications such as retinopathy, nephropathy and neuropathy or worsening of complications if they already exist (Schaefer-Graf et al., 2018; Sugrue and Zera, 2018). Women with GDM have a ~7-fold increased risk of developing T2DM (Bellamy et al., 2009) and ~4-fold increased risk of developing cardiovascular and coronary artery disease (Harreiter et al., 2014).

Adequate glycaemic control is critical to reduce the risk of adverse pregnancy outcomes. Glycated haemoglobin (HbA1c) is the gold standard for monitoring glucose control, however, it is a poor predictor of acute blood glucose changes during pregnancy and is therefore not recommended as a glucose monitoring tool for pregnant women (American Diabetes Association, 2022). It is advised that HbA1c be used as a secondary indicator of glycaemic management during pregnancy, following glucose self-monitoring (American Diabetes Association, 2022). However, the effectiveness of self-monitoring is highly dependent on patient compliance (Cosson et al., 2017) and can be expensive if there is a high burden of disease (Lombard, 2011). There is a need

for more acceptable, effective and affordable tools to monitor glycaemic control during pregnancy and to identify women at risk of adverse pregnancy outcomes.

Studies have reported that serum biochemical and molecular markers and knowledge of diabetes is associated with glycaemic control and adverse pregnancy outcomes in women with DIP (Adam et al., 2018; Garcia-Beltran et al., 2022; Guo et al., 2018; Kapustin et al., 2020). Serum biochemical markers including adipokines and sex hormone binding globulin (SHBG) have been reported to be dysregulated during pregnancy and have been associated with poor glycaemic control and adverse outcomes (Kapustin et al., 2020; Pheiffer et al., 2021; Xargay-Torrent et al., 2018). In recent years, molecular mechanisms have been explored as potential biomarkers of metabolic dysregulation and glucose homeostasis. Of those, microRNAs (miRNAs) small, non-coding RNAs that modulate gene expression through post-transcriptional mechanisms (Esteller, 2011), have garnered considerable interest. Studies have shown that miRNAs play an important role in regulating the metabolic and developmental processes during pregnancy (Krützfeldt and Stoffel, 2006; Sayed and Abdellatif, 2011).

Effective management of DIP is important to reduce and prevent adverse pregnancy outcomes (Brown et al., 2018; Hod et al., 2015). Monitoring blood glucose levels is a common way to measure how well DIP managed, but less emphasis is placed on determining how well a woman understands the key components required to meet glycaemic targets. Studies have shown that poor diabetic knowledge is associated with poor adherence to self-management (Shams et al., 2016; Worku et al., 2015) and glycaemic control (Martis et al., 2018; Ong et al., 2014), thereby increasing the risk of adverse pregnancy outcomes. Adequate diabetes knowledge may aid in adherence to glucose monitoring targets and achieving adequate glycaemic control during pregnancy.

Limited studies in Africa have investigated the association between DIP and adverse pregnancy outcomes. Furthermore, there are no studies that have explored the association between biomarkers (both biochemical and molecular), knowledge of diabetes, glycaemic control, and adverse pregnancy outcomes in South Africa.

1.2. Problem statement

The prevalence of DIP is increasing rapidly both nationally and internationally. South Africa has high rates of obesity and T2DM particularly in women of reproductive age which contributes to the high prevalence of DIP (Black et al., 2013; Onubi et al., 2016). Furthermore, South Africa has a high prevalence of maternal and child mortality and morbidity attributed to pregnancy and childbirth (Mabaso et al., 2014; Van Zyl and Levitt, 2018). Therefore, targeting DIP presents an opportunity to improve maternal and child health outcomes. The identification of novel, simple, accessible, and affordable biomarkers for glucose monitoring and prediction of adverse pregnancy complications that are applicable to low- to middle-income countries (LMICs), such as South Africa will positively impact maternal and child health.

1.3. Rationale

Identifying biochemical and molecular biomarkers that are associated with glycaemic control and adverse pregnancy outcomes, as well as diabetes-related knowledge gaps in LMICs like South Africa, can aid in identifying women who are at a higher risk of pregnancy complications. This, in turn, can facilitate intervention strategies to reduce the prevalence of adverse pregnancy outcomes, further reducing the burden of non-communicable diseases in an already resource-limited country. Literature has shown that biochemical and molecular markers and diabetes knowledge are associated with adequate glycaemic control and reduced pregnancy complications (Adam et al., 2018; Garcia-Beltran et al., 2022; Guo et al., 2018; Kapustin et al., 2020). Therefore,

the identification of altered levels of adiponectin, leptin, SHBG and miRNAs, as well as developing tools to assess knowledge of GDM, can provide prospective markers of glycaemic control and adverse pregnancy outcomes during DIP. Adiponectin, leptin, SHBG and miRNAs can be analysed from small quantities of blood, which may be more acceptable to patients. Furthermore, they can be recognized sub-clinically, prior to disease presentation, indicating the ability to predict pregnancy difficulties. Questionnaires assessing knowledge of diabetes can be easily administered through interviews during clinical visits. Together, these markers will facilitate management strategies for women with DIP and minimise costs of non-communicable diseases to the health system.

1.4. Hypothesis

1. We hypothesize that the type of DIP (T1DM, T2DM and GDM) is associated with adverse pregnancy outcomes and pregestational T1DM and T2DM is associated with more frequent maternal and fetal pregnancy outcomes.
2. We hypothesize that maternal adiponectin, leptin and SHBG levels during pregnancy are associated with neonatal birth outcomes.
3. We hypothesise that maternal circulating miRNA patterns correlate with glycaemic control and maternal and neonatal birth outcomes.
4. We hypothesise that a questionnaire to assess diabetes knowledge will adequately assess knowledge of GDM, nutrition and physical activity in women with GDM.

1.5. Aims and objectives

Overarching aim

To compare adverse pregnancy outcomes by DIP type and explore the candidacy of adiponectin, leptin, SHGB, miRNAs and diabetes knowledge to serve as markers of glycaemic control during pregnancy and perinatal outcomes in pregnancies complicated by T1DM, T2DM and GDM (Figure 1.1).

Aim 1

To determine the association between the type of diabetes during pregnancy (T1DM, T2DM and GDM), glucose control and maternal and fetal pregnancy outcomes.

Objectives

1. To conduct a systematic review of published articles on the association between DIP and adverse pregnancy outcomes.
2. To investigate the effect of DIP and pre-pregnancy obesity on obstetric and perinatal outcomes at Steve Biko Academic Hospital (SBAH).

Aim 2

To investigate the association between maternal serum adiponectin, leptin and SHBG concentrations and DIP, glucose concentrations, body mass index (BMI), and neonatal birth outcomes.

Objectives

1. To correlate and compare serum adiponectin, leptin and SHBG levels with glucose concentrations and body weight.
2. To correlate and compare serum adiponectin, leptin and SHBG levels with neonatal birth outcomes.

Aim 3

To investigate the association between miRNAs and glucose concentrations, BMI, and neonatal birth outcomes.

Objectives

1. Profile miRNAs in maternal serum using quantitative real-time polymerase chain reaction (qRT-PCR).
2. Compare and correlate miRNA patterns with glycaemic control and neonatal birth outcomes.
3. Conduct Receiver Operating Characteristics (ROC) analysis to assess predictive ability of miRNAs for neonatal birth outcomes.

Aim 4

To develop a questionnaire to assess diabetes knowledge in South African women with GDM.

Objectives

1. To develop a questionnaire to assess knowledge on GDM, nutrition, physical activity and blood glucose management.
2. To assess knowledge on GDM, nutrition, physical activity and blood glucose management.

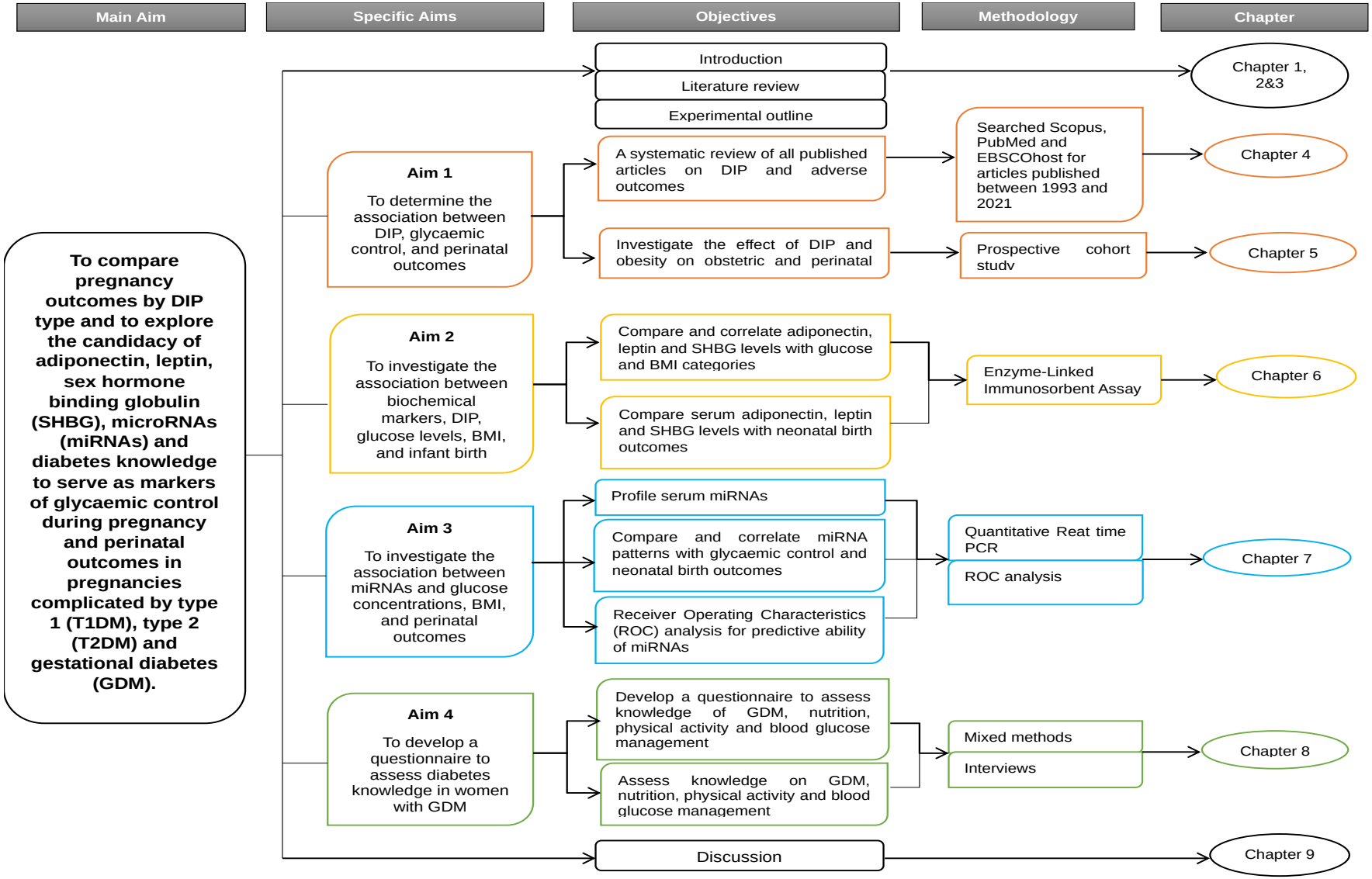

Figure 1.1. Flow diagram illustrating aims, objectives and methodology in each chapter.

CHAPTER 2

LITERATURE REVIEW

2.1. Diabetes in pregnancy

In 2021, it was estimated that approximately 21.1 million live births, globally representing 16.7% of births, were attributed to DIP. Of these, pregestational type 1 (T1DM) and type 2 (T2DM) account for 10.6% of cases, while T1DM and T2DM first diagnosed in pregnancy accounts for 9.1% of cases and gestational diabetes (GDM) accounts for 80.3% of cases (International Diabetes Federation, 2021). These figures might be an underestimation considering that many women with DIP remain undiagnosed, especially in resource-limited settings. Low- to middle-income countries (LMICs) such as those in Africa and South-East Asia carry the highest prevalence of undiagnosed diabetes cases. In Africa, the prevalence of DIP is 9.6% (International Diabetes Federation, 2021). There are no statistics on the prevalence of pregestational diabetes, warranting more epidemiological studies on types of DIP. In South Africa, the prevalence of GDM ranges from 1.8% to 25% (Adam & Rheeder, 2017; Basu et al., 2010; Jackson, 1979; Macaulay et al., 2018; Notelovitz, 1969; Ranchod et al., 1991) depending on the screening strategies and diagnostic criteria used throughout (Dias et al., 2019).

2.2. Pathophysiology

Hyperglycaemia is a common feature of all types of maternal diabetes; however, the underlying pathophysiology differs (Figure 2.1). Maternal T1DM is an autoimmune condition that results in the destruction of beta- (β) cells in the islets of Langerhans in the pancreas leading to insulin deficiency. These women become insulin deficient and rely on exogeneous insulin administration to achieve blood glucose control (Harris, 2005). In contrast, maternal T2DM is primarily attributed to obesity, insulin resistance, and the failure of insulin-sensitive tissues to respond effectively to insulin. Impaired insulin production by pancreatic β-cells subsequently results in hyperglycaemia (Shand et al., 2008). Unfortunately, due to limited access to healthcare, a large number of pregestational T2DM cases are first diagnosed during pregnancy (Chivese et al., 2019). GDM is a milder form of hyperglycaemia that results from maternal metabolic adaption during pregnancy. GDM is thought to occur in women who lack the ability to compensate for normal insulin resistance during pregnancy (Hjort et al., 2019). This process is mediated by maternal hormones such as oestrogen,

progesterone, cortisol and placental-derived hormones such as placental growth hormone, placental lactogen and prolactin (Newbern and Freemark, 2011).

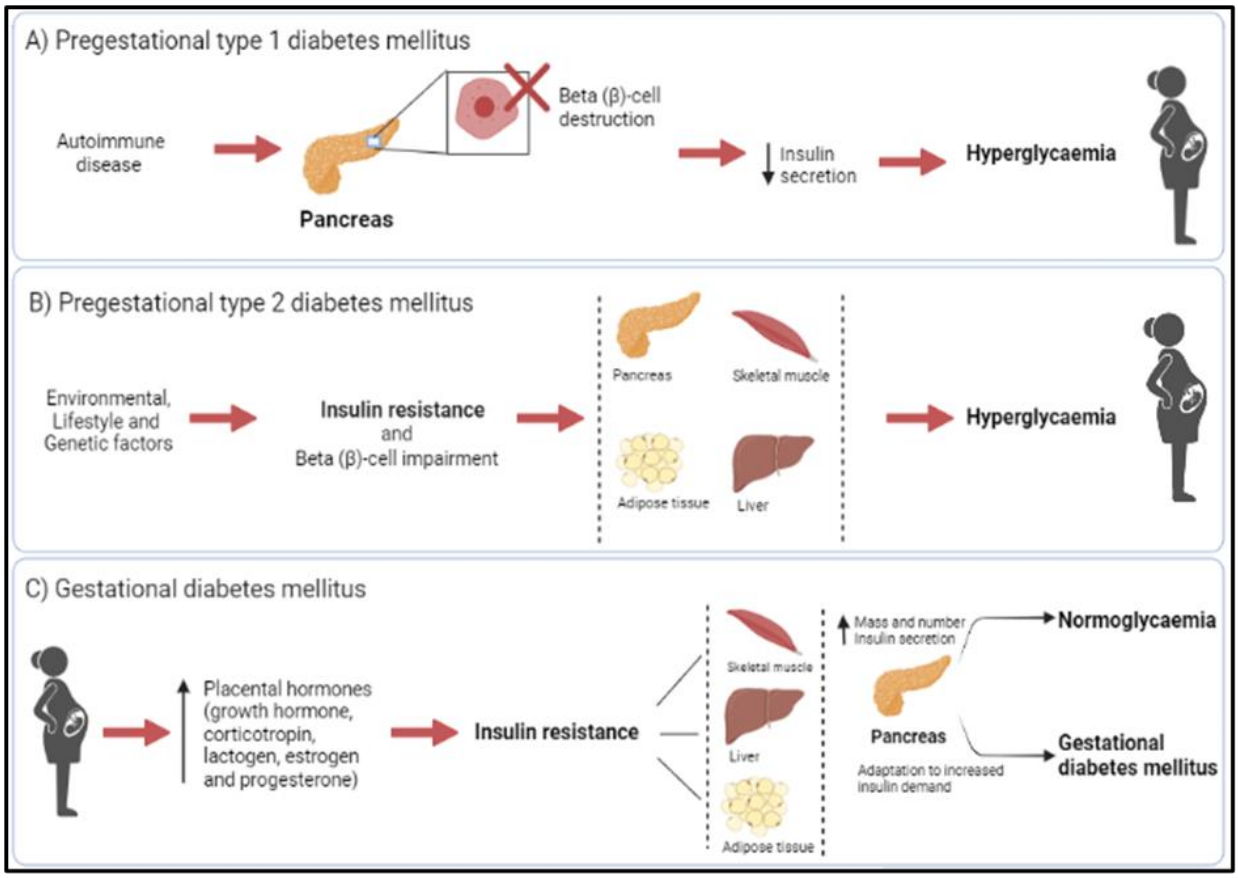

Figure 2.1. Schematic diagram illustrating the pathophysiology of maternal diabetes.

Pregestational type 1 diabetes (T1DM) occurs due to auto-immune destruction of pancreatic β-cells resulting in insulin deficiency and hyperglycaemia, B) Pregestational type 2 diabetes (T2DM) is due to environmental, lifestyle, and genetic factors that impair the pancreatic β-cell function and induce insulin resistance in peripheral tissues such skeletal muscle, liver, and adipose tissue, which results in hyperglycaemia. C) Gestational diabetes (GDM) occurs due to increases in maternal placental hormones leading to insulin resistance in peripheral tissues. Pancreatic β-cells boost insulin secretion and synthesis to compensate for insulin resistance, resulting in normoglycaemia; failing to do so causes hyperglycaemia, which in turn causes GDM (Masete, 2021).

2.3. Adverse pregnancy complications

Adverse pregnancy outcomes occur in ~10% to 20% of normal pregnancies (Lane-Cordova et al., 2019). Short-term maternal adverse outcomes include preeclampsia, gestational hypertension, and caesarean section (CS). Fetal adverse outcomes include congenital abnormalities, macrosomia,

large for gestational age (LGA), small for gestational age (SGA), preterm birth (PTB) and stillbirth (Burlina et al., 2019; McCance, 2011; Mitanchez et al., 2015). Although adverse pregnancy outcomes present differently, the majority share a common pathophysiology related to defective placental function and vascular development including endothelial dysfunction, inflammation, and vasospasms (Lane-Cordova et al., 2019). Adverse pregnancy outcomes have been found to impact up to 25% of newborns born to mothers with diabetes (Eriksson, 2009). The exact mechanisms underlying fetal complications are not completely elucidated, however, it has been hypothesized that maternal hyperglycaemia triggers reactive oxygen species (ROS), which damages deoxyribonucleic acid (DNA) in the fetus, causing apoptosis, proliferation, and inflammation and congenital abnormalities (A. Ornoy et al., 2015; Shub and Lappas, 2020). Congenital anomalies are defined as structural or functional abnormalities including metabolic disorders, present at birth which result from defective embryogenesis or intrinsic abnormalities in the development process (Francine et al., 2014). Pregnant women with diabetes have a ~20% risk of developing gestational hypertension and/or preeclampsia, especially those with underlying microvascular complications, pre-existing hypertension, or poor glycaemic control (Sullivan et al., 2011). Mothers with gestational hypertension or preeclampsia are at an increased risk of carrying an SGA fetus (Hung et al., 2018; Y. Liu et al., 2021; Panaitescu et al., 2017). SGA is defined as babies born with a birth weight less than the 10th percentile for their gestational age. SGA is associated with a higher risk for perinatal complications such as PTB, hypoglycaemia, perinatal asphyxia, and impaired immune function. In addition, SGA babies have an increased risk of long-term complications, including chronic kidney disease, coronary heart disease, hyperlipidaemia, and hypertension (Osuchukwu and Reed, 2023). Hyperglycaemia during pregnancy also increases the placental transport of glucose and other nutrients from the mother to the baby, resulting in fetal hyperinsulinaemia, LGA (fetal growth > 90th (Kiserud et al., 2018)) and subsequently macrosomia (Barnes-Powell, 2007; Sugrue and Zera, 2018). Macrosomia is defined as babies weighing more than 4 kg and has been associated with numerous perinatal and maternal complications, childhood obesity and long-term risk of developing T2DM, hypertension, and obesity in adulthood (Araujo Júnior et al., 2017). PTB is defined as births before 37 completed weeks of gestation (WHO, 1977). PTB affects about 11% of pregnancies

worldwide and ~90% of PTBs occur in LMICs (Walani, 2020). PTB is the leading cause of death in children under five and is responsible for more than a million infant deaths annually (Heinonen et al., 2015). When compared to term infants, preterm neonates have a high risk of poor neurodevelopment, cognitive impairment, and behavioural and emotional problems (Moreira et al., 2014).

In the long term, mothers with pregestational diabetes are more likely to develop diabetic complications such as retinopathy, neuropathy, and nephropathy, or have these complications worsen if they already exist (Schaefer-Graf et al., 2018; Sugrue and Zera, 2018). Women with GDM have a ~7-fold higher risk of developing T2DM (Bellamy et al., 2009) with ~70% of women with GDM developing T2DM within three years in high-risk populations (Kim et al., 2002). Furthermore, these women have a ~4-fold increased risk of developing cardiovascular and coronary artery disease (Harreiter et al., 2014). A recent study conducted in South Africa found that after 5–6 years, 31% of women with GDM develop T2DM, while, 7% and 13% of women developed impaired fasting glucose and impaired glucose tolerance, respectively (Chivese et al., 2019).

Children born to mothers with diabetes have a higher risk of developing obesity (Pitchika et al., 2018) and T2DM in later life due to the metabolic imbalance experienced *in utero* (Kanguru et al., 2014). According to Soepnel et al. ~10.5% of children born to mothers with GDM are overweight or obese at 3 to 6 years of age (Soepnel et al., 2021). The Developmental Origins of Health and Disease concept, which proposes that the mother's lifestyle and nutrition during pregnancy has long-term consequences on children's health, is assumed to mediate these health outcomes (Fleming et al., 2018). The Barker hypothesis explains how the intrauterine environment influences fetal programming (Barker, 1997). According to the hypothesis, maternal malnutrition affects fetal growth throughout pregnancy, resulting in physiological and metabolic alterations caused by fetal adaptation. The changes that the fetus undergo throughout pregnancy may result in the development of diseases such as coronary heart disease, diabetes, and hypertension in adulthood (Barker, 1997). Maternal malnutrition, obesity, or hyperglycaemia increases fetal risk of developing chronic diseases later in life, supporting the early origins of chronic diseases hypothesis. Although

the molecular mechanisms that underlie *in utero* programming remain unknown, epigenetic mechanisms such as DNA methylation have been widely implicated (Ruchat et al., 2013).

All types of DIP are associated with adverse complications. Pregestational diabetes is associated with more frequent and severe outcomes compared to GDM (Al-Nemri et al., 2018; Malaza et al., 2023, 2022; Van Zyl and Levitt, 2018). The first trimester is a critical period for organogenesis, and it is hypothesised that preconception hyperglycaemia and the longer time of exposure to hyperglycaemia *in utero* may contribute to the complications associated with pregestational diabetes (Dornhorst and Banerjee, 2010). One of the largest birth cohort studies to date, the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study, which followed 23,316 women during pregnancy, showed that even mild hyperglycaemia during pregnancy is associated with adverse outcomes such as high birth weight, CS, and neonatal hypoglycaemia (Metzger et al., 2008). As a result, attaining optimal glucose control throughout pregnancy is critical to reduce the risk of pregnancy complications (McCance, 2015).

2.4. Diagnostic strategies

Maternal diabetes can be diagnosed using fasting or random blood glucose levels and the oral glucose tolerance test (OGTT). Prior to pregnancy, T1DM and T2DM is diagnosed using fasting plasma glucose (FPG) ≥ 7.0 mmol/l, random plasma glucose or 2-h plasma glucose ≥ 11.1 mmol/l on the OGTT, or glycated haemoglobin (HbA1c) ≥ 6.5%, however, for T1DM, multiple autoantibodies (islet cell autoantibodies and autoantibodies to GAD (GAD65), insulin, the tyrosine phosphatases IA-2 and IA-2b, and zinc transporter 8 (ZnT8)) or presentation with diabetic ketoacidosis (DKA) are also used for diagnosis (American Diabetes Association, 2022). Pregestational diabetes is usually confirmed by self-report, medical records, or medication usage during the first prenatal visit. According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, overt DIP is diagnosed using FPG ≥ 7.0 mmol/l, random plasma glucose or 2-h plasma glucose ≥ 11.1 mmol/l on the OGTT, or HbA1c ≥ 6.5% (International Association of Diabetes in Pregnancy Study Group (IADPSG) Working Group on Outcome

Definitions et al., 2015). Currently, the most widely used method to diagnosis GDM includes risk factor based screening including age older than 35 years old, obesity, family history of diabetes, history of stillbirth, macrosomia, or previous GDM, followed by blood glucose testing using the OGTT (Dias et al., 2019). The screening and diagnosis strategies of GDM are contentious, with some countries using selective risk factor-based screening, while others use universal screening (Dias et al., 2019). Currently, there is no standard accepted diagnostic criteria, however, the majority of international institutions advocate for the IADPSG criteria ("International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy," 2010). The IADPSG recommends universal screening for GDM during pregnancy. Screening is performed around 24- 28 weeks gestation for women at risk. The IADPSG recommends a 1-step 75-g OGTT at 24 weeks based on the HAPO study ("International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy," 2010; Boyd E Metzger et al., 2008). In South Africa, there are still differences in the protocols used for the diagnosis of GDM between hospitals and provinces (Dias et al., 2019). In 2017 Society of Endocrinology, Metabolism and Diabetes in South Africa (SEMDSA) endorsed universal screening as recommended by the IADPSG. Currently, risk factor-based selective screening is predominately used in South Africa despite the IADPSG recommendation for universal screening, where all pregnant women must be screened for GDM at 24-28 weeks of gestation (Adams and Rheeder, 2017). Universal screening is costly and labour-intensive for LMICs such as South Africa. Adam et al. reported that there would be a substantial increase in the prevalence of GDM if the IADPSG universal screening is adopted given that risk factors are a poor screening tool for GDM in South Africa (Adam and Rheeder, 2017).

2.5. Management

Pregnant women with DIP must adhere to strict glucose monitoring since good glycaemic control is essential to lower the risk of adverse outcomes for mothers and their unborn infants (American Diabetes Association, 2022). Adequate glycaemic control may be achieved through 1) health and diabetes education, 2) lifestyle modification, 3) stringent glucose self-monitoring and 4)

pharmacological therapy before and during pregnancy (Sugrue and Zera, 2018). According to the American Diabetes Association, recommended glucose targets during pregnancy are: fasting glucose 3.9–5.3 mmol/L and either 1-h postprandial glucose 6.1–7.8 mmol/L or 2-h postprandial glucose 5.6–6.7 mmol/L (American Diabetes Association, 2022). For women with pregestational diabetes, achieving optimal blood glucose control while minimising hypoglycaemia is essential. Preconception counselling and care is crucial to decrease the risk of adverse outcomes, especially congenital abnormalities. During pregnancy, women with T2DM transition from oral hypoglycaemic medication to insulin or receive a combination of the two if optimal glucose control is not achieved. Women with T1DM continue with their daily multiple insulin injections or continuous subcutaneous insulin infusion therapy with adjustments when necessary (American Diabetes Association, 2022). For women with GDM, nutritional therapy and appropriate physical activity is the first-line treatment strategy, with pharmaceutical therapy initiated if lifestyle modification is not sufficient to maintain glucose control (Zhang et al., 2019). All women with DIP are required to conduct blood glucose self-monitoring at home.

HbA1c is the gold standard to monitor glucose control over a longer period. During pregnancy, HbA1c level below 6% is recommended to prevent adverse outcomes without causing hypoglycaemia (American Diabetes Association, 2022). Several studies have reported that a high HbA1c (>7%) is associated with adverse pregnancy outcomes (Gandhi et al., 2008; Gold et al., 1998; Immanuel et al., 2021; Lemaitre et al., 2022; Rey et al., 1999). However, HbA1c is a poor predictor during pregnancy due to the slow biochemical rate and physiological alterations in red blood cell production. Additionally, HbA1c may not accurately reflect postprandial hyperglycaemia during pregnancy (Law et al., 2017; Rafat and Ahmad, 2012). It is advised that HbA1c be used as a secondary indicator of glycaemic control during pregnancy, following glucose self-monitoring (American Diabetes Association, 2022).

Monitoring glucose levels during pregnancy is complicated by different cut-off values for tight glycaemic control. Various testing procedures, including weekly venous blood tests and daily self-monitoring of capillary blood glucose are used to monitor glucose. Therefore, there is a need for a standard measure to monitor glycaemia (Langer and Conway, 2000). These difficulties offer an

opportunity to investigate alternative approaches to monitor glycaemic levels in pregnancies affected by diabetes. There is a need for sensitive biomarkers to aid in monitoring glycaemia control. These biomarkers may predict the risk of adverse outcomes for both the mother and child, thereby facilitating strategies to improve health outcomes. Researchers have investigated several biomarkers for monitoring blood glucose levels during pregnancy, such as glycated albumin, fructosamine, and 1,5-anhydroglucitol. However, these biomarkers have not yet proven effective in clinical settings (Hashimoto and Koga, 2015).

2.6. Biomarkers

Biomarkers are defined as precisely measured and evaluated indications of typical biological and pathological processes or pharmacological reactions to a treatment intervention (Strimbu and Tavel, 2010). An ideal biomarker should have the following qualities: 1) affordability, 2) ease of measurement in non-invasive biological specimens like blood and urine, 3) sensitivity and specificity to diagnose disease and distinguish between diseased and healthy states, 4) time efficient, 5) contribution to disease prognosis, and 6) should shed light on the underlying disease mechanisms and should be biologically credible (Aronson and Ferner, 2017). The use of biological markers has recently become more practical because of technological advancements Liu et al., 2021). The measurement of biomarkers in biological samples including whole blood, plasma, serum, and urine could aid in elucidating the pathophysiology of disease.

2.6.1. Biochemical markers

Maternal hormones are involved in various physiological processes that impact both maternal health and fetal development. Studies indicate that adiponectin and leptin levels are associated with diabetes development and pregnancy outcomes (Kapustin et al., 2020; Perichart-Perera et al., 2017; Pheiffer et al., 2021; Spranger et al., 2003). Adipose tissue is an important endocrine organ, regulating appetite, energy expenditure, metabolism and supporting the physiological demands of pregnancy through adipokine secretion (Kabbani et al., 2023). Adiponectin and leptin are key

adipokines that play crucial roles in orchestrating metabolic adaption during pregnancy. Dysregulation of these adipokines is associated with pregnancy complications and adverse birth outcomes (Briffa et al., 2015). The observation that human placenta expresses adiponectin, leptin and leptin receptors suggests that these adipokines are potential regulators of trophoblast functions during implantation (D'Ippolito et al., 2012).

Adiponectin is secreted by adipose tissue and has many biological functions. It reduces inflammation by suppressing pro-inflammatory cytokines, improves lipid metabolism, insulin sensitivity, and glucose regulation. Additionally, it promotes the conversion of white adipose tissue to brown adipose tissue (Pheiffer et al., 2021), which is associated with healthier body fat distribution, reduced central obesity and liver fat accumulation (Wibmer et al., 2021). Adiponectin regulates glucose metabolism by enhancing insulin signaling in skeletal muscle and reducing gluconeogenesis in the liver (Aye et al., 2013). Changes in serum adiponectin levels are closely related to changes in maternal insulin sensitivity during pregnancy. In early gestation, the levels of serum adiponectin are higher than in the pre-pregnancy state. However, as pregnancy progresses, the levels of adiponectin in serum decline (Mazaki-Tovi et al., 2007). The decline in adiponectin levels in late pregnancy could potentially promote the shunting of nutrients to the fetus (Aye et al., 2013). Studies have shown that both maternal and cord blood adiponectin levels are associated with fetal growth (Mazaki-Tovi et al., 2005; Sivan et al., 2003). Additionally, studies have reported that maternal adiponectin levels are associated with PTB and birthweight (Lomakova et al., 2022; Mazaki-Tovi et al., 2009). Yeung et al. reported that adiponectin levels were associated with PTB and SGA (Yeung et al., 2015). Leptin is commonly known as the "satiety hormone" and it plays a crucial role in regulating energy balance in the body (Myers et al., 2008). It is produced in the white adipose tissue (Pereira et al., 2015) and binds to receptors located in the hypothalamus of the brain, particularly in the arcuate nucleus. Leptin signals the brain when the body has enough energy reserves (Myers et al., 2008). Additionally, leptin plays a crucial role in transporting nutrients, specifically amino acids, from the mother to the fetus via the placenta (Jansson et al., 2002). It increases the activity of the sodium-dependent neutral amino acid transporter (SNAT), which facilitates amino acid delivery to the fetus. In diabetic pregnancies, SNAT activity is enhanced,

resulting in excessive amino acid transfer from the mother to the fetus. This, in turn, leads to increased fetal growth (Jansson et al., 2002). Studies have shown that dysregulated maternal and cord blood leptin levels are associated with fetal growth (Kyriakakou et al., 2008; Lepercq et al., 2003; Nezar et al., 2009; Stefaniak and Dmoch-Gajzlerska, 2021; Visentin et al., 2014; Zareaan et al., 2017) and higher leptin levels associated with birthweight (Mazaki-Tovi et al., 2005; Shroff et al., 2013). Additionally, studies have demonstrated that maternal leptin levels are associated with PTB (Rabiepoor et al., 2019; Shroff et al., 2013).

While leptin and adiponectin are well-studied, other adipokines and hormones may also influence metabolic processes during pregnancy. A study by Lui et al. reported that lower levels of sex hormone-binding globulin (SHBG) were associated with lower adiponectin and higher leptin levels independent of testosterone (Liu et al., 2017). Additionally, adiponectin is reported to increase SHBG production through the upregulation of hepatocyte nuclear factor 4 α (HNF-4α) in HepG2 cells (Simó et al., 2014) SHBG is a glycoprotein produced in the liver that transports sex steroids, particularly testosterone and oestrogen, in the bloodstream. Its production is negatively regulated by insulin (Simó et al., 2015). Studies have reported that maternal SHBG levels were negatively correlated with neonatal birthweight and size (Simmons, 1995; Xargay-Torrent et al., 2018). Morisset et al. reported that maternal SHBG concentrations were a significant predictor of neonatal birthweight independent of maternal diabetes and pre-pregnancy BMI (Morisset et al., 2011). The early identification of women at risk of pregnancy complications and adverse outcomes could facilitate intervention strategies to improve pregnancy outcomes. In recent years, epigenetic markers have attracted increased interest as biomarkers of glucose homeostasis (Dhawan and Natarajan, 2019) and pregnancy complications (Barchitta et al., 2017).

2.6.2. Epigenetics

Epigenetics refers to alterations in gene expression that occur without changes in the DNA sequence (Jablonka and Lamb, 2002). Epigenetic mechanisms include DNA methylation (Holliday, 2006), chromatin remodelling (Esteller, 2011) and non-coding RNAs (Esteller, 2011). Non-coding

RNAs comprise both long and short non-coding RNAs, including microRNAs (miRNAs), which can influence the expression of genes involved in a variety of biological processes in either a positive or negative way (Esteller, 2011; Holliday, 2006). Epigenetic alterations occur due to the combination of genetic and environmental factors, including diet, physical inactivity, age, smoking and alcohol consumption and environmental pollutants (Alegría-Torres et al., 2011) which contributes to their key role in the pathophysiology of complex, multifactorial disorders (Fleming et al., 2018). Mounting evidence have implicated dysregulation of epigenetic pathways in the pathophysiology of numerous disorders including T1DM, T2DM, and GDM (Gluckman et al., 2009). Since epigenetic markers in tissue may be reflected in peripheral blood, they have attracted considerable attention as potential biomarkers of disease (Willmer et al., 2018). Epigenetic modifications are reversible; therefore, identification of these changes may provide a window for intervention strategies to correct dysregulated patterns and prevent or improve disease prognosis (Hjort et al., 2019).

2.6.2.1. MiRNAs

MiRNAs are small, highly conserved non-coding RNA molecules between 18-22 nucleotides long that regulate gene expression through post-transcriptional mechanisms. They bind to the 3' untranslated region (UTR) of target messenger RNA (mRNA) and induce gene silencing through translational repression or mRNA degradation (Guo et al., 2010). Since the discovery of *C. elegans* in 1993 (Lee et al., 1993), over 2500 miRNAs have been identified in humans, and collectively they regulate one third of the genome (Zhang and Wang, 2017). Numerous biological processes, such as cell division, proliferation, apoptosis, and development, as well as metabolic processes including glucose homeostasis, insulin signalling, pancreatic beta-cell function, lipid metabolism, and inflammation, have been shown to be regulated by miRNAs (Chen and Wang, 2013; O'Brien et al., 2018). Dysregulation of miRNA expression has been associated with human diseases such as obesity, diabetes, heart disease (Carson and Lawson, 2018), and cancer (Sharma et al., 2010). It has been shown that whole blood, plasma, serum, platelets, erythrocytes, and nucleated blood cells contain circulating miRNAs (Creemers Esther E. et al., 2012) including exosomes released from

cells (Zhang et al., 2015). Extracellular miRNAs have been demonstrated to function as signalling molecules to mediate cell-to-cell communication, serving as potential biomarkers for a number of diseases (Wang et al., 2016). Circulating miRNAs are suitable candidates for biomarkers because they are easy to collect, have been shown to be stable under various storage conditions, and can be evaluated using precise and sensitive assays such as qRT-PCR (Guay and Regazzi, 2013).

2.6.2.2. MiRNA biosynthesis and mechanism of action

MiRNA biogenesis is a complex process that begins in the nucleus of the cell (Figure 2.2). RNA polymerase II (and possibly RNA polymerase III) transcribes miRNA genes to pri-miRNAs, which are transcribed from intergenic areas within the genome (van Rooij, 2011). Pri-miRNAs fold into hairpin structures containing imperfectly base-paired stems, which are processed into 60- to 100-nucleotide hairpins called pre-miRNAs by the microprocessor complex, consisting of a RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8) and a ribonuclease III enzyme, Drosha. DGCR8 recognises N6-methyladenylated GGAC and other motifs within the pri-miRNA, while Drosha cleaves the pri-miRNA duplex at the base of the hairpin structure. The pre-miRNAs are transported from the nucleus to the cytoplasm by exportin 5 (XPO5)/RanGTP complex and are cleaved by the endonuclease Dicer to produce miRNA-miRNA duplexes (O'Brien et al., 2018; van Rooij, 2011). The mature miRNA is integrated into the RNA-induced silencing complex (RISC), which identifies targets within the mRNA genes and induces post transcriptional gene silencing (Leitão and Enguita, 2022; O'Brien et al., 2018). MiRNAs binds to the 3′ untranslated region (UTR) of their target mRNA inducing mRNA degradation and deadenylation or translational repression (O'Brien et al., 2018). The conserved sequence located at positions 2-8 at the UTR, known as the seed region, forms the basis for complementarity, which underlies the interaction between mRNA and miRNA. Evidence suggests that miRNAs may potentially aid in protein synthesis, despite the fact that they are most commonly associated with gene silencing (Vasudevan et al., 2007; Ørom et al., 2008). MiRNAs may interact with other epigenetic processes such as DNA methylation (Pheiffer et al., 2016), and influence nuclear transcript stability and alternative splicing events when reimported into the nucleus (Roberts, 2014).

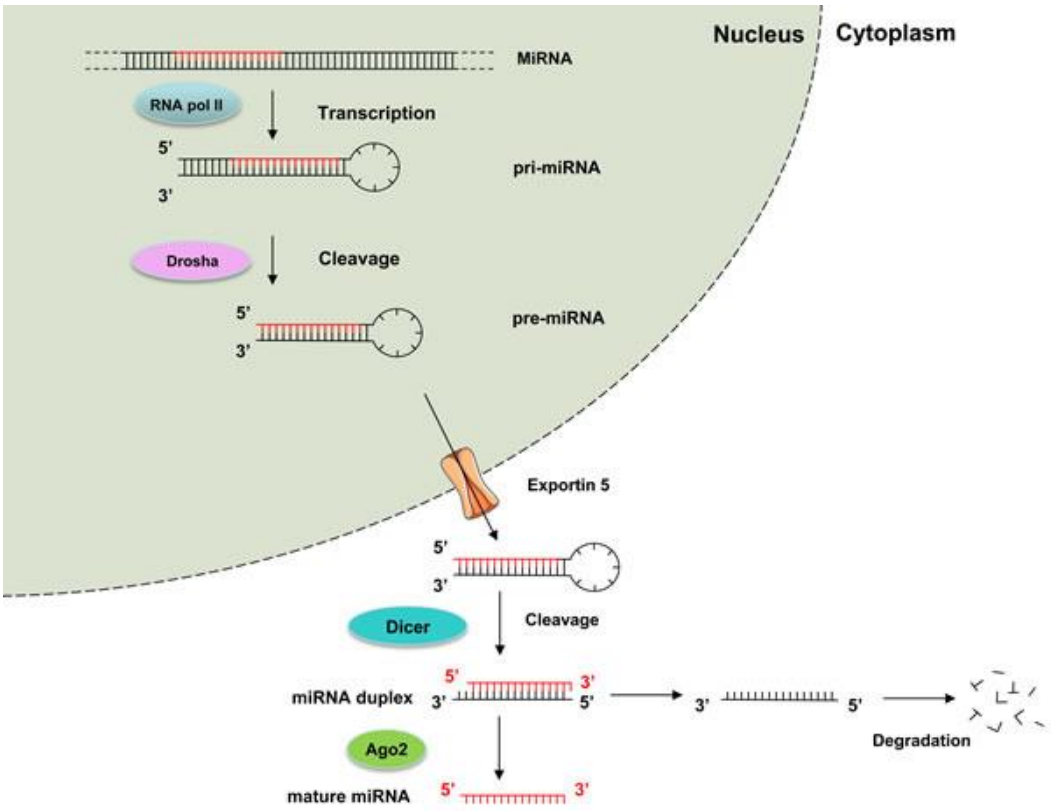

Figure 2.2. A diagram illustrating the biogenesis of miRNAs and mechanism of action.

RNA Polymerase (pol II or III) transcribes miRNA transcripts (pri-miRNA), which are then converted to pre-miRNAs by microprocessors (Drosha and DGCR8) and exported to the cytoplasm by exportedin-5 and RanGTP. Dicer and the related binding protein TRBP convert pre-miRNAs into short miRNA duplexes. The 5' mature miRNA strand is bound by the RNA-induced silencing complex (miRISC)-associated argonaut protein (Ago2), which then silences the messenger RNA target region (Masliah-Planchon et al., 2015). Abbreviations: pri- primary; pre- precursor; RanGTP- transporter protein; TRBP- transactivation response element RNA-binding protein; Ago- argonaut; RNA- ribonucleic acid; RISC- RNA-induced silencing complex; mRNA- messenger RNA (Masliah-Planchon et al., 2015).

2.6.3. MiRNAs and glycaemic control

Tang et al. showed that high glucose levels were associated with increased expression of miR-124a, miR-107, and miR-30d, and lower levels of miR-296, miR-484, and miR-690. Furthermore, the authors showed that overexpression of miR-30d, induced insulin gene expression, whereas inhibition of miR-30d prevented glucose-stimulated insulin gene transcription (Tang et al., 2009). MiR-30d stimulated the expression of insulin by activating MafA in pancreatic β-cells and protecting

β-cells from proinflammatory cytokines (Agbu and Carthew, 2021; Zhao et al., 2012). The expression of miR-375, miR-127-3p, miR-184, and miR-122 in β cells were also shown to be positively correlated with insulin biosynthesis, while miR-127-3p and miR-184 were negatively correlated with glucose-stimulated insulin secretion in non-diabetic donors. However, these relationships were absent in β cells from glucose intolerant donors (HbA1c ≥6.1%) (Bolmeson et al., 2011). Studies have demonstrated that the expression of miR-222-3p is dysregulated in patients with T1DM and T2DM compared to non-diabetic controls, and that miR-222-3p expression is associated with glycaemic control (Ahmed et al., 2018; Candia et al., 2017). The impact of a Mediterranean diet (MetDiet) on women with GDM during pregnancy to 2-3 years postpartum was investigated by Valerio and colleagues (Valerio et al., 2022). They showed that women in the intervention group had significantly lower HbA1c levels and glucose concentrations in the second trimester, which was associated with increased expression of miR-222-3p. According to the authors, antioxidants in the MetDiet altered the inflammatory cytokine profile associated with insulin resistance, and increased the expression of miR-222-3p (Valerio et al., 2022). Physical activity and dietary interventions have been shown to improve glycaemic control and the expression of multiple miRNAs (Improta Caria et al., 2018; Léniz et al., 2021; Silva et al., 2020; Valerio et al., 2022), warranting further experiments to explore miRNAs as potential biomarkers of glycaemic control in pregnancies complicated by diabetes.

2.6.4. MiRNAs during pregnancy

MiRNAs play an important role in regulating metabolic and developmental processes during pregnancy (Krützfeldt and Stoffel, 2006; Sayed and Abdellatif, 2011). MiRNAs are expressed in human placenta and their expression is regulated by factors such as hypoxia, signalling pathways, and epigenetic modification (Tsochandaridis et al., 2015). There are more than 500 miRNAs expressed in the human placenta, which has a distinct miRNA expression pattern (Morales-Prieto et al., 2014). MiRNAs are released into maternal bloodstream via exosomes originating from placental syncytiotrophoblasts (Valadi et al., 2007). During pregnancy, exosomes originating from

the placenta play a crucial role in establishing immune tolerance in the developing fetus. Pregnant women have higher levels of exosomes compared to non-pregnant women (Toth et al., 2007).

2.6.4.1. MiRNAs and adverse outcomes

MiRNAs may indicate pathological pregnancy conditions, such as miscarriage, fetal growth restriction or SGA, PTB, macrosomia, or low birth weight (Barchitta et al., 2017). The role of miRNAs in SGA has not been fully elucidated. However, studies have suggested pathways for several miRNAs. A study by Tang et al. reported that miRNA-141 contributes to SGA through regulation of pleomorphic adenoma gene 1 (PLAG1) expression, where PLAG1 is significantly decreased in placental tissue linked to SGA compared to controls (Tang et al., 2013). Increased levels of miR-424 have been found in intrauterine fetal growth restriction (IUGR) placenta. This miRNA is a critical mediator in oxygen-dependent pathways and is physiologically overregulated in the placenta during abnormal vascular development (Huang et al., 2013). MiR-210-3p is a placental miRNA and is a hypoxia sensor located in the intron of the hypoxia-inducible *AK123483* gene (Lycoudi et al., 2015). Its expression increases in response to low oxygen tension and is upregulated in hypoxia-associated diseases such as cancer and pregnancy-related disorders (Fu et al., 2013). Studies have shown that dysregulated maternal and placental levels of miR-210-3p were associated with SGA (Kochhar et al., 2022; Shchurevska and Zhuk, 2021). Mouillet et al. showed that in SGA pregnancies, certain plasma miRNAs, including miR-27a, miR-30d, miR-141, and miR-200c, are regulated by hypoxia and were dysregulated. Similarly, miR-205, miR-424, miR-451, and miR-491, as well as miRNAs from the C19MC cluster, which are primarily expressed by the placenta, show similar patterns of regulation (Mouillet et al., 2010). Other studies have reported dysregulated maternal levels of miR-20a-5p in pregnancies with SGA compared to AGA fetuses (Hromadnikova et al., 2022; Rodosthenous et al., 2017).

Dysregulated maternal and placental miRNAs have been associated with PTB (Elovitz et al., 2015; Enquobahrie et al., 2016; Hromadnikova et al., 2017; Wommack et al., 2018). Elovitz et al. reported 99 differentially expressed cervical cell miRNAs between women with PTB compared to women

with term birth (Elovitz et al., 2014). These authors reported dysregulated serum miR-4695 and miR-200a, miR-665 and miR-887 levels in women with PTB compared to women with term birth (Elovitz et al., 2015). Studies have also reported dysregulated whole blood and placental miR-210-3p expression in pregnancies with PTB compared to controls (Hromadnikova et al., 2022; Mayor-Lynn et al., 2011) and that first trimester peripheral blood miR-210-3p predicts PTB (Enquobahrie et al., 2016; Winger et al., 2020, 2017). The differential expression of plasma miRNA clusters c14mc and c19mc were reported to be predictive of PTB (Wommack et al., 2018). A study by Menon et al. reported dysregulation of exosomal let-7a-2-5p, miR-520a-3p, miR-520a-3p, miR-483-3p, miR-130b-3p, miR-4433b-3p, miR-142-5p, miR-342-3p and miR-222-3p in women with PTB compared to women with normal birth (Menon et al., 2019). Sanders et al. showed significant overexpressed of cervical cell miR-21, miR-30e, miR-142, miR-148b, miR-29b, and miR-223 in women who had PTB compared to women with term deliveries (Sanders et al., 2015).

Dysregulated maternal circulating and placental miRNAs have been associated with macrosomia (Jiang et al., 2015; Kochhar et al., 2022; Li et al., 2015). Li et al. conducted a study to investigate the role of placental and serum miRNA17-92 cluster in macrosomia. The authors found that miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a were upregulated in the placenta of mothers with macrosomic babies, while miR-17, miR-18a, miR-19a, and miR-92a were downregulated in the serum of mothers with macrosomic babies. The difference between placental and serum expression of these miRNAs in macrosomia might be due to altered exosome-dependent or independent release of miRNAs into the maternal serum because of reduced apoptosis in the placenta. Receiver operation curve (ROC) analysis suggested the combination of miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a had high diagnostic sensitivity and specificity for macrosomia (Li et al., 2015). Another study showed higher levels of plasma miR-661, miR-523-3p, miR-125a-5p, and miR-30a-3p and lower levels of miR-181a-5p, miR-200c-3p, miR-143-3p, miR-221-3p, miR-16-5p, miR-141-3p, miR-18a-5p, and miR-451 in plasma of women who delivered macrosomic neonates. ROC analysis also suggested that miR-523-3p, miR-200c-3p and miR-141-3p had high diagnostic sensitivity and specificity for macrosomia (Ge et al., 2015). Several studies have reported dysregulated miR-21, miR-29a-5p, miR-126-3p, miR-27b, and miR-486-5p

in the serum of women who delivered macrosomic neonates compared to healthy controls (Jiang et al., 2015; Miura et al., 2015; Ni et al., 2023; Ortiz-Dosal et al., 2020; Zhang et al., 2016). Conversely, other studies have reported no association between miRNAs and macrosomia (Kochhar et al., 2022; Ortiz-Dosal et al., 2020). MiRNA expression varies in different ethnicities (Becker and Lockwood, 2013), sample types (Ge et al., 2015; Zhu et al., 2014) and lab techniques used (Becker and Lockwood, 2013), this might account for the discrepancies between the studies.

Taken together, several studies have investigated miRNAs as potential biomarkers of adverse pregnancy outcomes, with conflicting results. Therefore, additional research is needed to evaluate the potential miRNAs as biomarkers of adverse pregnancy outcomes and their clinical usefulness.

2.7. Knowledge

Effective management of GDM is critical to mitigate pregnancy complications and prevent adverse outcomes (Brown et al., 2018; Hod et al., 2015). Blood glucose regulation is fundamental to GDM management. GDM management involves dietary modification, regular physical activity, stringent glucose monitoring and the use of insulin or metformin to adequately regulate blood glucose levels. Studies have shown that adequate glucose control reduced the risk of short-term adverse pregnancy outcomes (González-Quintero et al., 2007; Guo et al., 2019; H. Yu et al., 2014) and long-term cardiovascular disease risk (Yefet et al., 2019). Clinically, successful GDM management is assessed by monitoring blood glucose levels, with less emphasis placed on a woman's comprehension of the critical factors required to meet glycaemic targets. According to studies on T2DM, inadequate diabetes education is associated with poor glycaemic control (Al-Qazaz et al., 2011; Worku et al., 2015), while other studies reported that poor diabetes related knowledge results in poor adherence to self-management and glucose self-monitoring (Ong et al., 2014; Shams et al., 2016). Similarly, a study by Hussain et al. reported that in women with GDM, diabetes knowledge was associated with good glycaemic control (Hussain et al., 2015). A study analysing enablers and barriers to glucose management in women with GDM found that understanding the relevance of nutrition and exercise is crucial to achieving glycaemic targets (Martis et al., 2018). Understanding

GDM and the importance of nutrition and physical exercise is critical for optimal glucose control, leading to improved pregnancy and health outcomes for both the mother and the baby. Tools such as questionnaires to assess diabetes knowledge are crucial in identifying knowledge gaps in pregnant women with GDM, which may aid in developing interventions to improve glucose management and improve pregnancy outcomes in women with GDM.

CHAPTER 3

EXPERIMENTAL OUTLINE

3.1. Study design

3.1.1. Participant recruitment

This study is part of a larger prospective cohort study investigating epigenetic mechanisms in women with DIP conducted at the high-risk antenatal clinic at Steve Biko Academic Hospital (SBAH), Pretoria, Gauteng, South Africa. The study was approved by the University of Pretoria Health Science Research Ethics Committee (ethics numbers: 41/2021). At SBAH, the diabetes antenatal clinic manages referrals from local endocrine and internal medicine or antenatal clinics in the cluster. The referring clinics use the risk factor-based selective screening approach ("The 2017 SEMDSA Guidelines for the Management of Type 2 Diabetes | Journal of Endocrinology, Metabolism and Diabetes of South Africa," n.d.), which includes screening for risk factors, such as family history of diabetes mellitus, previous GDM, advanced maternal age, obesity and previous adverse pregnancy outcome, including congenital abnormality, recurrent miscarriages, delivery of a stillborn child, delivery of a baby ≥ 4 kg in a previous pregnancy or persistent glycosuria (Benhalima et al., 2019). The procedure for participant selection is illustrated in Figure 3.1. This study enrolled 239 women with pregestational T1DM and T2DM, GDM, and normoglycaemia (oral glucose tolerance test (OGTT) negative) at ≤ 28 weeks of gestation and included women who had singleton pregnancies, were between 18 and 42 years of age, of black African ethnicity and human immunodeficiency virus (HIV) negative. The study excluded women with multiple pregnancies. Written informed consent was obtained from all participants. Women with normoglycaemia were recruited at the antenatal clinic at SBAH. Oral glucose tolerance test (OGTT) was performed due to the women meeting the risk factors for GDM screening. DIP was classified as follows:

- Women were classified as normoglycaemic following negative OGTTs.

- If diabetes was diagnosed before or during pregnancy and women had positive anti-glutamic acid decarboxylase (GAD) antibodies or presented with diabetic ketoacidosis (DKA), it was classified as pregestational T1DM.

- If diagnosed with overt diabetes during pregnancy (fasting plasma glucose level ≥ 7.0 mmol/l, random plasma glucose or 2-h plasma glucose ≥ 11.1 mmol/l after the OGTT, or glycated haemoglobin (HbA1c) was ≥ 6.5% or if diabetes was diagnosed before pregnancy, it was classified as pregestational T2DM.

- If carbohydrate intolerance was first diagnosed during pregnancy according to The International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria at 24-28 weeks gestation (fasting plasma glucose level 5.1-6.9 mmol/l, or 1-h plasma glucose ≥ 10 mmol/l or 2-h plasma glucose 8.5-11.0 mmol/l after a 2-h 75-g OGTT, it was classified as GDM.

At enrollment, data collected included demographics, anthropometry, obstetric history and care, and diabetes care according to standard clinical procedures. Serum was collected to measure leptin, adiponectin, and SHBG concentrations using the commercial human enzyme-linked immunosorbent assay (ELISA) (Merck, Darmstadt, Germany), and 12 selected serum miRNAs were quantified using quantitative real time PCR (qRT-PCR) (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The women were followed up until delivery. At delivery, data collected included fetal growth, gestational age (GA) at delivery (weeks), the onset of labour, route of delivery, birth weight (g), neonatal outcome, and Apgar score at 5 minutes.

The development of the diabetes knowledge and perceptions questionnaire is illustrated in Chapter 8 (Figure 8.1). Briefly, an exploratory mixed-method study was conducted at SBAH. The knowledge of GDM questionnaire was developed by adapting three developed questions and in consultation with an expert panel. It was tested in eight pregnant women with pregestational (T1DM and T2DM) or GDM. Thereafter, amendments were made as appropriate, and the questionnaire was re-tested in twenty women with GDM.

3.2. Systematic review- Chapter 4

Chapter 4 a systematic review summarised and synthesised studies that have compared adverse pregnancy outcomes in pregnancies complicated by pregestational diabetes and GDM.

3.3. Maternal and fetal outcomes- Chapter 5

Chapter 5 investigated the association between type of diabetes during pregnancy (T1DM, T2DM, and GDM) and perinatal outcomes (maternal and fetal pregnancy outcomes) in women with T1DM (n=13), T2DM (n=65), GDM (n=39) and normoglycaemia (n=66).

3.4. Biochemical Markers- Chapter 6

Chapter 6 investigated the correlation between serum biochemical markers, glucose levels, body weight and neonatal birth outcomes in pregnant women with T1DM (n=23), T2DM (n=60), GDM (n=46), and normoglycaemia (n=46). Biochemical markers included total adiponectin, leptin and SHBG.

3.5. MiRNAs- Chapter 7

Chapter 7 investigated the correlation between serum miRNAs and glycaemic control, and neonatal birth outcomes in women with T1DM (n=23), T2DM (n=58), GDM (n=47), and normoglycaemia (n=56) were selected. Selected miRNAs included miR-124-3p, miR-126-3p, miR-128-3p, miR-155-5p, miR-19a-3p, miR-19b-3p, miR-20a-5p, miR-210-3p, miR-222-3p, miR-27a-3p, miR-29a-3p, miR-30d-5p.

*Detailed descriptions of the methodologies are outlined in the respective chapters

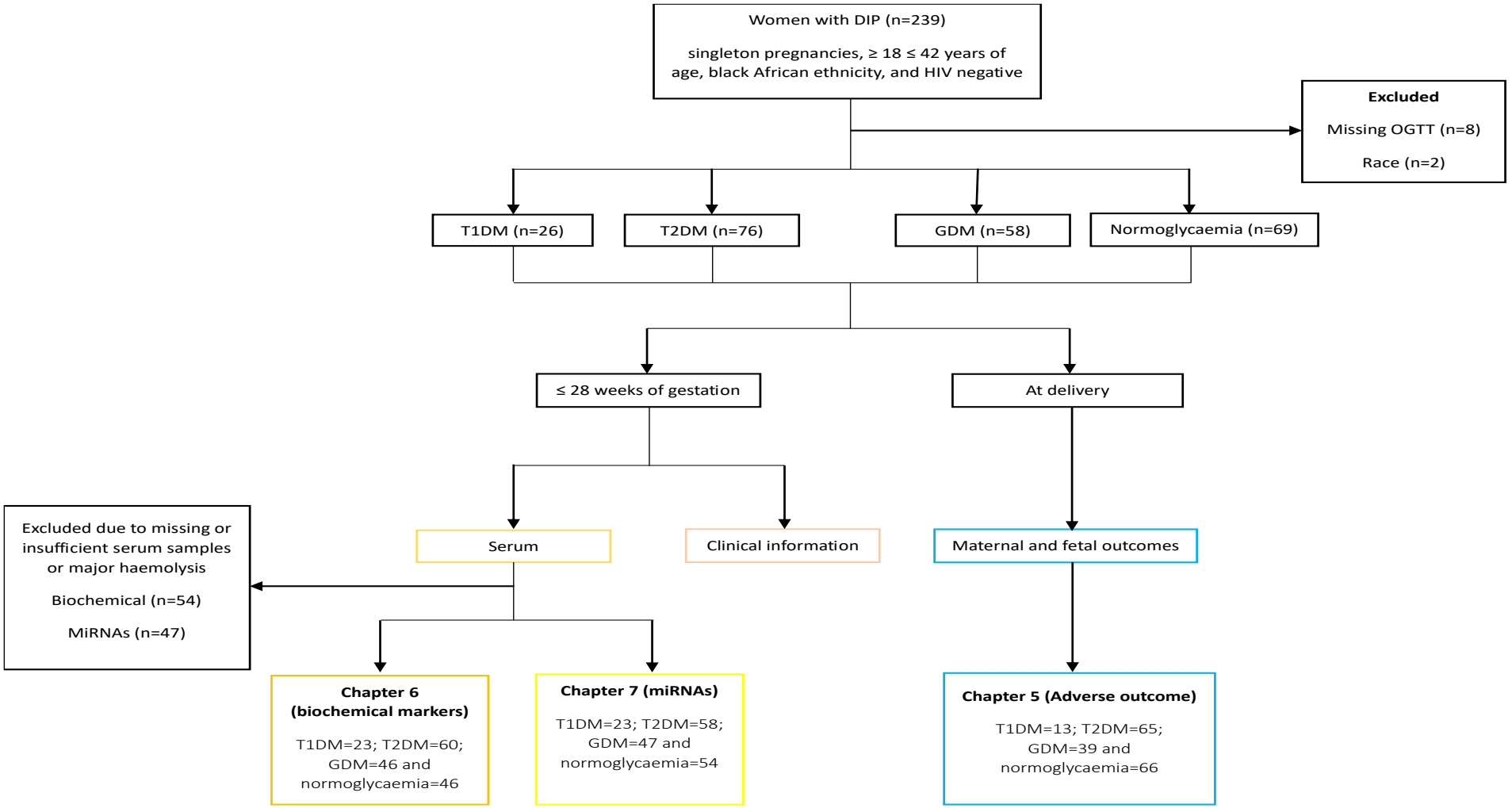

Figure 3.1: Flow diagram illustrating experimental outline

CHAPTER 4

SYSTEMATIC REVIEW TO COMPARE ADVERSE PREGNANCY OUTCOMES IN WOMEN WITH PREGESTATIONAL DIABETES AND GESTATIONAL DIABETES

Adapted from:

Malaza N, Masete M, Adam S, Dias S, Nyawo T, Pheiffer C. A Systematic Review to Compare Adverse Pregnancy Outcomes in Women with Pregestational Diabetes and Gestational Diabetes. *International Journal of Environmental Research and Public Health*. 2022; 19(17):10846. https://doi.org/10.3390/ijerph191710846

4.1. Abstract

Background. Pregestational type 1 (T1DM) and type 2 (T2DM) diabetes mellitus and gestational diabetes mellitus (GDM) are associated with increased rates of adverse maternal and neonatal outcomes. Adverse outcomes are more common in women with pregestational diabetes compared to GDM, although conflicting results have been reported.

Objective. This systematic review aims to summarise and synthesise studies that have compared adverse pregnancy outcomes in pregnancies complicated by pregestational diabetes and GDM.

Methods. Three databases, Pubmed, EBSCOhost and Scopus were searched to identify studies that compared adverse outcomes in pregnancies complicated by pregestational T1DM and T2DM, and GDM. A total of 20 studies met the inclusion criteria and are included in this systematic review. Thirteen pregnancy outcomes including caesarean section (CS), preterm birth (PTB), congenital anomalies, pre-eclampsia, neonatal hypoglycaemia, macrosomia, neonatal intensive care unit admission (NICU), stillbirth, Apgar score, large for gestational age, induction of labour, respiratory distress syndrome and miscarriages were compared.

Results. Findings from this review confirm that pregestational diabetes is associated with more frequent pregnancy complications than GDM.

Conclusion. Taken together, this review highlights the risks posed by all types of maternal diabetes and the need to improve care and educate women on the importance of maintaining optimal glycaemic control to mitigate these risks.

4.2. Introduction

According to estimates from the International Diabetes Federation (IDF), maternal diabetes was associated with 21.1 million (16.7%) live births worldwide in 2021. Of these, 80.3% were caused by gestational diabetes mellitus (GDM), a milder form of hyperglycaemia that develops in the second trimester, 10.6% were attributable to pregestational type 1 (T1DM) and type 2 (T2DM) diabetes mellitus, whilst T1DM and T2DM first diagnosed in pregnancy accounted for 9.1% of cases (International Diabetes Federation, 2021). Normal pregnancy is characterised by insulin resistance and requires an increased pancreatic β-cell response in order to maintain normoglycaemia (Dahlgren, 2006). GDM develops in women who are unable to mount a compensatory β-cells response, leading to hyperglycaemia. Increasing maternal age, along with increasing rates of obesity and diabetes worldwide, have led to the rising rates of DIP (International Diabetes Federation, 2021; Langer, 2018; Schaefer-Graf et al., 2018). Obesity has been identified as a significant risk factor for maternal diabetes. A meta-analysis of 20 studies reported that women who were overweight (2.1-fold), obese (3.6-fold) or severely obese (8.6-fold) had a higher risk of developing diabetes compared to normal-weight pregnant women (Chu et al., 2007).

Maternal diabetes is associated with pregnancy complications and increased rates of adverse maternal and neonatal outcomes (Johns et al., 2018; Sugrue and Zera, 2018). Short-term complications include macrosomia, large for gestational age (LGA), respiratory distress syndrome (RDS), neonatal hypoglycaemia, neonatal intensive care unit (NICU) admission, intrauterine growth restriction, congenital anomalies, preterm birth (PTB), preeclampsia and caesarean section (CS) while in the long-term both mothers and their babies have an increased risk of metabolic disease (Burlina et al., 2019; McCance, 2015; Mitanchez et al., 2015). Women with GDM have a ~7-fold increased risk of developing T2DM (Bellamy et al., 2009) and a ~4-fold increased risk of developing cardiovascular and coronary artery disease after pregnancy (Harreiter et al., 2014), while pregestational diabetes predisposes women to developing diabetes-related complications such as retinopathy and nephropathy or may accelerate the course of these complications if they already exist (Aguiree et al., 2013; Schaefer-Graf et al., 2018; Sugrue & Zera, 2018).

It is widely reported that all types of maternal diabetes are associated with pregnancy complications, although adverse outcomes are more common in women with pregestational diabetes (Al-Nemri et al., 2018; Tinker et al., 2020; Van Zyl and Levitt, 2018; Yamamoto et al., 2018). As adverse pregnancy outcomes are closely related to poor glycaemic control and the first trimester being a critical period for organogenesis, it is speculated that preconception hyperglycaemia and the longer time of exposure to hyperglycaemia *in utero* may contribute to the complications associated with pregestational diabetes (Dornhorst and Banerjee, 2010).

Despite the large body of evidence that associates pregestational diabetes with more frequent adverse pregnancy outcomes than GDM (Barakat et al., 2010; Gualdani et al., 2021; Hyari et al., 2013; Asher Ornoy et al., 2015; Peticca et al., 2009; Shefali et al., 2006), conflicting results have been reported (Hamedi, 2005; Hyari et al., 2013; Mahmood Buhary et al., 2016; Persson and Fadl, 2014; Van Zyl and Levitt, 2018). This review aims to summarise and synthesise studies that have compared adverse pregnancy outcomes in pregnancies complicated by pregestational diabetes and GDM. Three databases, Pubmed, Scopus and EBSCOhost were searched to identify eligible studies, which were summarised and synthesised using systematic review methods. Commonly reported adverse pregnancy outcomes in literature (Negrato et al., 2012) were selected for inclusion in this review. These include congenital anomalies, preeclampsia, neonatal hypoglycaemia, macrosomia, NICU admission, stillbirth, Apgar score, large for gestational age (LGA), induction of labour (IOL), respiratory distress syndrome (RDS) and miscarriages.

4.3. Methods

This systematic review was conducted adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009) (Supplementary Table S1).

4.3.1. Search strategy and study selection

Three databases, Pubmed, Scopus and EBSCOhost were searched for studies reporting on maternal diabetes and pregnancy outcomes, published between January 1993 and December 2021. The search terms included "type 1 diabetes mellitus" or "type 1 diabetes" or "diabetes mellitus type 1" or "diabetes type 1" and "type 2 diabetes" or "type 2 diabetes mellitus" and "pre-gestational diabetes" or "gestational diabetes" or "'diabetes in pregnancy" and "pregnancy complications" or "perinatal outcomes" or "adverse outcomes" or "pregnancy outcomes" and were adapted to each database. An experienced information scientist was consulted to ensure that the search terms were relevant and optimally arranged. References were managed in Zotero 5.0.96.2. After the removal of duplicate studies, two reviewers (NM and MM) independently screened articles for eligibility. Disagreements or uncertainties were resolved by discussion and consensus or in consultation with a third reviewer (CP). Additionally, references from selected articles were screened for potentially relevant articles.

4.3.2. Inclusion and exclusion criteria

Studies that compared pregnancy outcomes in one or two types of maternal diabetes only, those focusing on other forms of diabetes (maternal onset of diabetes in young (MODY), etc.), abstracts, review articles, letters, case reports, intervention studies and those not written in English, were excluded. Review articles were screened to identify eligible studies that may have been missed using our search strategy. Studies reporting on adverse outcomes in pregnancies complicated by T1DM, T2DM and GDM were included. This systematic review was conducted to answer the following question:

Is there an association between maternal diabetes type and the frequency of adverse pregnancy outcomes?

This was achieved using the following:

Participants – Pregnant women with GDM

Intervention – No intervention was used in this study

Comparator – Pregnant women with pregestational T1DM and T2DM

Outcome – Pregnancy outcomes

4.3.3. Data extraction and quality assessment

Data that were extracted and recorded included author details (name and date of publication), study details (aim and design, study period and GDM diagnostic criteria), sample size, characteristics of the population (ethnicity), country and pregnancy outcomes in the different diabetic groups. Two reviewers (NM and MM) independently appraised the study quality and risk of bias using the Newcastle-Ottawa Scale. The Newcastle-Ottawa Scale is used to assess the quality of non-randomized studies, such as case-control and cohort studies (Wells et al., 2000). It assesses study quality based on three study parameters: selection, comparability, and outcomes, which are divided into eight specific items that can be scored as one or two points with points totalling nine (Supplementary Table S3). Disagreements between the two reviewers were resolved by consulting a third reviewer (CP). A study was classified as having a low risk of bias (7 to 9), moderate (5 to 6), or high risk of bias (1 to 4) based on the total score.

4.3.4. Definitions of pregnancy outcomes

Caesarean section refers to the delivery of a fetus through an incision in the abdominal wall and uterus (Mashamba, 2021). PTB is defined as birth before 37 weeks of gestation (Walani, 2020). Congenital anomalies are defined as structural or functional anomalies that occur during intrauterine

life as determined by the ultrasound scan and laboratory tests (Asher Ornoy et al., 2015). Preeclampsia is defined as a systolic blood pressure of ≥ 140 mm Hg or diastolic blood pressure of ≥ 90 mm Hg on two occasions at least 4 hours apart; or shorter interval timing of systolic blood pressure of ≥ 160 mm Hg or diastolic blood pressure of ≥ 110 mm Hg, determined after 20 weeks of gestation (Karrar and Hong, 2023). Macrosomia is defined as giving birth to babies weighing > 4 kg (Negrato et al., 2012). Stillbirth is fetal death after 24 weeks of gestation or fetus > 500g (Smith and Fretts, 2007). LGA is defined as birth weight > 90th percentile for age (Damhuis et al., 2021). Neonatal hypoglycaemia is defined as a plasma glucose value <1.65 mmol/L in the first 24 hours of life and <2.5 mmol/L onwards (Stomnaroska-Damcevski et al., 2015). NICU admission refers to the admission of a newborn to an intensive care unit for specialised care due to a critical condition or illness (Carter et al., 2012). Miscarriage refers to fetal death before 24 weeks of gestation or fetus < 500g (Abdelazim et al., 2017). Induction of labour refers to the process that involves mechanical or surgical means to initiate uterine contractions (Tenore, 2003). The Apgar score is used to assess the wellbeing of a neonate at 1 minute and 5 minutes after birth (Simon et al., 2017). Respiratory distress syndrome is defined as the need to supplement oxygen to the neonate to maintain a saturation over 85% within the first 24 hours after birth (De Luca et al., 2017).

4.4. Results

4.4.1. Selected studies

A total of 2164 studies were identified from the search strategy. An additional three articles were identified by reviewing the reference lists of relevant articles and reviews resulting in 2167 articles. After removing duplicates, 1958 article titles and abstracts were screened for eligible full-text articles. We excluded studies that compared one or two types of maternal diabetes only, interventional studies, those not written in English, review articles, letters, case reports and abstracts. A total of 20 studies, published between January 1993 and December 2021, met the inclusion criteria and are discussed in this review (Figure 4.1).

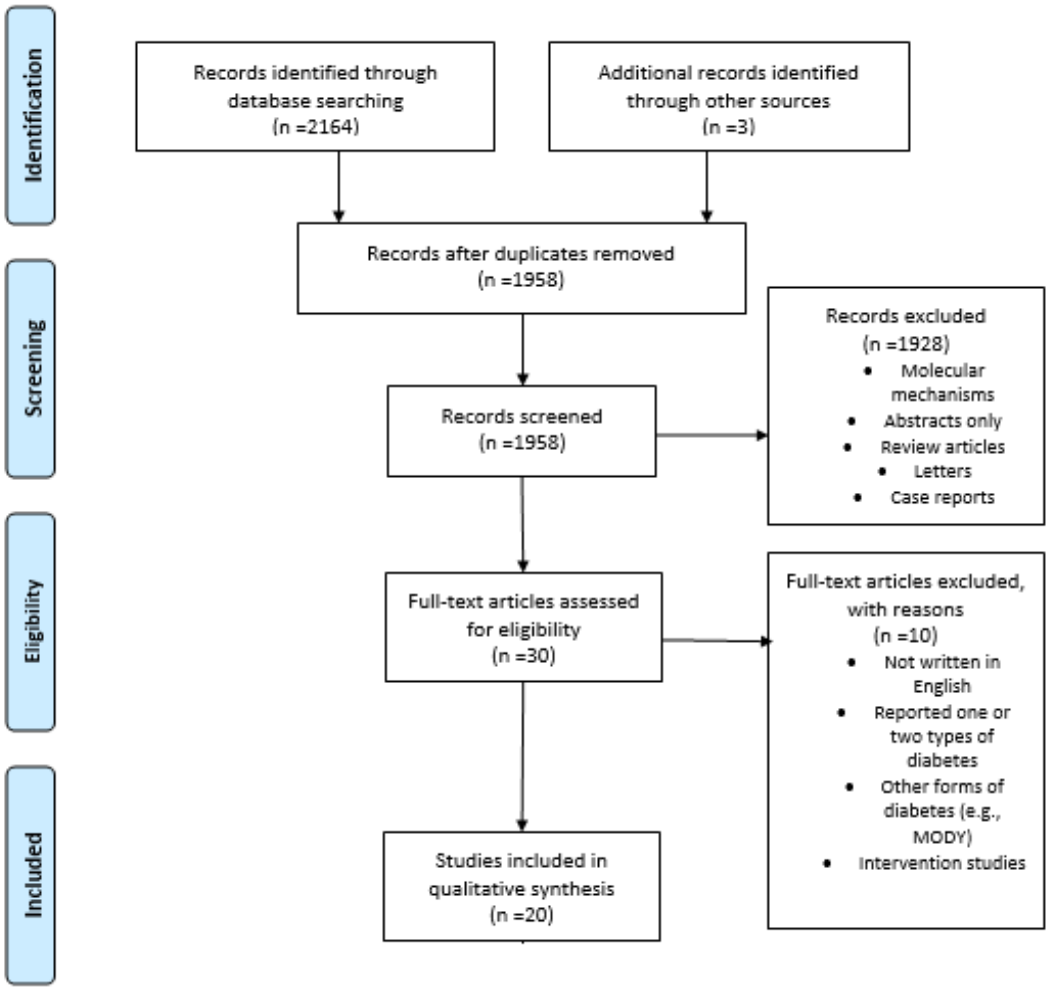

Figure 4.1. Flow diagram for the search criteria; MODY- maturity onset diabetes of the young.

4.4.2. Characteristics of included studies

Twenty articles published between 1993 and 2021 were included in the review (n= 196 232 participants; Supplementary Table S2). These studies were conducted across five continents (Europe, Asia, North America, Africa and Australia). Sixteen studies were retrospective, two were prospective, one was cross-sectional, and one was unspecified. Nine studies reported adverse outcomes for pregestational diabetes, combining data for T1DM and T2DM (Abu-Heija et al., 2015; Barakat et al., 2010; El Mallah et al., 1997; Gui et al., 2014; Hamedi, 2005; Hyari et al., 2013; Shand et al., 2008; Shefali et al., 2006; Tinker et al., 2020), while 11 studies reported data for T1DM and T2DM separately (Al-Nemri et al., 2018; Capobianco et al., 2020; Gualdani et al., 2021; Huddle et al., 1993; López-de-Andrés et al., 2020; Peticca et al., 2009; Soepnel et al., 2019; Stogianni et al., 2019; Van Zyl and Levitt, 2018; Wang et al., 2019; Yamamoto et al., 2018). These studies reported on various maternal and neonatal short-term pregnancy adverse outcomes, of which 13 are summarised in this review. These selected adverse outcomes are amongst the most common in literature (Negrato et al., 2012). None of the studies investigated long-term maternal outcomes in women with T1DM, T2DM and GDM.

The studies in this review used different diagnostic criteria for GDM, which included the International Association of Diabetes and Pregnancy Study Groups Consensus Panel, 2010 (IADPSG; n=2), American Diabetes Association (ADA; n=2), National Diabetes Data Group (NDDG; n=2), O'Sullivan and Mahan (n=1), Spanish Group for Diabetes (n=1), Australasian Diabetes in Pregnancy society (ADIPS; n=2), World Health Organization 1998/1999 (n=2). Five studies used institution based diagnostic criteria, while three studies did not report which diagnostic criteria were used. Pregestational diabetes was determined through hospital records and/or by the medication taken by patients. The studies were conducted in different populations which included: Omani, Saudi, African, Non-Hispanic black, Australian, Asian, Middle Eastern, Indian, Caucasian, and Hispanic. Many of the studies were retrospective and did not report the time of assessment of pregnancy outcomes. Twelve studies included in this review defined one or more of the adverse

outcomes, however, definitions and/or cut-offs varied across studies, while eight studies did not define outcomes.

Congenital anomalies included cardiovascular, central nervous system, cleft lip and palate, trisomy 21, gastrointestinal, musculoskeletal, and urogenital anomalies/malformations and were referred to differently across studies which included: congenital anomalies/malformations/abnormalities, birth defects, congenital defects, fetal anomalies/malformations, and neonatal deformities. For this review, these were collectively referred to as congenital anomalies. Moreover, the majority (92.31%) of the studies that reported on congenital anomalies reported the overall incidence and not the incidence of the individual congenital anomalies in their comparisons. Due to significant heterogeneity between studies and the low quality assessment scores for a few studies, a meta-analysis was not performed, as this may lead to an inaccurate estimate of overall effect size (Ioannidis et al., 2007).

4.4.3. Quality assessment of included studies

The quality of the 20 studies included in this review ranged from unsatisfactory to very good with scores ranging from 4 to 7 and an average score of 5.5. Three studies scored unsatisfactory (4), seven studies scored fair (5), six studies scored good (6), and four studies scored very good (7) (Supplementary Table S4). The studies that rated good and very good were due to controlling for confounding factors, while studies that rated fair and unsatisfactory were affected by not controlling for confounders. Most of the studies included in this review were retrospective, therefore were not able to control for confounders. Due to the narrative nature of this review, all studies were included for analysis despite their risk of bias rating.

4.4.4. Qualitative synthesis

Of the nine studies that compared combined data for pregestational T1DM and T2DM with GDM, the most common adverse outcome reported was CS (n=7), followed by PTB (n=7), congenital anomalies (n=7), preeclampsia (n=6), neonatal hypoglycaemia (n=5), macrosomia (n=4), NICU

admission (n=4), stillbirth (n=4), Apgar score (n=4), LGA (n=3) RDS (n=3) and IOL (n=2). Of the eleven studies that separately compared pregestational T1DM and T2DM with GDM, the most common adverse outcome reported was CS (n=10), followed by PTB (n=7), macrosomia (n=7), congenital anomalies (n=6), preeclampsia (n=4), stillbirth (n=4), neonatal hypoglycaemia (n=3), IOL (n=3) , Apgar score (n=3), LGA (n=3), miscarriage (n=2), NICU (n=2), and RDS (n=1). Certain studies subdivided GDM into true GDM (fasting glucose < 7 mmol/L and oral glucose tolerance test (OGTT) 2 h < 11.1 mmol/L) and overt GDM (fasting glucose ≥ 7 mmol/L or OGTT 2 h ≥ 11.1 mmol/L).

CS, PTB, and congenital anomalies were the most reported adverse outcomes, while the least reported outcomes were IOL, RDS and miscarriage. Other adverse outcomes reported included preeclampsia, neonatal hypoglycaemia, macrosomia, NICU admissions, stillbirths, LGA and Apgar scores. Most of the adverse outcomes were higher in pregestational T1DM and T2DM compared to GDM. However, there were a few adverse outcomes which were more common in GDM compared to pregestational T1DM and/or T2DM (Table 4.1). For the purpose of this review, we focused on outcomes for true GDM.

Caesarean section (CS). Of the studies that compared pregestational diabetes (combined T1DM and T2DM) with GDM, four studies reported higher rates of CS in pregestational diabetes compared to GDM (Abu-Heija et al., 2015; Barakat et al., 2010; Hamedi, 2005; Shand et al., 2008), while similar rates were reported in two studies (El Mallah et al., 1997; Shefali et al., 2006). Hyari et al. 2013 reported slightly higher rates of CS in women with GDM compared to pregestational diabetes. Of the studies that compared pregestational T1DM and T2DM separately with GDM, six studies reported higher rates of CS in T1DM and T2DM compared to GDM (Capobianco et al., 2020; Gualdani et al., 2021; López-de-Andrés et al., 2020; Stogianni et al., 2019; Van Zyl & Levitt, 2018; Yamamoto et al., 2018). Al-Nemri reported higher rates of elective CS in pregestational T1DM (25.0%) and T2DM (34.3%) compared to GDM (15.7%), but similar rates for emergency CS (li et et al., 2018). Petticca et al., 2009 reported higher rates of CS in pregestational T1DM (51.6%) compared to pregestational T2DM (38.0%) and GDM (38.0%), with the latter diabetes types showing similar rates of CS (Peticca et al., 2009). Soepnel et al. reported higher rates of CS in pregestational T2DM (78.4%) compared to T1DM (67.1%) and GDM (67.8%), with the latter

showing similar rates (Soepnel et al., 2019). In contrast, Huddle et al. reported a higher rate of CS in GDM (56.0%) compared to pregestational T1DM (39.8%), but similar rates in GDM compared to pregestational T2DM (55.5%) (Huddle et al., 1993). Taken together, these results demonstrate that CS is more common in women with pregestational T1DM and T2DM than women with GDM.

Preterm birth (PTB). Of the studies that compared pregestational diabetes (combined T1DM and T2DM) with GDM, five studies reported higher rates of PTB in pregestational diabetes compared to GDM (Abu-Heija et al., 2015; El Mallah et al., 1997; Gui et al., 2014; Hyari et al., 2013; Shand et al., 2008), while two studies reported higher rates in GDM compared to pregestational diabetes (Barakat et al., 2010; Hamedi, 2005). Of the studies that compared pregestational T1DM and T2DM separately with GDM, six studies reported higher rates of PTB in pregestational T1DM and T2DM compared to GDM (Capobianco et al., 2020; Gualdani et al., 2021; López-de-Andrés et al., 2020; Peticca et al., 2009; Van Zyl & Levitt, 2018; Yamamoto et al., 2018). Stogianni et al. reported higher rates of PTB in pregestational T2DM (46.0%) compared to pregestational T1DM (35.0%) and GDM (12.0%), and higher rates in pregestational T1DM compared to GDM (Stogianni et al., 2019). These results show that PTB is more common in women with pregestational T1DM and T2DM than women with GDM.

Congenital anomalies. Higher rates of congenital anomalies were reported in pregestational diabetes (combined T1DM and T2DM) compared to GDM in four studies (Hamedi, 2005; Hyari et al., 2013; Shefali et al., 2006; Tinker et al., 2020), while Barakat et al., 2010 reported higher rates in GDM (8.9%) compared to pregestational diabetes (5.6%) (Barakat et al., 2010). In contrast, two studies reported no significant difference in the rates of congenital anomalies between pregestational diabetes and GDM (Abu-Heija et al., 2015; Gui et al., 2014). When comparing T1DM and T2DM separately with GDM, four studies reported higher rates of congenital anomalies in pregestational T1DM and T2DM compared to GDM (Al-Nemri et al., 2018; Huddle et al., 1993; Peticca et al., 2009; Van Zyl & Levitt, 2018). Of these, two reported higher rates of congenital anomalies in pregestational T2DM compared to pregestational T1DM and GDM, and higher rates in pregestational T1DM compared to GDM (Al-Nemri et al., 2018; Huddle et al., 1993). In contrast, two studies reported no significant difference in rates of congenital anomalies between the three

diabetic groups (Gualdani et al., 2021; Soepnel et al., 2019). Although discrepant results are reported, most studies showed that congenital anomalies are more common in neonates born to mothers with pregestational T1DM and T2DM than neonates born to mothers with GDM.

Preeclampsia. Higher rates of preeclampsia were reported in pregestational diabetes (combined T1DM and T2DM) compared to GDM in three studies (Abu-Heija et al., 2015; Gui et al., 2014; Shand et al., 2008), while two studies reported higher rates in GDM compared to pregestational diabetes (Hamedi, 2005; Hyari et al., 2013). El Mallah et al. reported no difference in the rates of preeclampsia between pregestational diabetes (1.4%) and GDM (2.0%) (El Mallah et al., 1997). Preeclampsia was also compared in pregnant women with pregestational T1DM and T2DM separately with GDM. Higher rates of preeclampsia were reported in pregestational T1DM compared to T2DM and GDM in three studies, with the latter occurring at similar rates (Capobianco et al., 2020; Peticca et al., 2009; Van Zyl & Levitt, 2018). Soepnel et al. reported no significant difference in the rates of preeclampsia across the three diabetic groups (Soepnel et al., 2019). Taken together, preeclampsia is more common in women with pregestational T1DM and T2DM than GDM and more common in pregestational T1DM.

Neonatal hypoglycaemia. Three studies reported higher rates of neonatal hypoglycaemia in pregestational diabetes (combined T1DM and T2DM) compared to GDM (Abu-Heija et al., 2015; Hamedi, 2005; Shand et al., 2008), while two studies reported no difference in the rates of neonatal hypoglycaemia between pregestational diabetes and GDM (El Mallah et al., 1997; Hyari et al., 2013). When comparing neonatal hypoglycaemia between T1DM and T2DM separately with GDM, Yamamoto et al., 2018 reported higher rates in T1DM (27.5%) and T2DM (18.3%) compared to GDM (5.0%) (Yamamoto et al., 2018) and Huddle at al. reported higher rates of neonatal hypoglycaemia in neonates born to mothers with pregestational T1DM (4.2%) and GDM (4.2%) compared to neonates born to mothers with pregestational T2DM (3.6%) (Huddle et al., 1993). However, Al-Nemri et al., 2018 reported no difference in the rates of neonatal hypoglycaemia across the three diabetic groups (Al-Nemri et al., 2018). These results show that rates of neonatal hypoglycaemia are more common in neonates born to mothers with pregestational T1DM and T2DM compared to neonates born to mothers with GDM.

Macrosomia. Higher rates of macrosomia were reported in pregestational diabetes (combined T1DM and T2DM) compared to GDM in three studies (Barakat et al., 2010; El Mallah et al., 1997; Hyari et al., 2013), while Abu-Heija et al., 2015 reported no significant difference in the rates of macrosomia between pregestational diabetes (10.3%) and GDM (4.9%) (Abu-Heija et al., 2015). Macrosomia was also reported when comparing T1DM and T2DM separately with GDM. Two studies reported higher rates in T1DM and T2DM compared to GDM (Capobianco et al., 2020; Soepnel et al., 2019). Peticca et al. reported higher rates of macrosomia in T1DM (17.2%) and GDM (12.2%) compared to T2DM (11.1%) (Peticca et al., 2009), while Van Zyl and Levitt reported higher rates of macrosomia in GDM (9.2%) compared to pregestational T1DM (8.5%) and T2DM (8.2%) (Van Zyl & Levitt, 2018). However, three studies reported no significant difference in the rates of macrosomia between the three diabetic groups (Al-Nemri et al., 2018; Gualdani et al., 2021; Stogianni et al., 2019). Altogether, these studies indicate that macrosomia is more common in neonates born to mothers with pregestational diabetes T1DM and T2DM compared to GDM.

NICU admissions. When NICU admissions were compared between pregestational diabetes (combined T1DM and T2DM) and GDM, four studies reported higher rates of NICU admissions in pregestational diabetes compared to GDM (Abu-Heija et al., 2015; Barakat et al., 2010; Gui et al., 2014; Shand et al., 2008). NICU admissions were also reported when comparing T1DM and T2DM separately with GDM. Yamatoto et al., 2018 reported higher rates of NICU admissions in T1DM (55.5%) and T2DM (31.0%) compared to GDM (14.0%) (Yamamoto et al., 2018), while A-Nemri et al. reported higher rates of NICU admissions in pregestational T1DM (66.7%) compared to pregestational T2DM (16.0%) and GDM (10.2%), with the latter showing similar rates (Al-Nemri et al., 2018). These results demonstrate that NICU admissions are more common in neonates born to mothers with pregestational diabetes T1DM and T2DM compared to neonates born to mothers GDM.

Stillbirth. When stillbirth was compared between pregestational diabetes (combined T1DM and T2DM) and GDM, higher rates of stillbirth were reported in pregestational diabetes compared to GDM in two studies (El Mallah et al., 1997; Shand et al., 2008). However, two studies reported no difference in the rates of stillbirths between pregestational diabetes and GDM (Barakat et al., 2010;

Gui et al., 2014). When comparing T1DM and T2DM separately with GDM, higher rates of stillbirths were reported in pregestational T1DM and T2DM compared to GDM in three studies (Huddle et al., 1993; Peticca et al., 2009; Van Zyl and Levitt, 2018; Wang et al., 2019), while Huddle et al. reported higher rates in T2DM (4.7%) compared to T1DM (3.3%) and GDM (4.0%) with the latter occurring at a similar rate (Huddle et al., 1993). Altogether, these results demonstrate that stillbirths are more common in neonates born to mothers with pregestational T1DM and T2DM compared to neonates born to mothers with GDM.

Apgar score. Low Apgar scores (<7) were compared between pregestational diabetes (combined T1DM and T2DM) and GDM. Barakat et al. reported higher rates of low Apgar scores in pregestational diabetes (24.1%) compared to GDM (22.1%) (Barakat et al., 2010), while three studies reported no difference in the rates of low Apgar scores between pregestational diabetes and GDM (Abu-Heija et al., 2015; El Mallah et al., 1997; Shand et al., 2008). Low Apgar scores were also reported when comparing T1DM and T2DM separately with GDM. Gualdani et al. reported lower Apgar scores in T1DM (5.4%) compared to T2DM (2.5%) and GDM (1.3%) (Gualdani et al., 2021), while two studies reported similar rates of low Apgar scores in T1DM and T2DM, although higher than GDM (Peticca et al., 2009; Stogianni et al., 2019). These findings indicate that low Apgar scores present at a similar rate in neonates across the three diabetic groups.

Large for gestational age (LGA). Two studies reported higher rates of LGA in GDM compared to pregestational diabetes (combined T1DM and T2DM) (Hamedi, 2005; Shefali et al., 2006), while Shand et al. reported higher rates of LGA in pregestational diabetes (35.0%) compared to GDM (15.9%) (Shand et al., 2008). LGA was also reported when comparing T1DM and T2DM separately with GDM. Two studies reported higher rates of LGA in T1DM and T2DM compared to GDM (Stogianni et al., 2019; Yamamoto et al., 2018). In contrast, Gualdani et al. reported no significant difference between the three diabetic groups (Gualdani et al., 2021). Altogether, the results show that LGA is more common neonates born to mothers with pregestational T1DM and T2DM compared to neonates born to mothers with GDM.

Induction of labour (IOL). Two studies reported no difference in the rates of IOL between pregestational diabetes and GDM (Abu-Heija et al., 2015; Shand et al., 2008). In the comparison of T1DM and T2DM separately with GDM, López-de-Andrés et al. reported higher rates of IOL in pregestational T1DM (29.6%) and T2DM (30.4%) compared to GDM (22.6%) (López-de-Andrés et al., 2020), while Peticca et al. reported higher rates of IOL in T1DM (44.7%) and GDM (38.3%) compared to T2DM (36.6%) (Peticca et al., 2009). In contrast, Van Zyl and Levitt reported higher rates of IOL in GDM (30.0%) compared to T1DM (11.8%) and T2DM (18.6%) (Van Zyl and Levitt, 2018). These results show that IOL occurs at similar rates in women with pregestational T1DM and T2DM and GDM.

Respiratory distress syndrome (RDS). When comparing pregestational diabetes (combined T1DM and T2DM) and GDM, higher rates of RDS were reported in pregestational diabetes compared to GDM in two studies (Abu-Heija et al., 2015; Hamedi, 2005), while Barakat et al. reported higher rates in GDM (2.8%) compared to pregestational diabetes (1.6%) (Barakat et al., 2010). In the comparison of T1DM and T2DM separately with GDM, Al-Nemri et al. reported higher rates of RDS in T1DM (44.4%) compared to T2DM (13.9%) and GDM (13.5%) with similar rates occurring in the latter (Al-Nemri et al., 2018). These results demonstrate that RDS is more common in neonates born to mothers with pregestational T1DM and T2DM than neonates born to mothers with GDM.

Miscarriage. When comparing T1DM and T2DM separately with GDM, higher rates of miscarriage were reported in T1DM compared to T2DM and GDM in two studies (Soepnel et al., 2019; Van Zyl and Levitt, 2018). These results indicate that miscarriages are more common during pregestational T1DM compared to pregestational T2DM and GDM.

Table 4.1. The frequency of adverse pregnancy outcomes

Adverse outcome	**Increased in Pregestational diabetes**	**Increased in GDM**	**No difference**
Caesarean section	(Abu-Heija et al., 2015; Barakat et al., 2010; Capobianco et al., 2020; Gualdani et al., 2021; Hamedi, 2005; López-de-Andrés et al., 2020; Peticca et al., 2009; Shand et al., 2008; Soepnel et al., 2019; Stogianni et al., 2019; Van Zyl and Levitt, 2018; Yamamoto et al., 2020)	(Huddle et al., 1993; Hyari et al., 2013)	(El Mallah et al., 1997; Shefali et al., 2006)
Preterm birth	(Abu-Heija et al., 2015; Capobianco et al., 2020, 2020; El Mallah et al., 1997; Gualdani et al., 2021; Gui et al., 2014; Hyari et al., 2013; López-de-Andrés et al., 2020; Peticca et al., 2009; Shand et al., 2008; Stogianni et al., 2019; Van Zyl and Levitt, 2018; Yamamoto et al., 2020)	(Barakat et al., 2010; Hamedi, 2005)	

Congenital anomalies	(Al-Nemri et al., 2018; Hamedi, 2005; Huddle et al., 1993; Hyari et al., 2013; Peticca et al., 2009; Shefali et al., 2006; Tinker et al., 2020b; Van Zyl & Levitt, 2018)	(Barakat et al., 2010)	(Abu-Heija et al., 2015; Gualdani et al., 2021; Gui et al., 2014; Soepnel et al., 2019)
Preeclampsia	(Abu-Heija et al., 2015; Capobianco et al., 2020; Gui et al., 2014; Peticca et al., 2009; Shand et al., 2008; Van Zyl and Levitt, 2018)	(Hamedi, 2005; Hyari et al., 2013)	(El Mallah et al., 1997; Soepnel et al., 2019)
Neonatal hypoglycaemia	(Abu-Heija et al., 2015; Hamedi, 2005; Huddle et al., 1993; Shand et al., 2008; Yamamoto et al., 2020)		(Al-Nemri et al., 2018; El Mallah et al., 1997; Hyari et al., 2013)
Macrosomia	(Barakat et al., 2010; Capobianco et al., 2020; El Mallah et al., 1997; Hyari et al., 2013; Peticca et al., 2009; Soepnel et al., 2019)	(Van Zyl & Levitt, 2018)	(Abu-Heija et al., 2015; Al-Nemri et al., 2018; Gualdani et al., 2021; Stogianni et al., 2019)

NICU admission	(Abu-Heija et al., 2015; Al-Nemri et al., 2018; Barakat et al., 2010; Gui et al., 2014; Shand et al., 2008; Yamamoto et al., 2020)		
Stillbirth	(El Mallah et al., 1997; Huddle et al., 1993; Peticca et al., 2009; Shand et al., 2008; Van Zyl and Levitt, 2018; Wang et al., 2019)		(Barakat et al., 2010; Gui et al., 2014)
Apgar score	(Barakat et al., 2010; Gualdani et al., 2021; Peticca et al., 2009; Stogianni et al., 2019)		(Abu-Heija et al., 2015; El Mallah et al., 1997; Shand et al., 2008)
Large for gestational age	(Shand et al., 2008; Stogianni et al., 2019; Yamamoto et al., 2020)	(Hamedi, 2005; Shefali et al., 2006)	(Gualdani et al., 2021)
Induction of labour	(Abu-Heija et al., 2015; López-de-Andrés et al., 2020; Peticca et al., 2009)	(Van Zyl & Levitt, 2018)	(Shand et al., 2008)
Respiratory distress syndrome	(Abu-Heija et al., 2015; Al-Nemri et al., 2018; Hamedi, 2005)	(Barakat et al., 2010)	

Miscarriage	(Soepnel et al., 2019; Van Zyl and Levitt, 2018)		

4.5. Discussion

Adverse outcomes associated with maternal diabetes are reported to be more common in women with pregestational diabetes compared to GDM, although conflicting results have been reported (Al-Nemri et al., 2018; Hamedi, 2005; Huddle et al., 1993; Soepnel et al., 2019; Stogianni et al., 2019; Tinker et al., 2020; Van Zyl and Levitt, 2018). In this systematic review, we summarise and synthesise studies that have compared adverse pregnancy outcomes in pregnancies complicated by pregestational diabetes and GDM. Findings from this review confirm that both pregestational diabetes and GDM are associated with pregnancy complications including CS, PTB, congenital anomalies, preeclampsia, neonatal hypoglycaemia, macrosomia, NICU admission, stillbirth, Apgar score, LGA, IOL, RDS and miscarriage. Although conflicting results were reported in a few studies, the majority of studies report that adverse outcomes are more common in pregnancies complicated by pregestational diabetes than GDM. This review did not identify studies that compared long-term adverse outcomes in women with pregestational diabetes and GDM.

Thirteen perinatal complications, CS, PTB, congenital anomalies, preeclampsia, neonatal hypoglycaemia, macrosomia, NICU admission, stillbirth, Apgar score, LGA, IOL, RDS and miscarriage, which are amongst the most common maternal and fetal adverse outcomes reported in the literature, were compared in this review. CS was the most common adverse outcome reported. Although it is accepted that not all CS may be considered an adverse pregnancy outcome (Canelón and Boland, 2020), it is often recommended by health care providers as a strategy to reduce the risk of perinatal complications associated with maternal diabetes (Magro-Malosso et al., 2017; Sanchez-Ramos et al., 2002). PTB is defined as birth before 37 completed weeks of gestation (Walani, 2020) and is the leading cause of mortality in children younger than five years. Infants who survive PTB often present with poor neurodevelopment and cognitive disabilities (Moreira et al., 2014) and behavioural and emotional difficulties (Russell et al., 2007).

Congenital anomalies, which refer to structural or functional malformations that occur during intrauterine life, is associated with hyperglycaemia during the period of organogenesis that occurs in the first trimester of pregnancy. Depending on the criteria (especially criteria prior to 2010) used for GDM diagnosis, hyperglycaemia first diagnosed during pregnancy might have been misdiagnosed or classified as GDM (Gupta et al., 2020). Maternal hyperglycaemia leads to the increased production

of reactive oxygen species (ROS), resulting in DNA and membrane damage and the subsequent induction of apoptosis, causing malformations in major organs of the developing fetus (Ornoy et al., 2015). Preeclampsia is characterised by hypertension which usually develops after 20 weeks of gestation (Steegers et al., 2010) and is considered the leading cause of maternal morbidity and mortality among women who have diabetes (Ghulmiyyah and Sibai, 2012). The condition is thought to occur due to endothelial dysfunction, dyslipidaemia, and inflammation associated with diabetes (Barden et al., 2004; Jesmin et al., 2011).

Macrosomia refers to giving birth to babies weighing more than 4 kg and is considered the most common adverse outcome associated with maternal diabetes (Johns et al., 2018; Negrato et al., 2012). The condition is thought to occur due to increased placental transport of glucose and other nutrients from the mother to the fetus, resulting in accelerated growth (Barnes-Powell, 2007; Sugrue and Zera, 2018). Macrosomia is associated with several complications including, neonatal hypoglycaemia and premature birth (Magro-Malosso et al., 2017; Sanchez-Ramos et al., 2002). Abnormal placental supply of nutrients results in abnormal fetal growth, including fetal growth restriction (FGR) and fetal overgrowth and is associated with increased neonatal mortality. LGA refers to a fetus that weighs in > 90th percentile of the birth chart (Damhuis et al., 2021). LGA is associated with increased rate of CS and neonatal hypoglycaemia, including a longer hospital stay in mothers with diabetes (Weissmann-Brenner et al., 2012; Yang et al., 2006). Neonatal hypoglycaemia is defined as a plasma glucose value <1.65 mmol/l in the first 24 hours of life and <2.5 mmol/L onwards (Stomnaroska-Damcevski et al., 2015). Hypoglycaemia in neonates occurs due continuous placental transport of glucose and other nutrients from the mother to the fetus which results in hyperinsulinaemia which leads to a fall in glucose levels during and post-delivery (Alemu et al., 2017; Harding et al., 2017). Hyperinsulinism is very common in infants of mothers with diabetes (Stomnaroska-Damcevski et al., 2015). Hyperinsulinaemia in the fetus may also lead to RDS at birth. RDS is defined by need to supplement neonatal oxygen to maintain a saturation over 85% within the first 24 hours after birth and also radiological features (De Luca et al., 2017). The development of RDS has been attributed to the inhibitory effects of insulin on the expression of surfactant proteins A and B in lung epithelial cells, resulting in decreased production of surfactants and delayed pulmonary maturation (De Luca et al., 2017; Li et al., 2019; Persson and Fadl, 2014).

Placental abnormalities and congenital malformations are major risk factors for stillbirth and neonatal death, which represent the extreme end of the spectrum of complications in diabetic pregnancies (Wang et al., 2019). Stillbirth is defined as death of a fetus at ≥ 22 weeks of gestation or birth weight of ≥ 500 g (Smith and Fretts, 2007). Unexplained stillbirths at term in maternal diabetes are attributed to maternal hyperglycaemia and fetal hyperinsulinaemia, fetal hypoxia and acidaemia, and cardiomyopathy due to glycogen deposition in the myocardium (Mathiesen et al., 2011; Starikov et al., 2015). Maternal diabetes has also been associated with increased risk of miscarriages and habitual abortions (Kilshaw et al., 2017; Talaviya and Suvagiya, 2011). Animal models have shown that maternal diabetes affects the pre-implantation in the embryo developmental stages. In vivo and in vitro studies show that hyperglycaemia leads to an overexpression of *Bax,* (Bcl-2-associated X) which is a death promoting protein associated with increased apoptotic morphological changes and is reversed by insulin (Regan and Rai, 2000). In women with diabetes, IOL is recommended to minimise birth complications associated with macrosomia and the risk for stillbirth (Coates et al., 2020). A Cochrane review by Boulvain et al. 2001 showed that induction of labour lowered the prevalence of macrosomia without increasing the risk of caesarean section (Boulvain et al., 2001).

Furthermore, poor glucose control in the third trimester may lead to perinatal asphyxia and low Apgar scores (Mimouni et al., 1988; Wahabi et al., 2012). Apgar score is a clinical method used to assess the wellbeing of a neonate at 1 minute and 5 minutes after birth. The Apgar score assesses elements such as skin colour/tone, heart rate, reflexes, muscle tone, and respiration (Simon et al., 2017). Apgar scores may predict long-term neurological disabilities in infants (Mimouni et al., 1988; Wahabi et al., 2012). Fetal complications are associated with increased admissions to the neonatal intensive care unit (NICU), which is therefore often used as an indicator of adverse pregnancy outcomes (Murphy et al., 2007; Owens et al., 2015).

Limitations of the studies included in this review may hinder our ability to draw significant conclusions. There was heterogeneity across studies in terms of population characteristics, diagnostic criteria used, the definitions used for pregnancy outcomes (e.g., PTB, Apgar scores) and different medication regimens (diet, metformin, insulin). It has been widely reported that ethnicity (Farrar et al., 2015; Fujimoto et al., 2013), advanced maternal age (Guarga Montori et al., 2021), diet (De La Torre et al., 2019), socioeconomic status (Campbell et al., 2018) and medication regimen (Stogianni et al., 2019)

influence pregnancy outcomes. Furthermore, most studies were retrospective and were dependent on the accuracy of medical records and databases, which may negatively affect study accuracy (Mercieca-Bebber et al., 2016). Many of the included studies had poor risk of bias scores, which were mainly affected by the lack of accounting for confounding factors which may have affected the accuracy of study findings. Excluding studies with unsatisfactory ratings from the analysis, did not affect the overall conclusions of the review, and similarly to studies with satisfactory and high risk of bias scores, showed that adverse outcomes were more common in pregestational T1DM and T2DM compared to GDM. Therefore, all the studies were included as the data were deemed valuable for the purpose of this narrative review.

Despite the inconclusive results from this review, it is evident that pregestational diabetes poses a greater risk for pregnancy complications than GDM and emphasises the importance of maintaining optimal glucose control during the preconception period. Maternal metabolic factors may program physiological adaptation to pregnancy, thereby affecting pregnancy outcomes (Catalano and Demouzon, 2015; Stephenson et al., 2018). The importance of preconception health is increasingly acknowledged as a key determinant of pregnancy success, with increasing attention shifting to preconception intervention (Stephenson et al., 2018). A population-based study in Canada reported that a 10% weight reduction in the preconception period decreased the risk of developing GDM, preeclampsia, preterm delivery, macrosomia, and stillbirth (Schummers et al., 2015). Another study showed that women who underwent bariatric surgery prior to conception had a lower risk of developing GDM, hypertensive disorders and macrosomia (Yi et al., 2015). Furthermore, increased physical activity before conception is associated with lower risk of GDM (Zhang et al., 2006; Tobias et al., 2011) and preeclampsia (Aune et al., 2014). Taken together, these studies demonstrate a strong relationship between preconception health and pregnancy outcomes. The mechanisms that underlie these links are not known, but are likely to involve an array of genetic, epigenetic, and environmental factors that interact to affect physiological adaptation during pregnancy.

While acknowledging the importance of preconception health and optimal glucose control during pregnancy, the importance of GDM prevention should not be underestimated. As with pregestational diabetes, albeit less common, GDM was also associated with several adverse pregnancy outcomes. Importantly, these complications can be avoided by preventing the development of GDM. During

pregnancy, lifestyle modifications that include diet and physical activity have been shown to prevent GDM (Guo et al., 2019; Koivusalo et al., 2016; Tobias et al., 2011; Wang et al., 2017). Although not addressed in this review, recent studies have highlighted the occurrence of early-onset GDM, defined as GDM that can be detected in women before 24 weeks of gestation (Immanuel and Simmons, 2017). These women have an increased risk of adverse pregnancy outcomes compared to women with "normal" GDM diagnosed at 24-26 weeks (Bashir et al., 2019; Boriboonhirunsarn et al., 2021), and highlights the need to diagnose early pregnancy glycaemia as recently reported by McIntyre et al. (McIntyre et al., 2016).

4.6. Conclusions and future perspectives

Findings from this review confirm that adverse pregnancy outcomes are more common in women with pregestational diabetes compared to women with GDM. These findings highlight the importance of preconception health and the need to educate women of reproductive age who have diabetes or who are at risk of diabetes about the importance of pre-pregnancy care and maintaining good glycaemic control to improve pregnancy health and reduce the risk of adverse pregnancy outcomes. Another important finding of the review is the high rates of adverse outcomes observed in women with GDM, and the need for intervention strategies to prevent the development of GDM. Majority of studies included in this review were retrospective. In addition, we did not identify articles that investigated long-term adverse outcomes in women with pregestational T1DM and T2DM, and GDM. Therefore, there is a need for prospective, longitudinal studies in future to compare more accurately short-and long-term adverse pregnancy outcomes across diabetes types. PTB was one of the most common adverse outcomes reported in this review. The optimal timing of delivery for women with pregestational diabetes is not known due to lack of published trials (Biesty et al., 2018) therefore, there is a need for more studies to determine the optimal time to deliver babies born to mothers with diabetes as this will reduce the complications associated with preterm delivery.

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Conflicts of Interest

The authors declare no conflicts of interest.

CHAPTER 5

COMPARISON OF OBSTETRIC AND PERINATAL OUTCOMES IN WOMEN WITH DIABETES AT STEVE BIKO ACADEMIC HOSPITAL

Adapted from:

Malaza, N., Pheiffer, C., Dias, S. and Adam, S., 2023. Comparison of obstetric and perinatal outcomes in women with diabetes at Steve Biko Academic Hospital. *South African Journal of Obstetrics and Gynaecology*, *29*(1).

5.1. Abstract

Background. Diabetes and obesity in pregnancy have been associated with increased rates of adverse maternal and neonatal outcomes compared with women with normoglycaemia and normal weight.

Objective. To investigate the effect of diabetes and pre-pregnancy obesity on obstetric and perinatal outcomes.

Methods. This study included women with pregestational diabetes types 1 (T1DM) and 2 (T2DM), gestational diabetes (GDM) and normoglycaemia, who received care at the Steve Biko Academic Hospital antenatal clinic between 2017 and 2022. The women were followed up until delivery. Data collected included obstetric history and care, diabetes, obstetric and perinatal outcomes.

Results. A total of 183 women were recruited: 13 (7.1%) with T1DM, 65 (35.5%) with T2DM, 39 (21.3%) with GDM and 66 (36.1%) normoglycaemic controls. Women with T2DM and GDM were older ($p<0.01$) and more likely to have a history of chronic hypertension ($p=0.025$) compared with controls. Women with GDM were more likely to be obese than their T1DM counterparts ($p=0.036$). T1DM and T2DM were associated with higher rates of preterm birth (PTB) than controls ($p=0.002$). The frequency of GDM was significantly higher in women with obesity ($p=0.039$). The frequency of caesarean section before the onset of labour was higher in women with a weight ≥80 kg compared with women with a weight <80 kg ($p=0.015$).

Conclusion. Diabetes in pregnancy is associated with adverse obstetric and perinatal outcomes. Therefore, adequate glucose control should be accompanied by preconceptual weight optimisation to reduce adverse outcomes during pregnancy.

5.2. Introduction

Diabetes mellitus is a common pregnancy complication that poses a serious health threat to maternal and child health (International Diabetes Federation, 2021). Diabetes in pregnancy (DIP) can be classified as pregestational type 1 (T1DM) or 2 (T2DM) diabetes; T1DM or T2DM first diagnosed during pregnancy; or gestational diabetes mellitus (GDM), a milder form of carbohydrate intolerance that first develops during pregnancy, with glucose homeostasis usually restored within 6 weeks after delivery. DIP affects about 16.7% (21.1 million) live births worldwide. Among these, pregestational T1DM and T2DM account for 10.6% of cases, T1DM and T2DM first detected in pregnancy account for 9.1% of cases and GDM accounts for 80.3% of cases (International Diabetes Federation, 2021). South Africa (SA) is a low-to-middle-income country (LMIC) with high rates of DIP. Recent studies reported that the prevalence of GDM varied from 9.1% to 25.6%, depending on the diagnostic criteria (Dias et al., 2019).

All types of DIP are associated with an increased risk of short-and long-term adverse outcomes for mother and child (Table 5.1), especially when glycaemic control is suboptimal. The severity and frequency of these adverse outcomes are higher in women with pregestational diabetes compared with GDM. Achieving adequate glycaemic control and appropriate gestational weight gain is critical to prevent pregnancy complications and adverse outcomes (Schaefer-Graf et al., 2018).

Table 5.1. Short- and long-term outcomes of DIP

Short-term	**Long-term**
Maternal	
Preeclampsia, PTB, CS, miscarriage,* obstructed labour, PPH (Malaza et al., 2022)	Worsening of diabetic retinopathy and nephropathy*, diabetes mellitus, cardiovascular diseases (Bellamy et al., 2009; Harreiter et al., 2014; Sugrue and Zera, 2018)
Neonatal	
Congenital anomalies,* respiratory distress syndrome, jaundice, neonatal hypoglycaemia, macrosomia, NICU admission (Malaza et al., 2022)	Adiposity/obesity, diabetes mellitus, cardiovascular risk, cognitive impairment (Araujo Júnior et al., 2017; Moreira et al., 2014; Osuchukwu and Reed, 2023; Soepnel et al., 2021)

CS- caesarean section; PTB- preterm birth; PPH- postpartum haemorrhage; NICU- neonatal intensive care unit.

*Specific to pregestational diabetes.

Obesity is considered a major risk factor for DIP, with an increasing number of epidemiological studies supporting this association (Chu et al., 2007). In addition, obesity has also been reported to independently increase the risk of maternal and fetal adverse outcomes (Ehrenberg et al., 2004). In SA, the estimated prevalence of obesity in women of reproductive age is 35.2% (Nglazi and Ataguba, 2022), highlighting the potential negative effects of obesity on both maternal and child health. Studies have shown that an increase in both maternal weight and body mass index (BMI) before and during pregnancy is associated with adverse pregnancy outcomes (Brost et al., 1997; Jatta et al., 2021; Parveen et al., 2018). However, in resource-limited settings where measuring weight is more practical, the use of maternal weight instead of BMI to assess the risk of adverse outcomes related to weight during pregnancy might be a more viable option. This is substantiated by a study conducted by Wolfe et al (Wolfe et al., 1991).

This study aimed to investigate the effect of DIP and obesity on obstetric and perinatal outcomes in women attending the diabetic antenatal clinic at a tertiary hospital in Tshwane, South Africa.

5.3. Methods

5.3.1. Study population

We conducted a prospective study including women with pregestational T1DM or T2DM, GDM and normoglycaemia (negative oral glucose tolerance test (OGTT)) who attended the high-risk antenatal clinic at Steve Biko Academic Hospital (SBAH), Pretoria, Gauteng, South Africa between May 2017 and March 2022. The study was approved by the University of Pretoria Health Science Research Ethics Committee (ethics numbers: 41/2021). This study is part of a larger study investigating epigenetic mechanisms in women with DIP. At SBAH, the diabetes antenatal clinic manages referrals from local endocrine and internal medicine or antenatal clinics in the cluster. The referring clinics use the risk factor-based selective screening approach ("The 2017 SEMDSA Guidelines for the Management of Type 2 Diabetes | Journal of Endocrinology, Metabolism and Diabetes of South Africa," n.d.), which includes screening for risk factors, such as family history of diabetes mellitus, previous GDM, advanced maternal age, obesity and previous adverse pregnancy outcome, including congenital abnormality, recurrent miscarriages, delivery of a stillborn child, delivery of a baby ≥ 4 kg in a previous pregnancy or persistent glycosuria (Benhalima et al., 2019).

Women were included in the study if they had singleton pregnancies, were aged between 18 - 42 years, were of black African ethnicity, were at ≤ 28 weeks' gestation and were HIV negative. DIP was categorised as T1DM if diagnosed prior to pregnancy or if first diagnosed in pregnancy and was confirmed by the presence of positive antibodies or the occurrence of diabetic ketoacidosis, which is determined in consultation with an endocrinologist. A T2DM diagnosis was made if it was identified prior to pregnancy or if overt diabetes was diagnosed during pregnancy (fasting plasma glucose level ≥7.0 mmol/L, random plasma glucose or 2-h plasma glucose ≥11.1 mmol/L on the OGTT; or glycated haemoglobin (HbA1c) ≥ 6.5%). GDM was diagnosed if carbohydrate intolerance was first diagnosed during pregnancy according to the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria at 24 - 28 weeks' gestation (fasting plasma glucose level 5.1 - 6.9 mmol/L or 1-h plasma glucose ≥10 mmol/L or 2-h plasma glucose 8.5 - 11.0 mmol/L after a 2-h 75-g oral glucose tolerance test (OGTT)) ("International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy," 2010). Women were recruited as normoglycaemic controls if they had a negative OGTT. Women were

followed up until delivery. Data collected included demographics, anthropometric measures, obstetric history and care, diabetes care and fetal outcomes, according to standard clinical care.

5.3.2. Clinical information and anthropometry

Gestational age (GA) was determined using early ultrasound when available; otherwise, it was determined based on menstrual history or late ultrasound. Maternal weight at the first antenatal visit was recorded as pre-pregnancy weight was not available. Due to missing height measurements, maternal BMI data were limited. Consequently, both weight and BMI were collected. Obesity was defined as a BMI ≥30 kg/m^2 as per Institute of Medicine guidelines (ACOG, 2020). Dietary counselling and education on diabetes were provided by a trained dietician. Post diagnosis, some women with GDM were initiated on metformin in consultation with specialists, while others were started on a low-carbohydrate diet (dependent on OGTT levels). Women were counselled on maintaining glycaemic targets, including fasting/pre-prandial glucose levels of ≤ 5.3 mmol/L and 2-h post-prandial glucose levels of ≤ 6.7 mmol/L. All women with DIP monitored their glucose levels at home. Women were required to test their glucose with an On-Call Plus glucometer (On Call, Mexico) at least five times a day, at various times during the week: 30 minutes before each meal (fasting), 2 hours after each meal (post-prandial), at bedtime and 02h00. Poor glycaemic control was defined as > 25% of glucose values outside of the recommended range based on home glucose monitoring. The overall gestational glycaemic control is based on an average of more than three antenatal visits determined by an experienced maternal-fetal specialist.

5.3.3. Clinical definitions

Obstetric and perinatal outcomes included GA at delivery (weeks), onset of labour, route of delivery, birthweight (kg), neonatal outcome and Apgar score at 5 minutes. GA at delivery was categorised into preterm (≤ 37 weeks) and term delivery (> 37 weeks). Fetal growth was classified as small for gestational age (SGA) if fetal growth < 10th centile and large for gestational age (LGA) if fetal growth > 90th centile. Birthweight was defined as low birth weight (501 - 2 500 g), normal weight (2 500 - 4 000 g) and macrosomia (> 4 kg).

5.3.4. Statistical analysis

Data were captured in Microsoft Office Excel 2010 (Microsoft Corp., US) and analysed using STATA 17 (Stata Corp., US). Baseline characteristics were summarised using descriptive statistics. A skewness-kurtosis test was performed to assess normality. Continuous variables are presented as the median and interquartile range (IQR), while categorical variables are expressed as counts and percentages. Continuous data were compared using the Kruskal-Wallis test, followed by Dunn's *post hoc* multiple comparisons test. Categorical data were compared using Pearson's chi- squared ($\chi 2$) test with the Bonferroni *post hoc* test. For counts less than 5, Fisher's exact test was used. Statistical significance was defined as $p<0.05$.

5.4. Results

The general characteristics of the population according to diabetes type are summarised in Table 5.2. A total of 183 women were recruited, including 13 (7.1%) with T1DM, 65 (35.5%) with T2DM, 39 (21.3%) with GDM and 66 (36.1%) who were classified as normoglycaemic. Women with T2DM and GDM were older (p<0.01), had higher BMI (p<0.05) and had a history of chronic hypertension (p=0.025) compared with the control group. Obesity results were based on 74.31% of BMI data. Women with GDM had a significantly higher frequency of obesity compared with women with T1DM (80.6% v. 36.4%) but were not different to women with T2DM and controls. Women with T1DM and T2DM had significantly higher glycated haemoglobin (HbA1c) compared with those with GDM (p<0.001). At enrollment, more women with T1DM were on insulin treatment compared with those with T2DM (76.9% v. 15.0%; p<0.01), while more women with T2DM were on metformin compared with women with GDM (53.3% v. 26.5%; p<0.05). At delivery, 67.2% of women with T2DM and 18.2% of women with T1DM were managed with a combination of metformin and insulin compared with 15.2% of women with GDM (p<0.001; Table 5.2).

Obstetric and perinatal outcomes were compared in the combined diabetic group (T1DM, T2DM, GDM) compared with controls. Overall, the frequency of PTB was higher in the diabetic group compared with the controls (51.5% v. 17.1%; p<0.001). However, no between-group differences were noted in the other obstetric and perinatal outcomes. Next, the diabetic group was stratified into T1DM, T2DM and GDM and controls. Obstetric and perinatal outcomes in the sub-groups were compared with the control group (Table 5.3). The frequency of PTB was higher in women with T1DM (66.7% v. 17.1%; p<0.05) and T2DM (54.4% v. 17.1%; p<0.05). However, there was no significant difference between the GDM and control groups. No between-group differences were observed in the other obstetric and perinatal outcomes.

Next, the effect of obesity and weight on obstetric and perinatal outcomes was investigated. The frequency of GDM was significantly higher in women with obesity compared with women without obesity (30.5% v. 11.5%; p=0.036) (Table 5.4). The frequency of caesarean section (CS) performed before the onset of labour was higher in women weighing ≥80 kg compared with women weighing <80 kg (45.6% v. 26.0%; p<0.05). The frequency of T1DM was lower in the ≥80 kg weight category compared with the <80 kg weight category (13.6% v. 3.6%; p<0.05), while the frequency of GDM was

higher in the ≥80 kg compared with the <80 kg weight category (27.9% v. 12.1%; p<0.05) (Table 5.5). The frequency of spontaneous onset of labour was higher in the <80 kg weight category compared to the ≥80 kg and <120 kg weight category (52% v. 28.6; p<0.05). The rate of low Apgar scores at 5 minutes was significantly higher in the ≥120 kg group compared with the ≥80 and <120 kg groups (33.3% v. 4.2%; p<0.05) (Table 5.5).

Table 5.2. Participant characteristics according to glucose tolerance

Variable	**Normoglycaemia (n=66)**	**T1DM (n=13)**	**T2DM (n=65)**	**GDM (n=39)**	**p-value**
Age (years)	31 (27-36)[a,b]	29 (27-32)	35 (30-37)[a]	35 (32-38)[b]	<0.001
BMI (kg/m^2)	32.3 (28.2-39.7)[c,d]	24.5 (23.1-33.6)	31.6 (27.6-38.1)[c]	37.8 (31.2-42.2)[d]	<0.001
Obesity (≥30 kg/m^2)	23 (60.5)	4 (36.4)[c]	30 (55.6)	25 (80.6)[c]	0.036
Weight (kg)	82.2 (71.1-94.5)[c,a]	69.9 (60.4-85.0)[c,d,e]	84.7 (72.4-98.9)[d,b]	98.1 (81.9-112.0)[a,e,b]	<0.001
Weight (≥ 80 kg)	30 (49.2)	4 (30.8)	39 (60.9)	27 (69.2)	0.034
Weight (≥ 120 kg)	5 (8.2)	0 (0.0)	2 (3.1)	4 (10.3)	0.332
GA at recruitment (weeks)	22.0 (19.0-24.0)[c]	17.0 (15.0-21.0)[c,e]	21.0 (16.0-25.0)[f]	26.0 (24-27.0)[e,f]	<0.001
HbA1c (%)	4.9 (5.2-5.5)[e,f]	9.7 (9.0-11.0)[e,a,g]	7.6 (6.3-9.3)[f,a,h]	5.7 (5.4-6.1)[g,h]	<0.001
Glycaemic control: Poor		7 (63.6)[c]	23 (41.1)[d]	5 (15.6)[c,d]	0.006

Good		4 (36.4)	33 (58.9)	27 (84.4)	
Poor obstetric history:	32 (61.5)	2 (16.7)	19 (31.1)	10 (27.0)	<0.001
History of hypertension in pregnancy: Yes	4 (6.1)[b,c]	2 (15.4)	23 (35.9)[b]	12 (30.8)[c]	<0.001
Medication:					
At enrollment					
Insulin		10 (76.9)[e,f]	9 (15.0)[e]	0 (0.0)[f]	
Metformin		0 (0.0)[c]	32 (53.3)[c,d]	9 (26.5)[d]	<0.001
Diet		0 (0.0)[c]	7 (11.7)[b,c]	24 (28.7)[c]	
Metformin + Insulin		3 (23.1)	12 (20.0)	1 (2.9)	
At delivery					
Insulin		9 (81.8)[c,d]	5 (8.6)[c]	0 (0.0)[d]	
Metformin		0 (0.0)[c]	5 (8.6)	13 (39.4)[c]	<0.001
Diet		0 (0.0)[c]	2 (3.4)[d]	15 (16.7)[c,d]	
Metformin + Insulin		2 (18.2)[c]	39 (67.2)[c,d]	5 (15.2)[d]	

Data are presented as the median and interquartile range (25th and 75th percentiles) and counts (%). Statistical differences between groups were assessed with the Kruskal Wallis test with Dunn's post hoc multiple comparisons test, the Chi squared test with Bonferroni post hoc multiple comparisons test and Fisher's exact test for counts less 5. Similar superscripts denote statistical significance between groups. $^{a,b}p<0.01$; $^{c,d}p<0.05$; $^{e,f,g,h}p<0.001$. *Poor obstetric history was based on 78.79% of obstetric history data.

Abbreviations: BMI- body mass index, GA- gestational age, HbA1c- glycated haemoglobin, T1DM- type 1 diabetes, T2DM- type 2 diabetes, GDM- gestational diabetes mellitus.

Table 5.3. Obstetric and perinatal outcomes in sub-groups of diabetes compared with normoglycaemic controls

	Normoglycaemic controls (N=66)	**T1DM (N=13)**	**T2DM (N=65)**	**GDM (N=39)**	***p*-value**
PTB (≤37 weeks), *n* (%)	d=35 6 (17.1)[a,b]	d=9 6 (66.7)[a]	d=57 31 (54.4)[b]	d=31 13 (41.9)	0.002
Onset of labour, *n* (%) Induction of labour[#] CS Spontaneous onset of labour (n=46)	d=32 7 (21.9) 8 (25.0) 17 (53.1)	d=7 2 (28.6) 1 (14.3) 4 (57.1)	d=50 13 (26.0) 20 (40.0) 17 (34.0)	d=29 8 (27.6) 13 (44.8) 8 (27.6)	0.356
Route of delivery, *n* (%) Normal vaginal delivery Elective CS Emergency CS (n=46)	d=34 16 (47.1) 7 (20.6) 11 (32.4)	d=10 3 (30.0) 2 (20.0) 5 (50.0)	d=57 16 (28.1) 18 (31.6) 23 (40.4)	d=32 13 (40.6) 10 (31.3) 9 (28.1)	0.514
Fetal growth,* *n* (%) SGA (<10th centile) AGA LGA (>90th centile)		d=9 0 6 (66.7) 3 (33.3)	d=55 4 (7.3) 32 (58.2) 19 (34.5)	d=32 2 (6.3) 25 (78.1) 5 (15.6)	0.339

Birthweight (g), *n* (%) 501 – 2 500 2 500 - 4 000 >4 000	d=35 7 (20.0) 26 (74.3) 2 (5.7)	d=10 1 (10.0) 9 (90.0) 0 (0.0)	d=58 14 (24.1) 40 (69.0) 4 (6.9)	d=32 5 (15.6) 27 (84.4) 0(0.0)	0.659
Stillbirth , *n* (%)	d=35 0 (0.0)	d=10 1 (10.0)	d=58 4 (6.9)	d=32 1 (3.1)	0.227
Apgar score at 5 min, *n* (%) <7	d=55 2 (5.7)	d=10 1 (10.0)	d=56 5 (8.9)	d=31 4 (12.9)	0.743

Statistical differences between groups were assessed with the Chi squared test with Bonferroni multiple comparisons method and Fisher's exact test for counts less 5. Similar superscripts denote statistical significance among groups. [a,b,c,d]$p<0.05$.

[#]Induction of labour by either medical, mechanical or surgical means (or a combination thereof) was used to achieve labour. Failed inductions were considered if a patient was not in active labour within 24 hours.

*No fetal growth data for controls because of a lack of postnatal follow-up.

Abbreviations: D=denominator, T1DM- type 1 diabetes mellitus; T2DM- type 2 diabetes mellitus; GDM- gestational diabetes mellitus; PTB- preterm birth; CS- caesarean section; BMI- body mass index; SGA- small for gestational age; AGA- appropriate for gestational age; LGA- large for gestational age; min- minute.

Table 5.4. Effect of obesity on obstetric and perinatal outcomes

	Non-obese (*n*=54)	**Obese (*n*=82)**	***p*-value**
PTB (≤37 weeks), *n* (%)	d=40 21 (52.5)	d=53 24 (45.3)	0.491
Onset of labour, *n* (%) Induction of labour* CS Spontaneous onset of labour	d=36 7 (19.4) 11 (30.6) 18 (50.0)	d=45 14 (31.1) 19 (42.2) 12 (26.7)	0.094
Route of delivery, *n* (%) Normal vaginal delivery Elective CS Emergency CS	d=40 14 (35.0) 10 (25.0) 16 (40.0)	d=54 18 (33.3) 20 (37.0) 16 (29.6)	0.409
Normoglycaemic control, *n* (%) T1DM, *n* (%) T2DM, *n* (%) GDM, *n* (%)	d=52 15 (28.8) 7 (13.5) 24 (46.2) 6 (11.5)[a]	d=82 23 (28.0) 4 (4.9) 30 (36.6) 25 (30.5)[a]	0.036
Fetal growth, *n* (%)	d=30	d=47	

SGA (<10th centile) AGA LGA (>90th centile)	3 (10.0) 19 (63.3) 8 (26.7)	3 (6.4) 29 (61.7) 15 (31.9)	0.759
Birthweight (g), *n* (%) 501 – 2 500 2 500 – 4 000 >4 000	d=41 7 (17.1) 32 (78.0) 2 (4.9)	d=55 9 (16.4) 44 (80.0) 2 (3.6)	1.000
Stillbirth, *n* (%)	d=41 3 (7.3)	d=55 3 (5.5)	1.000
Apgar score at 5 min, *n* (%) <7	d=40 4 (10.0)	d=53 5 (9.4)	1.000

Statistical differences between groups were assessed with the Chi squared test with Bonferroni multiple comparisons method and Fisher's exact test for counts less 5.

*Induction of labour by either medical, mechanical or surgical means (or a combination thereof) was used to achieve labour. Failed inductions were considered if a patient was not in active labour within 24 hours.

Similar superscripts denote statistical significance. $p<0.05$.

Abbreviations: D=denominator, PTB- preterm birth; CS- caesarean section; T1DM- type 1 diabetes mellitus; T2DM- type 2 diabetes mellitus; GDM- gestational diabetes mellitus; SGA- small for gestational age; AGA- appropriate for gestational age; LGA- large for gestational age; min- minute.

Table 5.5. Effect of stratified weight on outcomes

	Weight <80 kg (*n*=70)	**Weight ≥80 kg - <120 kg (*n*=101)**	**Weight ≥120 kg (*n*=11)**	***p*-value**
PTB (≤37 weeks), *n* (%)	d=54 27 (50.0)	d=72 27 (37.5)	d=6 3 (50.0)	0.353
Onset of labour, *n* (%) Induction of labour* CS Spontaneous onset of labour	d=50 11 (22.0) 13 (26.0) 26 (52.0)[a]	d=63 17 (27.0) 28 (44.4) 18 (28.6)[a]	d=5 2 (40.0) 3 (60.0) 0 (0.0)	0.040
Route of delivery, *n* (%) Normal vaginal Elective CS Emergency CS	d=54 21 (38.9) 11 (20.4) 22 (40.7)	d=73 25 (34.2) 24 (32.9) 24 (32.9)	d=6 1 (16.7) 3 (50.0) 2 (33.3)	0.398
Fetal growth, *n* (%) SGA (<10th centile) AGA LGA (>10th centile)	d=32 3 (9.4) 23 (71.9) 6 (18.8)	d=57 3 (5.3) 36 (63.2) 18 (31.6)	d=6 0 (0.0) 3 (50.0) 3 (50.0)	0.454
Birthweight (g)	d=55	d=74	d=6	

501 – 2 500 2 500 – 4 000 >4 000	13 (23.6) 41 (74.5) 1 (1.8)	14 (18.9) 55 (74.3) 5 (6.8)	0 (0.0) 6 (100.0) 0 (0.00)	0.391
Neonatal outcome, *n* (%) Alive Stillbirth	d=55 52 (94.5) 3 (5.5)	d=74 71 (95.9) 3 (4.4)	d=6 6 (100.0) 0 (0.0)	0.803
Apgar score at 5 min, *n* (%) <7	d=54 7 (13.0)	d=72 3 (4.2)[a]	d=6 2 (33.3)[a]	0.025

Statistical differences between groups were assessed with the Chi squared test with Bonferroni multiple comparisons method and Fisher's exact test for counts less 5.

*Induction of labour by either medical, mechanical or surgical means (or a combination thereof) was used to achieve labour. Failed inductions were considered if a patient was not in active labour within 24 hours.

Similar superscripts denote statistical significance. $p < 0.05$.

Abbreviations: D=denominator, PTB- preterm birth; GA- gestational age; CS- caesarean section; SGA- small for gestational age; AGA- appropriate for gestational age; LGA- large for gestational age; AGA- appropriate for gestational age, min- minute.

5.5. Discussion

Literature has shown that diabetes and obesity in pregnancy are associated with adverse pregnancy outcomes for both the mother and child. Therefore, this study aimed to investigate the effect of diabetes and obesity in pregnancy on adverse obstetric and perinatal outcomes. The main findings of the study are 1) higher rates of PTB in women with T1DM and T2DM compared with the control group, 2) higher frequency of GDM in women with obesity compared with women without obesity, 3) higher risk of CS before the onset of labour in women who weighed more than 80 kg compared with women who weighed less than 80 kg and *iv*) lower rates of spontaneous onset of labour and higher rates of low Apgar scores in women who weighed more than 120 kg compared with women who weighed between 80 kg and 120 kg.

Our study showed that T1DM and T2DM were associated with higher rates of PTB compared with the control group. These findings are consistent with those of previous studies that also reported elevated rates of PTB in women with pregestational T1DM and T2DM compared with women with GDM and the control group (Gualdani et al., 2021; Peticca et al., 2009; Van Zyl and Levitt, 2018). In contrast, a systematic review reported studies that showed higher or similar risks of PTB in women with GDM compared with women with pregestational diabetes (Malaza et al., 2022). The optimal timing of delivery for women with DIP is contentious. Some recommendations suggest that in women with pregestational diabetes, especially those with vascular complications or suboptimal glycaemic control, early delivery (before 38.5 weeks gestation) is the better option (Graves, 2007). However, a 2018 Cochrane systematic review that aimed to determine the optimal timing of delivery for women with pregestational diabetes concluded that there were insufficient data to adequately determine the timing of delivery due to the lack of published trials (Biesty et al., 2018). Accordingly, the clinical decision regarding the timing of delivery in women with diabetes depends on several maternal and fetal factors, as well as the associated risk of adverse outcomes. Surprisingly, our study did not show differences in other obstetric and perinatal outcomes among the diabetes groups. This may be attributed to early delivery. Therefore, in our population, early

delivery might be a better option to reduce adverse outcomes that may occur at term delivery. The higher frequency of GDM in women with obesity, compared with their non-obese counterparts, is evidence that obesity is an independent risk factor for the development of GDM (Ehrenberg et al., 2004). A meta-analysis including 20 studies reported that women who were overweight (2.1-fold), obese (3.6-fold) or severely obese (8.6-fold) had a significantly higher risk of developing diabetes compared with normal-weight pregnant women (Chu et al., 2007). Furthermore, the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study reported that a high maternal BMI is associated with an increased risk of adverse outcomes, independent of glycaemic status (Group, 2010). Factors such as advanced maternal age, high rates of diabetes and obesity have contributed to increasing rates of GDM (Schaefer-Graf et al., 2018). In 2021, the International Diabetes Federation (IDF) reported that of the 16.2% of live births affected by maternal hyperglycaemia, 80.5% were due to GDM, while the prevalence of GDM was estimated to be 14.1% in Africa (International Diabetes Federation, 2021). Studies have shown that physical activity and weight loss prior to conception significantly reduced the risk of developing GDM (Schummers et al., 2015; Tobias et al., 2011). This emphasises the importance of preconception health for women of reproductive age who are either overweight or obese. Initiatives to encourage weight loss prior to pregnancy and to maintain appropriate gestational weight gain to reduce the risk of developing GDM and subsequent adverse outcomes are recommended.

The increased risk of CS before labour onset in women who weigh more than 80 kg compared with women who weigh less than 80 kg, and the reduced likelihood of spontaneous labour onset in women who weigh more than 120 kg, further demonstrates the negative impact of maternal weight on obstetric outcomes. Abdominal operative delivery in women with obesity is known to present significant problems such as anaesthetic difficulties, infections and greater blood loss, which pose a risk to both the mother and neonate (Creanga et al., 2022). Brost *et al*. reported that even after controlling for confounders, any increase in maternal weight and BMI before and during pregnancy was associated with an increased risk of CS. They reported that for each incremental

BMI unit (1 kg/ m^2) increase, there was an approximate 7.8% rise in the likelihood of CS (Brost et al., 1997). This complication is thought to be due to an increase in pelvic soft tissue, resulting in the narrowing of the birth canal, leading to difficulty in delivery (Creanga et al., 2022). A study conducted in Norway reported that European/Central Asian women who were overweight or obese were at an increased risk of elective CS compared with Norwegian women without overweight and obesity, while sub-Saharan African women who were overweight or obese had the highest risk for emergency CS compared with normal-weight women from Norway (Jatta et al., 2021). A study by Wolfe *et al*. reported that calculating maternal BMI offers no advantage over simply using maternal weight in the initial risk assessment of outcomes related to maternal weight (Wolfe et al., 1991). This practice should be considered for risk assessment of pregnant women instead of BMI, especially in busy, resource-limited settings.

Increased rates of low Apgar scores in women weighing more than 120 kg compared with women weighing between 80 and 120 kg are consistent with studies that showed negative effects of higher maternal weight and BMI on neonatal outcomes. There is evidence that the 5-minute Apgar score is a good predictor and indicator of infant survival and low Apgar scores at either 1, 5 or 10 minutes are associated with long-term neurological disabilities in infants (Straube et al., 2010). A study conducted in Pakistan reported that increasing maternal BMI was strongly associated with low Apgar scores at birth and NICU admissions (Parveen et al., 2018). Another study conducted in Germany found that women with obesity had a higher percentage of giving birth to neonates with a low Apgar score at 1 minute; however, no differences in Apgar scores were observed at 5 and 10 minutes among different BMI groups (Stepan et al., 2006). Since evidence has shown that Apgar scores are crucial indicators of neonatal and subsequent infant outcomes, knowledge of risk factors, especially modifiable risk factors such as maternal weight, that are associated with a low Apgar score, is important in reducing associated neonatal adverse outcomes.

The relationship between obesity and diabetes and their effect on pregnancy outcomes has been established. Globally, non- communicable diseases such as diabetes and obesity are negatively

associated with maternal and perinatal health. A study by Rosenberg *et al*. suggested that diabetes and excess maternal weight can adversely affect maternal and delivery outcomes through two different pathways. The first pathway involves the contribution of diabetes and excess weight to the development of preeclampsia, which can trigger PTB and CS. The second pathway pertains to the increased risk of macrosomia in neonates born to women with either diabetes, obesity or both. Babies with macrosomia often contribute to labour dystocia, which can result in an increased indication for CS delivery (Rosenberg et al., 2005).

5.6. Strengths and limitations

The strength of our study lies in its ability to demonstrate the negative effect of maternal diabetes and obesity on obstetric and perinatal outcomes. The limitations of the study include a small sample size and restriction to a specific ethnic group, namely black African ethnicity, which restrict the generalisability of our findings. A larger study that includes multiple ethnicities is needed to further validate our results. Also, the study had limited maternal BMI data due to missing height measurements. Therefore, we reported on both BMI and weight, as weight is easily obtained. Nevertheless, despite the low number of BMI measurements, we still observed the effects of both BMI and weight on obstetric and perinatal outcomes. Additionally, because some of the women who were recruited had not yet delivered at the time of analysis, or because we were unable to obtain delivery information for women who did not deliver at Steve Biko and were unreachable, the total denominators do not add up to the total due to missing delivery information. Lastly, hypertension was not categorised into chronic, gestational or preeclampsia.

5.7. Conclusion

This study showed that pregestational diabetes is associated with high rates of PTB and obesity is associated with the development of GDM, high rates of CS and low Apgar scores at 5 minutes.

Adequate glycaemic control and weight loss prior to pregnancy, as well as appropriate gestational weight gain, have been shown to reduce the risk of adverse pregnancy outcomes. Therefore, clinicians should prioritise pre-pregnancy glycaemic control and weight optimisation. Additionally, pregnant women with DIP should be advised about the importance of glycaemic control to reduce adverse pregnancy outcomes. Pregnant women with obesity should be counselled on the importance of appropriate gestational weight gain to prevent the development of GDM. Good antenatal care and education are essential to reduce adverse pregnancy outcomes for mothers with diabetes and obesity.

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Author Contributions

S Adam and N Malaza, - Conceptualization and original draft; N Malaza, S Adam, C Pheiffer, S Dias - manuscript writing and approval of the final draft. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

CHAPTER 6

EVALUATION OF SERUM ADIPONECTIN, LEPTIN AND SEX HORMONE BINDING GLOBULIN LEVELS IN SOUTH AFRICAN WOMEN WITH DIABETES IN PREGNANCY

This chapter will be submitted as a research article to *The Journal of Diabetes & Metabolic Disorders*. **Malaza N**, Adam S, Masete M, Moloto P, Dias S, Pheiffer C.

6.1. Abstract

Background. Adipokines such as adiponectin and leptin exhibit opposite regulation, with lower levels of adiponectin and higher levels of leptin associated with pregnancy, obesity and diabetes. Recently, sex hormone-binding globulin (SHBG) has been linked to both leptin and adiponectin in obesity and diabetes. However, limited studies have investigated the interplay between these hormones during pregnancy.

Objective. To explore the relationship between these hormones and their potential impact on adverse pregnancy outcomes in South African women with diabetes in pregnancy.

Methods. A prospective cohort study was conducted at Steve Biko Academic Hospital, Pretoria, South Africa between 2017 and 2023. The study included 229 pregnant women (69 with normoglycaemia, 26 with type 1 diabetes (T1DM), 76 with type 2 diabetes (T2DM), and 58 with gestational diabetes (GDM)). Serum hormone levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results. Lower levels of adiponectin and higher levels of leptin were associated with body mass index (BMI) and glucose concentrations, while SHBG was negatively correlated with glycated haemoglobin levels. Women with T2DM and GDM had lower levels of adiponectin, and higher levels of leptin compared to women with T1DM. SHBG levels were lower in women with T2DM compared to those with GDM. In terms of pregnancy outcomes, lower leptin levels were associated with large for gestational age (LGA), macrosomia (birthweight more than 4 kg) and preterm birth (PTB), while lower levels of SHBG were associated with macrosomia and levels were negatively correlated with neonatal birthweight.

Conclusion. This study showed that maternal adiponectin, leptin and SHBG levels vary with adiposity and diabetes type. Furthermore, leptin and SHBG concentrations were associated with fetal growth and birth outcomes. Future research in this area may allow further insight into the complex interactions between these hormones and their implications for maternal and child health.

6.2. Introduction

The incidence of diabetes in pregnancy (DIP) has risen dramatically and continues to pose a serious health threat, particularly in low- and middle-income countries (LMICs). In 2021, an estimated 16.7% (21.1 million) of live births worldwide were impacted by DIP (International Diabetes Federation, 2021). DIP is associated with an increased risk of adverse outcomes for mother and child, which include preeclampsia, preterm birth (PTB), congenital anomalies, large for gestational age (LGA), respiratory distress syndrome (RDS), and macrosomia, particularly when glycaemic control is suboptimal (Malaza et al., 2022).

Maternal hormones are involved in various physiological processes that impact both maternal health and fetal development. Imbalances in these hormones can affect fetal growth and contribute to variations in birthweight. Adipose tissue is an important endocrine organ during pregnancy, regulating appetite, energy expenditure, metabolism and supporting the physiological demands of pregnancy through adipokine secretion. Adiponectin and leptin are key adipokines that play crucial roles in orchestrating metabolic adaption during pregnancy (Briffa et al., 2015). Dysregulation of these adipokines is associated with pregnancy complications and adverse birth outcomes. Adiponectin is an insulin-sensitising hormone with anti-inflammatory effects, and regulation of glucose metabolism (Achari and Jain, 2017; Li et al., 2009). Decreased levels of adiponectin have been associated with obesity, insulin resistance and diabetes (Adam et al., 2018; Pheiffer et al., 2021). In addition, lower adiponectin concentrations are associated with pregnancy complications including GDM, PTB, and abnormal intrauterine growth (Lomakova et al., 2022; Vyas et al., 2019). Leptin, often referred to as the satiety hormone, regulates food intake and energy expenditure by binding to specific receptors in the hypothalamus. High levels of leptin have been associated with obesity and diabetes, and with PTB, low birthweight, small head circumference and low Apgar scores (Manderson et al., 2003; Rabiepoor et al., 2019)

While leptin and adiponectin are well-studied, other adipokines and hormones may also influence metabolic processes during pregnancy. Sex hormone-binding globulin (SHBG) is a glycoprotein

produced in the liver that transports sex steroids, particularly testosterone and oestrogen, in the bloodstream. Its production is negatively regulated by insulin (Simó et al., 2015). Low levels of SHBG have been associated with diabetes (Simmons, 1995; Spencer et al., 2005) and with pregnancy and birth complications such as GDM, miscarriages and abnormal neonate birthweight (Spencer et al., 2005; Yu et al., 2004).

Recently, low levels of SHBG have been shown to be associated with lower adiponectin and higher leptin levels, suggesting a complex interplay between these hormones and their impact on pregnancy outcomes (Liu et al., 2017). However, limited studies have investigated the association between these hormones during pregnancy. This study aimed to explore the relationship between maternal adiponectin, leptin and SHBG and their potential impact on adverse pregnancy outcomes in South African women with DIP. To the best of our knowledge, this is the first study to investigate the association between maternal adiponectin, leptin and SHBG levels and birth outcomes in South Africa.

6.3. Methods

6.3.1. Study design and population

A prospective study was conducted at the high-risk antenatal clinic at Steve Biko Academic Hospital, Pretoria, Gauteng, South Africa between May 2017 and April 2023 (n=229). The study population consisted of pregnant women with pregestational T1DM (n=26) or T2DM (n=52), T2DM first diagnosed in pregnancy (new T2DM) (n=24), GDM (n=58), and normoglycaemia (n=69). All women provided written informed consent prior to enrolment and the study was approved by the University of Pretoria Health Science Research Ethics Committee (ethics number: 41/2021). The eligibility criteria included 1) age between 18 and 42 years, 2) ≤ 28 weeks of gestation, 3) black African ethnicity, 4) human immunodeficiency virus (HIV) negative, and 5) singleton pregnancy. The gestational age (GA) was determined by ultrasound if it was available or calculated from the first day of the last normal menstrual period. DIP was categorized as pregestational T1DM or T2DM if diagnosed prior to pregnancy based on medical records, medication, positive anti-glutamic acid decarboxylase (GAD) antibodies (Zaharieva et al., 2017) or presentation with diabetic ketoacidosis (Lizzo et al., 2023). GDM and normoglycaemia were diagnosed using the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria at ≤ 28 weeks gestation (fasting plasma glucose level 5.1-6.9 mmol/L, or 1-h plasma glucose ≥ 10 mmol/L or 2-h plasma glucose 8.5-11.0 mmol/L) after a 2-h 75-g oral glucose tolerance test (OGTT). New T2DM was diagnosed during pregnancy if fasting plasma glucose level ≥7.0 mmol/L, random plasma glucose or 2-h plasma glucose ≥11.1 mmol/L on the OGTT; or glycated haemoglobin (HbA1c) ≥ 6.5%. (International Association of Diabetes and Pregnancy Study Groups Consensus Panel, 2010). Normoglycaemia was confirmed by negative OGTT results. Women with DIP had home glucose monitoring with an On-Call Plus glucometer (On Call, Cuauhtémoc, Mexico) and were required to test their glucose levels at least five times a day. Demographic and obstetric history and care information were collected using standard procedures.

6.3.2. Anthropometry

Maternal weight and height were measured at the first antenatal visit. Body mass index (BMI) was calculated using the standard equation: weight [kg]/height^2 [m^2]]. Underweight (BMI < 18.5 kg/m^2), normal weight (BMI = 18.5–24.9 kg/m^2), overweight (BMI = 25.0–29.9 kg/m^2), and obesity (BMI ≥ 30 kg/m^2) were defined according to the Institute of Medicine guidelines (ACOG, 2020).

6.3.3. Clinical definitions

Gestational age was determined by early fetal ultrasound at ≤24 weeks gestation or by last normal menstrual period. Fetal growth was defined as small for gestational age (SGA) if fetal growth was < 10th centile, appropriate for gestational age (AGA) if fetal growth was between 10th and 90th percentile and large for gestational age (LGA) if fetal growth was > 90th centile (Kiserud et al., 2018). Women were considered to have preterm delivery/birth if GA at delivery was ≤ 37 weeks (WHO, n.d.). Birthweight was defined as normal weight (< 4 kg), and macrosomia (≥ 4 kg) as previously defined (World Health Organization, 2004). Apgar score at 5 minutes: low Apgar score was defined as less than 7 and a normal Apgar score as 7 and above (Simon et al., 2017).

6.3.4. Blood collection

Maternal blood samples were collected at recruitment (≤ 28 weeks gestation), centrifuged at 4000 rpm (Hemle Z206A, Benchmark Scientific Inc., New Jersey, USA) at 4°C for 15 min and aliquots of serum were frozen immediately at −40°C then shipped to the South African Medical Research Council and stored at −80°C until analysis. Whole blood was sent to the Steve Biko Academic hospital National Health Laboratory Services (NHLS, Pretoria, South Africa) for measurement of glycated haemoglobin (HbA1c) levels.

6.3.5. Biochemical markers

Due to missing or insufficient serum samples or major haemolysis, 54 participants were excluded from biochemical markers analysis. The concentrations of adiponectin (ng/mL), leptin (ng/mL) and SHBG (nmol/L) were measured using enzyme-linked immunosorbent assay (ELISA) with commercial kits (total human adiponectin, human leptin and human SHBG ELISA, Merck, Darmstadt, Germany) according to the manufacturer's protocol. Data were analyzed using My Assay sigmoidal four-parameter logistic regression accessible at https://www.myassays.com/four-parameter-fit.assay. The intra-assay coefficient of variation (CV) was < 10% for all analysis.

6.3.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Software (IBM, Armonk, New York, USA). The Shapiro-Wilk test was used to test for normality. Categorical data are expressed as count (n) and percentage (%). The Mann-Whitney test or the Kruskal-Wallis test with Dunn's multiple comparison was used to compare variables across groups. The Chi-square test followed by Bonferroni *posthoc* analysis was used to compare categorical variables. The Spearman's rank test was used to evaluate the relationship between serum adiponectin, leptin and SHBG concentrations with biochemical parameters and neonate outcomes. A $p < 0.05$ was considered statistically significant.

6.4. Results

The general characteristics of the population according to diabetes type are summarized in Table 6.1. Data for women with pregestational T2DM and T2DM diagnosed for the first-time during pregnancy were combined based on the assumption that women with new T2DM had undiagnosed preexisting T2DM. Women with T2DM ($p<0.01$) and GDM ($p<0.001$) were older than women with T1DM and controls, while women with GDM weighed more than women with T1DM ($p<0.001$), T2DM ($p<0.01$), and controls ($p<0.001$). As expected, women with pregestational T1DM and T2DM presented to the clinic earlier ($p<0.001$) and had higher HbA1c levels ($p<0.001$) than women with GDM. More women with T2DM and GDM had a history of hypertension in pregnancy compared to controls ($p<0.05$).

Table 6.1. Participant characteristics according to diabetes type

Variable	Controls (n=69)	T1DM (n=26)	T2DM (n=76)	GDM (n=58)	p-value
Age (years)	31.0 (27.0-36.6)[a,b]	29.0 (27.0-32.0)[c,d]	35.0 (30.0-37.0)[a,c]	35.5 (32.0-38.0)[b,d]	<0.001
BMI (kg/m^2)	31.8 (27.7-39.5)[a]	28.3 (23.8-33.5)[b]	31.9 (28.8-37.6)[c]	38.7 (32.8-43.7)[a,b,c,]	<0.001
Weight (kg)	82.9 (72.1-98.6)[b]	71.0 (60.4-84.9)[e,d]	84.4 (72.7-94.4)[e,f]	102.2 (85.0-112.7)[b,d,f]	<0.001
GA at recruitment (weeks)	22 (20-25)[e,a]	17 (14-21)[e,b,f]	21 (17-25)[d]	25 (24-26)[a,b,d]	<0.001
0-h OGTT (mmol/L)	3.9 (3.7-4.3)[b,d]	-	[#]7.6 (6.9-9.3)[d,a]	5.4 (5.1-6)[b,a]	<0.001
1-h OGTT (mmol/L)	5.6 (4.4-6.8)[b,d]	-	[#]12.9 (11.4-14.5)[d,a]	9.9 (8.2-11)[b,a]	<0.001
2-h OGTT (mmol/L)	5.1 (4.5-6.5)[b,d]	-	[#]12.8 (11.2-15.9)[d,g]	8.8 (6.7-9.5)[b,g]	<0.001
HbA1c (%)	5.2 (5.0-5.4)[b,d,c]	9.3 (7.6-10.1)[b,g,e]	7.3 (6.3-8.9)[d,i]	5.7 (5.4-6.1)[g,i,h]	<0.001
History of hypertension in pregnancy (Yes)	d=48 4 (7.7)[e,h]	d=23 3 (5.8)	d=70 27 (38.5)[e]	d=47 18 (34.6)[h]	<0.001

Data are presented as the median and interquartile range (25th and 75th percentiles) and counts (%). Statistical differences between groups were assessed with the Kruskal Wallis test with Dunn's post hoc multiple comparisons test, the Chi-squared test with Bonferroni post hoc multiple comparisons test. [#]- OGTT is for newly diagnosed T2DM. Similar superscripts denote statistical significance, [e,h]p<0.05, [a,c,f]p <0.01, [b,d,g,i]p<0.001. Abbreviations: BMI- body mass index, D=denominator, GA- gestational age, GDM- gestational diabetes mellitus, HbA1c- glycated haemoglobin, h- hour, OGTT- oral glucose tolerance test, T1DM- type 1 diabetes, T2DM- type 2 diabetes.

Adiponectin, leptin and SHBG concentrations are correlated with adiposity and glucose concentrations

We evaluated the correlation between maternal serum adiponectin, leptin and SHBG levels with body weight, BMI, and glucose concentrations (Table 6.2). The correlation of glucose concentrations was only conducted for individuals with new T2DM, GDM, and normoglycemic controls, whereas the HbA1c correlation was conducted for all groups. Serum adiponectin levels were negatively correlated with body weight (r=-0.175, p=0.026), BMI (r=-0.245, p=0.004), and fasting plasma glucose concentrations (r=-0.243, p=0.019). Serum leptin levels positively correlated with body weight (r=0.482, p<0.001), BMI (r=0.412, p<0.001) and fasting plasma glucose (r=0.214, p=0.028). Serum SHBG negatively correlated only with HbA1c (r=-0.238, p=0.029).

Table 6.2. Correlations between serum biochemical markers, body weight and glucose concentrations

	Spearman's correlation co-efficient		
Variable	**Adiponectin**	**Leptin**	**SHBG**
Bodyweight (kg)	-0.175*	0.482***	-0.151
BMI (kg/m^2)	-0.245**	0.412***	-0.170
0-h OGTT (mmol/L)	-0.243*	0.214*	-0.170
1-h OGTT (mmol/L)	-0.196	0.158	-0.119
2-h OGTT (mmol/L)	-0.130	0.156	-0.129
HbA1c (%)	0.038	-0.163	-0.238*

Correlation analysis was conducted using Spearman's rank test. Abbreviations: BMI-body mass index, OGTT- oral glucose tolerance test, h- hour, HbA1c- glycated haemoglobin.

*p <0.05, **p <0.01, ***p <0.001.

Adiponectin, leptin and SHBG concentrations are associated with obesity and DIP

The relationship between serum adiponectin, leptin and SHBG levels with DIP and obesity was evaluated. Adiponectin levels were decreased in women with T2DM and GDM compared to T1DM (p=0.067 and p=0.009, respectively) and levels were decreased in GDM compared to controls (p=0.011; Figure 6.1A). Leptin levels was not associated with DIP (Figure 6.1B). SHBG was decreased in women with T2DM compared to GDM (p=0.021) and controls (p=0.016; Figure 1C). Adiponectin levels were decreased in women who were overweight and obese compared to women with normal weight (p=0.07 and p=0.023, respectively (Figure 6.2A). Leptin levels were increased in women who were overweight and obese compared to normal weight (p=0.023 and p<0.001, respectively) (Figure 6.2B). However, SHBG levels were not different across the obesity status (Figure 6.2C).

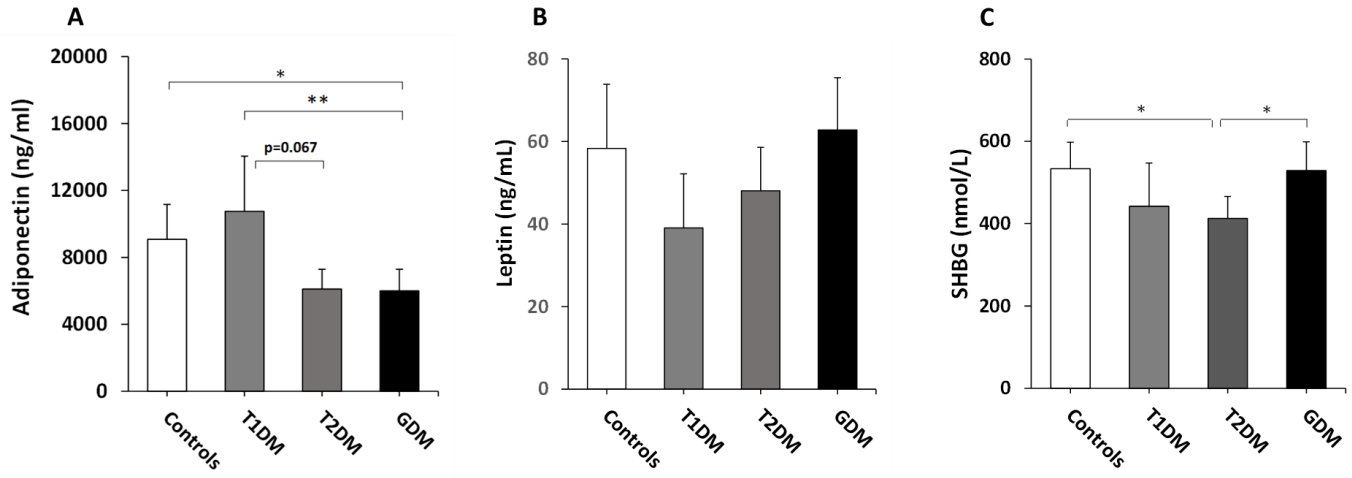

Figure 6.1. Serum adiponectin, leptin and SHBG levels across levels of glycaemia.

Serum adiponectin (A), leptin (B) and SHBG (C) levels were measured in the serum of pregnant women with T1DM (n=23), T2DM (n=59), GDM (n=47) and normoglycaemia (n=51) using ELISA. Data are represented as the mean ± standard error of mean (SEM). Abbreviations: GDM- gestational diabetes mellitus, SHBG- sex hormone binding globulin, T1DM- type 1 diabetes, T2DM- type 2 diabetes.

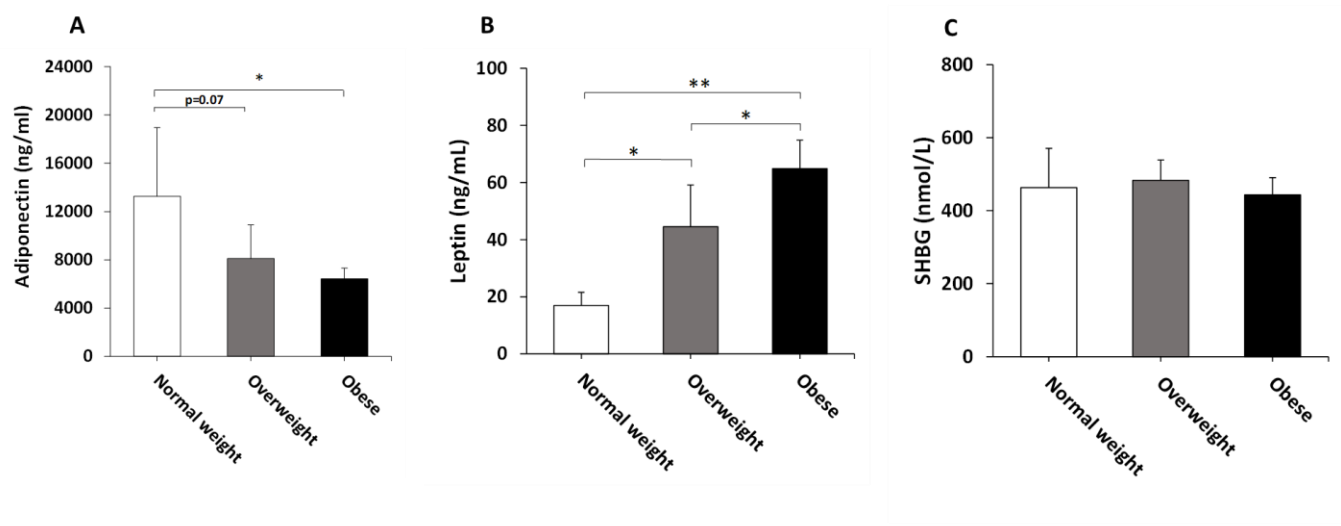

Figure 6.2. Serum adiponectin, leptin and SHBG levels by levels of maternal body weight.

Serum adiponectin, leptin and SHBG levels were measured in the serum of pregnant women with normal weight (n=15), overweight (n=36), and obesity (n=100) using ELISA. Data are represented as the mean ± standard error of mean (SEM). Abbreviations: SHBG- sex hormone binding globulin.

Maternal leptin and SHBG concentrations are associated with birth outcomes

The association between maternal serum adiponectin, leptin and SHBG levels with birth outcomes was evaluated. The results demonstrated that leptin levels were decreased in women with LGA babies compared to women with AGA babies (p=0.036; Figure 6.3B), in PTB compared to term birth (p=0.004; Figure 6.4B), and macrosomia compared to birthweight < 4 kg, although, not statistically significant for the latter (p=0.060; Figure 6.5B). SHBG levels negatively correlated with birthweight (r=-0.263, p=001) and was decreased in women who gave birth to babies with macrosomia compared to a birthweight <4 kg (p=0.025; Figure 6.5C). No association between serum hormones with neonate sex and Apgar score was observed (Figure 6.6A-C and Figure 6.7A-C).

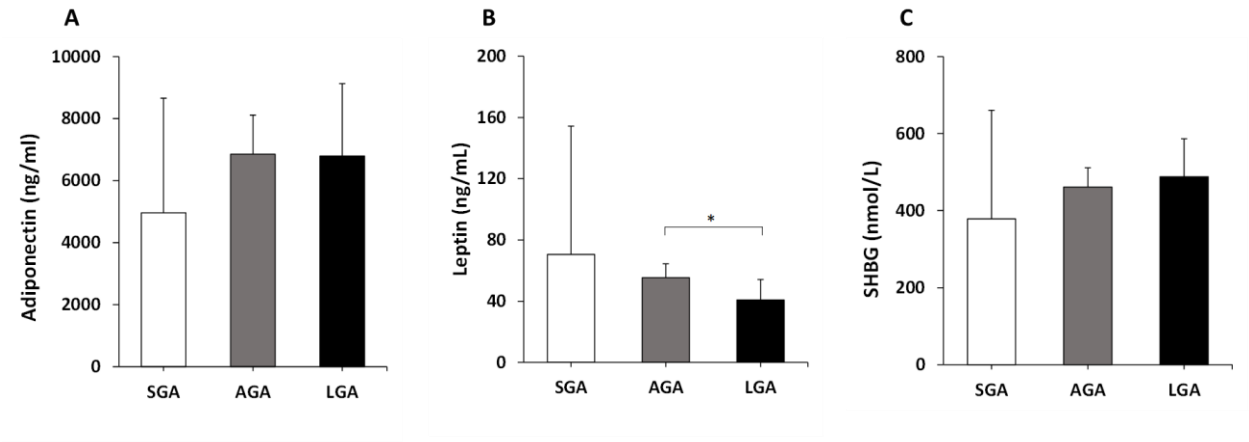

Figure 6.3. Association between serum adiponectin, leptin and SHBG with fetal growth.

Serum adiponectin (A), leptin (B) and SHBG (C) levels were measured in mothers with SGA (n=4), AGA (n=72) and LGA (n=33) babies using ELISA. Data are represented as the mean ± standard error of mean (SEM). Abbreviations: AGA- appropriate for gestational age, LGA- large for gestational age, SGA- small for gestational age, SHBG- sex hormone binding globulin.

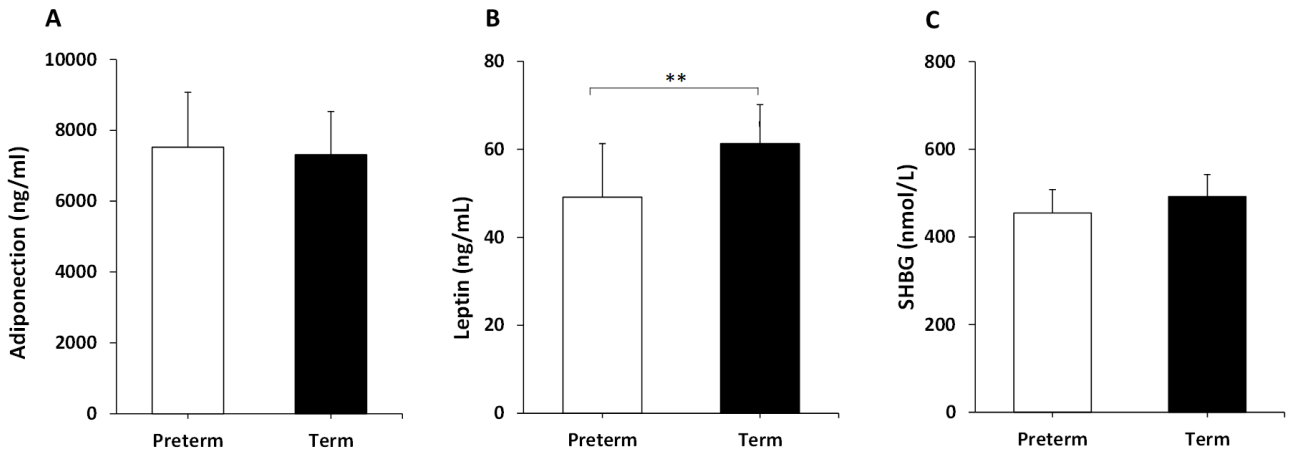

Figure 6.4. Association between serum adiponectin, leptin and SHBG levels with gestational age at birth. Serum adiponectin (A), leptin (B) and SHBG (C) levels were measured in mothers who gave birth to preterm (n=67) and term (n=95) using ELISA. Data are represented as the mean ± standard error of mean (SEM). Abbreviations: SHBG- sex hormone binding globulin.

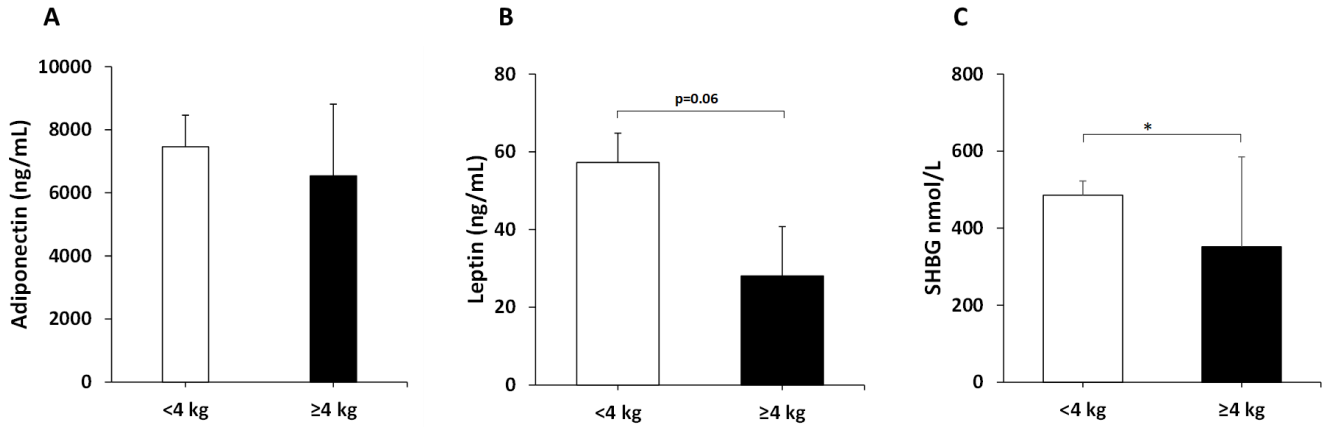

Figure 6.5. Association between serum adiponectin, leptin and SHBG with birthweight.
Serum adiponectin (A), leptin (B) and SHBG (C) levels were measured in mothers with babies' birthweight <4 kg (n=153) and birthweight ≥4 kg (n=8) using ELISA. Data are represented as the mean ± standard error of the mean (SEM). Abbreviations: SHBG- sex hormone binding globulin.

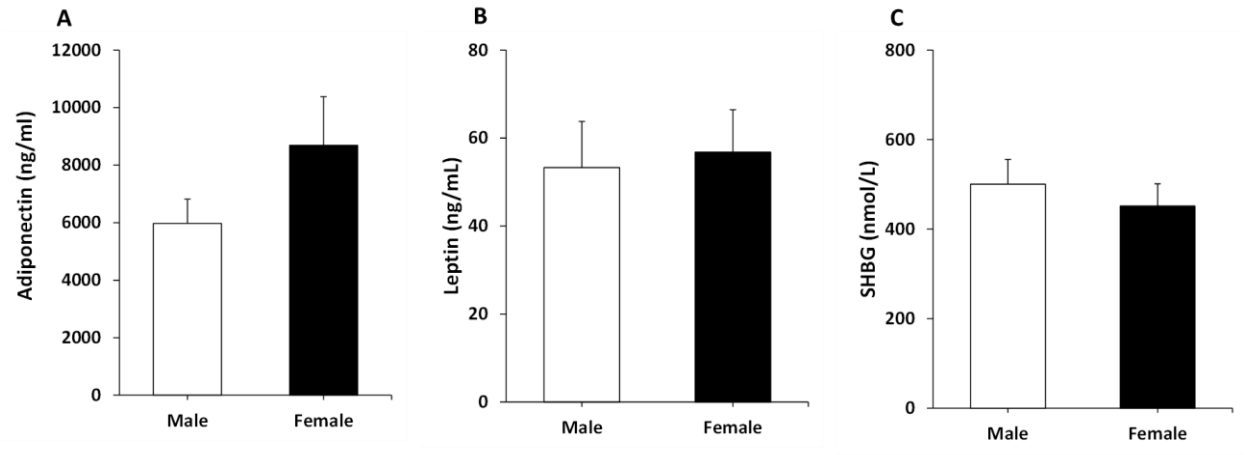

Figure 6.6. Association between serum adiponectin, leptin and SHBG levels with neonate sex. Serum adiponectin (A), leptin (B) and SHBG (C) levels were measured in mothers with male (n=78) and female (n=82) neonates using ELISA. Data are represented as the mean ± standard error of the mean (SEM). Abbreviations: SHBG- sex hormone binding globulin.

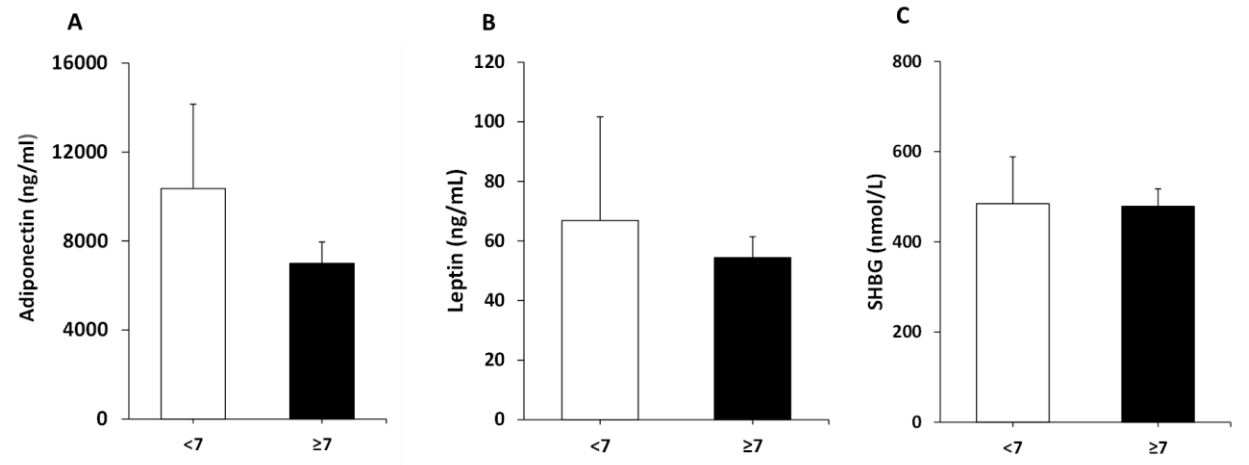

Figure 6.7. Association between serum adiponectin, leptin and SHBG levels with neonate Apgar scores. Serum adiponectin (A), leptin (B) and SHBG (C) levels were measured in mothers with neonates with Apgar scores <7 (n=18) and neonates with Apgar scores ≥7 kg (n=143) using ELISA. Data are represented as the mean ± standard error of the mean (SEM). Abbreviations: SHBG- sex hormone binding globulin.

6.5. Discussion

Adiponectin and leptin are key adipokines that play important roles in orchestrating metabolic adaptation during pregnancy (Briffa et al., 2015). Recently, low levels of SHBG have been associated with higher leptin and lower adiponectin levels, suggesting a complex interplay between these hormones and their impact on pregnancy outcomes (Liu et al., 2017). Studies have observed this association even after adjusting for BMI or body composition (Gannagé-Yared et al., 2006; Liu et al., 2017; Vanbillemont et al., 2012), demonstrating a complex interplay outside of obesity. However, most of these studies were conducted in males and the results might be different in women and pregnancy. Moreover, studies have shown decreased levels of adiponectin and SHBG, together with increased leptin levels during obesity and insulin resistance (Adam et al., 2018; Kapustin et al., 2020; Morisset et al., 2011). These adipokines and SHBG are present in the placenta, cord blood and fetal tissue suggesting their involvement in fetal development and neonatal outcomes (Mazaki-Tovi et al., 2005; Mellati et al., 2010; Simmons, 1995). Using a prospective study design, we investigated the association between maternal serum adiponectin, leptin and SHBG levels at study entry with hyperglycaemia and obesity in pregnancy and neonate birth outcomes in South African pregnant women. Our main findings show that lower leptin levels were associated with LGA, PTB, and macrosomia. Lower levels of SHBG were associated with macrosomia and levels negatively correlated with birthweight.

Adiponectin is an insulin-sensitising adipokine that is decreased in the presence of obesity, insulin resistance and diabetes (Pheiffer et al., 2021). Adiponectin regulates glucose metabolism by stimulating insulin signaling in skeletal muscle and decreasing gluconeogenesis in the liver (Aye et al., 2013). In adipose tissue, adiponectin suppresses pro-inflammatory cytokines, improves lipid metabolism, insulin sensitivity and glucose homeostasis, and promotes white adipose browning and adipose tissue expansion (Pheiffer et al., 2021). Our findings showed that adiponectin levels were lower in women with GDM compared to normoglycaemia and T1DM. Low adiponectin levels contribute to insulin resistance and the development of GDM, and our findings

are consistent with studies that have reported decreased adiponectin levels in women with GDM compared to women with normoglycaemia (Mierzyński et al., 2018; Spranger et al., 2003). Furthermore, our findings are also aligned with studies that have reported lower levels of adiponectin in patients with T2DM compared to T1DM (Maahs et al., 2009), with the latter showing levels comparable to or higher than non-diabetic controls (Maahs et al., 2007; Martos-Moreno et al., 2006). The differences in adiponectin levels between T1DM and T2DM may be attributed to the distinct pathophysiological mechanisms underlying these two forms of diabetes. T1DM is an autoimmune disease that leads to the destruction of insulin-producing pancreatic beta cells (DiMeglio et al., 2018), whereas T2DM is primarily characterized by insulin resistance, leading to impaired glucose regulation (Pendergrass et al., 1995). Studies have demonstrated that the changes in adiponectin levels observed in individuals with diabetes are more strongly associated with excessive adipose tissue or obesity than with hyperglycemia (Al-Hamodi et al., 2014; Neuparth et al., 2013). The negative relationship between adiponectin levels and BMI, as observed in this study, has been widely reported (Chan et al., 2004; Fuglsang et al., 2006; Kawano and Arora, 2009). Obesity is associated with chronic low-grade inflammation and insulin resistance (Kawano and Arora, 2009), which may partly explain lower levels of adiponectin during obesity. In contrast, some studies have reported no correlation between adiponectin levels and BMI during pregnancy (Worda et al., 2004). The discrepancies between studies may be attributed to variations in gestational age, as adiponectin levels have been observed to change with the progress of pregnancy (Fuglsang et al., 2006; Worda et al., 2004), and the presence of heterogeneity in population characteristics (Kawano and Arora, 2009).

Several lines of evidence support the involvement of adiponectin in fetal development and metabolism. Adiponectin receptors are present in the placenta and have been detected in both maternal and cord blood and fetal tissue, indicating its ability to cross the placenta (Caminos et al., 2005; Corbetta et al., 2005; Lindsay et al., 2003). Accordingly, studies have reported an association between maternal adiponectin levels and fetal outcomes including fetal growth, PTB

and birthweight (Lomakova et al., 2022; Mazaki-Tovi et al., 2009, 2005), suggesting its potential as a predictive biomarker for neonatal outcomes. However, in contrast to these studies, we did not see an association between maternal adiponectin levels and fetal growth, PTB, and birthweight. Lomakova et al. investigated the association between fasting serum adiponectin levels and the risk of preterm delivery in multi-ethnic healthy pregnancy women at < 20 weeks of gestation and adiponectin levels were determined using the human adipokine 2-plex magnetic bead panel. The authors found that decreased maternal adiponectin levels were associated with an increased risk of preterm delivery (Lomakova et al., 2022). Mazaki-Tovi et al. investigated the association between cord and maternal serum adiponectin, fetal growth and preterm delivery in healthy participants and adiponectin levels were determined using ELISA and radioimmunoassay (RIA) kits, respectively. The studies found lower levels of maternal serum total adiponectin were associated with PTB (Mazaki-Tovi et al., 2009), however no association was observed between cord serum adiponectin and LGA (Mazaki-Tovi et al., 2005). Discrepancies between studies might be attributed to different gestational ages across studies (Paradisi et al., 2010), heterogeneity in population characteristics (Kawano and Arora, 2009; Lomakova et al., 2022), different assay techniques (Rutkowski and Scherer, 2014) and our small sample size which limits the statistical power to detect associations. Further work is required to elucidate the mechanisms linking adiponectin to birth outcomes.

Leptin is often referred to as the satiety hormone and plays a critical role in regulating energy homeostasis (Myers et al., 2008). It is produced in white adipose tissue (Pereira et al., 2015) and binds to receptors on the hypothalamus in the brain, specifically in the arcuate nucleus, signaling to the brain when the body has sufficient energy stores (Myers et al., 2008). Dysfunction in the leptin signaling pathway has been linked to obesity and metabolic disorders (Briffa et al., 2015). Our results are consistent with others who have similarly reported higher leptin levels in women with obesity during pregnancy (Misra and Trudeau, 2011). Obesity creates a chronic inflammatory environment, increased leptin levels and leptin resistance, leading to the inability to reach satiety,

increased appetite, and a reduced ability to regulate body weight (Zhang and Scarpace, 2006). Higher maternal leptin levels have been demonstrated in pregnancies complicated by T2DM and GDM compared to T1DM and controls (Kapustin et al., 2020). However, our study did not show variations in leptin according to hyperglycaemia, which is consistent with a study by Higgins et al, who similarly failed to observe an association between leptin levels and DIP (Higgins et al., 2013). The relationship between leptin and diabetes is complex and requires further investigation to determine the precise role of leptin in the development of insulin resistance and progression to diabetes.

Leptin plays a crucial role in the transport of nutrients, specifically amino acids, from the mother to the fetus via the placenta (Jansson et al., 2002). Leptin increases the activity of the system A sodium-dependent neutral amino acid transporter (SNAT), which facilitates amino acid delivery to the fetus. In diabetic pregnancies, SNAT activity is enhanced, resulting in excessive amino acid transfer from the mother to the fetus, leading to increased fetal growth (Jansson et al., 2002). The placental leptin receptor OB–Rb, found in the syncytiotrophoblast, is downregulated in obese pregnancies at term. This suggests that high levels of maternal leptin may result in placental leptin resistance, as the body tries to enhance foetal growth in conditions of excess energy. In obese pregnancies, placental leptin resistance may disrupt the expression and signaling of important pathways that are involved in foetal and placental growth and development (Tessier et al., 2013). As such, studies have reported that increased leptin levels are associated with LGA (Mazaki-Tovi et al., 2005; Shroff et al., 2013), PTB (Rabiepoor et al., 2019; Shroff et al., 2013) and birthweight (Clausen et al., 2005) in contrast, our study reported lower maternal leptin levels in mothers with LGA and macrosomic babies. Others have similarly reported that decreased leptin levels were associated with birthweight (Manderson et al., 2003) and PTB (Ogein et al., 2023). Mazaki-Tovi et al. investigated the association between cord serum leptin (determined using RIA technique) and LGA. The study found increased cord leptin in neonates with LGA (Mazaki-Tovi et al., 2005). Shroff et al. investigated maternal serum leptin (determined using RIA technique) and LGA in a

multi-ethnic population at 16–27 weeks gestation. The study found higher maternal leptin levels in mothers of LGA infants, however, the association was attenuated after controlling for pre-pregnancy BMI (Shroff et al., 2013). Therefore, the discrepancies between the studies might be attributed to heterogeneity in population characteristics (Paracchini et al., 2005) and different assay techniques and sample types (Ma et al., 1996; Maffei et al., 1995).

SHBG is a glycoprotein produced by the liver that transports sex steroids in plasma, and its production is negatively regulated by insulin (Simó et al., 2015). Similar to previous studies (Kopp et al., 2001; Muka et al., 2016; Spencer et al., 2005), our study showed decreased levels of SHBG in women with T2DM compared to women with GDM and controls. Our study did not find an association between SHBG levels and obesity, which is in contrast with previous studies (Morisset et al., 2011; Xargay-Torrent et al., 2018). Morisset et al. reported a negative correlation between SHBG levels and BMI and showed that BMI was a major determinant of SHBG levels in women with GDM (Morisset et al., 2011). During pregnancy, women with pre- and gestational obesity showed lower levels of SHBG, which were associated with cardiometabolic risk factors including c-reactive protein, blood pressure, triglycerides, high molecular weight adiponectin and measures of glucose metabolism and control (Xargay-Torrent et al., 2018).The differences between the studies might be due to heterogeneity in population characteristics (Heald et al., 2005; Luo et al., 2021) or to the gestational age at the time of assessment because concentrations change as pregnancy progresses (Luo et al., 2021).

There is scarce and controversial data on the association between SHBG and neonatal outcomes such as birthweight. Maternal obesity is associated with low SHBG levels, which influences insulin resistance in the mother and developing fetus (Liu et al., 2013). This subsequently leads to fetal adiposity and high birthweight (Catalano et al., 2009). Our study observed a negative correlation between maternal SHBG levels and birthweight, and low SHBG levels were observed in mothers who gave birth to neonates with macrosomia (birthweight ≥ 4 kg). Other studies have similarly reported a negative correlation between SHBG levels and neonatal birthweight and other

measures of neonate size (Simmons, 1995; Xargay-Torrent et al., 2018). Morisset et al. reported that maternal SBHG concentrations were a significant predictor of neonatal birthweight independent of maternal diabetes and pre-pregnancy BMI (Morisset et al., 2011). In contrast, other studies found no association between SHBG levels and neonatal birthweight (Carlsen et al., 2006; Wuu et al., 2002). The differences between the studies might be due to heterogeneity in population characteristics (Heald et al., 2005; Luo et al., 2021) and different assay techniques (Bukowski et al., 2000). The association between SHBG levels and neonates birthweight has not been fully elucidated.

6.6. Strengths and limitations

To our knowledge, this is the first study to investigate the association between maternal adiponectin, leptin and SHBG levels with neonate birth outcomes in South African women with DIP. The strength of the study is that we were able to demonstrate a correlation between maternal hormones with LGA, birthweight and PTB. Limitations of the study include the small sample size and restriction to black African ethnicity, which limits the generalisability of our findings to other populations. Due to a small sample size, multivariate analysis was not conducted. In future studies with a larger sample, multivariate analysis will be performed to identify factors independently associated with pregnancy outcomes. Blood samples were not collected at the same gestational age (14-26 weeks of gestation) in the different glycaemic groups, which might affect the concentrations of the evaluated markers and confound results. Therefore, future studies should use blood samples collected at a similar gestational age (e.g. blood samples for pregestational diabetes can be collected later in pregnancy to coincide with GDM blood collection). Maternal adiponectin and leptin have been shown to increase from the beginning of gestation until mid-gestation and decline thereafter, while SHBG is reported to increase with gestation until delivery. The evaluation of total adiponectin only is another limitation given that variants such as high molecular weight (HMW) adiponectin have been shown to mediate insulin-sensitising effects and

that low levels of HMW adiponectin are associated with GDM (Retnakaran et al., 2007). However, other studies have reported that both total and HMW adiponectin are associated with PTB (Mazaki-Tovi et al., 2009), while work in our laboratory showed a high correlation between total and HMW adiponectin levels (Masete, 2021). Previous work showed that adiponectin levels may be impacted by human immunodeficiency virus (HIV) status (Dias et al., 2021), therefore future studies should investigate adiponectin levels in both HIV negative and positive pregnant women.

6.7. Conclusion

This study showed that serum maternal leptin levels were associated with LGA, neonate birthweight and PTB. Additionally, SHBG levels were negatively correlated with neonate birthweight and low levels were associated with birthweight ≥ 4 kg. These biomarkers are differentially expressed in maternal diabetes and obesity and are also associated with neonatal outcomes. Identification of dysregulated maternal biomarkers associated with adverse birth outcomes may aid in developing intervention strategies to improve child health.

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Author Contributions

C Pheiffer and N Malaza, - Conceptualization and original draft; N Malaza, S Adam, C Pheiffer, S Dias, M Masete - manuscript writing and approval of the final draft. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

CHAPTER 7

MATERNAL CIRCULATING MICRORNAS IN SOUTH AFRICAN PREGNANT WOMEN ARE ASSOCIATED WITH FETAL GROWTH AND BIRTHWEIGHT

This chapter will be submitted as a research article to the *Journal of Molecular Medicine*.

Malaza N, Masete M, Adam S, Dias S, Pheiffer C.

7.1. Abstract

Background. An adverse intrauterine environment is associated with abnormal fetal growth and macrosomia, both of which have been associated with pregnancy complications and future risk of metabolic dysfunction. The early identification of these conditions allows for timely intervention to mitigate pregnancy complications and prevent future metabolic disease. Circulating microRNAs (miRNAs) have attracted considerable attention as modulators of biological function and potential biomarkers of adverse pregnancy outcomes.

Objective. This study aimed to identify maternal serum miRNA associated with adverse pregnancy outcomes.

Methods. A prospective cohort study was conducted at Steve Biko Academic Hospital, Pretoria, South Africa between 2017 and 2023. The study included 232 high risk pregnant women (69 with normoglycaemia, 27 with type 1 diabetes (T1DM), 78 with type 2 diabetes (T2DM), and 58 with gestational diabetes (GDM)). Quantitative real-time PCR was used to identify maternal miRNAs associated with fetal outcomes.

Results. Four miRNAs (miR-124-3p, miR-128-3p, miR-20a-5p and miR-210-3p) were associated with small for gestational age (SGA) and were able to predict SGA, miR-124-3p (AUC=0.815), miR-128-3p (AUC=0.760), miR-20a-5p (AUC=0.841) and miR-210-3p (AUC=0.779). MiR-210-3p was associated with macrosomia and demonstrated good predictive ability (AUC=0.779). MiR-222-3p was increased in women with good glycaemic control compared to women with poor glycaemic control during pregnancy. MiR-27a-3p was negatively correlated with the oral glucose tolerance test (OGTT) 1-h and 2-h values.

Conclusion. This study showed associations between maternal circulating miRNAs, fetal growth and birth weight and demonstrated the ability of these miRNAs to predict SGA and macrosomia.

These miRNAs hold potential as biomarkers of these outcomes, however, validation of our findings in a different population is required.

7.2. Introduction

According to the World Health Organization (WHO), each year about 300 thousand mothers die due to pregnancy complications and childbirth while 2.5 million babies die during their first month of life ("[Maternal and newborn] - Mortality/causes of death," n.d.). Adverse pregnancy outcomes occur in ~10% to 20% of pregnancies (Lane-Cordova et al., 2019) and several risk factors such as obesity, diabetes and hypertension have been associated with these adverse outcomes ("Global Week for Action on NCDs | Figo," n.d.). Adverse pregnancy outcomes include GDM, stillbirth, caesarean section (CS), preterm birth (PTB), macrosomia, small for gestational age (SGA) and hypertensive disorders of pregnancy (Malaza et al., 2022; Yee et al., 2022). Although adverse pregnancy outcomes present differently, the majority share a common pathophysiology related to defective placental function and vascular development including endothelial dysfunction, inflammation, and vasospasms (Lane-Cordova et al., 2019). Adverse pregnancy outcomes have been established as risk factors for cardiovascular disease (Silverberg et al., 2018; Tanz et al., 2017) and increased future risk for metabolic disorders in both the mother and child (Parikh et al., 2021). Strategies such as pre-pregnancy weight loss (Schummers et al., 2015), adequate pregestational and gestational glycaemic control (González-Quintero et al., 2007), and health knowledge (Hussain et al., 2015) are critical to prevent pregnancy complications and adverse neonatal outcomes.

MicroRNAs (miRNAs) are small, highly conserved non-coding RNA molecules between 18-22 nucleotides in length that regulate gene expression through post-transcriptional mechanisms (Guo et al., 2010). Recently, miRNAs have attracted considerable attention as modulators of biological function and disease pathophysiology (Masete, 2021). MiRNAs regulate diverse biological processes, including cell proliferation, differentiation, apoptosis and development (Chen and Wang, 2013) and metabolic processes such as glucose homeostasis, insulin signalling, pancreatic beta-cell function, lipid metabolism and inflammation (Guay et al., 2011; Poirier et al.,

2017). MiRNAs play an important role in regulating metabolic and developmental processes during pregnancy (Krützfeldt and Stoffel, 2006; Sayed and Abdellatif, 2011) and dysregulated levels have been associated with adverse outcomes including preterm birth (Hromadnikova et al., 2022; Mayor-Lynn et al., 2011), macrosomia (Jiang et al., 2015; Kochhar et al., 2022; Li et al., 2015), and restricted fetal growth (Hromadnikova et al., 2022; Vrijens et al., 2018; Yao et al., 2024). The identification of miRNAs associated with adverse outcomes could serve as biomarkers to facilitate interventions in high-risk women.

To date, no studies have investigated the association between miRNAs and neonatal outcomes in South Africa. This study aimed to identify maternal serum miRNAs associated with adverse pregnancy outcomes in a high-risk population of pregnant South African women. Circulating maternal miRNAs were quantified using quantitative real time PCR (qRT-PCR).

7.3. Methods

7.3.1. Study design and study population

A prospective study was conducted at the high-risk antenatal clinic at Steve Biko Academic Hospital, Pretoria, Gauteng, South Africa between May 2017 and April 2023 (n=232). The study population consisted of pregnant women with pregestational T1DM (n=27) or T2DM (n=78), GDM (n=58), and normoglycaemia (n=69). All women provided written informed consent prior to enrolment and the study was approved by the University of Pretoria Health Science Research (ethics Committee 41/2021). The eligibility criteria included 1) age between 18 and 42 years, 2) ≤ 28 weeks gestation, 3) black African ethnicity, 4) human immunodeficiency virus (HIV) negative and 5) singleton pregnancy. DIP was categorized as pregestational T1DM or T2DM if diagnosed prior to pregnancy based on medical records or medication, had positive anti-glutamic acid decarboxylase (GAD) antibodies (Zaharieva et al., 2017) or presented with diabetic ketoacidosis (DKA) (Lizzo et al., 2023). GDM and normoglycaemia were defined using the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria at 24-28 weeks gestation (fasting plasma glucose level 5.1-6.9 mmol/L, or 1-h plasma glucose ≥ 10 mmol/L or 2-h plasma glucose 8.5-11.0 mmol/L after a 2-h 75-g oral glucose tolerance test (OGTT). New T2DM was diagnosed during pregnancy if fasting plasma glucose level ≥7.0 mmol/L, random plasma glucose or 2-h plasma glucose ≥11.1 mmol/L on the OGTT; or glycated haemoglobin (HbA1c) ≥ 6.5%. (International Association of Diabetes and Pregnancy Study Groups Consensus Panel, 2010). Women with DIP had home glucose monitoring with an On-Call Plus glucometer (On Call, Cuauhtémoc, Mexico) and were required to test their glucose at least five times a day, with varying times over the week as follows: 30 minutes before each meal (fasting), 2 hours after each meal (postprandial), at bedtime, and at 2 am. Glycaemic targets of fasting/pre-prandial glucose were ≤ 5.3 mmol/L and 2-h post-prandial glucose were ≤ 6.7 mmol/L. Poor glycaemic control was defined as > 25% of glucose values outside of the recommended range based on home glucose monitoring. The overall gestational glycaemic control is based on an average of

more than three antenatal visits determined by an experienced maternal fetal specialist. Demographic and obstetric history and care information were collected using standard procedures.

7.3.2. Anthropometry

Maternal weight and height were measured at the first antenatal visit; and body mass index (BMI: weight [kg]/height^2 [m^2]]) was calculated using the standard equation. Underweight (BMI < 18.5 kg/m^2), normal weight (BMI = 18.5–24.9 kg/m^2), overweight (BMI = 25.0–29.9 kg/m^2) and obesity (BMI ≥ 30 kg/m^2) were defined according to the Institute of Medicine guidelines (ACOG, 2020).

7.3.3. Clinical definitions

The gestational age (GA) was determined by ultrasound if it was available or, alternatively calculated from the first day of the last menstrual period (LMP). Fetal growth was defined as small for gestational age (SGA) if fetal growth was < 10th percentile, appropriate for gestational age (AGA) if fetal growth was between 10th and 90th percentile and large for gestational age (LGA) if fetal growth > 90th percentile as previously defined (Kiserud et al., 2018). Women were considered to have preterm delivery/birth if GA at delivery was ≤ 37 weeks (WHO, n.d.). Birth weight was defined as normal weight (< 4 kg) and macrosomia (≥ 4 kg) (World Health Organization, 2004). Apgar score at 5 minutes: A low Apgar score was defined as less than 7 and a normal Apgar score as 7 and above (Simon et al., 2017).

7.3.4. Serum preparation

Maternal blood samples were collected at recruitment (≤ 28 weeks gestation), centrifuged at 4000 rpm (Hemle Z206A, Benchmark Scientific Inc., New Jersey, USA) at 4°C for 15 min and aliquots

of serum were frozen immediately at −40°C then shipped to the South African Medical Research Council and stored at −80°C until analysis.

7.3.5. MiRNA extraction

Due to missing or insufficient serum samples or major haemolysis, 47 participants were excluded from miRNA analysis. MiRNA-enriched total RNA was isolated from 200 µl of serum using the miRNeasy Serum/Plasma kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. MiRNeasy serum/plasma spike-in control cel-miR-39 (*Caenorhabiditis elegans* miR-39) (1.6 x 108 copies/µl) (Qiagen, Hilden, Germany) was added as an exogenous synthetic miRNA to control for technical variation during RNA extraction. Due to manufacturer changes, an RNA spike-in kit was used as the exogenous synthetic miRNA in ~50% of the samples. The RNA spike-in kit consists of three templates (UniSp2, UniSp4 and UniSp5) provided as a premix in one vial. All spike-in controls were diluted according to the manufacturer's instructions (Qiagen, Hilden, Germany). The distribution of patients with T1DM, T2DM, GDM and normoglycaemia were the same in RNA extractions with different spike-in controls therefore minimizing bias. The RNA quantity was assessed using the NanoDrop™ One/OneC Microvolume UV-Vis spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA) according to the manufacturer's instructions.

7.3.6. Quantitative Real Time PCR

RNA (2 µl) was reverse transcribed into complementary DNA (cDNA) using the miRCURY LNA RT Kit (Qiagen, Hilden, Germany), adding UniSp6 spike-in to assess reverse transcription efficiency. Reverse transcription reactions were conducted using a 2720 Thermal Cycler (Applied Biosystems, Foster City, USA). Thereafter, 3 µl of cDNA was used for quantitative real time PCR

(qRT-PCR) using the miRCURY LNA SYBR® Green PCR Kit and miRCURY LNA miRNA PCR assays for miRNAs of interest (Table S1) (Qiagen, Hilden, Germany). All samples were run in duplicates. PCR was conducted on the Quantstudio 7™ Flex Real-Time PCR System (Applied Biosystems, Foster City, USA). UniSp2 or cel-miR-39 (miRNA extraction efficiency) and UniSp6 (reverse transcription efficiency) were used as controls to normalise miRNA expression. The relative expression of miRNAs was calculated using the comparative $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008), where ΔΔCt was calculated by subtracting the mean ΔCt value of the control group from the mean ΔCt of the diabetic group; ΔCt was calculated as $Ct_{sample} - (Ct_{average\ normalising\ control})$ (Ct, threshhold cycle).

7.3.7. Statistical analysis

Statistical analysis was performed using IBM SPSS Software (IBM, Armonk, New York, USA). The Shapiro-Wilk test was used to test for normality. Categorical data are expressed as count (n) and percentage (%). The Mann-Whitney test or the Kruskal-Wallis test with Dunn's multiple comparison was used to compare variables across groups. The Chi-square test followed by Bonferroni *posthoc* analysis was used to compare categorical variables. The Spearman's rank test was used to evaluate the relationship between miRNAs, biochemical parameters and neonate outcomes. Receiver operating characteristic (ROC) curves were constructed to assess the diagnostic value of miRNAs for distinguishing between SGA and AGA and normal weight and macrosomia. The area under the curve (AUC) value and 95% confidence intervals (CI) were calculated to determine the specificity and sensitivity. AUC value > 0.7 was considered acceptable. A $p < 0.05$ was considered statistically significant.

7.4. Results

Participant characteristics according to diabetes type are summarised in Table 7.1. Data for women with pregestational T2DM and DM diagnosed for the first time during pregnancy were combined based on the assumption that women with new DM had undiagnosed preexisting T2DM. Women with T2DM ($p<0.01$) and GDM ($p<0.001$) were older than women with T1DM and controls, while women with GDM weighed more than women with T1DM ($p<0.001$), T2DM ($p<0.01$), and controls ($p<0.001$). As expected, women with pregestational T1DM and T2DM visited the clinic earlier ($p<0.001$) and had higher HbA1c levels ($p<0.001$) than women with GDM. More women with T2DM and GDM had a history of hypertension in pregnancy compared to controls ($p<0.05$).

Table 7.1. Participant characteristics according to diabetes type

Variable	Controls (n=69)	T1DM (n=27)	T2DM (n=78)	GDM (n=58)	p-value
Age (years)	31.0 (27.0-36.0)[a,b]	29.0 (27.0-33.0)[c,d]	35.0 (30.0-37.0)[a,c]	35.5 (32.0-38.0)[b,d]	<0.001
BMI (kg/m^2)	31.8 (27.6-39.4)[a]	27.8 (23.8-33.5)[b]	31.9 (28.4-37.7)[c]	38.9 (32.8-43.7)[a,b,c,e]	<0.001
Weight (kg)	82.2 (70.3-94.4)[b]	71.0 (61.1-84.7)[e,d]	84.9 (72.7-97.0)[e,f]	101.0 (85.0-112.7)[b,d,f]	<0.001
GA at recruitment (weeks)	22 (20-25)[e,a]	17 (14.5-21)[e,b,f]	21 (16-25)[d]	25 (24-26)[a,b,d]	<0.001
0-h OGTT (mmol/L)	3.9 (3.7-4.3)[b,d]	-	[#]7.6 (6.9-9.3)[d,a]	5.4 (5.1-6.0)[b,a]	<0.001
1-h OGTT (mmol/L)	5.6 (4.4-6.8)[b,d]	-	[#]12.9 (11.4-14.5)[d,a]	10.0 (8.2-11)[b,a]	<0.001
2-h OGTT (mmol/L)	5.1 (4.5-6.5)[b,d]	-	[#]12.8 (11.2-15.9)[d,g]	8.8 (6.7-9.5)[b,g]	<0.001
HbA1c (%)	5.2 (5.0-5.4)[b,d,c]	9.4 (8.0-9.9)[b,g,e]	7.3 (6.3-8.9)[d,i]	5.7 (5.4-6.1)[g,i,h]	<0.001
History of hypertension in pregnancy (Yes)	d=48 4 (8.3)[e,h]	d=24 3 (12.5)	d=72 27 (37.5)[e]	d=47 18 (33.3)[h]	<0.001

Data are presented as the median and interquartile range (25th and 75th percentiles) and counts (%). Statistical differences between groups were assessed with the Kruskal Wallis test with Dunn's post hoc multiple comparisons test, the Chi-squared test with Bonferroni post hoc multiple comparisons test. [#]- OGTT is for newly diagnosed T2DM. Similar superscripts denote statistical significance, [e,h]p<0.05, [a,c,f]p <0.01, [b,d,g,i]p<0.001.Abbreviations: BMI- body mass index, D=denominator, GA- gestational age, GDM- gestational diabetes mellitus, HbA1c- glycated haemoglobin, h- hour, OGTT- oral glucose tolerance test, T1DM- type 1 diabetes, T2DM- type 2 diabetes.

MiRNA expressions are associated with neonatal birth outcomes

The association between miRNA expression and neonatal birth outcomes was evaluated. The expression of MiR-20a-5p (p=0.047; Figure 7.1A) and miR-124-3p (p=0.062; Figure 7.1B) were increased in women with SGA neonates compared to women with AGA neonates. MiR-128-3p (Figure 7.1C) and miR-210-3p (Figure 7.1D) showed a similar trend, although this was not statistically significant. MiR-210-3p was increased in women who gave birth to neonates with macrosomia compared to women who gave birth to neonates with normal birth weight (p=0.020, Figure 7.2). No associations between the other miRNAs and neonatal birth outcomes were observed (Table S2-S6).

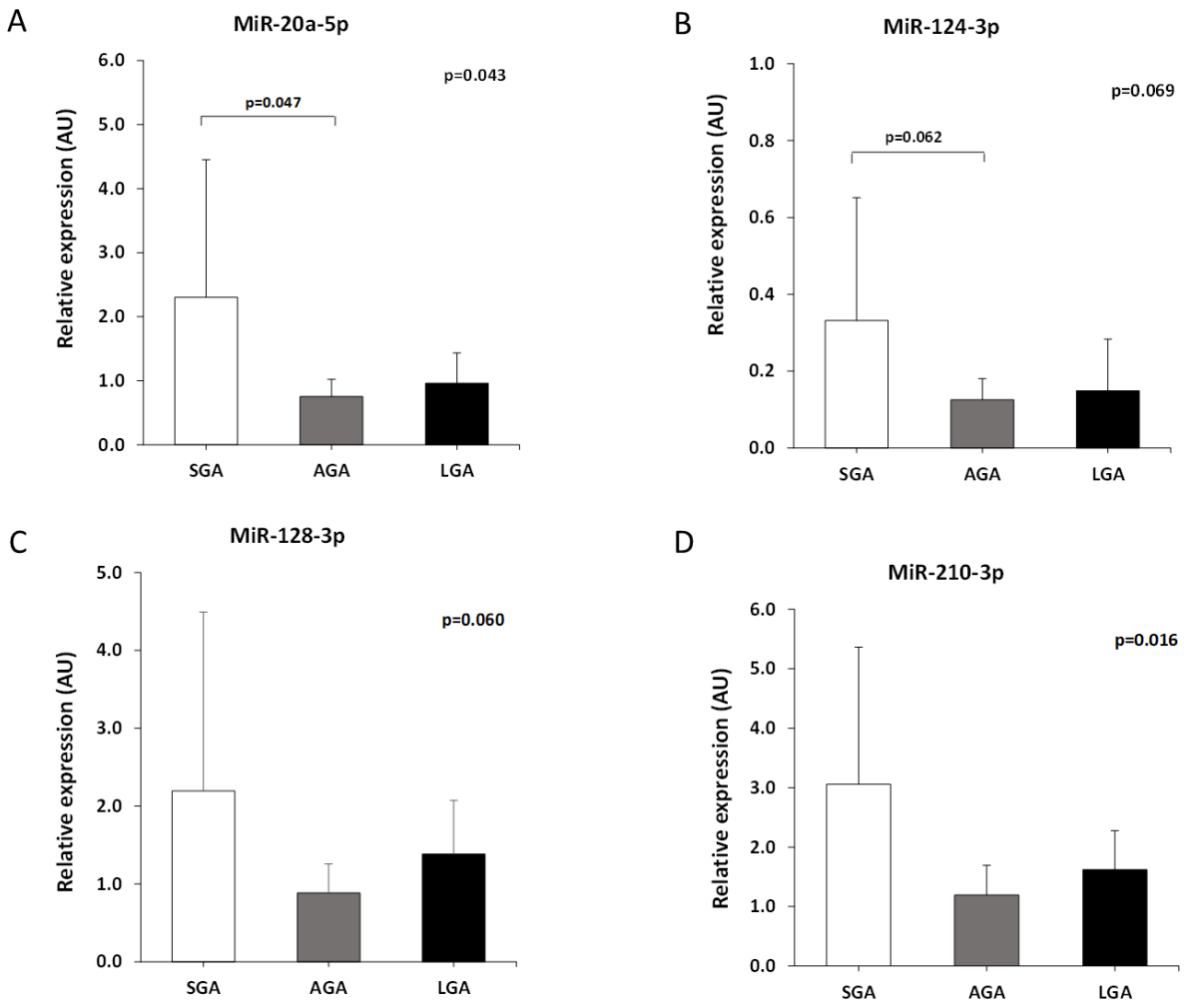


Figure 7.1. Association between miR-210-3p, miR-124-3p, miR-128-3p and miR-222-3p expression with fetal growth.

MiR-210-3p (A), miR-124-3p (B), miR-128-3p (C) and miR-222-3p (D) levels were measured in the serum of mothers with SGA (n=4), AGA (n=76) and LGA (n=29) neonates using quantitative real time PCR (qRT-PCR). Data are represented as the mean ± standard error of mean (SEM). Abbreviations: AGA- appropriate for gestational age, LGA- large for gestational age, SGA- small for gestational age.

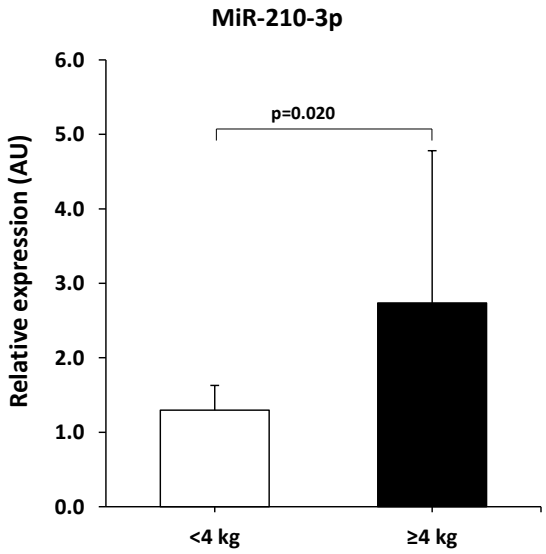
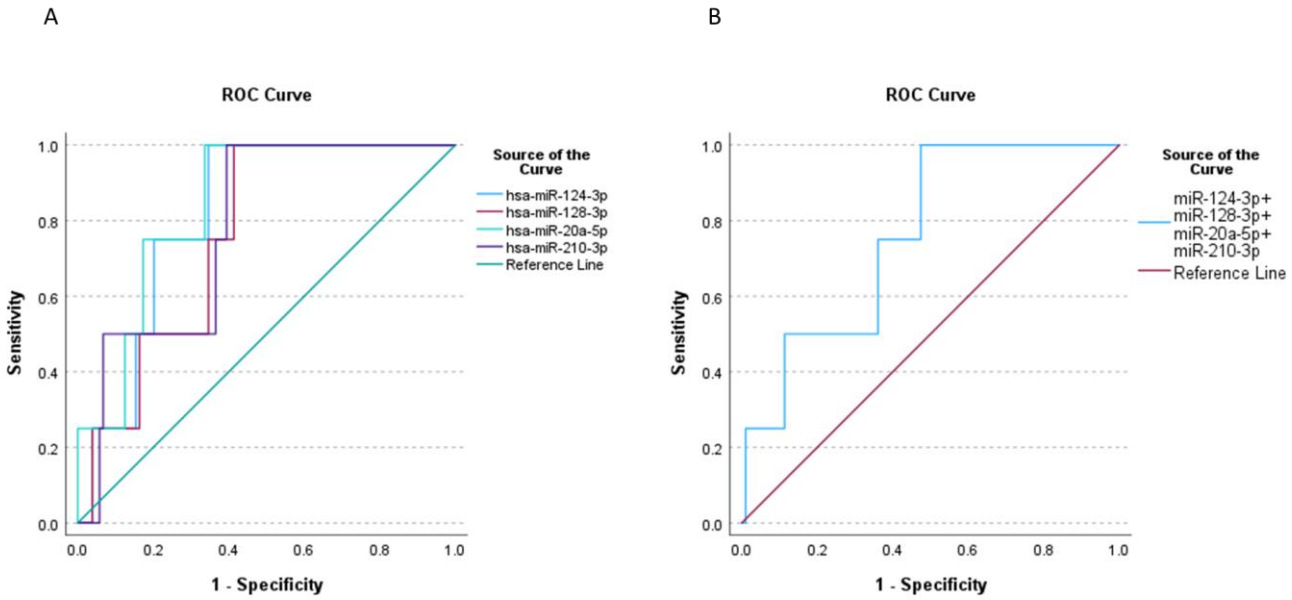


Figure 7.2. MiR-210-3p expression is associated with neonatal birth weight.

MiRNAs were measured in the serum of mothers who gave birth to neonates with birthweight <4 kg (n=165) and birthweight ≥4 kg (n=7) using quantitative real time PCR (qRT-PCR). Data are represented as the mean ± standard error of mean (SEM).

Differentially expressed miRNAs predict SGA and macrosomia

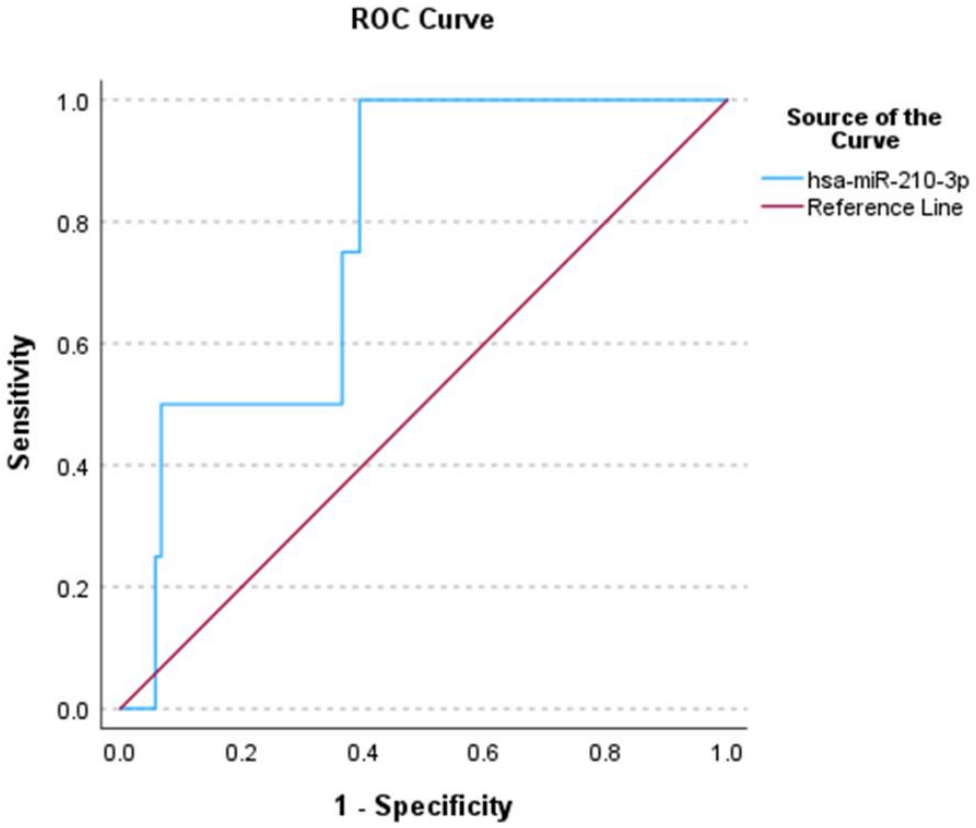
ROC curve analysis was carried out to evaluate the predictive performance of miR124-3p, miR-20a-5p and miR-210-3p in SGA (Figure 7.3) and macrosomia (Figure 7.4). MiR-124-3p had AUC=0.815, sensitivity and specificity=75%, respectively; miR-128-5p had AUC=0.760, sensitivity=75% and specificity=65.4%; miR-20a-5p had AUC=0.841, sensitivity and specificity=75%, respectively and miR-210-3p had AUC=0.779, sensitivity=75% and specificity=63.5% (Figure 7.3A). A combination of miR-124-3p, miR-128-3p, miR-20a-3p and miR-210-3p had AUC=0.760, sensitivity=75% and specificity=63.9% for identifying women at risk of carrying SGA fetuses (Figure 7.3B). MiR-210-3p had AUC=0.779, sensitivity=75% and specificity=63.5% (Figure 7.4).



Test Result Variable(s)	Area	Standard Error	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
miR-124-3p	0.815	0.063	0.000	0.691	0.939
miR-128-5p	0.760	0.081	0.001	0.600	0.919
miR-20a-5p	0.841	0.066	0.000	0.711	0.971
miR-210-3p	0.779	0.086	0.001	0.611	0.947
miR-124-3p+ miR-128-3p+ miR-20a-5p+ miR-210-3p	0.760	0.098	0.008	0.568	0.953

Figure 7.3. ROC curve analysis for the ability of miRNAs to predict SGA.

MiR-124-3p, miR-128-3p, miR-20a-5p and miR-210-3p expression to predict SGA (A). A combination of miR-124-3p, miR-128-3p, miR-20a-5p and miR-210-3p expression to predict SGA (B).



Test Result Variable(s):	Area	Standard Error	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
MiR-210-3p	0.779	0.086	0.001	0.611	0.947

Figure 7.4. ROC curve for miR-210-3p to predict macrosomia.

MiRNAs 222-3p and 30d-5p is associated with glycaemic control

To identify miRNAs associated with glycaemic control, miRNA levels in women with and without good glycaemic control were compared. Glycaemic control is measured throughout pregnancy until delivery, miRNAs were predictive of overall gestational glycaemic control (from study entry to delivery). The expression of miR-222-3p was decreased in women with poor glycaemic control

compared to women with good glycaemic control (p=0.046; Figure 7.5A). Similarly, the expression of miR-30d-5p was decreased in women with poor glycaemic control compared to women with good glycaemic control, however, the difference was not statistically significant (p=0.071; Figure 7.5B). No significant differences in the expression of other miRNAs and glycaemic control were observed (Table S7).

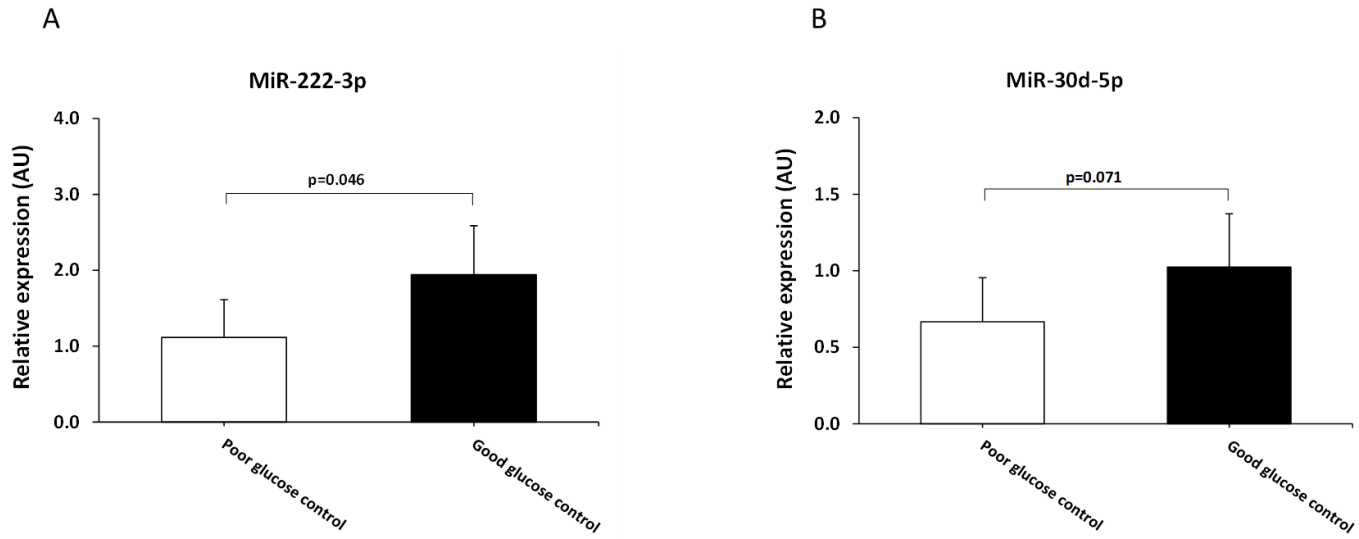


Figure 7.5. MiR-222-3p and miR-30-5p is associated with glucose control.

MiR-222-3p (A) and miR-30-5p (B) Expression levels were measured in the serum of pregnant women with poor (n=45) and good (n=65) glucose control using quantitative real time PCR (qRT-PCR). Data are represented as the mean ± standard error of mean (SEM).

MiR-27a-3p is correlated with glucose concentrations

The correlation of glucose concentrations at baseline was only conducted for individuals with newly diagnosed T2DM, GDM, and normoglycemic controls whereas the HbA1c correlation was conducted for all groups. MiR-27a-3p was negatively correlated with 1-h (r=-0.282, p=0.004) and

2-h (r=-0.246, p=0.012) OGTT glucose concentrations. No correlation between the other miRNAs and clinical characteristics were observed (Table S8).

Table 7.2. MiR-27a-3p is correlated with body weight and glucose concentrations

Variable	Spearman's correlation co-efficient	p-values
Bodyweight (kg)	-0.055	0.463
BMI (kg/m^2)	-0.003	0.966
0-h OGTT (mmol/L)	-0.136	0.171
1-h OGTT (mmol/L)	-0.282	0.004
2-h OGTT (mmol/L)	-0.246	0.012
HbA1c (%)	0.098	0.354

Correlation analysis was conducted using Spearman's rank test. Abbreviations: BMI-body mass index, OGTT- oral glucose tolerance test, h- hour, HbA1c- glycated haemoglobin.

$p < 0.05$ was considered statistically significant

7.5. Discussion

MiRNAs play an important role in regulating the metabolic and developmental processes during pregnancy and dysregulated miRNA expression has been associated with pregnancy complications (Krützfeldt and Stoffel, 2006; Sayed and Abdellatif, 2011). This study aimed to identify maternal serum miRNAs associated with adverse pregnancy outcomes in a high-risk population of pregnant South African women. Our main findings showed that 1) miR-124-3p, miR-128-3p, miR-20a-5p and miR-210-3p were increased in women with SGA fetuses compared to women with AGA fetuses, 2) miR-210-3p was higher in women who gave birth to neonates with macrosomia compared to women who delivered neonates weighing less than 4 kg , 3) miR-222-3p was decreased in women with poor glycaemic control compared to women with good glucose control and 4) miR-27a-3p was negatively correlated with 1-h OGTT and 2-h OGTT glucose concentrations.

SGA is defined as babies born with a birth weight less than the 10th percentile for their gestational age (Osuchukwu and Reed, 2023). Babies born SGA are at a higher risk for perinatal complications such as prematurity, hypoglycaemia, perinatal asphyxia, and impaired immune function. In addition, they have an increased risk of long-term complications, including chronic kidney disease, coronary heart disease, hyperlipidaemia, and hypertension (Osuchukwu and Reed, 2023). Differential expression of miRNAs has been associated with fetal growth (Hromadnikova et al., 2022; Kochhar et al., 2022; Yao et al., 2024). Our study showed that miR-124-3p, miR-128-3p, miR-20a-5p and miR-210-3p were associated with fetal growth. MiR-124-3p regulates various biological processes including apoptosis, tumour metastasis, angiogenic differentiation, and adipogenesis in human disorders (Ghafouri-Fard et al., 2021), although evidence on its association with fetal growth is scant. Our findings of increased miR-124-3p expression in women with SGA compared to AGA fetuses are consistent with previous studies (Di Pietro et al., 2018; Yao et al., 2024). Yao et al. demonstrated that overexpression of miR-124-

3p in pregnant mice inhibited endometrial cell proliferation and migration and decreased offspring size (Yao et al., 2024). Di Pietro et al showed that miR-124-3p was increased in the endometrium and serum of women with endometriosis compared to women without (Di Pietro et al., 2018), a condition that has been linked to a higher rate of SGA (Bruun et al., 2018). MiR-128-3p is encoded by two genes; miR-128-1 located in human chromosome 2q21.3 into R3HDM1 gene and miR-128-2 located in chromosome 3p22.3 into ARPP-21 gene (Kiel et al., 2024). MiR-128-3p is enriched in the central nervous system (Kiel et al., 2024) and has been reported to play an essential role in cell proliferation (Huo et al., 2019), inflammation (Zhu et al., 2022) and angiogenesis (Zhou et al., 2018). Although miR-128-3p has been extensively investigated in cancer and cancer-related diseases, there is limited data on its relation to pregnancy complications. MiR-128-3p regulates key genes, including pericentriolar material 1 (PCM-1) and PHD finger protein 6 (PHF6), in neuronal progenitor cells during embryonic brain development (Kiel et al., 2024). Our data showed the expression of miR-128-3p was increased in women who carried SGA fetuses compared to women who carried AGA fetuses. A study by Marzano et al. which investigated circulating miRNAs in Caucasian Italian SGA and AGA children with obesity and with normal weight reported that the expression of miR-128-3p was upregulated in obese-AGA children, however, no association was observed in with both obese and normal weight SGA children (Marzano et al., 2018). The differences in ethnicity, the source of miRNAs (maternal vs. children) and sample size might account for the differences in the results. Additionally, Dravet-Gounot et al. reported miR-128-3p expression was significantly downregulated in the lungs of rat offsprings with intrauterine growth restriction (Dravet-Gounot et al., 2017). The association between miR-128-3p and SGA needs to be further investigated. MiR-20a-5p was increased in women who carried SGA fetuses compared to women who carried AGA fetuses. MiR-20a-5p belongs to the miR-17-92 cluster, which is associated with physiological processes during pregnancy including angiogenesis and trophoblast development (Doebele et al., 2010). In the placenta, this miRNA plays a role in the regulation of the Eph receptor B4 (EPHB4) and ephrin-

B2 (EFNB2) expression in trophoblast and endothelial cells through the same "seed" sequence (Wang et al., 2012), suggesting a crucial role for miR-20a-5p in early placental development. Consistent with our results, Hromadnikova et al. similarly reported upregulation of miR-20a-5p in pregnancies with SGA compared to AGA fetuses. These authors further reported that miR-20a-5p in combination with miR-1-3p, miR-146a-5p, and miR-181a-5p was able to predict 75.7% of SGA pregnancies in the early stages of gestation (Hromadnikova et al., 2022). In contrast, another study that conducted a high throughput screening of extracellular microRNAs from 100 serum samples collected from pregnant women in their second trimester in Mexico City reported lower serum levels of miR-20b-5p in mothers with SGA fetuses compared to mothers with AGA fetuses (Rodosthenous et al., 2017). The discrepancies between studies might be due to study population characteristics, techniques (Becker and Lockwood, 2013) and GA at time of analysis because miRNA expression changes as pregnancy progresses. MiR-210-3p is a placental miRNA and is a hypoxia sensor located in the intron of the hypoxia-inducible *AK123483* gene (Lycoudi et al., 2015). In different cell types, the expression of miR-210-3p increases in response to low oxygen tension and is upregulated in hypoxia-associated diseases, such as cancer and pregnancy-related disorders (Fu et al., 2013). Our study showed that miR-210-3p was increased in the serum of women with SGA fetuses compared to women with AGA fetuses. Kochhar and colleagues similarly reported that higher levels of placental miR-210-3p were positively associated with SGA (Kochhar et al., 2022). A study that evaluated miR-210-3p in maternal and umbilical cord plasma showed no correlation between maternal plasma expression of miR-210-3p and fetal mass, however, a positive correlation was observed with umbilical cord blood miR-210-3p expression (Shchurevska and Zhuk, 2021). Conversely, a study by Vrijens et al. reported no association between placental expression of miR-210-3p and fetal growth indicators (Vrijens et al., 2018). Because miRNA expression varies in different ethnicities (Becker and Lockwood, 2013), sample types (Ge et al., 2015; Zhu et al., 2014) and lab techniques used (Becker and Lockwood, 2013), this might account for the discrepancies between the studies.

Due to their non-specificity where a single miRNA can regulate multiple genes (Barchitta et al., 2017), it has been suggested that a combination of miRNAs rather than individual miRNAs may serve as better biomarkers, however, our ROC analysis showed that the individual miRNAs (miR-124-3p, miR-20a-5p and miR-210-3p) except for miR-128-3p had better predictive ability for SGA compared to the combination of these miRNAs. This presents miRNAs as potential biomarkers for SGA, showing better predictability compared to clinical markers such as maternal weight, baseline HbA1c, and types of diabetes.

Macrosomia is defined as babies weighing more than 4 kg and has been associated with numerous perinatal and maternal complications, childhood obesity and a long-term risk of developing T2DM, hypertension, and obesity in adulthood (Araujo Júnior et al., 2017). Dysregulated maternal circulating and placental miRNAs have been associated with macrosomia (Jiang et al., 2015; Kochhar et al., 2022; Li et al., 2015). Our study showed that serum levels of miR-210-3p were increased in mothers who gave birth to neonates with macrosomia compared to women who gave birth to neonates with normal birth weight. In contrast, a study by Kochhar et al. found no association between placental levels of miR-210-3p and birth weight (Kochhar et al., 2022). The authors analysed miR-210-3p levels in placentas of Indian pregnant women at delivery. In contrast, our study measured miR-210-3p levels in the serum of Black pregnant women with diabetes during pregnancy at < 28 weeks of gestation. The difference in timing of measurement and ethnicity may account for the differences in the results obtained in both studies. Although evidence of the association between miR-210-3p and macrosomia is scant, miR-210-3p has been associated with obesity and diabetes (Chen et al., 2022; Gentile et al., 2015), with both conditions linked to macrosomia. Our ROC analysis demonstrated that miR-210-3p has a good predictive ability compared to clinical markers such as maternal weight, baseline HbA1c, and types of diabetes, suggesting a specific involvement of this miRNA in the pathogenesis of

macrosomia. However, the association between miR-210-3p expression with macrosomia needs further investigation in larger population sizes.

MiR-222-3p results in impaired insulin sensitivity by downregulation of insulin receptor substrate-1 (IRS-1) resulting in the inactivation of proteins in the insulin cascade and inhibition of glucose transporter type 4 (GLUT4) translocation (Li et al., 2020).Higher levels of miR-222-3p has been associated with diabetes (Ahmed et al., 2018; Ortega et al., 2014; Sadeghzadeh et al., 2020) and pregnancies with hyperglycaemia (Tagoma et al., 2018). Similar to previous studies, our study showed that increased maternal serum expression of miR-222-3p was associated with good glycaemic control throughout pregnancy. Ahmed et al. reported that miR-222-3p expression was increased in patients with T1DM and good glycaemic control compared to non-diabetic controls (Ahmed et al., 2018). Candia et al. showed that plasma miR-222-3p was decreased in patients with T2DM and increased with decreasing HbA1c (Candia et al., 2017). Consistent with our findings, another study that investigated the effect of a Mediterranean diet (MetDiet) in women with GDM followed up from pregnancy to 2-3 years post-delivery reported that women in the intervention group showed significant improvement in glucose concentrations and HbA1c levels in the second trimester, which was associated with increased expression of miR-222-3p (Valerio et al., 2022). The authors hypothesized that the antioxidants in the MetDiet enhanced miR-222-3p expression and improved the inflammatory cytokine profiles linked to insulin resistance (Valerio et al., 2022). Physical activity and dietary interventions have been shown to improve glycaemic control and to upregulate miR-222-3p expression (Improta Caria et al., 2018; Léniz et al., 2021; Valerio et al., 2022), warranting further experiments to explore this miRNA as a potential biomarker of glycaemic control in pregnancies complicated by diabetes. Although data on the association between miR-30d-5p and glycaemic control is scant, our study demonstrated that miR-30d-5p is increased in women with good glucose control. MiR-30d-5p has been observed to be highly expressed in pancreatic β-cells, suggesting that pancreatic β-cells are likely one of the

primary sources of this miRNA (Zhao et al., 2012). MiR-30d-5p is a glucose-regulated miRNA that induces insulin production through the activation of MafA in pancreatic β-cells and protect β-cell function from impairment caused by proinflammatory cytokines. The overexpression of this miRNA promotes the up-regulation of MafA, which increases insulin transcription (Agbu and Carthew, 2021; Zhao et al., 2012). In contrast to our results, a study by Ghaneh et al. reported no correlation between miR-30d-5p expression and glycaemic control (Ghaneh et al., 2023). The authors investigated the expression of miR-30d-5p in a small sample (n=52) of an Iranian population with intermediate hyperglycaemia and individuals with type 2 diabetes with hyperglycaemia. The discrepancies between the studies might be due to the different sample types (Ge et al., 2015; Zhu et al., 2014), study population characteristics and sample size (Becker and Lockwood, 2013). The association between miR-30d-5p expression and glycaemic in diabetic populations needs further investigation.

MiR-27a-3p is involved in pathways of glucose metabolism and insulin resistance. The increased expression of miR-27a-3p in L6 muscle cells reduces glucose uptake and decreases the expression of GLUT4, mitogen-activated protein kinase (MAPK)14, and phosphoinositide 3-kinase (PI3K) regulatory subunit beta expression (Zhou et al., 2016), promoting insulin resistance and resulting in the development of diabetes. In adipose tissue, the increased expression of miR-27a-3p inhibits peroxisome proliferator-activated receptor (PPAR)-γ expression, inducing insulin resistance (Chen et al., 2019). Our findings that maternal serum levels of miR-27a-3p were negatively correlated with glucose concentrations are in agreement with others (Ghoreishi et al., 2022; Li et al., 2015). A study conducted by Ghoreishi et al. reported decreased plasma expression of miR-27a-3p in patients with T2DM and its expression was negatively correlated with fasting plasma glucose concentrations (Ghoreishi et al., 2022). Li et al. reported downregulation of miR-27a-3p in the placenta of women with GDM compared to controls (Li et al., 2015). In contrast, other studies reported up-regulation of serum and exosome miR-27a-3p in

T2DM (Karolina et al., 2012; Wang et al., 2019). Karolina et al. reported a positive correlation of miR-27a-3p with fasting plasma glucose concentrations (Wang et al., 2019). The expression of miRNAs has been reported to vary between serum and plasma samples, as well as during pregnancy (Ge et al., 2015; Li et al., 2012), possibly contributing to the discrepant results between studies.

7.6. Strengths and limitations

To the best of our knowledge, this is the first study to investigate the association between miRNAs and neonatal birth outcomes in South African women. We were able to demonstrate an association between maternal miRNAs and neonatal birth outcomes, which paves the way for future research to explore these miRNAs in other populations. Limitations of the study include the small sample size and restriction to black African ethnicity, which limits the generalisability of our findings to other populations. Due to a small sample size, multivariate analysis was not conducted. In future studies with a larger sample, multivariate analysis will be performed to identify factors independently associated with pregnancy outcomes. Additionally, blood samples were not collected at the same gestational age (14-26 weeks of gestation) in the different glycaemic groups, which may affect the expression of the selected miRNAs and confound results. Therefore, future studies should use blood samples collected at a similar gestational age (e.g. blood samples for pregestational diabetes can be collected later in pregnancy to coincide with GDM blood collection). Previous work demonstrated that HIV infection modifies the expression of miR-20a-5p and miR-222-3p in women with GDM (Pheiffer et al., 2019), therefore future studies should investigate miRNA expression and neonatal birth outcomes in both HIV-negative and -positive pregnant women. In South Africa, an estimated 30% of all pregnancies are complicated by human immunodeficiency virus (HIV) infection (Woldesenbet et al., 2020), which needs to be considered when analysing miRNAs.

7.7. Conclusion

This study showed that increased expression of miRNAs 124-3p, miR-128-3p, 20a-5p and 210-3p were associated with SGA. Additionally, increased expression of miRNAs 20-5p and 210-3p were associated with birth weight ≥ 4 kg and increased miR-210-3p was associated with preterm birth. Furthermore, miR-222-3p were increased in women with good glycaemic control. These miRNAs could serve as potential biomarkers of pregnancy complications and adverse neonatal outcomes, facilitating intervention strategies to improve child health. However, validation of these miRNAs in larger sample sizes and different population groups are required.

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Author Contributions

C Pheiffer and N Malaza, - Conceptualization and original draft; N Malaza, S Adam, C Pheiffer, S Dias, M Masete - manuscript writing and approval of the final draft. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

CHAPTER 8

DEVELOPMENT OF A QUESTIONNAIRE TO ASSESS DIABETES KNOWLEDGE IN SOUTH AFRICAN WOMEN WITH GESTATIONAL DIABETES

8.1. Introduction

Gestational diabetes mellitus (GDM) is a serious pregnancy complication affecting many women globally. The condition is defined as glucose intolerance due to the inability to compensate for the insulin resistance that develops during pregnancy, with glucose homeostasis usually restored after delivery (Kanguru et al., 2014). Worldwide, the incidence of GDM has risen significantly, posing a notable public health concern. In 2021, the International Diabetes Federation (IDF) estimated that 16.7% of pregnancies globally were affected by DIP with 80.3% of these complicated by GDM (International Diabetes Federation, 2021). In Africa, the prevalence of GDM is estimated at 13.6% (Muche et al., 2019) while in South Africa, a low-middle income country (LMIC) in Africa, the prevalence of GDM is estimated to be 12.7% (Dickson et al., 2019).

GDM is associated with increased short- and long-term pregnancy complications for both mother and child. Short-term complications include preterm labour, preeclampsia, macrosomia, neonatal hypoglycaemia, growth restriction, and neonatal intensive care (NICU) admission (Malaza et al., 2022; O'Sullivan et al., 2011). In the long-term, women with GDM have an ~8.3-fold risk of developing type 2 diabetes (T2DM) (Dennison et al., 2021), with ~54% of women exposed to GDM developing T2DM within three years in high-risk populations (Walker et al., 2020). Women with GDM also have a ~4-fold increased risk of developing cardiovascular and coronary artery disease after pregnancy (Harreiter et al., 2014). According to a recent study conducted in South Africa, 31% of women with GDM acquired T2DM after 5–6 years, while 7% and 13% of women, respectively, experienced impaired glucose and fasting glucose tolerance (Chivese et al., 2019). Furthermore, another study in South Africa estimated that 10.5% of children born to mothers with GDM are overweight or obese by 3- to 6 years of age (Soepnel et al., 2021).

Effective management of GDM is critical to mitigate pregnancy complications and prevent adverse outcomes (Brown et al., 2018; Hod et al., 2015). At the core of GDM management is the regulation of blood glucose levels. This involves implementing dietary modifications, engaging in regular

physical activity, monitoring blood glucose levels consistently, and, in certain instances, insulin or metformin therapy may be used to meet glycaemic targets. A study by Yu et al. showed that continuous glucose monitoring resulted in adequate glucose control, therefore reducing the risk of preeclampsia, caesarean section, large for gestational age and macrosomia in women with GDM (Yu et al., 2014). González-Quintero et al. reported that controlled blood glucose in women with GDM led to fewer neonates with large for gestational age, macrosomia, hypoglycaemia and NICU admissions compared to women with uncontrolled glucose (González-Quintero et al., 2007). A study evaluating the use of an advanced mobile medical technology (mHealth) intervention (which is a mobile medical App used to educate and manage GDM patients) showed that women with GDM on the mHealth intervention showed good compliance and also good weight and blood glucose control resulting in reduced rates of pregnancy complications in both the mothers and fetuses (Guo et al., 2019). Yefet et al. reported that good glycaemic control in women with GDM was associated with a reduced long-term risk for cardiovascular disease (Yefet et al., 2019).

Clinically, the successful management of GDM is typically evaluated by monitoring blood glucose levels, with less attention paid to a woman's understanding of the essential factors necessary to achieve glycaemic targets. Several studies on T2DM have reported that poor diabetic knowledge is associated with poor glycaemic control (Al-Qazaz et al., 2011; Worku et al., 2015), while other studies reported that poor diabetes related knowledge results in poor adherence to self-management and glucose self-monitoring (Ong et al., 2014; Shams et al., 2016). Similarly, Hussain et al. reported that in women with GDM, diabetes knowledge was associated with glycaemia control (Hussain et al., 2015). A study evaluating enablers and barriers to glucose control in women with GDM reported that knowledge of the importance of nutrition and exercise is key to achieving glycaemic targets (Martis et al., 2018).

It is evident that understanding GDM and the importance of nutrition and physical activity, is crucial for effective management, preventing complications during pregnancy and promoting the overall well-being of both the mother and the baby. Despite the importance of GDM knowledge, there is a scarcity of studies that have investigated the understanding diabetes in pregnancy of amongst women with GDM in South Africa. The aim of this study was to develop a questionnaire to assess diabetes knowledge in South African women with GDM. Knowledge on GDM, nutrition, physical activity and blood glucose management were assessed. In addition, the questionnaire contained statements that participants used to rate their own feelings towards GDM.

8.2. Methods

8.2.1. Setting and study design

This study was conducted at Steve Biko Academic Hospital (SBAH), a tertiary academic hospital in Pretoria, Gauteng, South Africa between July 2022 and January 2023. SBAH has a combined high-risk diabetes antenatal clinic (obstetrics care, maternal-fetal medicine, diabetology, nutrition and diabetes education), where patients with diabetes are consulted weekly. Inclusion criteria for participants were pregnant women over the age of 18 years attending the antenatal clinic. DIP was categorized as pregestational type 1 diabetes (T1DM) or T2DM if diagnosed prior to pregnancy based on medical records or medication, had positive antibodies (Zaharieva et al., 2017) or presented with diabetic ketoacidosis (Lizzo et al., 2023). GDM was diagnosed using the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria at 24-28 weeks gestation (fasting plasma glucose level 5.1-6.9 mmol/L, or 1-h plasma glucose ≥ 10 mmol/L or 2-h plasma glucose 8.5-11.0 mmol/L) after a 2-h 75-g oral glucose tolerance test (OGTT) (International Association of Diabetes and Pregnancy Study Groups Consensus Panel, 2010). A mixed methods study design was used, which was conducted in three phases of questionnaire development, phase 1) literature review, expert panel consultation and primary questionnaire development, phase 2) testing in eight pregnant women with diabetes and phase 3) amendment and re-testing in 20 pregnant women with GDM (Figure 1). Approval for this study was obtained from the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (ethics no: 254/2022). All women provided written informed consent prior to enrolment. Questionnaires were administered in rooms that ensured patient privacy. Interviews were conducted in English by a multilingual researcher.

8.2.2. Questionnaire development

8.2.2.1. Phase 1

The questionnaire was developed by adapting questions from three developed questionnaires, one of which was validated: The knowledge of Gestational Diabetes questionnaire (Carolan-Olah and Vasilevski, 2021), the GDM knowledge questionnaire (Hussain et al., 2015) and the diabetes knowledge questionnaire, which was developed in the South African context (Mashige et al., 2008). The questionnaire was evaluated by an expert panel to determine if the questions 1) assessed knowledge of GDM effectively, 2) adequately covered all aspects of GDM knowledge and self-management, 3) were focused on the items covered during GDM education sessions in the antenatal clinic, and 4) evaluated whether the language usage was appropriate for the target population. The expert panel consisted of a nurse, endocrinologist, obstetrician, public health scientist, nutritionist, biokineticist and research scientist at SBAH who possess knowledge of the optimal language use in the target population. The expert panel assessed construct validity, and/or the ability to measure the factor it intends to measure (viz. knowledge of GDM, nutrition and physical activity).

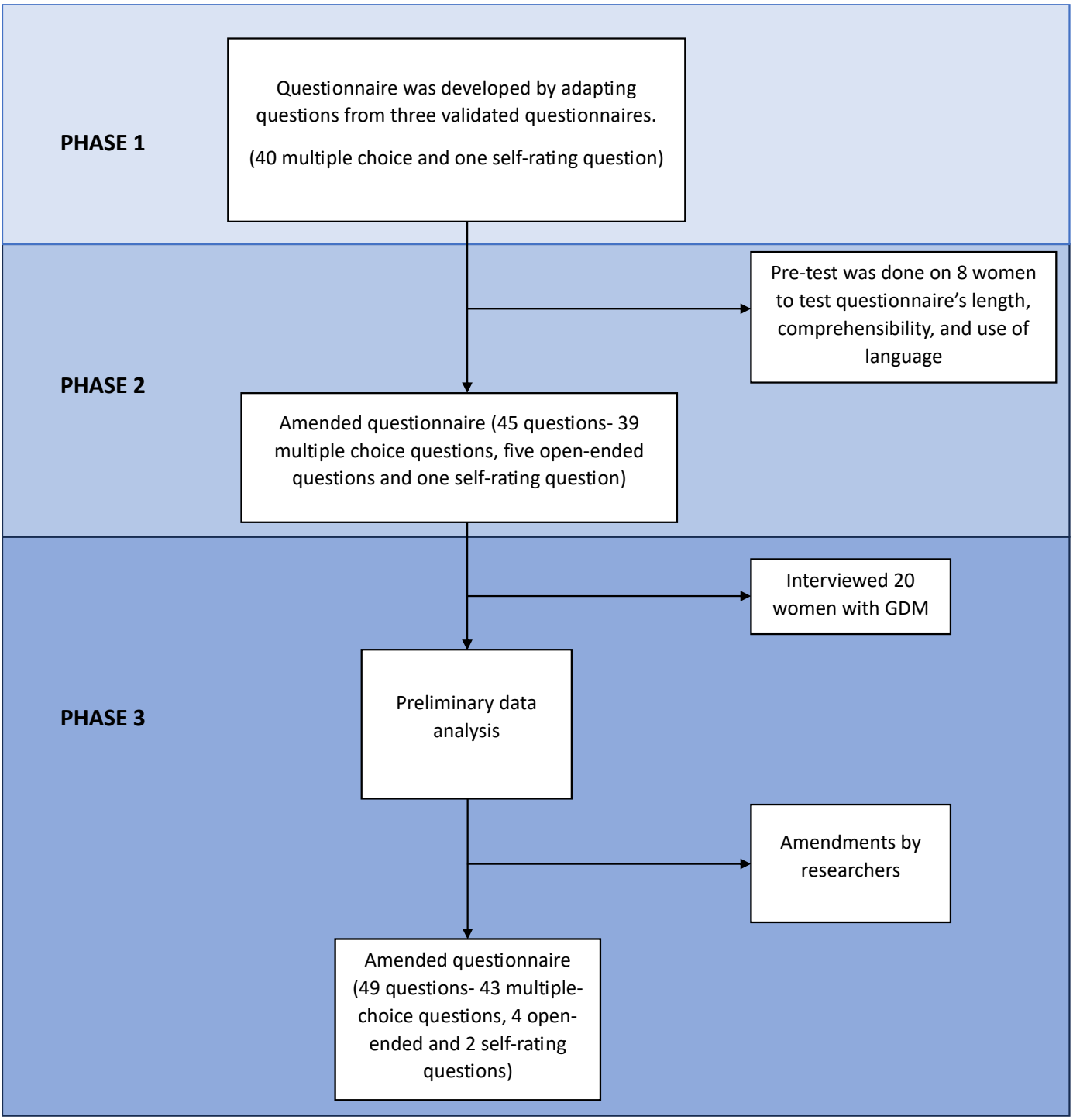

Figure 8.1. Flow diagram of questionnaire development.

8.2.2.2. Phase 2

For face validity, testing was conducted to determine the questionnaire's length (time to completion), comprehensibility, and language usage in women with DIP. The selected participants were diagnosed with either pregestational diabetes (n=4) or GDM (n=4). Women with other types of diabetes were included due to convenient sampling which was done only for testing time to complete, use of language and comprehension. The participants were requested to comment on the questionnaire's content and understandability. Self-reporting questions were included to assess the participants' perceptions of their diagnosis and its impact on their daily lives. Furthermore, during the interview, in consultation with a specialist, amendments to improve the understandability and comprehension of the questionnaire were made. Following amendments made, the questionnaire (version 2) was tested on women with GDM.

8.2.2.3. Phase 3

Twenty women with GDM were recruited at the SBAH high-risk antenatal clinic. Women were recruited at least two weeks post GDM diagnosis and were of all ethnicities. The participants were encouraged to ask any additional health information or questions regarding GDM, nutrition and physical activity after the completion of the interview. The participants' responses were entered into RedCap and coded for analysis. Quantitative statistical analysis was performed using STATA® version 14.0 (StataCorp, College Station, TX, USA). Numbers and percentages were used for quantitative data. After data analysis, additional changes were made to the questionnaire.

8.3. Results

8.3.1. Questionnaire development

8.3.1.1. Phase 1

Following literature review and input from the expert panel, a questionnaire comprising of 40 multiple-choice questions and one self-rating question, consisting of seven statements, was created. The questionnaire was categorised into four sections: 1) knowledge of GDM (eight questions), 2) knowledge on blood glucose testing (seven questions), 3) knowledge of diet, physical activity, and self-care after GDM diagnosis (18 questions), and 4) management of GDM (seven multiple-choice questions and one question with seven self-rating statements). Self-reporting questions were included to evaluate the participants' perceptions regarding their diagnosis and how that might affect their day-to-day lives.

We defined good knowledge as >60%, average knowledge between 40% and 60%, and poor knowledge as <40%. The grading of knowledge is not standard across the template questionnaires that were used, however all the studies used the percentage of correct responses to grade knowledge.

8.3.1.2. Phase 2

The questionnaire was modified after the testing phase, which involved reviewing the questions and language usage, and ensuring that it was understandable and comprehensible. Based on the review and in consultation with an expert panel, questions were removed, edited, or added. In section 1 (GDM knowledge) two open-ended questions were added at the beginning of the questionnaire because there was a need to assess basic knowledge on diabetes. The third option in question 7, which asked how GDM was treated, was updated to include metformin, instead of insulin only because the management of DIP includes both metformin and insulin. In section 2 (knowledge on blood glucose testing) two yes/no questions were added to ensure clarity and

certainty regarding the questions that follow. In section 3 (knowledge of diet, physical activity, and self-care of GDM), two open-ended questions were added because information that described participants' daily diet and the fast foods they consume was lacking. In section 4 (management of GDM), one question that enquired about participants' behavior at a social occasion because of GDM was moved to the self-rating statements because it would give better clarity on how participants believe they would behave. Additionally, three questions were eliminated due to them being repetitive. Two statements within the self-rating question were modified, and five new statements were included for clarity and assessment of participants' feelings about GDM. The sections on knowledge of testing blood glucose levels and management of GDM were combined into a new section (section 2), as the researchers realised that they addressed related questions. After all the modifications were made, the questionnaire had three sections with 39 multiple-choice questions, five open-ended questions, and one self-rating question with seven statements. The questionnaire was approved by the expert panel and used for phase 3.

8.3.1.3. Phase 3

The amended questionnaire was administered to 20 women with GDM and the responses are listed in Tables 8.1-8.4.

Section 1. GDM knowledge

Of the 20 women interviewed, 70% (n=14) were knew about the existence of multiple forms of diabetes in pregnancy (Q1) (Table 8.1). Only 20% (n=4) could correctly identify all three types, 10% (n=2) correctly identified either T2DM or GDM, and 25% (n=5) identified a combination of any two types (Q2). Eighty percent (n=16) of women correctly stated that GDM was diagnosed through a blood test, 15% (n=3) said that GDM was diagnosed using a combination of blood and

urine tests, and 5% (n=1) stated that GDM was diagnosed through urine testing only (Q3). Half of the women (50%, n=10) reported that their babies may be larger than average due to their condition, while 25% (n=5) reported that a combination of large birthweight, PTB, and NICU admission may affect their babies (Q4). Eighty percent (n=16) were able to identify one or more risk factor for GDM (Q5), while 75% (n=15) of women were aware of the pregnancy complications associated with GDM (Q6). The majority of women, 95% knew what uncontrolled blood sugar is (Q7) and knew at least one of the factors associated with GDM (Q8). All women identified at least one of the treatment strategies of GDM (Q9). Eighty-five percent (n=17) of women with GDM were aware that they needed a 6-week postpartum follow-up appointment after delivery (Q10).

Table 8.1. GDM knowledge

Question	**Number (%)**
1. Are you aware that there are different types of diabetes?	
Yes	14 (70.0)
No	6 (30.0)
2. Name them	
T1DM	0 (0.0)
T2DM	2 (10.0)
GDM	2 (10.0)
Combination of two	5 (25.0)
All three	4 (20.0)
I don’t know	7 (35.0)
3. How is gestational diabetes diagnosed	

Blood	16 (80.0)
Urine	1 (5.0)
Combination	3 (15.0)
I don't know	0 (0.0)
4. Because I have gestational diabetes, my baby may be:	
Larger than usual	10 (50.0)
Smaller than usual	0 (0.0)
Born early	0 (0.0)
Admitted to special care	0 (0.0)
Combination	5 (25.0)
Other response	1 (5.0)
I don't know	4 (20.0)
5. Women are more likely to develop gestational diabetes if they:	
Are overweight	2 (10.0)
Are over 35 years	3 (15.0)
Have a family history of diabetes	2 (10.0)
Previously had GDM	1 (5.0)
Combination	8 (40.0)
I don't know	4 (20.0)
6. Because I have gestational diabetes, I may:	
Need to come to the clinic more frequently	8 (40.0)
Need a caesarean section	1 (5.0)
Develop permanent diabetes later in life	2 (10.0)
Combination	4 (20.0)
I don't know	5 (25.0)

7. In uncontrolled diabetes the blood sugar is:	
Normal	0 (0.0)
Increased	14 (70.0)
Decreased	1 (5.0)
Combination	4 (20.0)
I don't know	1 (5.0)
8. Gestational diabetes is:	
Present during pregnancy	2 (10.0)
Disappears once the baby is born	5 (25.0)
May lead to diabetes in later life	8 (40.0)
Is not very serious	0 (0.0)
Combination	4 (20.0)
I don't know	1 (5.0)
9. Gestational diabetes may be treated with:	
Diet	1 (5.0)
Diet and exercise	1 (5.0)
Insulin/metformin	1 (5.0)
All of the above	14 (70.0)
Combination of 2	3 (15.0)
I don't know	0 (0.0)
10. When my baby is born:	
My diabetes will disappear	1 (5.0)
I don't need to worry about being diabetic anymore	1 (5.0)
Follow-up glucose test at my 6-week check-up	17 (85.0)
Combination	0 (0.0)

I don't know	1 (5.0)

Section 2. Knowledge on management and blood glucose testing during GDM

All of the women (n=20) correctly stated that regular blood glucose testing is important to manage GDM (Q11) and that regular glucose monitoring is important for the health of mother and child (Q12) (Table 8.2). Sixty-five percent (n=13) of women stated that controlling blood glucose levels would give their baby a healthy start, whilst 10% (n=2) indicated that controlling blood glucose had no effect on the baby (Q13). The majority of women knew when to test their glucose levels; 75% (n=15) of women said that they should test in the morning before breakfast, in the afternoon before lunch, 2-hours after meals, and at 2 am. Twenty percent (n=4) gave a combination of two/three of the responses, while 5% (n=1) of women said in the morning before breakfast only (Q14). Eighty-five percent (n=17) of women stated that they knew what their glucose levels should be (Q15), however, only 65% (n=13) knew what a normal fasting blood glucose level was (Q16), and only 60% (n=12) of the women knew what a normal 2-hour blood glucose level was (Q17). Twenty percent (n=4) of women did not know what to do when their blood glucose levels were high on one (Q18) or two (Q19) occasions in one week. The majority of women, 85% (n=17) stated that they should continue to check their blood glucose levels while sick (Q20). Most women (95%, n=19) reported that they check their glucose levels by using different fingers each day, while 5% (n=1) said they use the same finger every day (Q21).

Table 8.2. Knowledge on management and blood glucose testing during GDM

Question	**Number (%)**

11. Is regular glucose testing important during GDM?	
Yes	20 (100.0)
No	0 (0.0)
12. You should check your blood glucose levels:	
Regularly for the health of you and your baby	20 (100.0)
Occasionally	0 (0.0)
When you feel unwell	0 (0.0)
Before you go to see the doctor	0 (0.0)
Combination	0 (0.0)
I don't know	0 (0.0)
13. Controlling your blood glucose levels:	
Has no effect on baby	2 (10.0)
Will give a healthy start for baby	13 (65.0)
Has no effect on the pregnancy outcome	0 (0.0)
None of the above	1 (5.0)
Combination	1 (5.0)
Other response	1 (5.0)
I don't know	2 (10.0)
14. I should test my blood glucose level:	
In the morning before breakfast	1 (5.0)
In the afternoon before lunch	0 (0.0)
2 hours after meals	0 (0.0)
At 2am	0 (0.0)
Select all 4	15 (75.0)
Combination of 2/3	4 (20.0)

I don't know	0 (0.0)
15. Do you know what the glucose should be?	
Yes	17 (85.0)
No	3 (15.0)
16. A normal fasting (on an empty stomach) blood glucose level is:	
Less than 5 mmol/L	13 (65.0)
Less than 6 mmol/L	3 (15.0)
7 mmol/L or more	0 (0.0)
8 mmol/L or more	0 (0.0)
Combination	0 (0.0)
I don't know	4 (20.0)
17. A normal 2-hour (after eating) blood glucose level is:	
Less than 5 mmol/L	0 (0.0)
Less than 6.7 mmol/L	12 (60.0)
7 mmol/L or more	1 (5.0)
8 mmol/L or more	0 (0.0)
Combination	0 (0.0)
I don't know	7 (35.0)
18. What do I do if my blood glucose level is high on one occasion?	
Make a note in your diary	2 (10.0)
Check what you ate before the high blood glucose level	7 (35.0)
Go to the hospital	1 (5.0)
Combination	5 (25.0)
Other response	1 (5.0)
I don't know	4 (20.0)

19. What do I do if my blood glucose level is high on two occasions in one week?	
Make a note in your diary	4 (20.0)
Check what you ate before the high blood glucose level	5 (25.0)
Contact the diabetes educator	1 (5.0)
Go to the hospital	4 (20.0)
Combination	2 (10.0)
I don't know	4 (20.0)
20. Should I take my blood glucose level if I am feeling sick and haven't eaten?	
Yes, continue to take your blood glucose levels as usual	17 (85.0)
No, do not take your blood glucose levels until you are feeling better	0 (0.0)
I don't know	2 (15.0)
21. When you prick your finger, you should:	
Use the same finger every day	1 (5.0)
Use a different finger every day	19 (95.0)
It is not important	0 (0.0)
I don't know	0 (0.0)

Section 3. Knowledge on nutrition, physical activity and GDM management

The food women ate in a typical day included pap, rice, bread, vegetables, fruits, biscuits, chicken, beef (Q22) (Table 8.3). The majority of women, 90% (n=18) knew that the type of food they ate was important to control GDM (Q23). Of the women interviewed, 55% (n=11) stated that foods high in carbohydrates, starches, fats, and sugar should be avoided during GDM, 30% (n=6) stated that only food high in carbohydrates and starches should be avoided, 10% (n=2) indicated sugar

only, while 5% (n=1) indicated that only fats should be avoided (Q24). When asked about foods that can be consumed without restrictions, 55% (n=11) of women indicated vegetables, 30% (n=6) indicated a combination of vegetables, meat, and fruits, 5% (n=1) gave answers that were not listed as options, while 10% (n=2) did not know (Q25). Seventy percent (n=14) of women correctly identified carbohydrates/starches as the main dietary nutrient in pap, 5% (n=1) thought that pap contained protein, while 10% (n=2) thought that pap contained a mixture of carbohydrates/starches, protein, fat and sugar. Fifteen percent (n=3) of women did not know what the dietary content of pap was (Q26). Fifty percent (n=10) of women knew that adjusting the preparation process (cook, cool, and then reheat) could make pap safer for blood sugar control (Q27). Eighty-five percent (n=17) of women knew what the preferred types of carbohydrates are (Q28), while 90% (n=19) knew that fresh fruits and vegetables are better than juices, processed or canned options (Q29). The majority of women, 85% (n=17) reported eating fast food (Q30) such as fried chips, pizza, “kota”, sandwiches, fried chicken and burgers (Q31). Only one woman could not correctly identify a source of protein (Q32). When asked about the best type of chicken, 85% (n=17) of women indicated that skinless baked chicken is the best option to cook chicken (Q33). The majority of women (95%, n=19) knew what a balanced diet was (Q34). Of the women interviewed, 65% (n=13) stated that they would opt for another meal or snack when they feel hungry between meals, 30% (n=6) preferred to drink water and see if the hunger subsides, while 5% (n=1) chose to ignore the hunger altogether (Q35). All women (100%, n=20) agreed that engaging in physical activity was important to manage GDM (Q36), while only 85% (n=17) knew that exercise was important to help control the mother's blood glucose levels and benefit the baby's health (Q37). The majority of women (85%, n=17) indicated that walking and swimming is recommended during pregnancy, 5% (n=1) said running and skipping, while 10% (n=2) indicated a combination of running, skipping, walking, and swimming (Q38). Seventy-five percent (n=15) of women reported that mild exercise could be done during pregnancy, 20% (n=4) reported moderate exercise, and 5% (n=1) did not know (Q39). The majority of women, 70% (n=14) said

one should exercise for 30 minutes per day, 10% (n=2) said 10 minutes, while 5% (n=1) said 15 minutes. Ten percent (n=2) of women gave an option that was not provided such as “I just walk”, “it depends on the person” and 5% (n=1) did not know (Q40). When asked if overweight and unfit individuals should exercise, 85% (n=17) of women agreed that they should start slowly and increase gradually, 5% (n=1) felt that they should first lose weight and get fit before starting to exercise, while the remaining 10% (n=2) were unsure (Q41). When asked about ways to increase daily physical activity/exercise, 65% (n=13) of women suggested a combination of walking their children to school, taking the stairs, and walking to the shops. Fifteen percent (n=3) suggested walking their children to school only, 15% (n=3) suggested walking to the shopping centre only and 5% (n=1) stated taking the stairs instead of using the lift or elevator only (Q42). The majority of women (80%, n=16) thought that blood glucose could be controlled by eating a healthy, balanced diet and engaging in moderate exercise for 5-7 days per week, with each session lasting about 30 minutes. Another 10% (n=2) stated that a combination of a healthy diet, moderate exercise, and spending time resting, while 5% (n=1) suggested a healthy diet alone and 5% (n=1) did not provide a specific recommendation (Q43).

Table 8.3. knowledge on nutrition, physical activity and GDM management

Question	**Number (%)**
22. Describe what you eat in a typical day (open ended question)	-
23. Do you think that what you eat is important to control gestational diabetes?	
Yes	18 (90.0)
Other response	1 (5.0)

No	1 (5.0)
24. If you have gestational diabetes, you should avoid food containing high content of	
Carbohydrates/starches	6 (30.0)
Protein / meat	0 (0.0)
Fat	1 (5.0)
Sugar	2 (10.0)
Combination	11 (55.0)
I don't know	0 (0.0)
25. Which of the following food can be eaten without restriction during gestational diabetes?	
Sugar	0 (0.0)
Fruit	0 (0.0)
Vegetables	11 (55.0)
Meat	0 (0.0)
Combination	6 (30.0)
Other responses	1 (5.0)
I don't know	2 (10.0)
26. What is the type of dietary source mainly provided by pap?	
Carbohydrates/starches	14 (70.0)
Protein	1 (5.0)
Fat	0 (0.0)
Sugar	0 (0.0)
Combination	2 (10.0)
I don't know	3 (15.0)

27. How can you make pap safer for your blood sugar to eat?	
Cook and eat	4 (20.0)
Cook, cool and reheat	10 (50.0)
Add fats	0 (0.0)
Add lemon juice	1 (5.0)
Add vinegar	1 (5.0)
I don't know	4 (20.0)
28. The preferred type of carbohydrate/starchy foods are:	
White bread	0 (0.0)
Wholegrain bread	5 (25.0)
Foods that are high in fibre	7 (35.0)
Foods high in starch	0 (0.0)
Combination	5 (25.0)
I don't know	3 (15.0)
29. What form of fruits and vegetables are better?	
Fruit or vegetable juices	1 (5.0)
Processed or canned fruits and vegetables	0 (0.0)
Fresh fruit and vegetables	18 (90.0)
I don't know	1 (5.0)
30. How often do you eat fast food?	
Never	3 (15.0)
Once a week	8 (40.0)
More than three times a week	0 (0.0)
Other response	9 (45.0)
31. What fast food do you eat? (open ended question)	-

32. Protein intake can be obtained from:	
Meat	2 (10.0)
Fish	1 (5.0)
Nuts	1 (5.0)
Dairy such as milk or cheese	1 (5.0)
All of the above	10 (50.0)
Combination of 2	4 (20.0)
I don't know	1 (5.0)
33. What type of chicken is best?	
Skinless baked chicken	17 (85.0)
Skin-on chicken	0 (0.0)
Deep-fried chicken	0 (0.0)
Any chicken	1 (5.0)
I don't know	2 (10.0)
34. A balanced diet should have:	
More vegetables	7 (35.0)
Fewer carbohydrates/starches such as white bread	1 (5.0)
Low fat and low sugar choices	1 (5.0)
All of the above	10 (50.0)
Combination	0 (0.0)
I don't know	1 (5.0)
35. When you are hungry in between meals:	
Eat another meal/snack	13 (65.0)
Drink water and see if that helps	6 (30.0)
Try and ignore it	1 (5.0)

Go for a walk	0 (0.0)
Combination	0 (0.0)
I don't know	0 (0.0)
36. Is physical activity (movement) or exercise important to control gestational diabetes?	
Yes	20 (100.0)
No	0 (0.0)
37. Physical activity or exercise in gestational diabetes:	
Helps to control mother's blood glucose and improves baby's health	17 (85.0)
Is not helpful	0 (0.0)
Tires you out	0 (0.0)
Increases the risk of miscarriage during pregnancy	0 (0.0)
Combination	0 (0.0)
I don't know	3 (15.0)
38. With regard to exercise during pregnancy:	
Running and skipping are recommended	1 (5.0)
Walking and swimming are recommended	17 (85.0)
Combination	2 (10.0)
I don't know	0 (0.0)
39. How hard can you exercise during pregnancy?	
Mild exercise	15 (75.0)
Moderate exercise	4 (20.0)
Vigorous exercise	0 (0.0)
Until you are exhausted	0 (0.0)
I don't know	1 (5.0)

40. How long should you exercise per day?	
10 minutes	2 (10.0)
15 minutes	1 (5.0)
Till you get tired	0 (0.0)
30 minutes	14 (70.0)
Other response	2 (10.0)
I don't know	1 (5.0)
41. Should I exercise if I am overweight and unfit?	
No, you should not	0 (0.0)
Yes, you should start slowly and increase gradually	17 (85.0)
First you need to lose weight and get fit	1 (5.0)
I don't know	2 (10.0)
42. How can I increase my daily physical activity/exercise?	
Walk children to school	3 (15.0)
Take stairs instead of the lift or elevator	1 (5.0)
Walk to the shopping centre	3 (15.0)
Combination	13 (65.0)
I don't know	0 (0.0)
43. To control blood glucose effectively you should:	
Eat a healthy, balanced diet	1 (5.0)
Do moderate exercise 5-7 days a week for about 30 minutes a day	0 (0.0)
Spend most of the time resting	0 (0.0)
Eat a healthy, balanced diet with moderate exercise 5-7 days a week for about 30 minutes a day	16 (80.0)
Combination	2 (10.0)

I don't know	1 (5.0)

Section 4: Self-rating statements.

The responses for the self-rating statements (Q44.1- 44.7) are presented in Table 8.4 and Figure 9.2. Eighty five percent (n=17) of women strongly disagreed that they are ashamed of their diabetes diagnosis (Q44.1). They (40%, n=8) were unsure about whether a GDM diagnosis would make them miss work (Q44.2). Sixty five percent (n=13) of women strongly disagreed that their diabetes limited their career (Q44.3). Eighty percent (n=16) of women stated that they were satisfied with their glucose control (Q44.4) and that they did as instructed by health care professionals (Q44.5). The majority (60%, n=12) of women felt that GDM caused them not to enjoy their pregnancy (Q44.6), however 65% (n=13) disagreed that their pregnancies were abnormal (Q44.7).

When women were asked about how they felt about their GDM diagnosis, women expressed emotions that included fear (10%, n=2), anxiety (10%, n=2), shock (20%, n=4), sadness (40%, n=8), normal (10%, n=2) and stress (10%, n=2) (Q45).

Table 8.4. Self-rating statements

Statement	**Number (%)**
1. I would rather eat something unhealthy than tell someone that I have diabetes	
Strongly disagree	17 (85.0)
Disagree	2 (10.0)
Agree	0 (0.0)

Strongly agree	1 (5.0)
I don't know	0 (0.0)
2. I am worried about whether I will miss work	
Strongly disagree	5 (25.0)
Disagree	7 (35.0)
Agree	4 (20.0)
Strongly agree	2 (10.0)
I don't know	2 (10.0)
3. I feel diabetes limits my career	
Strongly disagree	13 (65.0)
Disagree	1 (5.0)
Agree	1 (5.0)
Strongly agree	3 (15.0)
I don't know	2 (10.0)
4. I feel satisfied with my blood glucose control	
Strongly disagree	2 (10.0)
Disagree	2 (10.0)
Agree	7 (35.0)
Strongly agree	9 (45.0)
I don't know	0 (0.0)
5. I record my blood glucose levels in my charts/diabetes diary when my health care personnel ask me to	
Strongly disagree	0 (0.0)
Disagree	1 (5.0)
Agree	7 (35.0)

Strongly agree	12 (60.0)
I don't know	0 (0.0)
6. Gestational diabetes has caused me not to enjoy my pregnancy	
Strongly disagree	3 (15.0)
Disagree	5 (25.0)
Agree	5 (25.0)
Strongly agree	7 (35.0)
I don't know	0 (0.0)
7. I feel that my pregnancy is an abnormal pregnancy	
Strongly disagree	8 (40.0)
Disagree	5 (25.0)
Agree	4 (20.0)
Strongly agree	2 (10.0)
I don't know	1 (5.0)
45. How did you feel when you were informed that you have gestational diabetes? (open ended question)	-

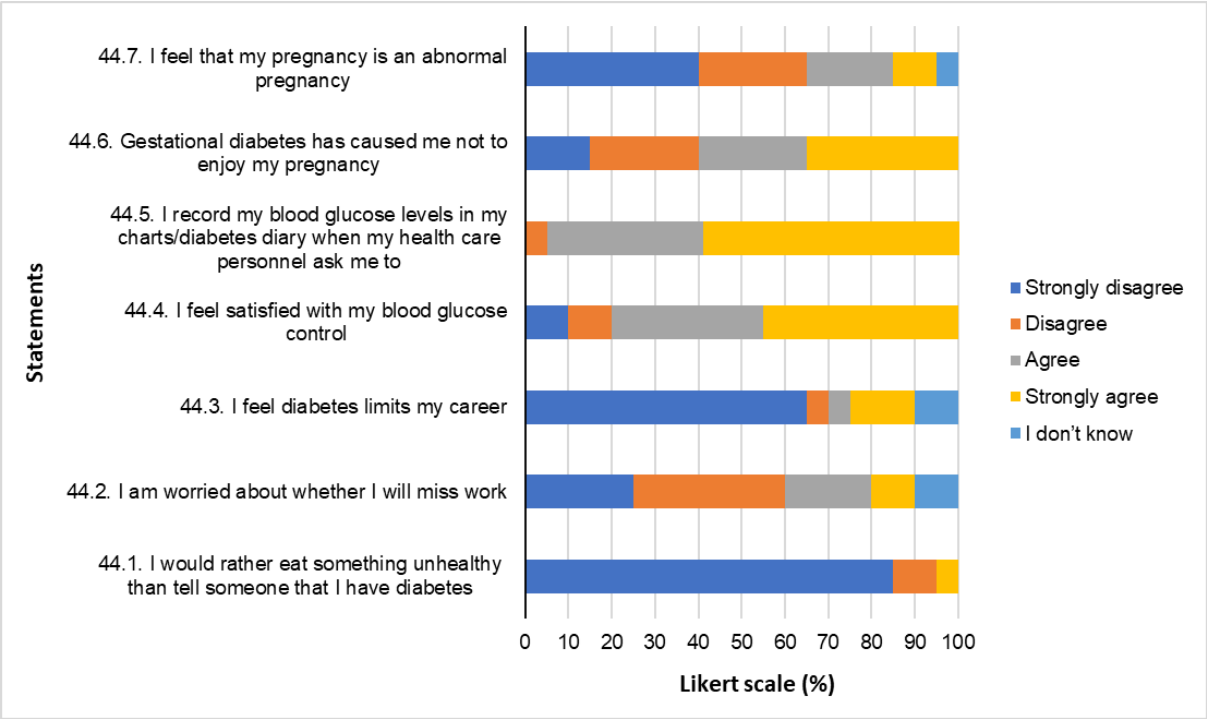

Figure 8.2. Responses to self-rating statements.

Based on the interviewee's responses to the questionnaire, changes were made in version 3 of the questionnaire. To improve section 1, (knowledge of GDM) two open-ended questions (Q1 and Q2) at the beginning of the questionnaire were replace by two different open-ended questions to evaluate basic knowledge of GDM and to understand the women's perspectives on what it meant to be diagnosed with GDM. One self-rating question, which allowed participants to rate their level of knowledge of GDM before starting with the multiple-choice questions was added (Q3). One multiple-choice question, which evaluated knowledge of the effect of GDM on the child's health post-delivery was added. Lastly, more choices were added, and amendments were made to questions 5 to 12 because more inclusive options to evaluate level of knowledge were needed. In section 2 (GDM management and blood glucose testing), several amendments were made. An open-ended question to support a yes/no answer to questions 13 to 17 was added to evaluate participants' understanding of the importance of certain aspects of GDM management. The

options for question 15 were changed and more choices to question 16 were added because more inclusive options to evaluate level of knowledge were needed. Additionally, sub-questions under question 20 to evaluate the participants' knowledge on what do at different glucose levels and amended the responses for question 21 for more clarity were included. In the section 3 (managing your health after being diagnosed with GDM) some updates to the questionnaire were made too. A multiple-choice question was added and question 24 was adapted into a table because it was easier to answer in tabular format. Questions 26-29 were rephrased and updated for better understanding and clarity. Lastly, more choices were added to questions 34, 37, and 40 because more options were needed for better understanding of level of knowledge. The version 3 of the questionnaire finally had 49 questions, including 4 open-ended, 2 self-rating, and 43 multiple-choice questions.

8.4. Discussion

This study describes the development of a questionnaire to assess diabetes knowledge in South African women with GDM. To our knowledge, this is the first study to develop a questionnaire to evaluate a woman's understanding of GDM in South Africa. In general, the questionnaire performed well in terms of content validity and was able to assess knowledge of diabetes in pregnant women with GDM. The majority of women in our study demonstrated adequate knowledge of the factors required to achieve glycaemic targets.

The questionnaire was developed by adapting three developed validated questionnaires (Carolan-Olah and Vasilevski, 2021; Hussain et al., 2015; Mashige et al., 2008), one of which was validated (Carolan-Olah and Vasilevski, 2021). The developed questionnaires in the above mentioned studies assessed knowledge on diabetes, nutrition, physical activity and diabetic complications either in the presence (Carolan-Olah and Vasilevski, 2021; Hussain et al., 2015) or absence (Mashige et al., 2008) of pregnancy. The questionnaire in our study assessed knowledge on GDM, its management, blood glucose testing, nutrition, physical activity, and self-care post-diagnosis. The original questionnaire was tested and re-tested and amended twice to correct the language usage and improve understandability.

The frequency of correct responses was high across all three sections of the questionnaire, demonstrating that in our population, women with GDM had good knowledge of their condition. These results are similar to a study by Carolan-Olah and Vasilevski (2021), which was conducted in a socially disadvantaged population. The study reported a high frequency of correct responses for the knowledge of GDM, GDM self-management and nutrition; the questions with multiple choice options were the most incorrectly answered (Carolan-Olah and Vasilevski, 2021). In contrast, Ogu et al. found that only one-third of the participants in their study knew about GDM risk factors, GDM screening, diagnosis, and treatment, and GDM consequences (Ogu et al.,

2020). The study was conducted in preselected rural and urban communities and households, where only one woman of reproductive age was interviewed per selected household.

GDM knowledge

Although women were not able to answer all the questions correctly, in general, the majority (83%) of women demonstrated basic knowledge about GDM. The majority of the women knew that there were different types of DIP and could name one of the three types. A study by Mashige et al. reported that 96% of participants knew that there were different types of diabetes, however, only mentioned two main types (T1DM and T2DM) and excluded women with GDM as this study was not in a pregnant population (Mashige et al., 2008). Other studies have reported that both diabetic and non-diabetic populations were unaware that there were different types of diabetes (Dias et al., 2010; Yun et al., 2007). These studies were conducted in much older populations (mean age 55 to 63 years) with incomplete basic education (secondary education). The lack of knowledge on diabetes in general and the different types of diabetes is ascribed to a failure to comprehend the information provided during education and counseling (Yun et al., 2007), as well as low levels of education (Dhyani et al., 2018; Zowgar et al., 2018). Our study showed that women knew that GDM was diagnosed using blood. Women who said that GDM was diagnosed using blood and urine might have thought that the urine collected (which is used for urine dipsticks, microscopy, cultures, and creatinine and proteins measurements) during their visit was used for their GDM diagnosis. The women were aware of the effect of GDM on the mother and baby, which is similar to Hussian et al. who showed good knowledge of the effect of GDM on the mother and baby (Hussain et al., 2015). In contrast, Carolan-Olah and Vasilevski reported poor knowledge of the effects of GDM on the mother and baby (Carolan-Olah and Vasilevski, 2021). The women in our study showed good knowledge on the risk factors of GDM similar to other studies (Hussain et al., 2015; Mahalakshmi et al., 2014). However, some studies have reported average knowledge

(Carolan-Olah and Vasilevski, 2021; Dhyani et al., 2018). These differences might be due to the variation in health education programs among different populations and countries. Our study showed good knowledge on what uncontrolled diabetes means and the different treatment options for GDM which is similar to what was reported by Carolan-Olah and Vasilevski (Carolan-Olah and Vasilevski, 2021). This might be due to the emphasis on the importance of maintaining adequate glycaemic control and self-monitoring in our setting.

Knowledge on management and blood glucose testing during GDM

In general, the majority (80.8%) of women were knowledgeable about blood glucose control during GDM. Our study showed that women were aware of the importance and benefits of regular glucose self-monitoring and knew when to test their blood glucose levels. Our study also showed that the majority of women had good knowledge on the normal fasting and 2-hour blood glucose values, which is similar to the findings by Carolan-Olah and Vasilevski (Carolan-Olah and Vasilevski, 2021). Hussain et al. also reported good knowledge, while Dhyani reported average knowledge for normal fasting glucose values (Dhyani et al., 2018; Hussain et al., 2015). Regarding knowledge on what to do when they experience hyperglycaemia either on one or two occasions, our study showed poor knowledge. In contrast, Hussain et al. and Dhyani et al. reported average knowledge on what to do during hypoglycaemia, a consequence of uncontrolled diabetes (Dhyani et al., 2018; Hussain et al., 2015). Due to the short-lived nature of GDM, women might not be familiar with self-management, therefore making lifestyle modifications challenging. According to Draffin et al. women stated that blood glucose monitoring was tiring, difficult and disrupted their normal daily routine, however, after several tries, they were able to maintain recommended targets. Women also tend to lie or even starve themselves to try maintain their glycaemic targets (Draffin et al., 2016). This shows the psychological impact GDM diagnosis, lifestyle modification and maintaining glucose targets has on women.

Knowledge on nutrition, physical activity and GDM management

Similar to the study by Hussain et al. which focused on rice (a staple in Malaysia) (Hussain et al., 2015), our study showed that the majority of women knew that the primary source of nutrition from pap (a staple porridge made from maize meal), is carbohydrates. Dhyani et al. reported an average level of knowledge regarding the main source of nutrition from rice (Dhyani et al., 2018). A study by Louie et al. reported that instead of complete avoidance or strict restriction, moderate intake of carbohydrates is usually recommended to achieve postprandial euglycaemia (Louie et al., 2015). In terms of foods that can be eaten without restriction, our study found good knowledge, similar to Hussain et al. who reported 83.1% correct score on foods to avoid when diagnosed with GDM (Hussain et al., 2015). In our study, women had good awareness of the preferred type of carbohydrate/starchy foods similar to Carolan-Olah and Vasilevski who reported 60.3% correct score (Carolan-Olah and Vasilevski, 2021). A systematic review found that a diet high in complex carbohydrates, specifically whole grains, may reduce the risk of some non-communicable diseases due to its high dietary fiber content (Reynolds et al., 2019). Tobias et al. found that a women's diet plays a crucial role in reducing the risk of postpartum diabetes. They discovered that women who adhere to a Mediterranean diet have a 40% lower risk of developing diabetes, especially those with a history of GDM (Tobias et al., 2012). However, researchers have reported that there are big dietary misconceptions in diabetic populations (Al-Saeedi et al., 2002; Zowgar et al., 2018).

Similar to Carolan-Olah and Vasilevski, our study demonstrated good knowledge about the benefits of physical activity/exercise in GDM and that walking and swimming are the recommended forms of exercise during pregnancy (Carolan-Olah and Vasilevski, 2021). In contrast, Dhyani et al reported average knowledge on the benefits of exercise in GDM and low knowledge on moderate exercise such as walking in women with GDM (Dhyani et al., 2018). Studies have reported that both diabetic and non-diabetic populations are aware that exercise

can decrease blood glucose values (Al-Mahrooqi et al., 2013; Lemes dos Santos et al., 2014), however non-adherence, lack of access to information or safe spaces for exercise might be the problem. Similar to Carolan-Olah and Vasilevski, who also revealed 78.4% selection for 30 minutes exercise per day, our study indicated that 30 minutes was the preferred length for exercise per day. Studies show that physical activities improve insulin resistance and limit gestational weight gain by increasing energy expenditure (Gilbert et al., 2019; Wang et al., 2016) and the majority of guidelines regarding exercise during pregnancy support 150 hours of moderate-intensity exercise, where 150 hours can be divided into 30 minute workouts for five days a week or smaller 10 minute workouts throughout the day (ACOG, 2022; WHO, 2020).

Some participants answered with responses that were not provided as options in the questionnaire which may indicate that certain responses needed to be revised or expanded. This has been considered in version three of the questionnaire and will further be refined in future versions.

Self-rating questions

The women in our study stated that they did not have a problem telling people about their GDM diagnosis especially regarding dietary concerns. In contrast, Martis et al. reported that women often only shared their diagnosis with their partners due to fear of being judged or scrutinized for behaviours such as dietary changes (Martis et al., 2018). Other studies reported that women felt different in social settings or at work due to adherence to GDM management recommendations (Lawson and Rajaram, 1994; Parsons et al., 2014; Toxvig et al., 2022). In our study, women expressed that they were satisfied with their glucose control, and that they monitored and recorded their glucose levels as requested by healthcare professionals. Martis et al. revealed that even though women were knowledgeable about their glucose targets and understood the

significance of adhering to them, they still expressed reluctance to monitor their glucose levels as advised by healthcare professionals (Martis et al., 2018). In contrast, Toxvig et al. reported that women felt that the recommendations made by healthcare professionals regarding glucose control made them feel safe and kept them disciplined (Toxvig et al., 2022). The majority of women in our study felt that GDM caused them not to enjoy their pregnancies. However, they disagreed that their pregnancies were abnormal. Similarly, several studies have shown that the drastic lifestyle changes and stringent glucose monitoring required in women with GDM made women not to enjoy their pregnancies (Draffin et al., 2016; Faal Siahkal et al., 2022; Martis et al., 2018; Toxvig et al., 2022). Women in these studies felt that their pregnancies were abnormal (Craig et al., 2020; Devsam et al., 2013; Lawson and Rajaram, 1994), which is on contrast to our study.

Anxiety

Nulliparous women with first GDM diagnosis or women with first GDM diagnosis following prior uncomplicated pregnancies expressed anxiety, shock, fear and stress when asked about how they felt about their GDM diagnosis. This is similar to previous research which showed that it is common for women to express anxiety, shock and fear following their GDM diagnosis (Draffin et al., 2016; Persson et al., 2010; Tait Neufeld, 2014). Women with a previous GDM diagnosis or with a strong family history of diabetes expressed only sadness and disappointment following their GDM diagnosis. However, they also expressed a sense of relief after receiving GDM counselling and support from healthcare professionals and family members. Studies have shown that health education and support improves not only pregnancy outcomes and disease management, but also reduces emotional distress following a diagnosis (Berkman et al., 2011; Gharachourlo et al., 2018; Ogu et al., 2020; Rosland et al., 2008).

In our study, women who were newly diagnosed had comparable levels of knowledge across all three sections of the questionnaire compared to women with recurring GDM. At SBAH women with recurring GDM are counselled on diabetes, education and nutrition with every diagnosis, therefore better overall diabetes knowledge is expected compared to newly diagnosed women. However, studies have indicated that women experience emotional stress and anxiety after giving birth as they adjust to motherhood (Muhwava et al., 2020; Obrochta et al., 2020), therefore this might reduce adherence to lifestyle modification and the knowledge attained during pregnancy. According to a recent systematic review, many women felt abandoned post-delivery due to the lack of individualized and continued care, limited options for delivery, and insufficient comprehensive follow-up. These authors found that these factors led to a sense of abandonment amongst women who experienced intense medical intervention during pregnancy only to receive little or no support afterwards (Craig et al., 2020). Consequently, it became challenging for them to attend postpartum check-ups and maintain a healthy lifestyle. This highlights the need for long-term lifestyle interventions to reduce the likelihood of GDM recurrence in subsequent pregnancies and the future development T2DM and cardiovascular diseases. Postnatal care may aid to identify women at risk for future pregnancy complications and promote healthy pregnancies (Adam et al., 2023). Several lifestyle modifications, such as the consumption of a nutritionally balanced diet, regular physical activity, and maintaining a healthy weight have been shown to be successful in reducing the risk of T2DM in women who had previous GDM (Hod et al., 2015). This supports the feasibility of utilising these interventions as effective strategies for women with a history of GDM. When counselling women on lifestyle modifications, it is important to not only provide guidance on what to do, but also on how to achieve and maintain their goals long-term (Adam et al., 2023). A promising tool for promoting long-term health in pregnant women with complications is the International Federation of Gynecology and Obstetrics (FIGO) pregnancy passport. This passport is given to women who have pre-existing or pregnancy-induced complications and are at risk of developing cardiometabolic complications after delivery. It facilitates the screening and proper

management of women who had pregnancy complications such as hypertensive disorders during pregnancy or GDM (Nguyen-Hoang et al., 2023).

8.5. Strengths and limitations

This is the first study to develop a questionnaire to evaluate diabetes knowledge in women with GDM in South Africa. The strength of this study is that it is based on a combination of three questionnaires, two were developed and one validated for GDM in similar low-middle income populations in Australia (Carolan-Olah and Vasilevski, 2021) and Malaysia (Hussain et al., 2015) and one was developed to assess diabetes knowledge in a similar South African population in Durban, Kwa-Zulu Natal (Mashige et al., 2008). These studies assessed similar diabetes content and assessed knowledge in populations with similar socioeconomic backgrounds attending public hospitals or healthcare facilities. Furthermore, our study describes in detail the development of a comprehensive questionnaire assessing GDM knowledge, GDM self-management, diet, and physical activity. There are limited studies evaluating all these factors to evaluate GDM knowledge. For example, Ogu et al, developed a questionnaire covering sociodemographic information, awareness and knowledge of GDM, knowledge of GDM screening and diagnosis, and knowledge of GDM complications but they did not detail the development process or the healthcare professionals involved (Ogu et al., 2020). Alayoub et al did not detail the development for their questionnaire evaluating the effectiveness of a GDM education program (Alayoub et al., 2018).

The study is limited by the small number of participants, who may not reflect the diverse population of women with social, economic, and cultural differences who attend SBAH. The study was also conducted in one hospital, which limits the generalizability of the results. At the time of the interview some participants had not attended their dietician appointments, which may have limited their knowledge of nutrition. Women with other forms of DIP during pregnancy may have had

more exposure which might affect the accuracy of the questionnaire. Therefore, the questionnaire would need to be evaluated in women with GDM only. The diabetic files only contain the year of diagnosis and not the full date of GDM diagnosis. This limited the accuracy of the average duration of diagnosis before the interview was initiated. The required limited two weeks since diagnosis was based on self-report. Women with other forms of DIP during pregnancy may have had more exposure to education which might affect the accuracy of the questionnaire. Therefore, the questionnaire would need to be evaluated in women with GDM only. Moreover, despite English being the primary language at SBAH and concerted efforts were made to ensure patients understood the questions, it is important to recognize the potential impact of language barriers. This study describes the development of a questionnaire, acknowledging the necessity for a thorough validation process to evaluate construct validity, internal consistency, and test-retest reliability in future. Furthermore, this study did not evaluate the knowledge of healthcare workers, who play a critical role in the management of GDM and provide continuous education and support.

8.6. Conclusion

Although additional refinements and validation are necessary, our preliminary findings present a comprehensive tool for evaluating diabetes knowledge in pregnant women with GDM in South Africa. This questionnaire may be useful in identifying knowledge gaps in pregnant women with GDM, which may aid in enhancing education programs and developing interventions to improve glucose management and improve pregnancy outcomes in women with GDM.

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Author Contributions

N Malaza, S Adam and C Pheiffer - Conceptualization and original draft; N Malaza, S Adam, C Pheiffer, S Dias, M Masete - manuscript writing and approval of the final draft. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

CHAPTER 9

DISCUSSION

9.1. Summary

Diabetes in pregnancy (DIP) is increasing globally and is associated with an increased risk of short- and long-term adverse pregnancy outcomes for both the mother and child (International Diabetes Federation, 2021). Maternal adiponectin, leptin and SHBG were reported to be associated with pregnancy complications (Lomakova et al., 2022; Manderson et al., 2003; Spencer et al., 2005). Additionally, miRNAs play an important role in regulating metabolic and developmental processes during pregnancy (Krützfeldt and Stoffel, 2006; Sayed and Abdellatif, 2011) and dysregulated levels have been associated with adverse outcomes including PTB (Hromadnikova et al., 2022; Mayor-Lynn et al., 2011), macrosomia (Kochhar et al., 2022; Li et al., 2015), and restricted fetal growth (Hromadnikova et al., 2022; Yao et al., 2024). There is limited data on the prevalence of adverse pregnancy outcomes and the association between biochemical and miRNA markers and neonatal birth outcomes in South Africa. This study aimed to 1) determine the prevalence of adverse outcomes in pregnant women with T1DM, T2DM and GDM 2) explore the candidacy of adiponectin, leptin, and SHBG and 3) miRNAs to serve as biomarkers of glycaemic control and neonatal birth outcomes in pregnancies complicated by T1DM, T2DM and GDM. Serum adiponectin, leptin, and SHBG levels were measured using ELISA and qRT-PCR was used to identify maternal miRNAs associated with neonatal birth outcomes. Results of each chapter are briefly summarised below, followed by integration and synthesis of the overall thesis findings, highlighting the significance and novelty of the study and how the study findings contribute to existing knowledge both locally and globally. Lastly, we discuss the strengths and limitations of the study, recommendations for future research and potential impact of biomarkers on health systems.

The first objective of the thesis was to assess the association between DIP and adverse outcomes globally (chapter 4) and in our South African population (chapter 5). Literature shows that all types of maternal diabetes are associated with adverse pregnancy outcomes, however, adverse

outcomes are more common in pregestational diabetes (Al-Nemri et al., 2018; Soepnel et al., 2019; Tinker et al., 2020). Adverse pregnancy outcomes are associated with inadequate glycaemic control. It is hypothesized that preconception hyperglycaemia and the longer time of exposure to hyperglycaemia in utero may contribute to the complications associated with pregestational diabetes (Dornhorst and Banerjee, 2010). There is a large body of evidence on the association of DIP and adverse outcomes, however, the results are conflicting. A systematic review was conducted to summarise and synthesise studies that have compared adverse pregnancy outcomes in pregnancies complicated by pregestational diabetes (T1DM and T2DM) and GDM globally. Studies published between 1993 and 2021 were included in the review. The review showed that all types of DIP were associated with adverse outcomes including CS, PTB, congenital anomalies, preeclampsia, neonatal hypoglycaemia, macrosomia, NICU admission, stillbirth, Apgar score, LGA, IOL, RDS and miscarriage, which are amongst the most common maternal and fetal adverse outcomes reported in the literature (Negrato et al., 2012). A few studies showed that CS (Capobianco et al., 2020; Hyari et al., 2013), PTB (Barakat et al., 2010; Hamedi, 2005), congenital anomalies (Barakat et al., 2010), preeclampsia (Hamedi, 2005; Hyari et al., 2013), macrosomia (Van Zyl and Levitt, 2018), LGA (Hamedi, 2005; Shefali et al., 2006), IOL (Van Zyl and Levitt, 2018) and RDS (Barakat et al., 2010) were more common in GDM than pregestational diabetes. The association between GDM and congenital anomalies might be due to the misclassification of hyperglycaemia first diagnosed during pregnancy as GDM and not pre-gestational diabetes due to diagnostic criteria used prior to 2010. However, the majority of the studies reported that most adverse pregnancy outcomes were more common in pregestational diabetes compared to GDM (Abu-Heija et al., 2015; Barakat et al., 2010; El Mallah et al., 1997; Gualdani et al., 2021; Gui et al., 2014; Hamedi, 2005; Huddle et al., 1993; Peticca et al., 2009; Shand et al., 2008; Soepnel et al., 2019; Stogianni et al., 2019; Van Zyl and Levitt, 2018; Wang et al., 2019; Yamamoto et al., 2020) (chapter 4). A prospective cohort study was conducted to investigate the association between DIP and obstetric and perinatal outcomes in women attending

a tertiary hospital in Tshwane, South Africa. This study compared obstetric and perinatal outcomes in women with DIP and obesity who attended the high-risk antenatal clinic at Steve Biko Academic Hospital (SBAH), Pretoria, Gauteng, South Africa between May 2017 and March 2022. This study showed that pregestational diabetes was associated with high rates of PTB and that obesity was associated with the development of GDM, high rates of CS and low Apgar scores at 5 minutes. This study suggests that adequate glycaemic control and weight loss prior to pregnancymay help to reduce the risk of adverse pregnancy outcomes, similar to previous studies (Mahmood Buhary et al., 2016; Schummers et al., 2015; Temple et al., 2002; Tobias et al., 2011; Yi et al., 2015).

The second objective (chapter 6) was to investigate the association between maternal serum biochemical markers, DIP, and neonatal birth outcomes. Maternal adiponectin, leptin and SHBG were reported to be associated with pregnancy complications (Lomakova et al., 2022; Manderson et al., 2003; Spencer et al., 2005). However, limited studies have investigated the association between these hormones and neonatal outcomes in pregnant South African women. The study explored the relationship between maternal adiponectin, leptin and SHBG assessed at study entry and their potential impact on adverse pregnancy outcomes in South African women with DIP. The study demonstrated that low maternal serum leptin levels were associated with LGA, neonate birthweight, and PTB. These adverse pregnancy outcomes are associated with an increased risk of long-term cardiometabolic disorders (“Global Week for Action on NCDs | Figo,” n.d.; Silverberg et al., 2018; Tanz et al., 2017). Low levels of SHBG were correlated with birthweight ≥ 4 kg. Neonatal macrosomia is associated with an increased risk of short-term complications such as CS, perineal trauma and shoulder dystocia (Araujo Júnior et al., 2017) and also increase the risk of childhood obesity and hypertension, and diabetes in adulthood (Scifres, 2021). Similar to previous studies (Manderson et al., 2003; Morisset et al., 2011; Ogein et al., 2023; Xargay-Torrent et al., 2018), these results suggest that differentially expressed biomarkers during maternal

diabetes and obesity are associated with neonatal outcomes and the identification of dysregulated maternal biomarkers associated with adverse birth outcomes may aid in developing intervention strategies to improve child health.

The third objective (chapter 7) was to investigate the association between miRNAs and glucose control and perinatal outcomes. MiRNAs play an important role in regulating metabolic and developmental processes during pregnancy (Krützfeldt and Stoffel, 2006; Sayed and Abdellatif, 2011) and dysregulated levels have been associated with neonatal outcomes (Hromadnikova et al., 2022; Kochhar et al., 2022; Mayor-Lynn et al., 2011; Yao et al., 2024). No studies have investigated the association between miRNAs and neonatal outcomes in South Africa. This study identified maternal serum miRNAs associated with adverse pregnancy outcomes in a high-risk population of pregnant South African women. The increased expression of maternal miRNAs 124-3p, 128-3p, 20a-5p, and 210-3p were associated with SGA. Moreover, the increased expression of miR-210-3p was associated with a birth weight ≥ 4 kg. Certain miRNAs are involved in multiple biological mechanisms and associated with multiple disorders, which might be the case with miR-210. Additionally, miR-210 might be associated with common gene pathways of growth such as the PI3K-Akt signaling pathway which is involved in survival and growth in response to extracellular signals (Kochhar et al., 2022). Additionally, this study showed that the expression of maternal miRNAs 222-3p and 30d-5p were decreased in women with poor glycaemic control throughout pregnancy. MiR-124-3p has been associated with β-cell dysfunction (Jiang et al., 2021), miR-128-3p has been associated with insulin resistance (Wang et al., 2019), miR-20a-5p and miR-210-3p have been associated with T2DM and GDM (Cao et al., 2017; Chen et al., 2022; Katayama et al., 2018; Pheiffer et al., 2018). These findings suggest that these miRNAs can serve as potential biomarkers of pregnancy complications and adverse neonatal outcomes in our population.

The fourth objective (chapter 8) was the develop a questionnaire to assess diabetes knowledge in South African women with GDM. Adequate glycaemic control in women with GDM is critical to reduce pregnancy complications and prevent adverse outcomes (González-Quintero et al., 2007; Yu et al., 2014). Additionally, a woman's understanding of the essential factors necessary to achieve glycaemic targets is important and several studies have associated diabetes knowledge with glycaemic control (Hussain et al., 2015; Martis et al., 2018; Shams et al., 2016; Worku et al., 2015). Despite the importance of GDM knowledge, there is a scarcity of studies that have investigated the understanding of DIP among women with GDM in South Africa. This study developed a questionnaire to assess diabetes knowledge in South African women with GDM. Knowledge of GDM, nutrition, physical activity and blood glucose management were also assessed. The findings of this study present a comprehensive tool for evaluating the knowledge of diabetes in pregnant women with GDM in South Africa. However, additional refinements and validation are necessary. This is the first study in South Africa to develop a questionnaire to assess diabetes knowledge in women with GDM. These results suggest that this questionnaire can be useful in identifying knowledge gaps in pregnant women with GDM. It can help in enhancing education programs and developing interventions to improve glucose management and pregnancy outcomes in women with GDM. Studies have highlighted that these developed questionnaires maybe used to effectively determine knowledge gaps, patients' informational needs and development or enhancement of education programs aimed at improving health literacy, self-management (Carolan-Olah and Vasilevski, 2021; Hussain et al., 2015) and disease complications (Mashige et al., 2008). A good understanding of GDM, nutrition, physical activity and self-management may result in improved glucose levels (de Barros et al., 2010; Klonoff, 2012), reduce the need for insulin (Moses et al., 2009) and subsequently reduction adverse outcomes.

9.2. Integration and synthesis

Collectively, our results showed that DIP is associated with adverse outcomes. Furthermore, not only pregestational diabetes but also obesity is associated with adverse outcomes. The independent negative association between obesity and diabetes and pregnancy outcomes has been established, with the combination of both further increasing the risk of complications (Roman et al., 2011; Rosenberg et al., 2005; Wahabi et al., 2014; Yogev and Visser, 2009). Consistent with previous studies (Gualdani et al., 2021; Peticca et al., 2009; Van Zyl and Levitt, 2018), pregestational diabetes was found to be associated with PTB. However, this correlation could be attributed to the clinical decision for iatrogenic preterm delivery in an effort to prevent adverse outcomes that may occur with prolonging the pregnancy in pregnancies with poor glycaemic control or additional complications. The optimal timing of delivery is still uncertain; however, the goal is to delay delivery to minimize neonatal adverse outcomes while delivering sufficiently early to avoid significant risks for fetal complications such as macrosomia, shoulder dystocia and stillbirth (Thung and Landon, 2013). To date, there is a lack of definitive evidence and the timing of delivery is complex. Therefore, the appropriate clinical management should be customized according to the patient's clinical condition (Maso et al., 2014). Some studies have recommended that women with pregestational diabetes with suboptimal glycaemic control deliver their baby early, before 38.5 weeks' gestation in order to reduce the risk of complications (Berger and Melamed, 2014; Graves, 2007; Thung and Landon, 2013). However, it is important to consider various factors such as clinical characteristics of the pregnancy, maternal health and expected neonatal outcomes when deciding the optimal gestational age for delivery (Catalano and Sacks, 2011). Additionally, our experimental findings suggest that levels of maternal leptin, SHBG and miRNAs 124-3p, 128-3p, 20a-5p, 210-3p, 222-3p and 30d-5p are dysregulated in women with DIP who experienced adverse pregnancy outcomes. Even though adiponectin was found to be associated with obesity and DIP, adiponectin levels were not associated with neonatal birth outcomes in our South African population. Therefore, larger studies are needed to confirm its

reliability as a biomarker of pregnancy outcomes in our population. Additionally, our ROC analysis suggests that maternal leptin and SHBG alone may not be reliable predictors of neonatal outcomes. Furthermore, individual miRNAs rather than the combination might be better predictors of neonatal birth outcomes in South African women with DIP. Although miRNA panels have been shown to have better predictability than single miRNAs, there are individual miRNAs that are specific to certain tissues and cells and can exhibit limited expression in particular organs (Chorley et al., 2021). This might be the reason why single miRNAs demonstrate good predictive ability in our population. The prospective monitoring of biochemical and epigenetic markers throughout pregnancy may provide very important additional information. It will allow the assessment of how factors such as gestational age, gestational glycaemic control, gestational weight gain, and various treatment options impact on the levels of these biomarkers and, in turn, how these factors and changes in biomarkers influence both maternal and fetal outcomes. This study highlights the strengths and challenges of using biochemical and molecular biomarkers to predict adverse pregnancy outcomes in high-risk pregnant women in South Africa. Strengths include minimal invasion, easy extraction, stability, sensitivity, and specificity while challenges include the lack of an ideal normalization strategy and time of collection. The study also emphasizes the need for further research to explore the candidacy of these biomarkers in identifying potential risks in South Africa and globally.

9.3. Novelty and significance of the study

To our knowledge, this is the first study to investigate the association between adiponectin, leptin, SHBG and birth outcomes and between miRNAs, glycaemic control and birth outcomes in South African women with DIP. Additionally, this is the first study to develop a tool to assess diabetes knowledge in South African women with GDM. Adverse pregnancy outcomes are associated with long-term risk for cardiometabolic disorders, which emphasises the need for candidate

biomarkers for glycaemic control and adverse pregnancy outcomes in high-risk pregnant women. There is limited human studies on the association between maternal miRNAs 124-3p and fetal growth (Di Pietro et al., 2018; Yao et al., 2024). Furthermore, miR-222-3p and miR-30d-5p were shown to be associated with glycaemic control in women with DIP despite limited availability of glycaemic control data in our population. Therefore, the associations that were observed in our study provide new insights into the potential of these biochemical and molecular markers as biomarkers for birth outcomes.

Biochemical and molecular biomarkers in maternal circulation associated with neonatal outcomes may offer potential as biomarkers obtained in the early stages of pregnancy to identify women at risk of adverse pregnancy outcomes (Barchitta et al., 2017; Farias et al., 2017; Lomakova et al., 2022; Tsochandaridis et al., 2015). This will help initiate intervention strategies early in pregnancy to mitigate the risk of short- and long-term adverse outcomes for both the mother and child in the South African population. This will further reduce the vicious cycle of cardiometabolic diseases in our population and reduce the burden on the health system. Glucose self-monitoring is currently used to evaluate glycaemic control during pregnancy. However, its effectiveness is highly dependent on patient compliance (Cosson et al., 2017) and can be expensive if there is a high burden of disease (Lombard, 2011). Therefore, these biomarkers offer simple, accessible, and affordable tests for glucose monitoring that are applicable to low to middle-income countries such as South Africa. MiRNAs can also be detected in the urine of women with DIP (Herrera-Van Oostdam et al., 2020), therefore, this may offer non-invasive methods for monitoring glycaemic control and predicting adverse pregnancy outcomes.

Additionally, this study successfully developed a reliable and all-inclusive tool for assessing diabetes knowledge in pregnant women with GDM in South Africa. The questionnaire can be helpful in pinpointing areas where pregnant women with GDM lack knowledge, which can aid in

enhancing education programs and developing interventions to improve glucose management, as well as improve pregnancy outcomes in women with GDM.

9.4. Strengths and limitations

One of the strengths of our study is its pragmatic approach. We recruited pregnant women from the surrounding communities who were attending the antenatal high-risk clinic under standard routine clinical practices in a primary healthcare setting. This increased the potential for biomarker discovery in realistic situations. Compared to studies that have investigated association between miR-210-3p, fetal growth and birth weight in 108 children (Marzano et al., 2018), 100 mothers (Rodosthenous et al., 2017), 80 placental samples (Kochhar et al., 2022), our study had a larger sample size of fetal growth (n=109) and birth weight (n=171). This study was also able to determine the predictive ability of these dysregulated miRNAs.

The study also had some limitations, including restricting the study to only HIV-negative women. HIV infection has been shown to modify the expression of certain miRNAs in women with DIP (Pheiffer et al., 2019). The small sample size and restriction to Black ethnicity limits the generalisability of our findings to other populations. Additionally, recruitment was done at different time points in gestation although at < 28 weeks gestations, which might affect the concentrations of biomarkers as they change with advancing gestation (Fuglsang et al., 2006; Luo et al., 2021). Physical activity and diet which are widely reported to influence miRNA expression (Improta Caria et al., 2018; Léniz et al., 2021; Valerio et al., 2022) were not considered for our study and could confound our analysis. During the interview of the questionnaire testing, it was noted that a few participants had not been able to attend their dietician appointments, which could have limited their knowledge of nutrition. Additionally, despite English being the primary language at SBAH and efforts being made to ensure that patients understood the questions, it is important to recognize that language barriers could potentially impact the accuracy of the responses.

9.5. Recommendations and future work

Longitudinal studies in a larger sample that includes both HIV-negative and positive multi-ethnic pregnant women are required to explore the candidacy of dysregulated adiponectin, leptin, SHBG and miRNAs as potential biomarkers for glycaemic control and adverse neonatal outcomes in this high-risk population. South Africa has high rates of HIV, especially in women of reproductive age (Woldesenbet et al., 2020), therefore, evaluating these potential biomarkers in the HIV-positive population will help mitigate the added risk of adverse pregnancy outcomes posed by the infection. We recommend that future studies monitor biochemical and epigenetic markers throughout pregnancy to understand how factors such as gestational age, glycaemic control, weight gain, and treatment options affect these markers and pregnancy outcomes. MiRNAs may be influenced by interactions between genes and the environment. Therefore, we recommend investigating potential biomarkers in various ethnicities and populations to identify robust markers that can be applicable both locally and globally. Furthermore, study participants must be recruited at the same GA to reduce the effect of GA on biomarker concentrations.

Additionally, the study only covers the development of the questionnaire, meaning that a thorough validation process to assess construct validity, internal consistency, and test-retest reliability is highly recommended in the future. Additionally, it is crucial to evaluate the level of knowledge and expertise of healthcare workers, who are responsible for managing GDM and providing continuous education and support.

9.6. Impact on the public health system

Our study adds to the growing body of evidence supporting the use of adipokines, sex hormones and miRNAs as biomarkers for glycaemic control and neonatal birth outcomes. However, further

validations in larger sample sizes and multi-ethnic populations are required to confirm their clinical candidacy. Adverse pregnancy outcomes perpetuate the vicious cycle of cardiometabolic diseases, specifically diabetes and obesity in our populations. Therefore, the integration of these biomarkers into the clinical setting may help improve the monitoring and management of pregnancy complications and reduce short- and long-term risk. This will further reduce the burden of non-communicable diseases in an already resource-limited healthcare system. The field of molecular biology and laboratory technologies is advancing quickly, which brings hope that advanced biomarkers may become cheaper, easier to use and clinically feasible. This could lead to the development of quick, cost-effective, point-of-care test that could accurately identify women who are at high risk of adverse pregnancy outcomes. Although there are new biomarkers that show promise, health systems are still hesitant to move away from their standard procedures, which makes it difficult to translate and implement these new biomarkers in a clinical setting. As a result, researchers, clinicians and healthcare personnel must work together closely to integrate and implement potential biomarkers into clinical practice.

APPENDICES

APPENDIX 1: PUBLICATIONS

International Journal of
Environmental Research and Public Health

MDPI

Systematic Review

A Systematic Review to Compare Adverse Pregnancy Outcomes in Women with Pregestational Diabetes and Gestational Diabetes

Nompumelelo Malaza [1,2], Matladi Masete [1,2], Sumaiya Adam [2,3], Stephanie Dias [1], Thembeka Nyawo [1,4] and Carmen Pheiffer [1,2,4,*]

1 Biomedical Research and Innovation Platform (BRIP), South African Medical Research Council, Tygerberg, Cape Town 7505, South Africa
2 Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Pretoria, Pretoria 0001, South Africa
3 Diabetes Research Centre, Faculty of Health Sciences, University of Pretoria, Pretoria 0001, South Africa
4 Centre for Cardio-Metabolic Research in Africa, Division of Medical Physiology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Cape Town 7505, South Africa
* Correspondence: carmen.pheiffer@mrc.ac.za

Abstract: Pregestational type 1 (T1DM) and type 2 (T2DM) diabetes mellitus and gestational diabetes mellitus (GDM) are associated with increased rates of adverse maternal and neonatal outcomes. Adverse outcomes are more common in women with pregestational diabetes compared to GDM; although, conflicting results have been reported. This systematic review aims to summarise and synthesise studies that have compared adverse pregnancy outcomes in pregnancies complicated by pregestational diabetes and GDM. Three databases, Pubmed, EBSCOhost and Scopus were searched to identify studies that compared adverse outcomes in pregnancies complicated by pregestational T1DM and T2DM, and GDM. A total of 20 studies met the inclusion criteria and are included in this systematic review. Thirteen pregnancy outcomes including caesarean section, preterm birth, congenital anomalies, pre-eclampsia, neonatal hypoglycaemia, macrosomia, neonatal intensive care unit admission, stillbirth, Apgar score, large for gestational age, induction of labour, respiratory distress syndrome and miscarriages were compared. Findings from this review confirm that pregestational diabetes is associated with more frequent pregnancy complications than GDM. Taken together, this review highlights the risks posed by all types of maternal diabetes and the need to improve care and educate women on the importance of maintaining optimal glycaemic control to mitigate these risks.

Keywords: type 1 diabetes mellitus; type 2 diabetes mellitus; gestational diabetes mellitus; adverse outcomes; pregnancy

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1. Introduction

Globally, it is estimated that 21.1 million (16.7%) live births in 2021 were associated with maternal diabetes [1]. Of these, 10.6% were due to pregestational type 1 (T1DM) and type 2 (T2DM) diabetes mellitus, 9.1% were due to T1DM or T2DM first detected in pregnancy and 80.3% were due to gestational diabetes mellitus (GDM), a milder form of hyperglycaemia that develops in the second trimester [1]. Normal pregnancy is characterised by insulin resistance and requires an increased pancreatic β-cell response in order to maintain normoglycaemia [2]. GDM develops in women who are unable to mount a compensatory β-cells response, leading to hyperglycaemia. Increasing maternal age, along with increasing rates of obesity and diabetes worldwide, has led to rising rates of diabetes in pregnancy [1,3,4]. Obesity has been identified as a significant risk factor for maternal diabetes. A meta-analysis of 20 studies reported that women who were overweight (2.1-fold), obese (3.6-fold) or severely obese (8.6-fold) had a higher risk of developing diabetes compared to normal-weight pregnant women [5].

Maternal diabetes is associated with pregnancy complications and increased rates of adverse maternal and neonatal outcomes [6,7]. Short-term complications include macrosomia, large for gestational age (LGA), respiratory distress syndrome (RDS), neonatal hypoglycaemia, neonatal intensive care unit (NICU) admission, intrauterine growth restriction, congenital anomalies, preterm birth, pre-eclampsia, caesarean section (C/S) and preterm birth while in the long-term both mothers and their babies have an increased risk of metabolic disease [8–10]. Women with GDM have a ~7-fold increased risk of developing T2DM [11] and a ~4-fold increased risk of developing cardiovascular and coronary artery disease after pregnancy [12], while pregestational diabetes predisposes women to developing diabetes-related complications such as retinopathy and nephropathy or may accelerate the course of these complications if they already exist [4,7,13].

It is widely reported that all types of maternal diabetes are associated with pregnancy complications; although, adverse outcomes are more common in women with pregestational diabetes [14–18]. As adverse pregnancy outcomes are closely related to poor glycaemic control and the first trimester being a critical period for organogenesis, it is speculated that preconception hyperglycaemia and the longer time of exposure to hyperglycaemia in utero may contribute to the complications associated with pregestational diabetes [19].

Despite the large body of evidence that associates pregestational diabetes with more frequent adverse pregnancy outcomes than GDM [20–25], conflicting results have been reported [17,22,26–28]. This review aims to summarise and synthesise studies that have compared adverse pregnancy outcomes in pregnancies complicated by pregestational diabetes and GDM. Three databases, Pubmed, Scopus and EBSCOhost were searched to identify eligible studies, which were summarised and synthesised using systematic review methods. Commonly reported adverse pregnancy outcomes in literature [29] were selected for inclusion in this review. These include congenital anomalies, pre-eclampsia, neonatal hypoglycaemia, macrosomia, NICU admission, stillbirth, Apgar score, large for gestational age (LGA), induction of labour (IOL), respiratory distress syndrome (RDS) and miscarriages.

2. Methodology

This systematic review was conducted adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [30] (Supplementary Table S1).

2.1. Search Strategy and Study Selection

Three databases, Pubmed, Scopus and EBSCOhost were searched for studies reporting on maternal diabetes and pregnancy outcomes, published between January 1993 and December 2021. The search terms included "type 1 diabetes mellitus" or "type 1 diabetes" or "diabetes mellitus type 1" or "diabetes type 1" and "type 2 diabetes" or "type 2 diabetes mellitus" and "pre-gestational diabetes" or "gestational diabetes" or "diabetes in pregnancy" and "pregnancy complications" or "perinatal outcomes" or "adverse outcomes" or "pregnancy outcomes" and were adapted to each database. An experienced information scientist was consulted to ensure that the search terms were relevant and optimally arranged. References were managed in Zotero 5.0.96.2 (Corporation for Digital Scholarship, Vienna, VA, USA). After the removal of duplicate studies, two reviewers (NM and MM) independently screened articles for eligibility. Disagreements or uncertainties were resolved by discussion and consensus or in consultation with a third reviewer (CP). Additionally, references from selected articles were screened for potentially relevant articles.

2.2. Inclusion and Exclusion Criteria

Studies that compared pregnancy outcomes in one or two types of maternal diabetes only, those focusing on other forms of diabetes (maternal onset of diabetes in young (MODY), etc.), abstracts, review articles, letters, case reports, intervention studies and those not written in English, were excluded. Review articles were screened to identify eligible

studies that may have been missed using our search strategy. Studies reporting on adverse outcomes in pregnancies complicated by T1DM, T2DM and GDM were included. This systematic review was conducted to answer the following question:

Is there an association between maternal diabetes type and the frequency of adverse pregnancy outcomes?

This was achieved using the following:

Participants—Pregnant women with GDM;
Intervention—No intervention was used in this study;
Comparator—Pregnant women with pregestational T1DM and T2DM;
Outcome—Pregnancy outcomes.

2.3. Data Extraction and Quality Assessment

Data that were extracted and recorded included author details (name and date of publication), study details (aim and design, study period and GDM diagnostic criteria), sample size, characteristics of the population (ethnicity), country and pregnancy outcomes in the different diabetic groups. Two reviewers (NM and MM) independently appraised the study quality and risk of bias using the Newcastle–Ottawa Scale. The Newcastle–Ottawa Scale is used to assess the quality of non-randomized studies, such as case-control and cohort studies [31]. It assesses study quality based on three study parameters: selection, comparability, and outcomes, which are divided into eight specific items that can be scored as one or two points with points totalling nine (Supplementary Table S3). Disagreements between the two reviewers were resolved by consulting a third reviewer (CP). A study was classified as having a low risk of bias (7 to 9), moderate (5 to 6) or high risk of bias (1 to 4) based on the total score.

2.4. Definitions of Pregnancy Outcomes

Caesarean section refers to the delivery of a foetus through an incision in the abdominal wall and uterus [32]. Preterm birth is defined as birth before 37 weeks of gestation [33]. Congenital anomalies are defined as structural or functional anomalies that occur during intrauterine life as determined by the ultrasound scan and laboratory tests [23]. Preeclampsia is defined as hypertension (>140/90 mm Hg) and proteinuria (>0.3 g of protein in a 24 h urine collection) developing after 20 weeks of gestation [34]. Macrosomia is defined as giving birth to babies weighing > 4000 g [29]. Stillbirth is foetal death after 24 weeks of gestation or foetus > 500 g [35]. LGA is defined as birth weight > 90th percentile for age [36]. Neonatal hypoglycaemia is defined as a plasma glucose value <1.65 mmol/L in the first 24 h of life and <2.5 mmol/L onwards [37]. NICU admission refers to the admission of a newborn to an intensive care unit for specialised care due to a critical condition or illness [38]. Miscarriage refers to foetal death before 24 weeks of gestation or foetus < 500 g [39]. Induction of labour refers to the process that involves mechanical or surgical means to initiate uterine contractions [40]. The Apgar score is used to assess the well-being of a neonate at 1 min and 5 min after birth [41]. Respiratory distress syndrome is defined as the need to supplement oxygen to the neonate to maintain a saturation over 85% within the first 24 h after birth [42].

3. Results

3.1. Selected Studies

A total of 2164 studies were identified from the search strategy. An additional three articles were identified by reviewing the reference lists of relevant articles and reviews resulting in 2167 articles. After removing duplicates, 1958 article titles and abstracts were screened for eligible full-text articles. We excluded studies that compared one or two types of maternal diabetes only, interventional studies, those not written in English, review articles, letters, case reports and abstracts. A total of 20 studies, published between

January 1993 and December 2021, met the inclusion criteria and are discussed in this review (Figure 1).

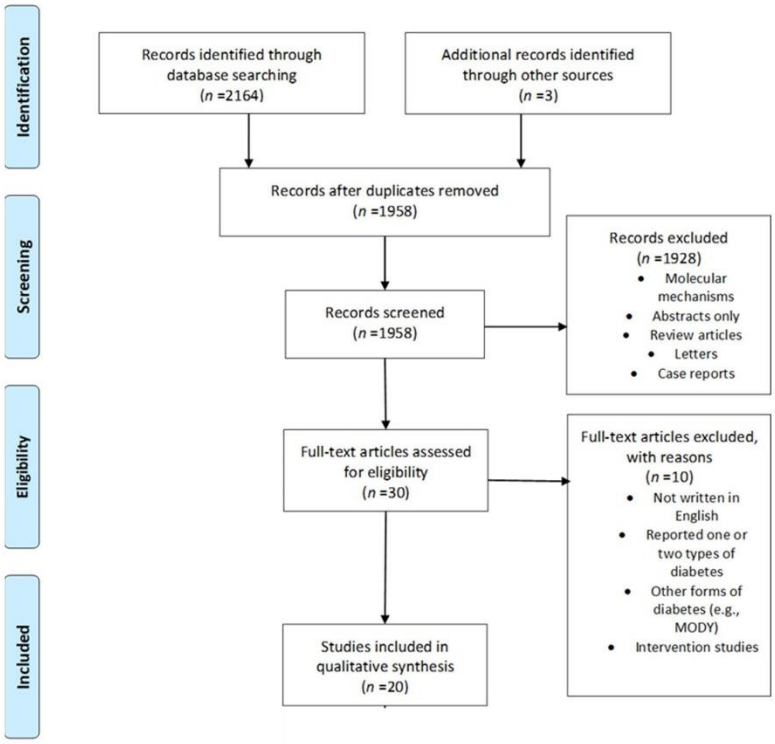

Figure 1. Flow diagram for the search criteria; MODY—maturity-onset diabetes of the young.

3.2. Characteristics of Included Studies

Twenty articles published between 1993 and 2021 were included in the review (n = 196,232 participants; Supplementary Table S2). These studies were conducted across five continents (Europe, Asia, North America, Africa and Australia). Sixteen studies were retrospective, two were prospective, one was cross-sectional and one was unspecified. Nine studies reported adverse outcomes for pregestational diabetes, combining data for T1DM and T2DM [16,20,22,25,27,43–46], while 11 studies reported data for T1DM and T2DM, separately [14,15,17,21,24,47–52]. These studies reported on various maternal and neonatal short-term pregnancy adverse outcomes, of which 13 are summarised in this review. These selected adverse outcomes are amongst the most common in the literature [29]. None of the studies investigated long-term maternal outcomes in women with T1DM, T2DM and GDM.

The studies in this review used different diagnostic criteria for GDM, which included the International Association of Diabetes and Pregnancy Study Groups Consensus Panel, 2010 (IADPSG; n = 2), American Diabetes Association (ADA; n = 2), National Diabetes Data Group (NDDG; n = 2), O'Sullivan and Mahan (n = 1), Spanish Group for Diabetes (n = 1), Australasian Diabetes in Pregnancy Society (ADIPS; n = 2) and World Health Organization 1998/1999 (n = 2). Five studies used institution-based diagnostic criteria, while three studies did not report which diagnostic criteria were used. Pregestational diabetes was determined through hospital records and/or by the medication taken by

patients. The studies were conducted in different populations, which included: Omani, Saudi, African, Non-Hispanic black, Australian, Asian, Middle Eastern, Indian, Caucasian and Hispanic. Many of the studies were retrospective and did not report the time of assessment of pregnancy outcomes. Twelve studies included in this review defined one or more of the adverse outcomes; however, definitions and/or cut-offs varied across studies, while eight studies did not define outcomes.

Congenital anomalies included cardiovascular, central nervous system, cleft lip and palate, trisomy 21, gastrointestinal, musculoskeletal, and urogenital anomalies/malformations and were referred to differently across studies, which included: congenital anomalies/malformations/abnormalities, birth defects, congenital defects, foetal anomalies/malformations, and neonatal deformities. For the purpose of this review, these were collectively referred to these as congenital anomalies. Moreover, the majority (92.31%) of the studies that reported on congenital anomalies reported the overall incidence and not the incidence of the individual congenital anomalies in their comparisons. Due to significant heterogeneity between studies and the low-quality assessment scores for a few studies, a meta-analysis was not performed, as this may lead to an inaccurate estimate of overall effect size [53].

3.3. Quality Assessment of Included Studies

The quality of the 20 studies included in this review ranged from unsatisfactory to very good with scores ranging from 4 to 7 and an average score of 5.5. Three studies scored unsatisfactory (4), seven studies scored fair (5), six studies scored good (6), and four studies scored very good (7) (Supplementary Table S4). The studies that rated good and very good were due to controlling for confounding factors, while studies that rated fair and unsatisfactory were affected by not controlling for confounders. The majority of the studies included in this review were retrospective and, therefore, were not able to control for confounders. Due to the narrative nature of this review, all studies were included for analysis despite their risk of bias rating.

3.4. Qualitative Synthesis

Of the nine studies that compared combined data for pregestational T1DM and T2DM combined with GDM, the most common adverse outcome reported was C/S (n = 7), followed by preterm birth (n = 7), congenital anomalies (n = 7), pre-eclampsia (n = 6), neonatal hypoglycaemia (n = 5), macrosomia (n = 4), NICU admission (n = 4), stillbirth (n = 4), Apgar score (n = 4), LGA (n = 3) RDS (n = 3) and IOL (n = 2). Of the eleven studies that separately compared pregestational T1DM and T2DM with GDM, the most common adverse outcome reported was C/S (n = 10), followed by preterm birth (n = 7), macrosomia (n = 7), congenital anomalies (n = 6), pre-eclampsia (n = 4), stillbirth (n = 4), neonatal hypoglycaemia (n = 3), IOL (n = 3), Apgar score (n = 3), LGA (n = 3), miscarriage (n = 2), NICU (n = 2) and RDS (n = 1). Certain studies subdivided GDM into true GDM (fasting glucose < 7 mmol/L and oral glucose tolerance test (OGTT) 2 h < 11.1 mmol/L) and overt GDM (fasting glucose $\geq$ 7 mmol/L or OGTT 2 h $\geq$ 11.1 mmol/L). For the purpose of this review, we focused on outcomes for true GDM.

C/S, preterm birth, and congenital anomalies were the most reported adverse outcomes, while the least reported outcomes were IOL, RDS and miscarriage. Other adverse outcomes reported included preeclampsia, neonatal hypoglycaemia, macrosomia, NICU admissions, stillbirths, LGA and Apgar scores. The majority of the adverse outcomes were higher in pregestational T1DM and T2DM compared to GDM. However, there were a few adverse outcomes that were more common in GDM compared to pregestational T1DM and/or T2DM (Table 1).

Table 1. The frequency of adverse pregnancy outcomes.

Adverse Outcome	Increased in Pregestational Diabetes	Increased in GDM	No Difference
Caesarean section	[15,17,20,21,24,27,43,46,48,50–52]	[22,47]	[25,44]
Preterm birth	[17,21,22,24,43–46,48,50–52]	[20,27]	
Congenital anomalies	[14,16,17,22,24,25,27,47]	[20]	[15,21,43,45]
Pre-eclampsia	[17,24,43,45,46,50]	[22,27]	[15,44]
Neonatal hypoglycaemia	[27,43,46,47,52]		[14,22,44]
Macrosomia	[15,20,22,24,44,50]	[17]	[14,21,43,48]
NICU admission	[14,20,43,45,46,52]		
Stillbirth	[17,24,44,46,47,49]		[20,45]
Apgar score	[20,21,24,48]		[43,44,46]
Large for gestational age	[46,48,52]	[25,27]	[21]
Induction of labour	[24,43,51]	[17]	[46]
Respiratory distress syndrome	[14,27,43]	[20]	
Miscarriage	[15,17]		

Caesarean section (C/S). Of the studies that compared pregestational diabetes (combined T1DM and T2DM) with GDM, four studies reported higher rates of C/S in pregestational diabetes compared to GDM [20,27,43,46], while similar rates were reported in two studies [25,44]. Hyari et al., 2013, reported slightly higher rates of C/S in women with GDM compared to pregestational diabetes [22]. Of the studies that compared pregestational T1DM and T2DM separately with GDM, six studies reported higher rates of C/S in T1DM and T2DM compared to GDM [17,21,48,50–52]. Al-Nemri reported higher rates of elective C/S in pregestational T1DM (25.0%) and T2DM (34.3%) compared to GDM (15.7%), but similar rates for emergency C/S [14]. Petticca et al., 2009, reported higher rates of C/S in pregestational T1DM (51.6%) compared to pregestational T2DM (38.0%) and GDM (38.0%), with the latter diabetes types showing similar rates of C/S [24]. Soepnel et al., 2018, reported higher rates of C/S in pregestational T2DM (78.4%) compared to T1DM (67.1%) and GDM (67.8%), with the latter showing similar rates [15]. In contrast, Huddle et al., 1993, reported a higher rate of C/S in GDM (56.0%) compared to pregestational T1DM (39.8%), but similar rates in GDM compared to pregestational T2DM (55.5%) [47]. Taken together, these results demonstrate that C/S is more common in women with pregestational T1DM and T2DM than in women with GDM.

Preterm birth. Of the studies that compared pregestational diabetes (combined T1DM and T2DM) with GDM, five studies reported higher rates of preterm birth in pregestational diabetes compared to GDM [22,43–46], while two studies reported higher rates in GDM compared to pregestational diabetes [20,27]. Of the studies that compared pregestational T1DM and T2DM separately with GDM, six studies reported higher rates of preterm birth in pregestational T1DM and T2DM compared to GDM [17,21,24,50–52]. Stogianni et al., 2019, reported higher rates of preterm birth in pregestational T2DM (46.0%) compared to pregestational T1DM (35.0%) and GDM (12.0%), and higher rates in pregestational T1DM compared to GDM [48]. These results show that preterm birth is more common in women with pregestational T1DM and T2DM than in women with GDM.

Congenital anomalies. Higher rates of congenital anomalies were reported in pregestational diabetes (combined T1DM and T2DM) compared to GDM in four studies [16,22,25,27], while Barakat et al., 2010, reported higher rates in GDM (8.9%) compared to pregestational diabetes (5.6%) [20]. In contrast, two studies reported no significant difference in the rates of congenital anomalies between pregestational diabetes and GDM [43,45]. When comparing

T1DM and T2DM separately with GDM, four studies reported higher rates of congenital anomalies in pregestational T1DM and T2DM compared to GDM [14,17,24,47]. Of these, two reported higher rates of congenital anomalies in pregestational T2DM compared to pregestational T1DM and GDM, and higher rates in pregestational T1DM compared to GDM [14,47]. In contrast, two studies reported no significant difference in rates of congenital anomalies between the three diabetic groups [15,21]. Although discrepant results are reported, the majority of studies showed that congenital anomalies are more common in neonates born to mothers with pregestational T1DM and T2DM than in neonates born to mothers with GDM.

Pre-eclampsia. Higher rates of pre-eclampsia were reported in pregestational diabetes (combined T1DM and T2DM) compared to GDM in three studies [43,45,46], while two studies reported higher rates in GDM compared to pregestational diabetes [22,27]. El Mallah et al., 1997, reported no difference in the rates of pre-eclampsia between pregestational diabetes (1.4%) and GDM (2.0%) [44]. Pre-eclampsia was also compared in pregnant women with pregestational T1DM and T2DM separately with GDM. Higher rates of pre-eclampsia were reported in pregestational T1DM compared to T2DM and GDM in three studies, with the latter occurring at similar rates [17,24,50]. Soepnel et al., 2019, reported no significant difference in the rates of pre-eclampsia across the three diabetic groups [15]. Taken together, pre-eclampsia is more common in women with pregestational T1DM and T2DM than GDM and more common in pregestational T1DM.

Neonatal hypoglycaemia. Three studies reported higher rates of neonatal hypoglycaemia in pregestational diabetes (combined T1DM and T2DM) compared to GDM [27,43,46], while two studies reported no difference in the rates of neonatal hypoglycaemia between pregestational diabetes and GDM [22,44]. When comparing neonatal hypoglycaemia between T1DM and T2DM separately with GDM, Yamamoto et al., 2020, reported higher rates in T1DM (27.5%) and T2DM (18.3%) compared to GDM (5.0%) [52] and Huddle at al., 1993, reported higher rates of neonatal hypoglycaemia in neonates born to mothers with pregestational T1DM (4.2%) and GDM (4.2%) compared to neonates born to mothers with pregestational T2DM (3.6%) [47]. However, Al-Nemri et al., 2018, reported no difference in the rates of neonatal hypoglycaemia across the three diabetic groups [14]. These results show that rates of neonatal hypoglycaemia are more common in neonates born to mothers with pregestational T1DM and T2DM compared to neonates born to mothers with GDM.

Macrosomia. Higher rates of macrosomia were reported in pregestational diabetes (combined T1DM and T2DM) compared to GDM in three studies) [20,22,44], while Abu-Heija et al., 2015, reported no significant difference in the rates of macrosomia between pregestational diabetes (10.3%) and GDM (4.9%) [43]. Macrosomia was also reported when comparing T1DM and T2DM separately with GDM. Two studies reported higher rates in T1DM and T2DM compared to GDM [15,50]. Peticca et al., 2009, reported higher rates of macrosomia in T1DM (17.2%) and GDM (12.2%) compared to T2DM (11.1%) [24], while Van Zyl and Levitt reported higher rates of macrosomia in GDM (9.2%) compared to pregestational T1DM (8.5%) and T2DM (8.2%) [17]. However, three studies reported no significant difference in the rates of macrosomia between the three diabetic groups [14,21,48]. Altogether, these studies indicate that macrosomia is more common in neonates born to mothers with pregestational diabetes T1DM and T2DM compared to GDM.

NICU admissions. When NICU admissions were compared between pregestational diabetes (combined T1DM and T2DM) and GDM, four studies reported higher rates of NICU admissions in pregestational diabetes compared to GDM [20,43,45,46]. NICU admissions were also reported when comparing T1DM and T2DM separately with GDM. Yamatoto et al., 2020, reported higher rates of NICU admissions in T1DM (55.5%) and T2DM (31.0%) compared to GDM (14.0%) [52], while A-Nemri et al., 2018, reported higher rates of NICU admissions in pregestational T1DM (66.7%) compared to pregestational T2DM (16.0%) and GDM (10.2%), with the latter showing similar rates [14]. These results demonstrate that NICU admissions are more common in neonates born to mothers

with pregestational diabetes T1DM and T2DM compared to neonates born to mothers with GDM.

Stillbirth. When stillbirth was compared between pregestational diabetes (combined T1DM and T2DM) and GDM, higher rates of stillbirth were reported in pregestational diabetes compared to GDM in two studies [44,46]. However, two studies reported no difference in the rates of stillbirths between pregestational diabetes and GDM [20,45]. When comparing T1DM and T2DM separately with GDM, higher rates of stillbirths were reported in pregestational T1DM and T2DM compared to GDM in three studies [17,24,49], while Huddle et al., 1993, reported higher rates in T2DM (4.7%) compared to T1DM (3.3%) and GDM (4.0%) with the latter occurring at a similar rate [47]. Altogether, these results demonstrate that stillbirths are more common in neonates born to mothers with pregestational T1DM and T2DM compared to neonates born to mothers with GDM.

Apgar score. Low Apgar scores (<7) were compared between pregestational diabetes (combined T1DM and T2DM) and GDM. Barakat et al., 2010, reported higher rates of low Apgar scores in pregestational diabetes (24.1%) compared to GDM (22.1%) [20], while three studies reported no difference in the rates of low Apgar scores between pregestational diabetes and GDM [43,44,46]. Low Apgar scores were also reported when comparing T1DM and T2DM separately with GDM. Gualdani et al., 2021, reported lower Apgar scores in T1DM (5.4%) compared to T2DM (2.5%) and GDM (1.3%) [21], while two studies reported similar rates of low Apgar scores in T1DM and T2DM, although higher than GDM [24,48]. These findings indicate that low Apgar scores present at a similar rate in neonates across the three diabetic groups.

Large for gestational age (LGA). Two studies reported higher rates of LGA in GDM compared to pregestational diabetes (combined T1DM and T2DM) [25,27], while Shand et al., reported higher rates of LGA in pregestational diabetes (35.0%) compared to GDM (15.9%) [46]. LGA was also reported when comparing T1DM and T2DM separately with GDM. Two studies reported higher rates of LGA in T1DM and T2DM compared to GDM [48,52]. In contrast, Gualdani et al., 2021, reported no significant difference between the three diabetic groups [21]. Altogether, the results show that LGA is more common in neonates born to mothers with pregestational T1DM and T2DM compared to neonates born to mothers with GDM.

Induction of labour (IOL). Two studies reported no difference in the rates of IOL between pregestational diabetes and GDM [43,46]. In the comparison of T1DM and T2DM separately with GDM, López-de-Andrés et al., 2020, reported higher rates of IOL in pregestational T1DM (29.6%) and T2DM (30.4%) compared to GDM (22.6%) [51], while Peticca et al., 2009, reported higher rates of IOL in T1DM (44.7%) and GDM (38.3%) compared to T2DM (36.6%) [24]. In contrast, Van Zyl and Levitt, 2018, reported higher rates of IOL in GDM (30.0%) compared to T1DM (11.8%) and T2DM (18.6%) [17]. These results show that IOL occurs at similar rates in women with pregestational T1DM and T2DM and GDM.

Respiratory distress syndrome (RDS). When comparing pregestational diabetes (combined T1DM and T2DM) and GDM, higher rates of RDS were reported in pregestational diabetes compared to GDM in two studies [27,43], while Barakat et al. reported higher rates in GDM (2.8%) compared to pregestational diabetes (1.6%) [20]. In the comparison of T1DM and T2DM separately with GDM, Al-Nemri et al. reported higher rates of RDS in T1DM (44.4%) compared to T2DM (13.9%) and GDM (13.5%) with similar rates occurring in the latter [14]. These results demonstrate that RDS is more common in neonates born to mothers with pregestational T1DM and T2DM than in neonates born to mothers with GDM.

Miscarriage. When comparing T1DM and T2DM separately with GDM, higher rates of miscarriage were reported in T1DM compared to T2DM and GDM in two studies [15,17]. These results indicate that miscarriages are more common during pregestational T1DM compared to pregestational T2DM and GDM.

4. Discussion

Adverse outcomes associated with maternal diabetes are reported to be more common in women with pregestational diabetes compared to GDM; although, conflicting results have been reported [14–17,27,47,48]. In this systematic review, we summarise and synthesise studies that have compared adverse pregnancy outcomes in pregnancies complicated by pregestational diabetes and GDM. Findings from this review confirm that both pregestational diabetes and GDM are associated with pregnancy complications including C/S, preterm birth, congenital anomalies, pre-eclampsia, neonatal hypoglycaemia, macrosomia, NICU admission, stillbirth, Apgar score, LGA, IOL, RDS and miscarriage. Although conflicting results were reported in a few studies, the majority of studies report that adverse outcomes are more common in pregnancies complicated by pregestational diabetes than GDM. This review did not identify studies that compared long-term adverse outcomes in women with pregestational diabetes and GDM.

Thirteen perinatal complications, C/S, preterm birth, congenital anomalies, pre-eclampsia, neonatal hypoglycaemia, macrosomia, NICU admission, stillbirth, Apgar score, LGA, IOL, RDS and miscarriage, which are amongst the most common maternal and foetal adverse outcomes reported in the literature, were compared in this review. C/S was the most common adverse outcome reported. Although it is accepted that not all C/S may be considered an adverse pregnancy outcome [54], it is often recommended by health care providers as a strategy to reduce the risk of perinatal complications associated with maternal diabetes [55,56]. Preterm birth is defined as birth before 37 completed weeks of gestation [33] and is the leading cause of mortality in children younger than five years. Infants who survive preterm birth often present with poor neurodevelopment and cognitive disabilities [57] and behavioural and emotional difficulties [58]. Congenital anomalies, which refer to structural or functional malformations that occur during intrauterine life, are associated with hyperglycaemia during the period of organogenesis that occurs in the first trimester of pregnancy. Maternal hyperglycaemia leads to the increased production of reactive oxygen species (ROS), resulting in DNA and membrane damage and the subsequent induction of apoptosis, causing malformations in major organs of the developing foetus [23]. Pre-eclampsia is characterised by hypertension, which usually develops after 20 weeks of gestation [34] and is considered the leading cause of maternal morbidity and mortality among women who have diabetes [59]. The condition is thought to occur due to endothelial dysfunction, dyslipidaemia, and inflammation associated with diabetes [60,61].

Macrosomia refers to giving birth to babies weighing more than 4 kg and is considered the most common adverse outcome associated with maternal diabetes [6,29]. The condition is thought to occur due to increased placental transport of glucose and other nutrients from the mother to the foetus, resulting in accelerated growth [7,62]. Macrosomia is associated with several complications including, neonatal hypoglycaemia and premature birth [55,56]. Abnormal placental supply of nutrients results in abnormal foetal growth, including foetal growth restriction (FGR) and foetal overgrowth, and is associated with increased neonatal mortality. LGA refers to a foetus that weighs in >90th percentile of the birth chart [36]. LGA is associated with an increased rate of C/S and neonatal hypoglycaemia, including a longer hospital stay in mothers with diabetes [63,64]. Neonatal hypoglycaemia is defined as a plasma glucose value < 1.65 mmol/L in the first 24 h of life and <2.5 mmol/L onwards [37]. Hypoglycaemia in neonates occurs due to continuous placental transport of glucose and other nutrients from the mother to the foetus, which results in hyperinsulinaemia, which leads to a fall in glucose levels during and post-delivery [65,66]. Hyperinsulinism is very common in infants of mothers with diabetes [37]. Hyperinsulinaemia in the foetus may also lead to RDS at birth. RDS is defined by the need to supplement neonatal oxygen to maintain a saturation of over 85% within the first 24 h after birth and also radiological features [42]. The development of RDS has been attributed to the inhibitory effects of insulin on the expression of surfactant proteins A and B in lung epithelial cells, resulting in decreased production of surfactants and delayed pulmonary maturation [28,42,67].

Placental abnormalities and congenital malformations are major risk factors for stillbirth and neonatal death, which represent the extreme end of the spectrum of complications in diabetic pregnancies [49]. Stillbirth is defined as the death of a foetus at ≥22 weeks of gestation or birth weight of ≥500 g [35]. Unexplained stillbirths at term in maternal diabetes are attributed to maternal hyperglycaemia and foetal hyperinsulinaemia, foetal hypoxia and acidaemia and cardiomyopathy due to glycogen deposition in the myocardium [68,69]. Maternal diabetes has also been associated with an increased risk of miscarriages and habitual abortions [70,71]. Animal models have shown that maternal diabetes affects pre-implantation in the embryo developmental stages. In vivo and in vitro studies show that hyperglycaemia leads to an overexpression of *Bax,* (Bcl-2-associated X), which is a death-promoting protein associated with increased apoptotic morphological changes and is reversed by insulin [72]. In women with diabetes, IOL is recommended to minimise birth complications associated with macrosomia and the risk for stillbirth [73]. A Cochrane review by Boulvain et al., 2001, showed that induction of labour lowered the prevalence of macrosomia without increasing the risk of caesarean section [74].

Furthermore, poor glucose control in the third trimester may lead to perinatal asphyxia and low Apgar scores [75,76]. Apgar score is a clinical method used to assess the wellbeing of a neonate at 1 min and 5 min after birth. The Apgar score assesses elements such as skin colour/tone, heart rate, reflexes, muscle tone and respiration [41]. Apgar scores may predict long-term neurological disabilities in infants [77,78]. Foetal complications are associated with increased admissions to the neonatal intensive care unit (NICU), which is therefore often used as an indicator of adverse pregnancy outcomes [77,78].

Limitations of the studies included in this review may hinder our ability to draw significant conclusions. There was heterogeneity across studies in terms of population characteristics, the diagnostic criteria used, the definitions used for pregnancy outcomes (e.g., preterm birth, Apgar scores) and different medication regimens (diet, metformin, and insulin). It has been widely reported that ethnicity [79,80], advanced maternal age [81], diet [82], socioeconomic status [83] and medication regimen [48] influence pregnancy outcomes. Furthermore, the majority of studies were retrospective and were dependent on the accuracy of medical records and databases, which may negatively affect study accuracy [84]. Many of the included studies had a poor risk of bias scores, which were mainly affected by the lack of accounting for confounding factors, which may have affected the accuracy of study findings. Excluding studies with unsatisfactory ratings from the analysis, did not affect the overall conclusions of the review, and similar to studies with a satisfactory and high risk of bias scores, showed that adverse outcomes were more common in pregestational T1DM and T2DM compared to GDM. Therefore, all the studies were included as the data were deemed valuable for the purpose of this narrative review.

Despite the inconclusive results from this review, it is evident that pregestational diabetes poses a greater risk for pregnancy complications than GDM and emphasises the importance of maintaining optimal glucose control during the preconception period. Maternal metabolic factors may program physiological adaptation to pregnancy, thereby affecting pregnancy outcomes [85,86]. The importance of preconception health is increasingly acknowledged as a key determinant of pregnancy success, with increasing attention shifting to preconception intervention [86]. A population-based study in Canada reported that a 10% weight reduction in the preconception period decreased the risk of developing GDM, pre-eclampsia, preterm delivery, macrosomia and stillbirth [87]. Another study showed that women who underwent bariatric surgery prior to conception had a lower risk of developing GDM, hypertensive disorders and macrosomia [88]. Furthermore, increased physical activity before conception is associated with a lower risk of GDM [89] and pre-eclampsia [90]. Taken together, these studies demonstrate a strong relationship between preconception health and pregnancy outcomes. The mechanisms that underlie these links are not known, but are likely to involve an array of genetic, epigenetic and environmental factors that interact to affect physiological adaptation during pregnancy.

While acknowledging the importance of preconception health and optimal glucose control during pregnancy, the importance of GDM prevention should not be underestimated. As with pregestational diabetes, albeit less common, GDM was also associated with several adverse pregnancy outcomes. Importantly, these complications can be avoided by preventing the development of GDM. During pregnancy, lifestyle modifications that include diet and physical activity have been shown to prevent GDM [89,91–93]. Although not addressed in this review, recent studies have highlighted the occurrence of early-onset GDM, defined as GDM that can be detected in women before 24 weeks of gestation [94]. These women have an increased risk of adverse pregnancy outcomes compared to women with "normal" GDM diagnosed at 24–26 weeks [95,96], and highlights the need to diagnose early pregnancy glycaemia as recently reported by McIntyre et al. [97].

5. Future Perspectives

The majority of studies included in this review were retrospective. In addition, we did not identify articles that investigated long-term adverse outcomes in women with pregestational T1DM and T2DM, and GDM. Therefore, there is a need for prospective, longitudinal studies in the future to more accurately compare short- and long-term adverse pregnancy outcomes across diabetes types. Preterm birth was one of the most common adverse outcomes reported in this review. The optimal timing of delivery for women with pregestational diabetes is not known due to a lack of published trials [98]; therefore, there is a need for more studies to determine the optimal time to deliver babies born to mothers with diabetes as this will reduce the complications associated with preterm delivery.

6. Conclusions

In conclusion, the findings from this review confirm that adverse pregnancy outcomes are more common in women with pregestational diabetes compared to women with GDM. These findings highlight the importance of preconception health and the need to educate women of reproductive age who have diabetes or who are at risk of diabetes about the importance of pre-pregnancy care and maintaining good glycaemic control to improve pregnancy health and reduce the risk of adverse pregnancy outcomes. Another important finding of the review is the high rates of adverse outcomes observed in women with GDM, and the need for intervention strategies to prevent the development of GDM.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph191710846/s1, Table S1: PRISMA guidelines, Table S2: Studies correlating types of diabetes in pregnancy and adverse outcomes, Table S3: Newcastle–Ottawa Scale, Table S4: Risk of bias scores.

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RESEARCH

Comparison of obstetric and perinatal outcomes in women with diabetes at Steve Biko Academic Hospital

N Malaza,[1,2] MSc (Med); **C Pheiffer,**[1,2] PhD (MPH); **S Dias,**[1] PhD; **S Adam,**[2,3] MB ChB, FCOG (SA), MMed (O&G), PhD (O&G)

[1] *Biomedical Research and Innovation Platform (BRIP), South African Medical Research Council, Tygerberg, South Africa*
[2] *Department of Obstetrics and Gynaecology, School of Medicine, Faculty of Health Sciences, University of Pretoria, South Africa*
[3] *Diabetes Research Centre, Faculty of Health Sciences, University of Pretoria, South Africa*

Corresponding author: *S Adam (sumaiya.adam@up.ac.za)*

Background. Diabetes and obesity in pregnancy have been associated with increased rates of adverse maternal and neonatal outcomes compared with women with normoglycaemia and normal weight.
Objective. To investigate the effect of diabetes and pre-pregnancy obesity on obstetric and perinatal outcomes.
Methods. This study included women with pregestational diabetes types 1 (T1DM) and 2 (T2DM), gestational diabetes (GDM) and normoglycaemia, who received care at the Steve Biko Academic Hospital antenatal clinic between 2017 and 2022. The women were followed up until delivery. Data collected included obstetric history and care, diabetes, obstetric and perinatal outcomes.
Results. A total of 183 women were recruited: 13 (7.1%) with T1DM, 65 (35.5%) with T2DM, 39 (21.3%) with GDM and 66 (36.1%) normoglycaemic controls. Women with T2DM and GDM were older ($p<0.01$) and more likely to have a history of chronic hypertension ($p=0.025$) compared with controls. Women with GDM were more likely to be obese than their T1DM counterparts ($p=0.036$). T1DM and T2DM were associated with higher rates of preterm delivery than controls ($p=0.002$). The frequency of GDM was significantly higher in women with obesity ($p=0.039$). The frequency of caesarean section before the onset of labour was higher in women with a weight ≥80 kg compared with women with a weight <80 kg ($p=0.015$).
Conclusion. Diabetes in pregnancy is associated with adverse obstetric and perinatal outcomes. Therefore, adequate glucose control should be accompanied by preconceptual weight optimisation to reduce adverse outcomes during pregnancy.

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Diabetes mellitus is a common pregnancy complication that poses a serious health threat to maternal and child health.[1] Diabetes in pregnancy (DIP) can be classified as pregestational type 1 (T1DM) or 2 (T2DM) diabetes; T1DM or T2DM first diagnosed during pregnancy; or gestational diabetes mellitus (GDM), a milder form of carbohydrate intolerance that first develops during pregnancy, with glucose homeostasis usually restored within 6 weeks after delivery. Globally, ~16.7% (21.1 million) of live births are affected by DIP. Among these, pregestational T1DM and T2DM account for 10.6% of cases, T1DM and T2DM first detected in pregnancy account for 9.1% of cases and GDM accounts for 80.3% of cases.[1] South Africa (SA) is a low-to-middle-income country (LMIC) with high rates of DIP. Recent studies reported that the prevalence of GDM varied from 9.1% to 25.6%, depending on the diagnostic criteria.[2]

All types of DIP are associated with an increased risk of short- and long-term adverse outcomes for mother and child (Table 1), especially when glycaemic control is suboptimal. The severity and frequency of these adverse outcomes are higher in women with pregestational diabetes compared with GDM. Achieving adequate glycaemic control and appropriate gestational weight gain is critical to prevent pregnancy complications and adverse outcomes.[3]

Obesity is considered a major risk factor for DIP, with an increasing number of epidemiological studies supporting this association.[4] In addition, obesity has also been reported to independently increase the risk of maternal and fetal adverse outcomes.[5] In SA, the estimated prevalence of obesity in women of reproductive age is 35.2%,[6] highlighting the potential negative effects of obesity on both maternal and child health. Studies have shown that an increase in both maternal weight and body mass index (BMI) before and during pregnancy is associated with adverse pregnancy outcomes.[7-9] However, in resource-limited settings where measuring weight is more practical, the use of maternal weight instead of BMI to assess the risk of adverse outcomes related to weight during pregnancy might be a more viable option. This is substantiated by a study conducted by Wolfe *et al.*[10]

This study aimed to investigate the effect of DIP and obesity on obstetric and perinatal outcomes in women attending the diabetic antenatal clinic at a tertiary hospital in Tshwane, South Africa.

Table 1. Short- and long-term outcomes of DIP

Short-term	Long-term
Maternal	
Pre-eclampsia, preterm birth, CS, miscarriage,* obstructed labour, PPH	Worsening of diabetic retinopathy and nephropathy*, diabetes mellitus, cardiovascular diseases
Neonatal	
Congenital anomalies,* respiratory distress syndrome, jaundice, neonatal hypoglycaemia macrosomia, NICU admission	Adiposity/obesity, diabetes mellitus, cardiovascular risk, cognitive impairment

CS = caesarean section; PPH = postpartum haemorrhage; NICU = neonatal intensive care unit.
*specific to pregestational diabetes.

Methods

We conducted a prospective study including women with pregestational T1DM or T2DM, GDM and normoglycaemia (negative oral glucose tolerance test (OGTT)) who attended the high-risk antenatal clinic at Steve Biko Academic Hospital (SBAH), Tshwane, Pretoria, between May 2017 and March 2022. The study was approved by the University of Pretoria Health Science Research Ethics Committee (ref. no. 191/2016 and 41/2021). This study is part of a larger study investigating epigenetic mechanisms in women with DIP. At SBAH, the diabetes antenatal clinic manages referrals from local endocrine and internal medicine or antenatal clinics in the cluster. The referring clinics use the risk factor-based selective screening approach,[11] which includes screening for risk factors, such as family history of diabetes mellitus, previous GDM, advanced maternal age, obesity and previous adverse pregnancy outcome, including congenital abnormality, recurrent miscarriages, delivery of a stillborn child, delivery of a baby ≥4 000 g in a previous pregnancy or persistent glycosuria.[12]

Women were included in the study if they had singleton pregnancies, were aged between 18 - 40 years, were of black African ethnicity, were at ≤28 weeks' gestation and were HIV negative. DIP was categorised as TIDM if diagnosed prior to pregnancy or if first diagnosed in pregnancy and was confirmed by the presence of positive antibodies or the occurrence of diabetic ketoacidosis, which is determined in consultation with an endocrinologist. A T2DM diagnosis was made if it was identified prior to pregnancy or if overt diabetes was diagnosed during pregnancy (fasting plasma glucose level ≥7.0 mmol/L, random plasma glucose or 2-hour plasma glucose ≥11.1 mmol/L on the OGTT; or glycated haemoglobin (HbA1c) ≥6.5%). GDM was diagnosed if carbohydrate intolerance was first diagnosed during pregnancy according to the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria at 24 - 28 weeks' gestation (fasting plasma glucose level 5.1 - 6.9 mmol/L or 1-hour plasma glucose ≥10 mmol/L or 2-hour plasma glucose 8.5 - 11.0 mmol/L after a 2-hour 75-g oral glucose tolerance test (OGTT)).[13] Women were recruited as normoglycaemic controls if they had a negative OGTT. Women were followed up until delivery. Data collected included demographics, anthropometric measures, obstetric history and care, diabetes care and fetal outcomes, according to standard clinical care. Gestational age (GA) was determined using early ultrasound when available; otherwise, it was determined based on menstrual history or late ultrasound. Maternal weight at the first antenatal visit was recorded as pre-pregnancy weight was not available. Due to missing height measurements, maternal BMI data were limited. Consequently, both weight and BMI were collected. Obesity was defined as a BMI ≥30 kg/m^2 as per Institute of Medicine guidelines.[14] Dietary counselling and education on diabetes were provided by a trained dietician. Post diagnosis, some women with GDM were initiated on metformin in consultation with specialists, while others were started on a low-carbohydrate diet (dependent on OGTT levels). Women were counselled on maintaining glycaemic targets, including fasting/pre-prandial glucose levels of ≤5.3 mmol/L and 2-hour post-prandial glucose levels of ≤6.7 mmol/L. All women with DIP monitored their glucose levels at home. Women were required to test their glucose with an On-Call Plus glucometer (On Call, Mexico) at least five times a day, at various times during the week: 30 minutes before each meal (fasting), 2 hours after each meal (post-prandial), at bedtime and 02h00. Poor glycaemic control was defined as having >25% of glucose values outside the recommended range based on home glucose monitoring.

Obstetric and perinatal outcomes included GA at delivery (weeks), onset of labour, route of delivery, birthweight (g), neonatal outcome and Apgar score at 5 minutes. GA at delivery was categorised into preterm (≤37 weeks) and term delivery (>37 weeks). Fetal growth was classified as small for gestational age (SGA) if fetal growth <10th centile and large for gestational age (LGA) if fetal growth >90th centile. Birthweight was defined as low birthweight (501 - 2 500 g), normal weight (2 500 - 4 000 g) and macrosomia (>4 000 g).

Statistical analysis

Data were captured in Microsoft Office Excel 2010 (Microsoft Corp., US) and analysed using STATA 17 (Stata Corp., US). Baseline characteristics were summarised using descriptive statistics. A skewness-kurtosis test was performed to assess normality. Continuous variables are presented as the median and interquartile range (IQR), while categorical variables are expressed as counts and percentages. Continuous data were compared using the Kruskal-Wallis test, followed by Dunn's *post hoc* multiple comparisons test. Categorical data were compared using Pearson's chi-squared (χ^2) test with the Bonferroni *post hoc* test. For counts less than 5, Fisher's exact test was used. Statistical significance was defined as $p<0.05$.

Results

The general characteristics of the population according to diabetes type are summarised in Table 2. A total of 183 women were recruited, including 13 (7.1%) with T1DM, 65 (35.5%) with T2DM, 39 (21.3%) with GDM and 66 (36.1%) who were classified as normoglycaemic. Women with T2DM and GDM were older ($p<0.01$), had higher BMI ($p<0.05$) and had a history of chronic hypertension ($p=0.025$) compared with the control group. Obesity results were based on 74.31% of BMI data. Women with GDM had a significantly higher frequency of obesity compared with women with T1DM (80.6% v. 36.4%) but were not different to women with T2DM and controls. Women with T1DM and T2DM had significantly higher glycated haemoglobin (HbA1c) compared with those with GDM ($p<0.001$). At enrollment, more women with T1DM were on insulin treatment compared with those with T2DM (76.9% v. 15.0%; $p<0.01$), while more women with T2DM were on metformin compared with women with GDM (53.3% v. 26.5%; $p<0.05$). At delivery, 67.2% of women with T2DM and 18.2% of women with T1DM were managed with a combination of metformin and insulin compared with 15.2% of women with GDM ($p<0.001$; Table 2).

Obstetric and perinatal outcomes were compared in the combined diabetic group (T1DM, T2DM, GDM) compared with controls. Overall, the frequency of preterm delivery was higher in the diabetic group compared with the controls (51.5% v. 17.1%; $p<0.001$). However, no between-group differences were noted in the other obstetric and perinatal outcomes. Next, the diabetic group was stratified into T1DM, T2DM and GDM and controls. Obstetric and perinatal outcomes in the sub-groups were compared with the control group (Table 3). The frequency of preterm birth was higher in women with T1DM (66.7% v. 17.1%; $p<0.05$) and T2DM (54.4% v. 17.1%; $p<0.05$). However, there was no significant difference between the GDM and control groups. No between-group differences were observed in the other obstetric and perinatal outcomes.

Next, the effect of obesity and weight on obstetric and perinatal outcomes was investigated. The frequency of GDM was significantly higher in women with obesity compared with women without obesity (30.5% v. 11.5%; $p=0.036$) (Table 4). The frequency of caesarian section (CS) performed before the onset of labour was

higher in women weighing ≥80 kg compared with women weighing <80 kg (45.6% v. 26.0%; $p<0.05$). The frequency of T1DM was lower in the ≥80 kg weight category compared with the <80 kg weight category (13.6% v. 3.6%; $p<0.05$), while the frequency of GDM was higher in the ≥80 kg compared with the <80 kg weight category (27.9% v. 12.1%; $p<0.05$) (Table 5). The frequency of spontaneous onset of labour was higher in the <80 kg weight category compared to the ≥80 kg and <120 kg weight category (52% v. 28.6; $p<0.05$). The rate of low Apgar scores at 5 minutes was significantly higher in the ≥120 kg group compared with the ≥80 and <120 kg groups (33.3% v. 4.2%; $p<0.05$) (Table 5).

Discussion

Literature has shown that diabetes and obesity in pregnancy are associated with adverse pregnancy outcomes for both the mother and child. Therefore, this study aimed to investigate the effect of diabetes and obesity in pregnancy on adverse obstetric and perinatal outcomes. The main findings of the study are *i*) higher rates of preterm delivery in women with T1DM and T2DM compared with the control group, *ii*) higher frequency of GDM in women with obesity compared with women without obesity, *iii*) higher risk of CS before the onset of labour in women who weighed more than 80 kg compared with women who weighed less than 80 kg and *iv*) lower rates of spontaneous onset of labour and higher rates of low Apgar scores in women who weighed more than 120 kg compared with women who weighed between 80 kg and 120 kg.

Our study showed that T1DM and T2DM were associated with higher rates of preterm delivery compared with the control group. These findings are consistent with those of previous studies that also reported elevated rates of preterm delivery in women with pregestational T1DM and T2DM compared with women with GDM and the control group.[15–17] In contrast, a systematic review reported deaths that showed higher or similar risks of preterm delivery in women with GDM compared with women with pregestational diabetes.[18] The optimal timing of delivery for women with DIP is contentious. Some recommendations suggest that in women with pregestational diabetes, especially those with vascular complications or suboptimal glycaemic control, early delivery (before 38.5 weeks' gestation) is the better option.[19] However, a 2018 Cochrane systematic review that aimed to determine the optimal timing of delivery for women with pregestational diabetes concluded that there were insufficient data to adequately determine the timing of delivery due to the lack of published trials.[20] Accordingly, the clinical decision regarding the timing of delivery in women with diabetes depends on several maternal and fetal factors, as well as the associated risk of adverse outcomes. Surprisingly, our study did not show differences in other obstetric and perinatal outcomes among the diabetes groups. This may be attributed to early delivery. Therefore, in our population, early delivery might be a better option to reduce adverse outcomes that may occur at term delivery.

The higher frequency of GDM in women with obesity, compared with their non-obese counterparts, is evidence that obesity is an independent risk factor for the development of GDM.[5] A meta-analysis including 20 studies reported that women who were overweight (2.1-fold), obese (3.6-fold) or severely obese (8.6-fold) had a significantly higher risk of developing diabetes compared with normal-weight pregnant women.[4] Furthermore, the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO)

Table 4. Effect of obesity on obstetric and perinatal outcomes

	Non-obese (n=54)	Obese (n=82)	p-value
Preterm delivery (≤37 weeks), *n* (%)	21 (52.5)	24 (45.3)	0.491
Onset of labour, *n* (%)			
Induction of labour*	7 (19.4)	14 (31.1)	0.094
CS	11 (30.6)	19 (42.2)	
Spontaneous onset of labour	18 (50.0)	12 (26.7)	
Route of delivery, *n* (%)			
Normal vaginal delivery	14 (35.0)	18 (33.3)	
Elective CS	10 (25.0)	20 (37.0)	0.409
Emergency CS	16 (40.0)	16 (29.6)	
Normoglycaemic control, *n* (%)	15 (28.8)	23 (28.0)	0.036
T1DM, *n* (%)	7 (13.5)	4 (4.9)	
T2DM, *n* (%)	24 (46.2)	30 (36.6)	
GDM, *n* (%)	6 (11.5)†	25 (30.5)†	
Fetal growth, *n* (%)			
SGA (<10th centile)	3 (10.0)	3 (6.4)	0.759
AGA	19 (63.3)	29 (61.7)	
LGA (>90th centile)	8 (26.7)	15 (31.9)	
Birthweight (g), *n* (%)			
501 – 2 500	7 (17.1)	9 (16.4)	1.000
2 500 – 4 000	32 (78.0)	44 (80.0)	
>4 000	2 (4.9)	2 (3.6)	
Stillbirth, *n* (%)	3 (7.3)	3 (5.5)	1.000
Apgar score at 5 min, *n* (%)			
<7	4 (10.0)	5 (9.4)	1.000

CS = caesarean section; T1DM = type 1 diabetes mellitus; T2DM = type 2 diabetes mellitus; GDM = gestational diabetes mellitus; SGA = small for gestational age; AGA= appropriate for gestational age; LGA = large for gestational age; min = minute.
*Induction of labour by either medical, mechanical or surgical means (or a combination thereof) was used to achieve labour. Failed inductions were considered if a patient was not in active labour within 24 hours.
†Similar superscripts denote statistical significance.

study reported that a high maternal BMI is associated with an increased risk of adverse outcomes, independent of glycaemic status.[21] Factors such as advanced maternal age, high rates of diabetes and obesity have contributed to increasing rates of GDM.[3] In 2021, the International Diabetes Federation (IDF) estimated that GDM affected 80.5% of live births, while the prevalence of GDM is estimated to be 14.1% in Africa.[1] Studies have shown that physical activity and weight loss prior to conception significantly reduced the risk of developing GDM.[22,23] This emphasises the importance of preconception health for women of reproductive age who are either overweight or obese. Initiatives to encourage weight loss prior to pregnancy and to maintain appropriate gestational weight gain to reduce the risk of developing GDM and subsequent adverse outcomes are recommended.

The increased risk of CS before labour onset in women who weigh more than 80 kg compared with women who weigh less than 80 kg, and the reduced likelihood of spontaneous labour onset in women who weigh more than 120 kg, further demonstrates the negative impact of maternal weight on obstetric outcomes. Abdominal operative delivery in women with obesity is known to present significant problems such as anaesthetic difficulties, infections and greater blood loss, which pose a risk to both the mother and neonate.[24] Brost *et al.*[8] reported that even after controlling for confounders, any increase in maternal weight and BMI before and during pregnancy was associated with an increased risk of CS. They reported that for each incremental BMI unit (1 kg/m^2) increase, there was an approximate 7.8% rise in the likelihood of CS. This complication is thought to be due to an increase in pelvic soft tissue, resulting in the narrowing of the birth canal, leading to difficulty in delivery.[24] A study conducted in Norway reported that European/Central Asian women who were overweight or obese were at an increased risk of elective CS compared with Norwegian women without overweight and obesity, while sub-Saharan African women who were overweight or obese had the highest risk for emergency CS compared with normal-weight women from Norway.[7] A study by Wolfe *et al.*[10] reported that calculating maternal BMI offers no advantage over simply using maternal weight in the initial risk assessment of outcomes related to maternal weight. This practice should be considered for risk assessment of pregnant women instead of BMI, especially in busy, resource-limited settings.

Increased rates of low Apgar scores in women weighing more than 120 kg compared with women weighing between 80 and 120 kg are consistent with studies that showed negative effects of higher maternal weight and BMI on neonatal outcomes. There is evidence that the 5-minute Apgar score is a good predictor and indicator of infant survival and low Apgar scores at either 1, 5 or 10 minutes are associated with long-term neurological disabilities in infants.[25] A study conducted in Pakistan reported that increasing maternal BMI was strongly associated with low Apgar scores at birth and NICU admissions.[9] Another study conducted in Germany found that women with obesity had a higher percentage of giving birth to neonates with a low Apgar score at 1 minute; however, no differences in Apgar scores were observed at 5 and 10 minutes among different BMI groups.[26] Since evidence has shown that Apgar scores are crucial indicators of neonatal and subsequent infant outcomes, knowledge of risk factors, especially modifiable risk factors such as maternal weight, that are associated

Table 5. Effect of stratified weight on outcomes

	Weight <80 kg (*n*=70)	Weight ≥80 kg - <120 kg (*n*=101)	Weight ≥120 kg (*n*=11)	*p*-value
GA at delivery, *n* (%)				
≤37 weeks	27 (50.0)	27 (37.5)	3 (50.0)	0.353
>37 weeks	27 (50.0)	45 (62.5)	3 (50.0)	
Onset of labour, *n* (%)				
Induction of labour*	11 (22.0)	17 (27.0)	2 (40.0)	0.040
CS	13 (26.0)	28 (44.4)	3 (60.0)	
Spontaneous onset of labour	26 (52.0)†	18 (28.6)†	0 (0.0)	
Route of delivery, *n* (%)				
Normal vaginal delivery	21 (38.9)	25 (34.2)	1 (16.7)	0.398
Elective CS	11 (20.4)	24 (32.9)	3 (50.0)	
Emergency CS	22 (40.7)	24 (32.9)	2 (33.3)	
Fetal growth, *n* (%)				
SGA (<10th centile)	3 (9.4)	3 (5.3)	0	0.454
AGA	23 (71.9)	36 (63.2)	3 (50.0)	
LGA (>10th centile)	6 (18.8)	18 (31.6)	3 (50.0)	
Birthweight (g)				
501 – 2 500	13 (23.6)	14 (18.9)	0	
2 500 – 4 000	41 (74.5)	55 (74.3)	6 (100.0)	0.391
>4 000	1 (1.8)	5 (6.8)	0	
Neonatal outcome, *n* (%)				
Alive	52 (94.5)	71 (95.9)	6 (100.0)	0.803
Stillbirth	3 (5.5)	3 (4.4)	0 (0.0)	
Apgar score at 5 min, *n* (%)				
<7	7 (13.0)	3 (4.2)‡	2 (33.3)‡	0.025
≥7	47 (87.0)	69 (95.8)§	4 (66.7)§	

GA = gestational age; CS = caesarean section; SGA = small for gestational age; AGA= appropriate for gestational age; LGA = large for gestational age; min = minute; AGA = appropriate for gestational age, min = minute.
*Induction of labour by either medical, mechanical or surgical means (or a combination thereof) was used to achieve labour. Failed inductions were considered if a patient was not in active labour within 24 hours.
†,‡,§Similar superscripts denote statistical significance among groups.

with a low Apgar score, is important in reducing associated neonatal adverse outcomes.

The relationship between obesity and diabetes and their effect on pregnancy outcomes has been established. Globally, non-communicable diseases such as diabetes and obesity are negatively associated with maternal and perinatal health. A study by Rosenberg *et al.*[27] suggested that diabetes and excess maternal weight can adversely affect maternal and delivery outcomes through two different pathways. The first pathway involves the contribution of diabetes and excess weight to the development of pre-eclampsia, which can trigger preterm delivery and CS. The second pathway pertains to the increased risk of macrosomia in neonates born to women with either diabetes, obesity or both. Babies with macrosomia often contribute to labour dystocia, which can result in an increased prompt for CS 4delivery.

The strength of our study lies in its ability to demonstrate the negative effect of maternal diabetes and obesity on obstetric and perinatal outcomes.

The limitations of the study include a small sample size and restriction to a specific ethnic group, namely black African ethnicity, which restrict the generalisability of our findings. A larger study that includes multiple ethnicities is needed to further validate our results. Also, the study had limited maternal BMI data due to missing height measurements. Therefore, we reported on both BMI and weight, as weight is easily obtained. Nevertheless, despite the low number of BMI measurements, we still observed the effects of both BMI and weight on obstetric and perinatal outcomes. Lastly, hypertension was not categorised into chronic, gestational or preeclampsia.

Conclusion

This study showed that pregestational diabetes is associated with high rates of preterm birth and obesity is associated with the development of GDM, high rates of CS and low Apgar scores at 5 minutes. Adequate glycaemic control and weight loss prior to pregnancy, as well as appropriate gestational weight gain, have been shown to reduce the risk of adverse pregnancy outcomes. Therefore, clinicians should prioritise pre-pregnancy glycaemic control and weight optimisation. Additionally, pregnant women with DIP should be advised about the importance of glycaemic control to reduce adverse pregnancy outcomes. Pregnant women with obesity should be counselled on the importance of appropriate gestational weight gain to prevent the development of GDM. Good antenatal care and education are essential to reduce adverse pregnancy outcomes for mothers with diabetes and obesity.

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Accepted 29 September 2023.

APPENDIX 2: ETHICS APPROVAL AND AMENDMENTS

Ethics approval certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

13 April 2023

Approval Certificate
Annual Renewal

Dear Miss NL Malaza,

Ethics Reference No.: 41/2021 – Line 3
Title: The identification of microRNA signatures associated with glycaemic control and pregnancy outcomes in pregnancies complicated by type 1, type 2 and gestational diabetes mellitus

The **Annual Renewal** as supported by documents received between 2023-03-15 and 2023-04-12 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-04-12 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2024-04-13.
- Please remember to use your protocol number (41/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Ethics Amendment

Permission to increase age at recruitment to ≥18 ≤42 (Ethics no: protocol 41/2021).

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

15 September 2022

Approval Certificate
Amendment

Dear Miss NL Malaza,

Ethics Reference No.: 41/2021 – Line 2
Title: The identification of microRNA signatures associated with glycaemic control and pregnancy outcomes in pregnancies complicated by type 1, type 2 and gestational diabetes mellitus

The **Amendment** as supported by documents received between 2022-08-15 and 2022-09-14 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2022-09-14 as resolved by its quorate meeting.

Please note the following about your ethics approval:
- Please remember to use your protocol number (41/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
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www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

APPENDIX 3: CONSENT FORM

PARTICIPANT'S INFORMATION LEAFLET AND INFORMED CONSENT

Title of study:

Name of researchers:

Institutions:

Dear Miss/Mrs_____Date:___/___/20___

Definitions:

Epigenetic programming refers to factors of the body that can be changed by environmental and/or lifestyle factors. For example, high glucose levels during pregnancy could affect things such as your health, the way you look, and the health and development of your child. We aim to identify these high glucose-induced changes in both mother *(maternal)* and offspring *(fetal)* to determine their association.

Invitation:

You are invited to volunteer for the research study. This information leaflet is to help you to decide whether you would like to participate. Before you agree to take part in this study you should fully understand what is involved. Please take your time to read the following information carefully and discuss it with others if you wish. If you have any questions which are not fully explained in this leaflet, do not hesitate to ask. You should not agree to take part unless you are completely happy about all the procedures involved. Thank you for reading this.

What is the purpose of the study?

You have been diagnosed as having either gestational diabetes during pregnancy or had a negative oral glucose tolerance test (i.e. did not have diabetes in this pregnancy) or had pre-existing type 1 or type 2

diabetes before pregnancy. The investigator would like you to consider taking part in the study to determine whether different types of diabetes during pregnancy affects the health outcomes for both mother and child compared to women who did not have diabetes in pregnancy. This study will provide information that could help to improve pregnancy health outcomes for mothers and their babies in future.

Why have I been chosen?

You have been chosen because you have high glucose levels during pregnancy, have pre-existing *diabetes (type 1 or type 2 diabetes)*, have a high risk of developing gestational diabetes during pregnancy or have a pregnancy with normal glucose levels.

To meet the inclusion criteria, you need to be:

- ✓ Between the ages of 18 and 42 years
- ✓ Of Black ethnic origin
- ✓ Having a singleton pregnancy *(pregnant with 1 baby)*
- ✓ Less than 28 weeks pregnant
- ✓ HIV negative

Procedures to be followed:

This study involves answering some questions *with regards to your past pregnancies*, your family medical history and general health related information. Thereafter, you will be asked to remove your shoes and outer clothing to have your weight, height, waist circumference and blood pressure measured by a trained doctor or registered nurse, as part of your routine ante-natal care and also as part of this research study. In addition, we will test your blood for glucose. If you do not have a history of diabetes you will be asked to come to the clinic so that we can do a glucose tolerance test. For this test, you will be asked to fast overnight for approximately 8-10 hours the day before the visit. On the day, you will be asked to drink a 75 g glucose (sugary) solution and your blood will be tested. In addition, we will collect urine and 3-4 ml (equivalent to 1 teaspoon) blood at < 28 weeks of pregnancy, placenta and cord blood will be collected at delivery, and urine and blood will be collected six weeks after delivery. Buccal swabs will be collected for babies at birth prior to discharge. This is a harmless procedure which involves swabbing the inside of the

cheek with a sponge. These samples will be stored and used for tests to determine if diabetes during pregnancy is related to health outcomes in mother and child. At six weeks after you give birth, you and your baby will be examined, and we will ask you questions regarding the health of you and your baby.

Collecting blood and ingestion of the glucose solution to test blood glucose levels is part of routine clinical care. If you have GDM or overt T1D or T2D you will be managed at specialized clinics by specialists.

None of these procedures are harmful to you or your baby.

Risk and discomfort involved:

There may be slight pain and bruising after taking blood. The glucose solution may make you feel nauseous.

Possible benefits of this study:

Many of the questions asked and tests are done routinely in pregnancy. If any of the test results are abnormal, you will be referred for appropriate care.

I understand that if I do not want to participate in this study, I will still receive standard treatment for my illness. I may withdraw from this study at any time.

Has the study received ethical approval?

This protocol has received ethical approval (ethics no: 41/2021) from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, telephone numbers 012 356 3084 / 012 356 3085. The study has been structured in accordance with the Declaration of Helsinki which deals with the recommendations guiding doctors in biomedical research involving human subjects. A copy of the Declaration may be obtained from the investigator should you wish to review it.

If you have any questions concerning this study, you should contact:

- **Prof Sumaiya Adam Tel: 0123542849**

- **Prof Carmen Pheiffer Tel: 0219380292 or**
- **Ms Nompumelelo Malaza Tel: 0662335090**

Compensation:

Your participation is entirely voluntary. You will not be paid to participate in this study.

Confidentiality:

All records obtained whilst in this study will be regarded as confidential. Results will be published in such a fashion that patients remain unidentifiable.

Consent to participate in this study:

I have read the above information, or it has been read to me in a language that I understand. I understand the above information before signing the consent form. The content and meaning of this information have been explained to me. I have been given the opportunity to ask questions and am satisfied that they have been answered satisfactorily. I understand that if I do not participate it will not alter my management of this pregnancy in any way. I hereby volunteer to take part in this study.

I have received a signed copy of this informed consent agreement.

_____	**___/___/20___**
Participant's Signature	**Date**
_____	**___/___/20___**
Person obtaining informed consent	**Date**
_____	**___/___/20___**
Witness	**Date**

Verbal participant informed consent (if person cannot read or write):

I, the undersigned _____, have read and have explained fully to the participant, named _____ and/or to her relative, the patient information leaflet, which has indicated the nature and purpose of the study in which I have asked the person to participate. The explanation I have given has mentioned both the possible risks and benefits of the study. The participant indicated that she understands that she will be free to withdraw from the study at any time for any reason without jeopardizing the further care of her pregnancy.

I hereby certify that the patient has agreed to participate in this study.

Participant's name: _____

Investigator's name: _____

Investigator's signature: _____

Witness's Name: _____

Witness's signature: _____

Date: ___/___/20___

APPENDIX 4: SUPPLEMENTARY FILES FOR CHAPTER 4

Table S1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines

Section/Topic	#	Checklist Item
TITLE		
Title	1	Identify the report as a systematic review, meta-analysis, or both.
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.
INTRODUCTION		
Rationale	3	Describe the rationale for the review in the context of what is already known.
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).
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.
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.
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.
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.
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).
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.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.
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.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).
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.
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.
RESULTS		
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see Item 12).
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).
DISCUSSION		
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., health care providers, users, and policy makers).
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias).
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.
FUNDING		
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.

doi:10.1371/journal.pmed.1000097.t001

Table S3. Newcastle–Ottawa Scale

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and
Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
a) yes, with independent validation ✸
b) yes, eg record linkage or based on self reports
c) no description
2) Representativeness of the cases
a) consecutive or obviously representative series of cases ✸
b) potential for selection biases or not stated
3) Selection of Controls
a) community controls ✸
b) hospital controls
c) no description
4) Definition of Controls
a) no history of disease (endpoint) ✸
b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
a) study controls for _____ (Select the most important factor.) ✸
b) study controls for any additional factor ✸ (This criteria could be modified to indicate specific
control for a second important factor.)

Exposure

1) Ascertainment of exposure
a) secure record (eg surgical records) ✸
b) structured interview where blind to case/control status ✸
c) interview not blinded to case/control status
d) written self report or medical record only
e) no description
2) Same method of ascertainment for cases and controls
a) yes ✸
b) no
3) Non-Response rate
a) same rate for both groups ✸
b) non respondents described
c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and
Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
a) truly representative of the average _____ (describe) in the community ✸
b) somewhat representative of the average _____ in the community ✸

Table S4. Risk of bias scores.

Study and year	Total quality score	Rating
van Zyl and Levitt 2018	5	Fair
Al-Nemri et al., 2018	5	Fair
Wang et al., 2019	4	unsatisfactory
Yamamoto et al., 2019	7	very good
Tinker et al., 2020	5	Fair
López-de-Andrés et al., 2020	6	Good
Shand et al., 2008	5	Fair
Stogianni et al., 2019	3	unsatisfactory
Gui et al., 2014	5	Fair
Soepnel et al., 2019	6	Good
Huddle et al .,1993	4	unsatisfactory
Gualdani et al., 2020	5	Fair
Capobianco et al., 2020	5	Fair
Abu-Heija et al., 2015	6	Good
Akhlaghi and Hamedi 2005	7	very good
Barakat et al., 2010	7	very good
EL Mallah et al., 1997	6	Good
Hyari et al., 2013	6	Good
Peticca et al., 2009	7	very good
Shefali et al. 2006	6	Good

*Supplementary table for chapter 4 is available on request it is too large to be included in this document:

Table S2: Studies correlating types of diabetes in pregnancy and adverse outcomes

APPENDIX 5: SUPPLEMENTARY FILES FOR CHAPTER 7

Table S1: MiRNAs of interest

Mature miRNA ID	GeneGlobe ID
hsa-miR-155-5p	YP00204308
hsa-miR-19a-3p	YP00205862
hsa-miR-20a-5p	YP00204292
hsa-miR-222-3p	YP00204551
hsa-miR-29a-3p	YP00204698
hsa-miR-9-5p	YP00204513
hsa-miR-124-3p	YP00206026
hsa-miR-126-3p	YP00204227
hsa-miR-210-3p	YP00204333
hsa-miR-30d-5p	YP00206047
hsa-miR-27a-3p	YP00206038
hsa-miR-128-3p	YP00205995

These miRNAs were selected because they have been previously shown to be dysregulated in DIP in our laboratory.

Table S2: Correlation between miRNAs, body weight and glucose concentrations

	Spearman's correlation co-efficient										
Variable	**miR-124-3p**	**miR-126-3p**	**miR-128-3p**	**miR-155-5p**	**miR-19a-3p**	**miR-19b-3p**	**miR-20a-5p**	**miR-210-3p**	**miR-222-3p**	**miR-29a-3p**	**miR-30d-5p**
Bodyweight (kg)	-0.057	-0.066	-0.034	-0.068	-0.089	-0.082	-0.128	-0.020	-0.026	-0.054	-0.059
BMI (kg/m^2)	-0.135	0.009	-0.014	-0.107	-0.041	-0.073	-0.070	-0.047	-0.046	-0.020	-0.030
0-h OGTT (mmol/L)	0.059	-0.081	0.006	0.101	-0.009	-0.005	-0.046	0.182	0.108	0.133	-0.093
1-h OGTT (mmol/L)	0.052	-0.146	-0.047	0.064	-0.080	-0.127	-0.087	0.115	0.096	0.027	-0.183
2-h OGTT (mmol/L)	0.013	-0.152	-0.135	-0.011	-0.127	-0.090	-0.130	0.072	-0.003	0.026	-0.167
HbA1c (%)	-0.088	-0.027	0.005	-0.025	-0.043	-0.080	-0.039	0.077	0.051	0.048	0.063

Correlation analysis was conducted using Spearman's rank test. Abbreviations: BMI-body mass index, OGTT- oral glucose tolerance test, h- hour, HbA1c- glycated haemoglobin.

Table S3: Association between miRNA expression and glucose control

	Glucose control		
	Poor (n=45)	**Good (n=63)**	**P-value**
MiRNA 124	1.308 (0.000-2.200)	1.600 (0.000-2.415)	0.537
MiRNA 126	0.549 (0.068-0.743)	0.846 (0.203-1.070)	0.151
MiRNA 128	0.867 (0.000-1.226)	0.782 (0.022-0.916)	0.722
MiRNA 155	0.869 (0.000-1.312)	1.216 (0.109-1.216)	0.238
MiRNA 19a	0.672 (0.085-0.667)	0.773 (0.137-1.031)	0.665
MiRNA 19b	0.692 (0.000-0.594)	0.699 (0.000-0.787)	0.479
MiRNA 20a	0.504 (0.039-0.541)	0.808 (0.105-0.789)	0.497
MiRNA 210	1.267 (0.000-1.574)	1.581 (0.099-1.817)	0.117
MiRNA 27a	0.585 (0.072-0.975)	0.693 (0.033-1.033)	0.800
MiRNA 29a	1.274 (0.038-1.438)	1.495 (0.302-1.963)	0.119

Data presented as mean (interquartile range). Poor glycaemic control was defined as > 25% of glucose values outside of the recommended range based on home glucose monitoring. $p < 0.05$

Table S4: Association between miRNA expression and fetal growth

	Fetal growth			
	SGA (n=4)	**AGA (n=75)**	**LGA (n=29)**	**P-value**

MiRNA 126	0.901 (0.521-1.280)	0.755 (0.115-1.021)	0.619 (0.205-0.860)	0.441
MiRNA 155	0.449 (0.107-0.791)	1.009 (0.000-1.048)	1.319 (0.000-1.791)	0.545
MiRNA 19a	2.029 (0.673-3.385)	0.696 (0.078-0.805)	0.644 (0.142-1.007)	0.184
MiRNA 19b	0.826 (0.000-1.651)	0.596 (0.092-0.869)	0.939 (0.432-2.496)	0.65
MiRNA 20a	1.464 (0.432-2.496)	0.594 (0.058-0.539)	0.801 (0.108-1.064)	**0.043**
MiRNA 222	1.612 (0.433-2.792)	1.190 (0.024-1.351)	1.700 (0.230-2.311)	0.199
MiRNA 27a	0.832 (0.524-1.140)	0.669 (0.003-0.980)	0.573 (0.149-0.100)	0.407
MiRNA 29a	1.022 (0.500-1.594)	1.511 (0.111-1.804)	1.193 (0.413-1.381)	0.925
MiRNA 30d	1.147 (0.311-1.982)	0.577 (0.025-0.638)	0.602 (0.084-0.753)	0.124

Data presented as mean (interquartile range). Abbreviations: AGA- appropriate for gestational age, LGA- large for gestational age, SGA- small for gestational age. $p< 0.05$

Table S5: Association between miRNA expression and ga at delivery

	GA at delivery		
	Preterm (n=47)	**Term (n=124)**	**P-value**
MiRNA 124	1.087 (0.000-0.831)	1.385 (0.000-2.334)	0.095
MiRNA 126	0.755 (0.214-1.212)	0.763 (0.153-1.050)	0.648
MiRNA 128	0.761 (0.000-0.940)	0.838 (0.000-0.985)	0.924
MiRNA 155	0.836 (0.000-1.033)	1.013 (0.000-1.287)	0.397

MiRNA 19a	0.787 (0.097-0.891)	0.749 (0.057-0.842)	0.488
MiRNA 19b	0.623 (0.064-0.727)	0.798 (0.015-0.777)	0.978
MiRNA 20a	0.811 (0.103-0.607)	0.673 (0.027-0.736)	0.334
MiRNA 222	1.112 (0.161-1.390)	1.191 (0.000-1.489)	0.954
MiRNA 27a	0.743 (0.140-1.038)	0.735 (0.003-0.979)	0.416
MiRNA 29a	1.233 (0.326-1.572)	1.341 (0.076-1.414)	0.253
MiRNA 30d	0.799 (0.095-1.263)	0.653 (0.027-0.804)	0.375

Data presented as mean (interquartile range). p< 0.05

Table S6: Association between miRNA expression and neonatal birth weight

	Birth weight		
	Normal weight <4 kg (162)	**Macrosomia ≥4 kg (7)**	**P-value**
MiRNA 124	1.297 (0.000-2.296)	1.399 (0.000-1.707)	0.965
MiRNA 126	0.771 (0.166-1.093)	0.704 (0.259-0.814)	0.913
MiRNA 128	0.817 (0.000-0.967)	0.989 (0.000-1.972)	0.902
MiRNA 155	0.970 (0.000-1.177)	0.863 (0.243-0.904)	0.811
MiRNA 19a	0.764 (0.085-0.837)	0.705 (0.051-1.103)	0.395
MiRNA 19b	0.724 (0.023-0.719)	1.462 (0.451-1.942)	0101
MiRNA 20a	0.693 (0.040-0.592)	1.219 (0.292-1.750)	**0.247**

MiRNA 210	1.261 (0.000-1.594)	2.064 (1.064-2.539)	**0.020**
MiRNA 222	1.138 (0.036-1.389)	2.019 (0.339-2.973)	0.295
MiRNA 27a	0.735 (0.044-0.990)	0.899 (0.045-1.490)	0.888
MiRNA 29a	1.317 (0.130-1.473)	1.440 (0.435-2.536)	0.429
MiRNA 30d	0.676 (0.039-0.863)	1.272 (0.170-1.779)	0.394

Data presented as mean (interquartile range). p< 0.05

Table S7: Association between miRNA expression and neonatal sex

	Neonatal sex		
	Male (n=81)	**Female (n=87)**	**P-value**
MiRNA 124	1.515 (0.000-2.775)	1.117 (0.000-1.512)	0.603
MiRNA 126	0.805 (0.208-1.106)	0.719 (0.097-0.913)	0.220
MiRNA 128	0.729 (0.000-0.961)	0.899 (0.000-1.045)	0.893
MiRNA 155	0.965 (0.000-1.318)	0.962 (0.000-1.037)	0.637
MiRNA 19a	0.842 (0.102-0.836)	0.690 (0.053-0.850)	0.476
MiRNA 19b	0.704 (0.000-0.759)	0.801 (0.033-0.777)	0.539
MiRNA 20a	0.777 (0.071-0.876)	0.663 (0.025-0.561)	0.252
MiRNA 210	1.322 (0.000-1.717)	1.276 (0.000-1.598)	0.493
MiRNA 222	1.126 (0.097-1.318)	1.224 (0.006-1.614)	0.675

MiRNA 27a	0.731 (0.074-1.080)	0.737 (0.003-0.909)	0.415
MiRNA 29a	1.411 (0.257-1.637)	1.239 (0.055-1.351	0.387
MiRNA 30d	0.704 (0.058-0.915)	0.698 (0.029-0.767)	0.597

Data presented as mean (interquartile range). p< 0.05

Table S8: Association between miRNA expression and Apgar scores

	Apgar score		
	<7 (n=17)	**≥7 (n=152)**	**P-value**
MiRNA 124	1.190 (0.000-2.635)	1.314 (0.000-2.154)	0.991
MiRNA 126	0.950 (0.181-1.323)	0.748 (0.164-1.050)	0.330
MiRNA 128	0.958 (0.044-0.867)	0.810 (0.000-1.006)	0.572
MiRNA 155	0.652 (0.000-0.797)	1.001 (0.000-1.216)	0.407
MiRNA 19a	0.457 (0.101-0.547)	0.795 (0.078-0.904)	0.371
MiRNA 19b	0.741 (0.133-0.605)	0.756 (0.003-0.798)	0.550
MiRNA 20a	0.877 (0.087-0.448)	0.697 (0.039-0.736)	0.884
MiRNA 210	0.884 (0.000-1.573)	1.340 (0.000-1.631)	0.346
MiRNA 222	0.555 (0.155-0.575)	1.244 (0.042-1.624)	0.212
MiRNA 27a	0.746 (0.116-0.996)	0.742 (0.042-1.007)	0.791
MiRNA 29a	1.377 (0.446-1.473)	1.316 (0.086-1.533)	0.458

MiRNA 30d	0.758 (0.023-0.590)	0.694 (0.048-0.885)	0.792

Data presented as mean (interquartile range). $p < 0.05$

APPENDIX 6: CONSENT FORM AND QUESTIONNAIRES FOR CHAPTER 8

Patient's Information Leaflet and Informed Consent

Study title: A study to investigate the relationship between knowledge, gestational weight gain and glycaemic control in women with gestational diabetes

Name of Institutions: Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Pretoria (UP)

Biomedical Research and Innovation Platform, South African Research Council

(SAMRC)

Study investigators: Sumaiya Adam and Fuziwe Zulu (UP)

Carmen Pheiffer and Stephanie Dias (SAMRC)

This informed consent form has two parts:

1. An Information Sheet where the research project is explained to you in simple terms.

2. A Consent section for your signature if you agree to take part in the study.

PART 1: INFORMATION SHEET

1. INTRODUCTION

The Department of Obstetrics and Gynaecology, University of Pretoria is currently doing a study in collaboration with the South African Medical Research Council to evaluate nutritional,

physical activity and diabetes knowledge in women with gestational diabetes mellitus. You have been approached as a potential participant in this study because you have been diagnosed with gestational diabetes and meet the criteria for the research study. This information leaflet is to help you to decide whether you would like to participate. Before you agree to take part in this study you should fully understand what is involved. Please take your time to read the following information carefully and discuss it with others if you wish.

If you have any questions about the study, please feel free to ask. We will take the time to explain. If you have any more questions at a later stage, you can at any point ask the doctor or the staff. You should not agree to take part unless you are completely happy about all the procedures involved. Thank you for reading this.

2. WHAT IS THE PURPOSE OF THE STUDY?

There is a large percentage of women in South Africa who develop diabetes mellitus during pregnancy. This is known as gestational diabetes mellitus. This disease can cause health problems for the mother and the baby if it is not managed in the pregnancy. The purpose of this study is to measure nutritional, physical activity and diabetes knowledge in women with gestational diabetes mellitus. This study is important and can provide information that could help

to improve pregnancy health outcomes for mothers and their babies in future. Taking part in this study will require you to answer a few questions on your knowledge about gestational diabetes, nutrition and physical activity. Participation in this study will not be harmful to you or your baby.

3. WHY HAVE I BEEN APPROACHED?

You have been chosen because you are older than 18 years and have been diagnosed with gestational diabetes within the last 4 weeks.

To meet the inclusion criteria, you need to be:

- Older than 18 years; diagnosed with gestational diabetes within 4 weeks

4. PROCEDURES TO BE FOLLOWED

This study involves answering some questions about your past pregnancies, your family medical history and general health related information. Thereafter, you will be asked to remove your shoes and outer clothing to have your weight, height, waist circumference, hip circumference, mid-thigh circumference and blood pressure measured by a trained doctor or registered nurse. In addition, we will test your blood for glucose. All these procedures are part of routine antenatal care and will be done irrespective of whether you partake in the research study. In addition, you will be asked to answer questions relating to your knowledge about diet, physical activity and gestational diabetes, as part of this research study. The estimated time of the questionnaire is approximately 45-60 minutes. None of these procedures are harmful to you or your baby. We will do our best to make you comfortable and put you at ease during the study.

INFORMATION SHEET AND INFORMED CONSENT (PATIENT)

5. VOLUNTARY PARTICIPATION

Your participation in this study is completely free and voluntary. It is your choice to decide whether you want to participate or not. If you choose to participate in the study, you can change your mind at any time and withdraw your consent whatever your reasons. If you do not want to participate in this study, you will continue to receive the normal standard of care by your usual doctor.

6. RISKS RELATED TO THE STUDY

All procedures are part of routine antenatal care, except for answering a few questions relating to your knowledge about diet, physical activity and gestational diabetes, as part of this research study. None of these procedures are harmful to you or your baby. We will do our best to make you comfortable and put you at ease during the study.

7. BENEFITS RELATED TO THE STUDY

The results of the study may contribute to improved pregnancy outcomes on your future pregnancies.

8. COMPENSATION

You will be entitled to compensation for transport costs and a snack for taking the extra time to complete the questionnaire.

9. CONFIDENTIALITY

Any information or data concerning you will be treated in a strict and confidential manner. All records obtained whilst in this study will be regarded as confidential. Your identity will never appear in any report or publication. Results will be published in such a manner that your identity remains unidentifiable and cannot be traced back to you.

10. WHO TO CONTACT

If you have any questions concerning this study, you should contact:

- Prof Sumaiya Adam Tel: 0123542849 or 0849511773
- Dr Fuziwe Zulu Tel: 0833534521

The conduct of the study is in accordance with the general principles of Good Clinical Practice and

Helsinki declaration

The study has been reviewed and approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Pretoria, telephone numbers 012 356 3084 / 012 356 3085

Approval number: ……

Date: ……

INFORMATION SHEET AND INFORMED CONSENT (PATIENT)

PART 2: WRITTEN AND SIGNED CONSENT

This Informed Consent is for (Name as written on Identity card / Birth certificate / Passport)

Name: ……

Surname: ……

Date of Birth: ……/……/……..

Phone No:…….

ID Number:

……/……/……/……/……/……/……/……/……/……/……/……/……

By signing, I agree that:

- I have read this consent document or it has been read to me in a language that I understand.
- The content and meaning of this information have been explained to me.
- I have been given the opportunity to ask questions and am satisfied that they have been answered satisfactorily.
- I understand that if I choose not to be in the study, or to leave the study at any time by informing the investigator, it will not alter the management of this pregnancy in any way.
- I understand that my participation in this study is completely voluntary

- I give permission to use and share my data as described in this document
- I will receive a copy of this consent form.
- I hereby volunteer to take part in this study.

Name of the person giving the consent: ……

Date: ……

Place: ……

Please write in block letters “READ AND APPROVED” Next to your signature

Signature……..

INFORMATION SHEET AND INFORMED CONSENT (PATIENT)

TO BE FILLED BY CLINICIAN OR PERSON OBTAINING CONSENT

I confirm that the subject was given an opportunity to ask questions and all the questions have been answered in the best of my ability. I confirm that the individual has not been coerced for consent and that consent has been given freely and voluntarily. A copy of this informed consent sheet has been given to the subject.

Name of clinician or person obtaining consent: ……

Date: ……. /……/……...

Signature……

Name of witness: ……..

Date: ……. /……/……...

Signature……..

VERBAL PARTICIPANT INFORMED CONSENT (IF PERSON CANNOT READ OR WRITE):

I, the undersigned …….., have read and have explained fully to the participant, named ……....and/or to her relative, the patient information leaflet, which has indicated the nature and purpose of the study in which I have asked the person to participate. The explanation I have given has mentioned both the possible risks and benefits of the study. The participant indicated that she understands that she will be free to withdraw from the study at any time for any reason without jeopardizing the further care of her pregnancy.

I hereby certify that the patient has agreed to participate in this study.

Participant's name: ……..

Investigator's name: ……..

Investigator's signature: ……..

Witness's name: ……..

Witness's signature: ……..

Date: ……. /……./……..

PATIENT QUESTIONNAIRE VERSION 1

PATIENT QUESTIONNAIRE

Questionnaire - Patient

Study title: A study to investigate the relationship between knowledge, gestational weight gain and glycaemic control in women with gestational diabetes

Name of Institutions: Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Pretoria (UP)
Biomedical Research and Innovation Platform, South African Research Council (SAMRC)

Study investigators: Sumaiya Adam and Fuziwe Zulu (UP)
Carmen Pheiffer and Stephanie Dias (SAMRC)

RECOMMENDATIONS FOR STUDY STAFF

1. Include participants after verification of inclusion and non-inclusion criteria.
2. Make sure that the informed consent was obtained.
3. The questionnaire consists of two parts, Please complete all parts
    a. Part A: Clinical information
    b. Part B: Gestational diabetes knowledge test

PART A

STEVE BIKO ACADEMIC HOSPITAL ANTENATAL CLINIC
DIABETIC PATIENT QUESTIONNAIRE

Date of Examination: ……/……/….

Examining Doctor: ……

Patient Name: ……

PATIENT STICKER

DEMOGRAPHICS AND SOCIAL HISTORY:

Date of Birth: ……/……/……..

Age: ……

Ethnic Group: Black / White / Indian / Coloured / Other ……

Home Language: ……..

Marital Status: Single / Married / Divorced /Widow / Other ……..

Current Employment: Unemployed / Housewife / Informal / Formal / Other ……

Employment: Full time / Part time

Social Grants: Yes / No

Access to: Water / Sanitation / Electricity

Educational level: None / Primary / Secondary / Diploma/Degree / Other ……..

Smoking: Never / Past – Year Stopped: ……./ Current – Number of cigarettes/week / Other……..

Alcohol: No/ Social / Binger (present/past) / Regular Units/day: **……**

Supplements/vitamins:

1. Describe what you eat in a typical day:

a. Breakfast
b. Lunch
c. Dinner
d. Snacks
e. Water intake

OBSTETRIC/GYNAECOLOGICAL HISTORY

Parity: ……...

Gravidity: ……

Miscarriages:……..

Ectopic: …….

Last Normal Menstrual Period (Current pregnancy): ……/……/……..

Gestational Age: ……

Expected Date of Delivery: ……/……/……..**by Early Sonar / Sure Dates / Uncertain**

Previous pregnancies:

YEAR	*GESTATIONAL AGE AT DELIVERY*	*ROUTE OF DELIVERY*	*BIRTH WEIGHT*	*OUTCOME*	*COMPLICATIONS / CONGENITAL ABNORMALITIES / PREVIOUS STILLBIRTH / MACROSOMIA / DIABETES / POLYCYSTIC OVARIAN SYNDROME*

Rhesus: Positive / Negative – Coombs …… **RPR:** Negative / Positive ……

HIV: Pos / Neg/ Unknown **CD4 count:…… HIV viral load: _____**

Antiretroviral drugs – Date of Commencement: …../……/…….. **Drugs:** ……...

Contraceptive Use: ……

Injectables:……

Infant Feeding: …….

DIABETES HISTORY

Type of diabetes: Type 1 / Type 2 / Secondary / Gestational / Uncertain……

Year Diagnosed:……..

How Diagnosed: Symptoms - Polyuria / Polydipsia / Weight loss / Other …….

OGTT result: 0 hour …… 1 hour …… 2 hour ……

OGTT adverse effects: Nauseous / Other……..

History of diabetes in previous pregnancies: Yes / No……..

Has a Dietician been consulted: Yes / No

Home Glucose monitoring: Yes / No **Glucometer:** Yes / No

HbA1C at conception: ……

Current Medications:

Diet Only / Sulphonylureas / Glucophage / Insulin / Acarboses / ACE inhibitors / Diuretics / ARVs / Other**:**

……

Any episodes of hypoglycaemia: Yes / No

Any episodes of DKAs: Yes / No

Details of hospital admissions: ……

……

What were you told about Gestational Diabetes prior to the OGTT? ……

……..

How did you feel when you were informed that you have Gestational diabetes? ……

……..

MEDICAL HISTORY

Other chronic illnesses:

Medications and Dosage:

……

……

Target organ damage: Eyes, kidneys, heart, nervous system

24-hour urine:

spot urine protein:creatinine ratio: _____

Protein: ……g/24h

Creatinine Clearance: ……ml/min

U/E: Urea……. Creatinine…….

FBC: Hb…….

Fundoscopy: _____

Allergies: ……

Other medical and surgical history: ……

FAMILY HISTORY:

Diabetes: Yes/ No – Relation…….

Hypertension: Yes/ No – Relation……..…

MI <60years: Yes/ No – Relation……

Other: Yes/ No – Relation……...

EXERCISE HISTORY

On average how many days a week do you exercise? _____days

On the days you do exercise, on average:

- How many minutes do you exercise for? _____minutes
- How hard are you exercising? / How intense are your exercise sessions? ___Low / ___Moderate/ ___High

Note: The talk test is an easy indicator of the intensity at which you are exercising.

- Low intensity- If you can sing several phrases of a song without breathing hard.
- Moderate intensity- if you can have a conversation and breathe comfortably whilst exercising.
- High intensity- if you must take a breath between every word you say whilst exercising.

CLINICAL EXAMINATION:

GENERAL:

Weight: ……kg Height: ……cm BMI: ……

MUAC: ……cm Waist circumference ……cm Hip circumference ……cm

Mid-thigh circumference ……cm

Jaundice / Anaemia / Cyanosis / Clubbing / Oedema / Lymphadenopathy / Skin/thyroid/breasts/ Normal

Details …….

CARDIOVASCULAR:

Blood Pressure: Clinic measurement (routine) ……../……..mmHg

Right (study)……../……..mmHg Left (study) ……../……..mmHg

Pulse: …….. b/min Rhythm: Normal / AF / Other ……...

Signs of CCF (crepitations, raised JVP, Oedema, S3): ……

RESPIRATORY: ……

ABDOMEN: ……

Glucose control:

- HbA1c ……g/dL
- Last 2 fasting values 1)…….mmol/l 2) ……mmol/l
- 7-day average (glucometer) ……mmol/l

PART B - Gestational Diabetes Mellitus (GDM) Questionnaire

KNOWLEDGE OF GESTATIONAL DIABETES

Below are some statements about diabetes. There may be more than one correct answer. After reading the statement please circle whatever answers you believe are true. If you do not know the answer please circle a number (I don't know).

These questions may have more than one correct answer

1. How is gestational diabetes diagnosed

1. blood test
2. urine
3. I don't know

2. Because I have gestational diabetes, my baby may be:

1. larger than usual
2. smaller than usual
3. born early
4. admitted to special care
5. I don't know

3. Women are more likely to develop gestational diabetes if they:

1. are overweight
2. are over 35 years
3. have a family history of diabetes
4. previously had GDM
5. I don't know

4. Because I have gestational diabetes, I may:

1. need to come to the clinic more frequently
2. need a caesarean section
3. develop permanent diabetes later in life
4. I don't know

5. In uncontrolled diabetes the blood sugar is:

1. normal
2. increased
3. decreased
4. I don't know

6. Gestational diabetes is:

1. present during pregnancy
2. disappears once the baby is born
3. may lead to diabetes in later life
4. is not very serious
5. I don't know

7. Gestational diabetes may be treated with:

1. diet
2. diet and exercise
3. insulin
4. all of the above
5. I don't know

8. When my baby is born:

1. my diabetes will disappear
2. I don't need to worry about being diabetic anymore
3. I should get a follow-up glucose test at my 6-week check-up
4. I don't know

KNOWLEDGE ON TESTING BLOOD GLUCOSE LEVEL

The following questions require you to circle **ONE** number only.

9. A normal fasting (on an empty stomach) blood glucose level is:

1. less than 5 mmol/L
2. less than 6 mmol/L
3. 7 mmol/L or more
4. 8 mmol/L or more
5. I don't know

10. A normal 2-hour (after eating) blood glucose level is:

1. less than 5 mmol/L
2. less than 6.7 mmol/L
3. 7 mmol/L or more
4. 8 mmol/L or more
5. I don't know

11. I should test my blood glucose level:

1. in the morning before breakfast
2. in the afternoon before lunch
3. 2 hours after meals
4. At 2am
5. I don't know

12. What do I do if my blood glucose level is high on one occasion?

1. make a note in your diary
2. check what you ate before the high blood glucose level
3. go to the hospital
4. I don't know

13. What do I do if my blood glucose level is high on two occasions in one week?

1. make a note in your diary
2. check what you ate before the high blood glucose level
3. contact the diabetes educator
4. go to the hospital
5. I don't know

14. Should I take my blood glucose level if I am feeling sick and haven't eaten?

1. yes, continue to take your blood glucose levels as usual
2. no, do not take your blood glucose levels until you are feeling better
3. I don't know

15. When you prick your finger, you should:

1. use the same finger every day
2. use a different finger every day
3. it is not important
4. I don't know

KNOWLEDGE OF DIET, PHYSICAL ACTIVITY AND LOOKING AFTER YOURSELF AFTER GDM DIAGNOSIS

These questions may have more than one correct answer

16. Do you think that what you eat is important to control gestational diabetes?

1. Yes
2. No

17. If you have gestational diabetes, you should avoid food containing high content of

1. Carbohydrates/starches
2. Protein / meat
3. Fat
4. Sugar

18. Which of the following food can be eaten without restriction during gestational diabetes

1. Sugar
2. Fruit
3. Vegetables
4. Meat

19. What is the type of dietary source mainly provided by pap?

1. Carbohydrates/starches
2. Protein
3. Fat
4. Sugar

20. How can you make pap safer for your blood sugar to eat?

1. Cook and eat
2. Cook, cool and reheat
3. Add fats
4. Add lemon juice
5. Add vinegar

21. The preferred type of carbohydrate/starchy foods are:

1. white bread
2. wholegrain bread
3. foods that are high in fibre
4. foods high in starch
5. I don't know

22. What form of fruits and vegetables are better?

1. fruit or vegetable juices
2. processed or canned fruits and vegetables
3. fruits with added fats, sugar and salt
4. fresh fruit and vegetables
5. I don't know

23. Protein intake can be obtained from:

1. meat
2. fish
3. nuts
4. dairy such as milk or cheese
5. all of the above
6. I don't know

24. What type of chicken is best?

1. skinless baked chicken
2. skin-on chicken
3. deep-fried chicken
4. any chicken
5. I don't know

25. A balanced diet should have:

1. more vegetables
2. fewer carbohydrates/starches such as white bread
3. low fat and low sugar choices
4. All of the above
5. I don't know

26. Is physical activity (movement) or exercise important to control gestational diabetes?

1. Yes
2. No

27. Exercise in gestational diabetes:

1. helps to control mother's blood glucose and improves baby's health
2. is not helpful
3. tires you out
4. increases the risk of miscarriage during pregnancy
5. I don't know

28. With regard to exercise during pregnancy:

1. running and skipping are recommended
2. walking and swimming are recommended
3. exercise is not recommended
4. I don't know

29. How hard can you exercise during pregnancy?

1. mild exercise
2. moderate exercise
3. vigorous exercise
4. until you are exhausted

5. I don't know

30. To control blood glucose effectively you should:

1. eat a healthy, balanced diet
2. do moderate exercise 5-7 days a week for about 30 minutes a day
3. spend most of your time resting
4. eat a healthy, balanced diet with moderate exercise 5-7 days a week, 30 minutes a day
5. I don't know

31. How long should you exercise per day?

1. 10 minutes
2. 15 minutes
3. till you get tired
4. 30 minutes (one 30-minute session or three 10-minute sessions)
5. I don't know

32. Should I exercise if I am overweight and unfit?

1. No, you should not
2. Yes, you should start slowly and increase gradually
3. First you need to lose weight and get fit
4. I don't know

33. How can I increase my daily physical activity/exercise?

1. walk children to school
2. take stairs instead of the lift or elevator
3. walk to the shopping centre
4. I don't know

MANAGEMENT OF GESTATIONAL DIABETES

34. You should check your blood glucose levels:

1. regularly for the health of you and your baby
2. occasionally
3. when you feel unwell
4. before you go to see the doctor
5. I don't know

35. Controlling your blood glucose levels:

1. has no effect on baby
2. will give a healthy start for baby
3. has no effect on the pregnancy outcome
4. none of the above
5. I don't know

36. If there is a social occasion, such as a party, you should:

1. not go
2. take a day off from diabetes and eat whatever is served at the party
3. eat nothing during the event
4. eat before you go and take a snack

5. I don't know

37. When your blood glucose levels are high:

1. try and work out the cause and make a note in your diary
2. just consider it to be one of those days
3. hope that tomorrow is better
4. exercise more
5. I don't know

38. You should exercise:

1. occasionally
2. only when you feel like it
3. daily for 30 minutes
4. only when blood glucose levels are high
5. I don't know

39. Gestational diabetes can be controlled by:

1. leaving it alone
2. continuing your normal routine
3. following a healthy diet and exercise
4. none of the above
5. I don't know

40. When you are hungry in between meals:

1. eat another meal
2. drink water and see if that helps
3. try and ignore it
4. go for a walk
5. I don't know

41. Rate the importance of the following modifications in the management of diabetes mellitus. Not important = [1], Slightly important = [2], Very important = [3], Do not know = [4]

	1	2	3	4
1. Eating healthy (diet)				
2. Regular exercise				
3. Regular blood level checks				
4. Maintaining an ideal body weight				
5. Taking medication regularly				
6. Routine medical check-ups				

42. Rate the following statements on quality of life. Strongly disagree = [1], Disagree = [2], Agree = [3], Strongly agree = [4], Do not know [5]

	1	2	3	4	5
1. I prefer to eat something I shouldn't, rather than tell someone that I have diabetes					
2. I'm worried about whether I will miss work					
3. I feel diabetes limits my career					
4. I feel satisfied with my blood glucose control					
5. 'I record my blood glucose levels in my chart/diabetes diary when my health care personnel ask me to					
6. I adjust insulin dose based on my blood glucose					
7. My diet is repetitious and not diversified					
8. Gestational diabetes has caused me not to enjoy my pregnancy					
9. I feel that my pregnancy is an abnormal pregnancy					

……

Thank you for your participation

PATIENT QUESTIONNAIRE

Questionnaire - Patient

Study title: A study to investigate the relationship between knowledge, gestational weight gain and glycaemic control in women with gestational diabetes

Name of Institutions: Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Pretoria (UP)
Biomedical Research and Innovation Platform, South African Research Council (SAMRC)

Study investigators: Sumaiya Adam and Fuziwe Zulu (UP)
Carmen Pheiffer and Stephanie Dias (SAMRC)

RECOMMENDATIONS FOR STUDY STAFF

4. Include participants after verification of inclusion and non-inclusion criteria.
5. Make sure that the informed consent was obtained.
6. The questionnaire consists of two parts, Please complete all parts
    a. Part A: Clinical information
    b. Part B: Gestational diabetes knowledge test

PART A

STEVE BIKO ACADEMIC HOSPITAL ANTENATAL CLINIC
DIABETIC PATIENT QUESTIONNAIRE

Date of Examination: ……/……/……

Examining Doctor: ……

PATIENT STICKER

DEMOGRAPHICS AND SOCIAL HISTORY:

Date of Birth: ……/……/……..

Age: ……

Home Language: ……..

Marital Status: Single / Married / Divorced /Widow / Other ……..

Current Employment: Unemployed / Housewife / Informal / Formal / Other ……

Employment: Full time / Part time

Social Grants: Yes / No

Access to: Water / Sanitation / Electricity

Educational level: None / Primary / Secondary / Diploma/Degree / Other ……..

Smoking: Never / Past – Year Stopped: ……./ Current – Number of cigarettes/week / Other……..

Alcohol: No/ Social / Binger (present/past) / Regular Units/day: **……**

Supplements/vitamins:

OBSTETRIC/GYNAECOLOGICAL HISTORY

Parity: ……...

Gravidity: ……

Miscarriages:……..

Ectopic: …….

Last Normal Menstrual Period (Current pregnancy): ……/……/……..

Gestational Age: ……

Expected Date of Delivery: ……/……/……..**by Early Sonar / Sure Dates / Uncertain**

Previous pregnancies:

YEAR	*GESTATIONAL AGE AT DELIVERY*	*ROUTE OF DELIVERY*	*BIRTH WEIGHT*	*OUTCOME*	*COMPLICATIONS / CONGENITAL ABNORMALITIES / PREVIOUS STILLBIRTH / MACROSOMIA / DIABETES / POLYCYSTIC OVARIAN SYNDROME*

Rhesus: Positive / Negative – Coombs …… **RPR:** Negative / Positive ……

HIV: Pos / Neg/ Unknown **CD4 count:…… HIV viral load: _____**

Antiretroviral drugs – Date of Commencement: …../……/…….. **Drugs:** ……...

Contraceptive Use: ……

Injectables:……

Infant Feeding: …….

DIABETES HISTORY

Type of diabetes: Type 1 / Type 2 / Secondary / Gestational / Uncertain……

Year Diagnosed:……..

How Diagnosed: Symptoms - Polyuria / Polydipsia / Weight loss / Other …….

OGTT result: 0 hour …… 1 hour …… 2 hour ……

OGTT adverse effects: Nauseous / Other……..

History of diabetes in previous pregnancies: Yes / No……..

Has a Dietician been consulted: Yes / No

Home Glucose monitoring: Yes / No **Glucometer:** Yes / No

HbA1C at conception: ……

Current Medications:

Diet Only / Sulphonylureas / Glucophage / Insulin / Acarboses / ACE inhibitors / Diuretics / ARVs / Other**:**

……

Any episodes of hypoglycaemia: Yes / No

Any episodes of DKAs: Yes / No

Details of hospital admissions: ……

……

MEDICAL HISTORY

Other chronic illnesses:

Medications and Dosage:

……

……

Target organ damage: Eyes, kidneys, heart, nervous system

24-hour urine:

spot urine protein:creatinine ratio: _____

Protein: ……g/24h

Creatinine Clearance: ……ml/min

U/E: Urea……. Creatinine…….

FBC: Hb…….

Fundoscopy: _____

Allergies: ……

Other medical and surgical history: ……

FAMILY HISTORY:

Diabetes: Yes/ No – Relation…….

Hypertension: Yes/ No – Relation……..…

MI <60years: Yes/ No – Relation……

Other: Yes/ No – Relation……...

EXERCISE HISTORY

On average how many days a week do you exercise? _____days

On the days you do exercise, on average:

- How many minutes do you exercise for? _____minutes
- How hard are you exercising? / How intense are your exercise sessions? ___Low / ___Moderate/ ___High

Note: The talk test is an easy indicator of the intensity at which you are exercising.

- Low intensity- If you can sing several phrases of a song without breathing hard.
- Moderate intensity- if you can have a conversation and breathe comfortably whilst exercising.
- High intensity- if you must take a breath between every word you say whilst exercising.

CLINICAL EXAMINATION:

GENERAL:

Weight: ……kg Height: ……cm BMI: ……

MUAC: ……cm Waist circumference ……cm Hip circumference ……cm

Mid-thigh circumference ……cm

Jaundice / Anaemia / Cyanosis / Clubbing / Oedema / Lymphadenopathy / Skin/thyroid/breasts/ Normal

Details …….

CARDIOVASCULAR:

Blood Pressure: Clinic measurement (routine) ……../……..mmHg

Right (study)……../……..mmHg Left (study) ……../……..mmHg

Pulse: …….. b/min Rhythm: Normal / AF / Other ……...

Signs of CCF (crepitations, raised JVP, Oedema, S3): ……

RESPIRATORY: ……

ABDOMEN: ……

Glucose control:

- HbA1c ……g/dL
- Last 2 fasting values 1)…….mmol/l 2) ……mmol/l
- 7-day average (glucometer) ……mmol/l

PART B - Gestational Diabetes Mellitus (GDM) Questionnaire

KNOWLEDGE OF GESTATIONAL DIABETES

Below are some statements about diabetes. There may be more than one correct answer. After reading the statement please circle whatever answers you believe are true. If you do not know the answer please circle a number (I don't know).

These questions may have more than one correct answer

Are you aware that there are different types of diabetes?

Can you name them?

1. How is gestational diabetes diagnosed

4. blood test
5. urine
6. I don't know

2. Because I have gestational diabetes, my baby may be:

6. larger than usual
7. smaller than usual
8. born early
9. admitted to special care
10. I don't know

3. Women are more likely to develop gestational diabetes if they:

6. are overweight
7. are over 35 years
8. have a family history of diabetes
9. previously had GDM
10. I don't know

4. Because I have gestational diabetes, I may:

5. need to come to the clinic more frequently
6. need a caesarean section
7. develop permanent diabetes later in life
8. I don't know

5. In uncontrolled diabetes the blood sugar is:

5. normal
6. increased
7. decreased
8. I don't know

6. Gestational diabetes is:

6. present during pregnancy
7. disappears once the baby is born

8. may lead to diabetes in later life
9. is not very serious
10. I don't know

7. Gestational diabetes may be treated with:

6. diet
7. diet and exercise
8. insulin/metformin
9. all of the above
10. I don't know

8. When my baby is born:

5. my diabetes will disappear
6. I don't need to worry about being diabetic anymore
7. I should get a follow-up glucose test at my 6-week check-up
8. I don't know

KNOWLEDGE ON GDM MANAGEMENT AND BLOOD GLUCOSE TESTING

The following questions require you to circle **ONE** number only.

9. Is regular glucose testing important during GDM?

1. Yes
2. No

10. You should check your blood glucose levels:

6. regularly for the health of you and your baby
7. occasionally
8. when you feel unwell
9. before you go to see the doctor
10. I don't know

11. Controlling your blood glucose levels:

6. has no effect on baby
7. will give a healthy start for baby
8. has no effect on the pregnancy outcome
9. none of the above
10. I don't know

12. I should test my blood glucose level:

6. in the morning before breakfast
7. in the afternoon before lunch
8. 2 hours after meals
9. At 2am
10. I don't know

13. Do you know what the glucose should be?

1. Yes
2. No

If no skip questions 14, 15, 16, 17 and 18

14. A normal fasting (on an empty stomach) blood glucose level is:

6. less than 5 mmol/L
7. less than 6 mmol/L
8. 7 mmol/L or more
9. 8 mmol/L or more
10. I don't know

15. A normal 2-hour (after eating) blood glucose level is:

6. less than 5 mmol/L
7. less than 6.7 mmol/L
8. 7 mmol/L or more
9. 8 mmol/L or more
10. I don't know

16. What do I do if my blood glucose level is high on one occasion?

5. make a note in your diary
6. check what you ate before the high blood glucose level
7. go to the hospital
8. I don't know

17. What do I do if my blood glucose level is high on two occasions in one week?

6. make a note in your diary
7. check what you ate before the high blood glucose level
8. contact the diabetes educator
9. go to the hospital
10. I don't know

18. Should I take my blood glucose level if I am feeling sick and haven't eaten?

4. yes, continue to take your blood glucose levels as usual
5. no, do not take your blood glucose levels until you are feeling better
6. I don't know

19. When you prick your finger, you should:

5. use the same finger every day
6. use a different finger every day
7. it is not important
8. I don't know

KNOWLEDGE OF DIET, PHYSICAL ACTIVITY AND LOOKING AFTER YOURSELF AFTER GDM DIAGNOSIS

These questions may have more than one correct answer

20. Describe what you eat in a typical day:

1. Breakfast
2. Lunch
3. Dinner
4. Snacks

5. Water intake

21. Do you think that what you eat is important to control gestational diabetes?

3. Yes
4. No

22. If you have gestational diabetes, you should avoid food containing high content of

5. Carbohydrates/starches
6. Protein / meat
7. Fat
8. Sugar

23. Which of the following food can be eaten without restriction during gestational diabetes

5. Sugar
6. Fruit
7. Vegetables
8. Meat

24. What is the type of dietary source mainly provided by pap?

5. Carbohydrates/starches
6. Protein
7. Fat
8. Sugar

25. How can you make pap safer for your blood sugar to eat?

6. Cook and eat
7. Cook, cool and reheat
8. Add fats
9. Add lemon juice
10. Add vinegar

26. The preferred type of carbohydrate/starchy foods are:

6. white bread
7. wholegrain bread
8. foods that are high in fibre
9. foods high in starch
10. I don’t know

27. What form of fruits and vegetables are better?

6. fruit or vegetable juices
7. processed or canned fruits and vegetables
8. fresh fruit and vegetables
9. I don’t know

28. How often do you eat fast food?

1. Never
2. Once a week
3. More than three times a week

29. What fast food do you eat?

30. Protein intake can be obtained from:

7. meat
8. fish
9. nuts
10. dairy such as milk or cheese
11. all of the above
12. I don't know

31. What type of chicken is best?

6. skinless baked chicken
7. skin-on chicken
8. deep-fried chicken
9. any chicken
10. I don't know

32. A balanced diet should have:

6. more vegetables
7. fewer carbohydrates/starches such as white bread
8. low fat and low sugar choices
9. All of the above
10. I don't know

33. When you are hungry in between meals:

6. eat another meal
7. drink water and see if that helps
8. try and ignore it
9. go for a walk
10. I don't know

34. Is physical activity (movement) or exercise important to control gestational diabetes?

3. Yes
4. No

35. Physical activity or exercise in gestational diabetes:

6. helps to control mother's blood glucose and improves baby's health
7. is not helpful
8. tires you out
9. increases the risk of miscarriage during pregnancy
10. I don't know

36. With regard to exercise during pregnancy:

5. running and skipping are recommended
6. walking and swimming are recommended
7. I don't know

37. How hard can you exercise during pregnancy?

6. mild exercise
7. moderate exercise
8. vigorous exercise
9. until you are exhausted
10. I don't know

38. How long should you exercise per day?

6. 10 minutes
7. 15 minutes
8. till you get tired
9. 30 minutes (one 30-minute session or three 10-minute sessions)
10. I don't know

39. Should I exercise if I am overweight and unfit?

5. No, you should not
6. Yes, you should start slowly and increase gradually
7. First you need to lose weight and get fit
8. I don't know

40. How can I increase my daily physical activity/exercise?

5. walk children to school
6. take stairs instead of the lift or elevator
7. walk to the shopping centre
8. I don't know

41. To control blood glucose effectively you should:

6. eat a healthy, balanced diet
7. do moderate exercise 5-7 days a week for about 30 minutes a day
8. spend most of your time resting
9. eat a healthy, balanced diet with moderate exercise 5-7 days a week, 30 minutes a day
10. I don't know

42. Rate the following statements on quality of life. Strongly disagree = [1], Disagree = [2], Agree = [3], Strongly agree = [4], Do not know [5]

	1	2	3	4	5
10. I would rather eat something unhealthy than tell someone that I have diabetes					
11. I am worried about whether I will miss work					
12. I feel diabetes limits my career					
13. I feel satisfied with my blood glucose control					
14. I record my blood glucose levels in my chart/diabetes diary when my health care personnel ask me to					
15. Gestational diabetes has caused me not to enjoy my pregnancy					
16. I feel that my pregnancy is an abnormal pregnancy					

43. How did you feel when you were informed that you have Gestational diabetes?

……
……

……

Thank you for your participation

PATIENT QUESTIONNAIRE VERSION 3

PATIENT QUESTIONNAIRE

Questionnaire - Patient

Study title: A study to investigate the relationship between knowledge, gestational weight gain and glycaemic control in women with gestational diabetes

Name of Institutions: Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Pretoria (UP)
Biomedical Research and Innovation Platform, South African Research Council (SAMRC)

Study investigators: Sumaiya Adam and Fuziwe Zulu (UP)
Carmen Pheiffer and Stephanie Dias (SAMRC)

RECOMMENDATIONS FOR STUDY STAFF

7. Include participants after verification of inclusion and non-inclusion criteria.
8. Make sure that the informed consent was obtained.
9. The questionnaire consists of two parts, Please complete all parts
    a. Part A: Clinical information
    b. Part B: Gestational diabetes knowledge test

PART A

STEVE BIKO ACADEMIC HOSPITAL ANTENATAL CLINIC
DIABETIC PATIENT QUESTIONNAIRE

Date of Examination: ……/……/……

Examining Doctor: ……

PATIENT STICKER

DEMOGRAPHICS AND SOCIAL HISTORY:

Date of Birth: ……/……/……..

Age: ……

Home Language: ……..

Marital Status: Single / Married / Divorced /Widow / Other ……..

Current Employment: Unemployed / Housewife / Informal / Formal / Other ……

Employment: Full time / Part time

Social Grants: Yes / No

Access to: Water / Sanitation / Electricity

Educational level: None / Primary / Secondary / Diploma/Degree / Other ……..

Smoking: Never / Past – Year Stopped: ……./ Current – Number of cigarettes/week / Other……..

Alcohol: No/ Social / Binger (present/past) / Regular Units/day: **……**

Supplements/vitamins:

OBSTETRIC/GYNAECOLOGICAL HISTORY

Parity: ……...

Gravidity: ……

Miscarriages:……..

Ectopic: …….

Last Normal Menstrual Period (Current pregnancy): ……/……/……..

Gestational Age: ……

Expected Date of Delivery: ……/……/……..**by Early Sonar / Sure Dates / Uncertain**

Previous pregnancies:

YEAR	*GESTATIONAL AGE AT DELIVERY*	*ROUTE OF DELIVERY*	*BIRTH WEIGHT*	*OUTCOME*	*COMPLICATIONS / CONGENITAL ABNORMALITIES / PREVIOUS STILLBIRTH / MACROSOMIA / DIABETES / POLYCYSTIC OVARIAN SYNDROME*

Rhesus: Positive / Negative – Coombs …… **RPR:** Negative / Positive ……

HIV: Pos / Neg/ Unknown **CD4 count:…… HIV viral load: _____**

Antiretroviral drugs – Date of Commencement: …../……/…….. **Drugs:** ……...

Contraceptive Use: ……

Injectables:……

Infant Feeding: …….

DIABETES HISTORY

Type of diabetes: Type 1 / Type 2 / Secondary / Gestational / Uncertain……

Year Diagnosed:……..

How Diagnosed: Symptoms - Polyuria / Polydipsia / Weight loss / Other …….

OGTT result: 0 hour …… 1 hour …… 2 hour ……

OGTT adverse effects: Nauseous / Other……..

History of diabetes in previous pregnancies: Yes / No……..

Has a Dietician been consulted: Yes / No

Home Glucose monitoring: Yes / No **Glucometer:** Yes / No

HbA1C at conception: ……

Current Medications:

Diet Only / Sulphonylureas / Glucophage / Insulin / Acarboses / ACE inhibitors / Diuretics / ARVs / Other**:**

……

Any episodes of hypoglycaemia: Yes / No

Any episodes of DKAs: Yes / No

Details of hospital admissions: ……

…….

MEDICAL HISTORY

Other chronic illnesses:

Medications and Dosage:

……

……

Target organ damage: Eyes, kidneys, heart, nervous system

24-hour urine:

spot urine protein:creatinine ratio: _____

Protein: ……g/24h

Creatinine Clearance: ……ml/min

U/E: Urea……. Creatinine…….

FBC: Hb…….

Fundoscopy: _____

Allergies: ……

Other medical and surgical history: ……

FAMILY HISTORY:

Diabetes: Yes/ No – Relation…….

Hypertension: Yes/ No – Relation……..…

MI <60years: Yes/ No – Relation……

Other: Yes/ No – Relation……...

EXERCISE HISTORY

On average how many days a week do you exercise? _____days

On the days you do exercise, on average:

- How many minutes do you exercise for? _____minutes
- How hard are you exercising? / How intense are your exercise sessions? ___Low / ___Moderate/ ___High

Note: The talk test is an easy indicator of the intensity at which you are exercising.

- Low intensity- If you can sing several phrases of a song without breathing hard.
- Moderate intensity- if you can have a conversation and breathe comfortably whilst exercising.
- High intensity- if you must take a breath between every word you say whilst exercising.

CLINICAL EXAMINATION:

GENERAL:

Weight: ……kg Height: ……cm BMI: ……

MUAC: ……cm Waist circumference ……cm Hip circumference ……cm

Mid-thigh circumference ……cm

Jaundice / Anaemia / Cyanosis / Clubbing / Oedema / Lymphadenopathy / Skin/thyroid/breasts/ Normal

Details …….

CARDIOVASCULAR:

Blood Pressure: Clinic measurement (routine) ……../…….mmHg

Right (study)……../…….mmHg Left (study) ……../…….mmHg

Pulse: …….. b/min Rhythm: Normal / AF / Other ……...

Signs of CCF (crepitations, raised JVP, Oedema, S3): ……

RESPIRATORY: ……

ABDOMEN: ……

Glucose control:

- HbA1c ……g/dL
- Last 2 fasting values 1)…….mmol/l 2) ……mmol/l
- 7-day average (glucometer) ……mmol/l

PART B - Gestational Diabetes Mellitus (GDM) Questionnaire

KNOWLEDGE OF GESTATIONAL DIABETES

Below are some statements about diabetes. There may be one or more correct answers. After reading the statements, please tick whatever answers you believe are true. If you do not know the answer, please tick the box (I don't know).

1. **You have been selected for this study as you have been diagnosed with GDM. What do you understand by GDM?**

_____
_____
_____

2. **What does your GDM diagnosis mean to you? How does the diagnosis of GDM make you feel?**

_____
_____
_____

3. **Rate your knowledge of GDM:**

☐ **1-very poor** ☐ **2-poor** ☐ **3-fair/average** ☐ **4-good** ☐ **5-very good**

You may choose more than one option that applies to your knowledge of GDM (if you want that), ELSE Choose the option that BEST describes your response to this question. (if you only want ONE sort-of-best answer to the question)

4. **How is gestational diabetes diagnosed**

☐ my history
☐ blood test
☐ urine
☐ I don't know

5. **Because I have gestational diabetes, my baby may be:**

☐ larger than usual
☐ growth restricted
☐ born premature before 34 weeks
☐ admitted to neonatal ICU
☐ diagnosed with congenital anomalies
☐ I don't know

6. **Because I have gestational diabetes, after giving birth my child may:**

☐ have an increased risk of obesity
☐ grow up to have diabetes

☐ have learning disabilities
☐ have seizures/fits

7. Women are more likely to develop gestational diabetes if they:
☐ are overweight
☐ are over 35 years
☐ have a family history of diabetes
☐ previously had GDM
☐ previously had a small baby
☐ I don't know

8. Because I have gestational diabetes, I may:
☐ need to come to the clinic more frequently
☐ need a caesarean section
☐ develop permanent diabetes later in life
☐ break my water before time (prematurely before 34 weeks)
☐ need to terminate my pregnancy
☐ I don't know

9. In uncontrolled diabetes the blood sugar is:
☐ decreased
☐ normal
☐ increased
☐ increased and decreased
☐ I don't know

10. Gestational diabetes
☐ develops during pregnancy
☐ develops before pregnancy
☐ disappears once the baby is born
☐ may lead to diabetes in later life
☐ may cause my baby to be born early
☐ I don't know

11. Gestational diabetes may be treated with:
☐ diet
☐ diet and exercise
☐ insulin/metformin
☐ all of the above
☐ I don't know

12. When my baby is born:
☐ my diabetes will disappear
☐ I have to continue to monitor my glucose 8-times a day
☐ I no longer have to follow a diet plan
☐ I should get a follow-up glucose test at my 6-week check-up

☐ I don't know

KNOWLEDGE ON GDM MANAGEMENT AND BLOOD GLUCOSE TESTING

The following questions require you to tick **ONE** box only. Select the MOST correct option.

13. Is regular glucose testing important during GDM?

☐ Yes
☐ No
Why? _____

14. You should check your blood glucose levels

☐ regularly for the health of you and your baby
☐ occasionally
☐ only when you feel unwell
☐ before you go to see the doctor
☐ I don't know

15. When I control my blood glucose levels:

☐ it does not affect the baby
☐ it will badly affect the baby
☐ it does not affect the pregnancy outcome
☐ none of the above
☐ I don't know

16. I should test my blood glucose level:

☐ in the morning before breakfast
☐ in the afternoon before lunch
☐ 2 hours after meals
☐ At 2 am
☐ All of the above
☐ I don't know

17. Do you know what the glucose should be?

☐ Yes
☐ No

If YES, kindly share the value/(s): _____mmol/l

If NO skip questions 18, and 19

18. A normal fasting (on an empty stomach) blood glucose level is:

☐ less than 5.3 mmol/L
☐ less than 7 mmol/L
☐ more than 7 mmol/L
☐ more than 9 mmol/L

19. A normal 2-hour (after eating) blood glucose level is:

☐ less than 5.3 mmol/L
☐ less than 6.7 mmol/L
☐ more than 7 mmol/L
☐ more than 9 mmol/L

20. What do I do if my blood glucose level is 8 mmol/L on one occasion?

☐ make a note in your diary
☐ check what you ate before the high blood glucose level
☐ inject additional insulin and recheck your glucose
☐ go to the hospital immediately
☐ I don't know

20.1. What do I do if my blood glucose level is 12 mmol/on one occasion?

☐ make a note in your diary
☐ check what you ate before the high blood glucose level
☐ inject additional insulin and recheck your glucose
☐ go to the hospital immediately
☐ I don't know

20.2. What do I do if my blood glucose level is 25 mmol/L on one occasion?

☐ make a note in your diary
☐ check what you ate before the high blood glucose level
☐ go to the hospital
☐ I don't know

21. What do I do if my blood glucose level is between 10-12 mmol/L on two occasions in one week?

☐ make a note in your diary
☐ check what you ate before the high blood glucose level
☐ inject additional insulin and recheck your glucose
☐ contact the diabetes educator
☐ go to the hospital immediately
☐ I don't know

22. Should I check my blood glucose level if I am feeling sick and/or haven't eaten?

☐ yes, continue to take your blood glucose levels as usual
☐ no, do not check your blood glucose levels until you are feeling better or have eaten
☐ I don't know

23. When you prick your finger, you should:

☐ use the same finger every day
☐ use a different finger every day
☐ it is not important
☐ I don't know

KNOWLEDGE OF DIET, PHYSICAL ACTIVITY AND LOOKING AFTER YOURSELF AFTER GDM DIAGNOSIS

These questions may have more than one correct answer

24. Describe what you eat in a typical day:

Breakfast	
Lunch	
Dinner	
Snacks	**Morning:** **Afternoon:** **Evening:**
Water intake	**_____ glasses** **_____ tea** **_____ coffee**

25. Do you think that what you eat is important to control gestational diabetes?

☐ Yes
☐ No

26. If you have gestational diabetes, you should avoid food containing high content of

☐ Carbohydrates/starches (pap, rice, samp, potatoes)
☐ Protein/meat (beef, chicken, beans)
☐ Fat
☐ I don't know

27. Which of the following food can you eat more of during gestational diabetes

☐ Carbohydrates/starches
☐ Fruit
☐ Vegetables
☐ Meat
☐ I don't know

28. What dietary source is mainly provided by pap/pasta/rice/samp/potato?

☐ Carbohydrates/starches
☐ Protein
☐ Fat

☐ I don't know

29. How can you make pap/pasta/rice/samp/potato safer for your blood sugar to eat?

☐ cook and eat
☐ cook, cool and reheat
☐ add fats
☐ add lemon juice
☐ add vinegar

30. The preferred type of carbohydrate/starchy foods are:

☐ white bread
☐ brown or wholegrain bread
☐ foods that are high in fibre
☐ fruits and/or vegetables
☐ pap/pasta/rice/samp
☐ I don't know

31. What form of fruits and vegetables are better?

☐ fruit juice or vegetable juice
☐ processed or canned fruits and vegetables
☐ fresh fruit and fresh vegetables
☐ I don't know

32. How often do you eat fast food?

☐ never
☐ once a week
☐ more than three times a week

33. What fast food do you eat and why?

_____

_____

_____

34. Protein intake can be obtained from:

☐ meat
☐ fish
☐ nuts
☐ dairy such as milk or cheese
☐ potatoes
☐ vegetables
☐ I don't know

35. What type of chicken is good for you?

☐ skinless baked chicken
☐ skin-on chicken
☐ deep-fried chicken
☐ any chicken

☐ I don't know

36. A balanced diet should have:
☐ more vegetables
☐ fewer carbohydrates/starches such as white bread
☐ low fat and low sugar choices
☐ all of the above
☐ I don't know

37. When you are hungry in between meals:
☐ eat another meal/snack: _____
☐ drink water and see if that helps
☐ try and ignore it
☐ go for a walk
☐ I don't know

38. Is physical activity (movement) or exercise important to control gestational diabetes?
☐ Yes
☐ No

39. Physical activity or exercise in gestational diabetes:
☐ helps to control the mother's blood glucose
☐ improves baby's health
☐ is not helpful and makes you tired
☐ increases the risk of miscarriage during pregnancy
☐ I don't know

40. Which exercise is recommended during pregnancy:
☐ running
☐ skipping
☐ walking
☐ swimming
☐ team sports like soccer/rugby/netball
☐ I don't know

41. How hard can you exercise during pregnancy?
☐ mild exercise
☐ moderate exercise
☐ vigorous exercise
☐ until you are exhausted
☐ I don't know

42. How long should you exercise per day?
☐ 10 minutes
☐ 15 minutes
☐ till you get tired
☐ 30 minutes (one 30-minute session or three 10-minute sessions)

☐ I don't know

43. How long should you exercise per week?

☐ 60 minutes
☐ 120 minutes
☐ 150 minutes
☐ 300 minutes
☐ I don't know

44. Should I exercise if I am overweight and unfit?

☐ No, you should not
☐ Yes, you should start slowly and increase gradually
☐ First you need to lose weight
☐ I don't know

45. How can I increase my daily physical activity/exercise?

☐ walk children to school
☐ take the stairs instead of the lift or elevator
☐ walk to the shopping centre
☐ I don't have to be physically active/exercise
☐ I don't know

46. To control blood glucose effectively you should:

☐ eat a healthy, balanced diet only
☐ do moderate exercise 5-7 days a week for about 30 minutes a day only
☐ spend most of your time resting
☐ eat a healthy, balanced diet with moderate exercise for 150 minutes throughout the week
☐ I don't know

47. Rate the following statements on the quality of life. Strongly disagree = [1], Disagree = [2], Agree = [3], Strongly agree = [4], Do not know [5]

	1	2	3	4	5
17. I would rather eat something unhealthy than tell someone that I have diabetes					
18. I am worried about whether I will miss work					
19. I feel diabetes limits my career					
20. I feel satisfied with my blood glucose control					
21. I record my blood glucose levels in my chart/diabetes diary when my healthcare personnel ask me to					
22. Gestational diabetes has caused me not to enjoy my pregnancy					
23. I feel that my pregnancy is abnormal					

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Thank you for your participation

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