THE DEVELOPMENT OF CHILDREN BORN TO MOTHERS WITH DIABETES MELLITUS IN PREGNANCY: GROWTH, BODY COMPOSITION, AND COGNITIVE AND MOTOR DEVELOPMENT

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Declaration

I, Larske Marit Soepnel, declare that this thesis is my own original, unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other university or institution.

Signature 

25th day of June, 2021

Dedication

This thesis is dedicated to my mom, Liliane, my dad, Niels,

and

My sisters, Brechtje and Sabine.

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Publications and contributions

Part of this work has been distributed in the form of publications. My contributions per paper are described under each paper.

Publications:

I. Soepnel L.M., Nicolaou V., Huddle K.R., Klipstein-Grobusch K., Levitt N.S. and Norris S.A. Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV. *Int J Gynecol Obstet.* 2019;147(3):404-412. https://doi.org/10.1002/ijgo.12956.

Contributions: I contributed to the conceptualization of this paper with the support of my supervisors. I helped build the online database for the retrospective data extraction and subsequently performed the data extraction from original hospital files. I also contributed to the statistical analysis, interpretation, and the manuscript writing.

II. Soepnel L.M., Nicolaou V., Slater C., Chidumwa G., Levitt N.S., Klipstein-Grobusch K., Norris S.A. Obesity and adiposity of 3- to 6-year-old children born to mothers with hyperglycaemia detected in pregnancy in an urban South African setting. *Annals of Human Biology*. 2021; 48(2):81-92. Doi: 10.1080/03014460.2021.1918245.

Contributions: I contributed to the conceptualization of the research presented in this paper, as presented in chapter 1 of this thesis. Furthermore, I developed the study protocol, devised the necessary standard operating procedures, and contributed to building the online database used for the prospective data collection required for this paper. I also contributed to the project management from inception to finalization, which included the financial, logistical, and day-to-day operations of the project, as well as data quality control. Working with a small team of colleagues (one fellow PhD student and two research assistants), I contacted participants and

obtained informed consent from parents/guardians and conducted the data collection for mother/child pairs. I carried out the procedure for the deuterium dilution method for each child, took care of appropriate storage of samples prior to their analysis, and oversaw the analysis of samples. I conducted the data management, statistical analysis, interpretation, and manuscript writing for this paper.

III. Soepnel L.M., Nicolaou V., Draper C.E., Levitt N.S., Klipstein-Grobusch K., Norris S.A. Cognitive and motor development in 3- to 6-year-old children born to mothers with hyperglycaemia first detected in pregnancy in an urban African population. *Maternal and Child Health Journal (In review, submitted November 2020).*

Contributions: My roles in this project's conceptualisation, inception, and project management were as described above under paper II. I was additionally responsible for carrying out the cognitive test for each child, running the results from these tests through the test software, and cleaning and statistically analysing the data. I also conducted the interpretation and wrote the manuscript.

Additional related publications/submissions arising from this work:

- Nicolaou V., **Soepnel L.M.,** Huddle K.R., Levitt N.S., Klipstein-Grobusch K., Norris S.A. Maternal and neonatal outcomes following the introduction of oral hypoglycaemic agents for gestational diabetes mellitus were comparable to insulin monotherapy in two historical cohorts. *SAMJ*. 2020;110(2): 154-158. https://dx.doi.org/10.7196/samj.2020.v110i2.14024

Contributions: I contributed to the conceptualisation, database building, and data extraction for this paper. I also assisted with writing/editing of the manuscript.

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

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- Boerstra B.A., **Soepnel L.M.,** Nicolaou V., Kolkenbeck-Ruh A., Kagura J., Ware L.J., Norris S.A., Klipstein-Grobusch K. The impact of maternal hyperglycaemia first detected in pregnancy on offspring blood pressure in Soweto, South Africa. (*Submitted to Journal of Hypertension).*

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The 18th International Congress of Endocrinology, 53rd SEMDSA Congress, Cape Town, South Africa (2018). Poster presentation: Soepnel, L.M.: The short-term maternal and neonatal outcomes following diabetes in pregnancy in Soweto, South Africa, over a six-year study period.

The Faculty of Health Sciences Research Day, University of the Witwatersrand, Johannesburg, South Africa (2018). Poster presentation: Soepnel, L.M.: The short-term maternal and neonatal outcomes after diabetic pregnancy in Soweto, South Africa, over a six-year study period.

The Faculty of Health Sciences Research Day, University of the Witwatersrand, Johannesburg, South Africa (2020). Oral presentation: Soepnel, L.M.: Body composition and growth of 3 to 6-year-old children born to mothers with hyperglycaemia diagnosed in pregnancy in an Urban South African setting.

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Nomenclature

ANOVA	Analysis of variance
ART	Antiretroviral therapy
BMI	Body mass index
BW	Birth weight
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	Confidence interval
CV	Coefficient of variance
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray Absorptiometry
DIP	Diabetes in pregnancy, subcategory of HFDP
DOHaD	Developmental origins of health and disease
DPHRU	MRC/Wits Developmental Pathways for Health Research Unit
FDC	Fixed-dose combination
FFM	Fat-free mass
FIGO	Federation of Gynaecology and Obstetrics
FMI	Fat mass index
GA	Gestational age
GDM	Gestational diabetes mellitus, subcategory of HFDP
GSEM	Generalised structural equation modelling
HAPO	Hyperglycaemia and Adverse Pregnancy Outcomes, Study Group
HbA1c	Glycated haemoglobin
HeLTI	Healthy Life Trajectories Initiative
HEU	HIV-exposed, uninfected
HFDP	Hyperglycaemia first detected in pregnancy
HIC	High-income country
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
IADPSG	International Association of the Diabetes and Pregnancy Study Groups
IAEA	International Atomic Energy Agency
IC	Information criteria
IDF	International Diabetes Foundation

IQR	Interquartile range
LBW	Low birth weight
LMIC	Low- or middle-income country
MBG	Mean blood glucose
MUAC	Mid-upper arm circumference
NCD	Non-communicable disease
NDDG	National Diabetes Data Group
OGTT	Oral glucose tolerance test
OHA	Oral hypoglycaemic agent
OR	Odds Ratio
PIH	Pregnancy-induced hypertension
PNM	Perinatal mortality
SD	Standard deviation
SEMDSA	Society for Endocrinology, Metabolism and Diabetes of South Africa
SES	Socioeconomic status
SSA	Sub-Saharan Africa
TBW	Total body water
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
UK	United Kingdom
USA	United States of America
WC	Waist circumference
WHO	World Health Organization

Abstract

Background

Globally, the prevalence of hyperglycaemia in pregnancy is increasing, driven by risk factors associated with the nutrition transition and an increasingly sedentary lifestyle. In spite of this, little is known about the short or long-term outcomes of children exposed to hyperglycaemia in pregnancy in sub-Saharan Africa (SSA). The pregnancy factors associated with birth outcomes in pregnancies complicated by either pre-existing diabetes, or hyperglycaemia first detected in pregnancy (HFDP) are not thoroughly understood, particularly in light of context-relevant factors such as maternal HIV infection. In addition to these short-term obstetric consequences, HFDP may adversely impact early cognitive development and childhood body composition, contributing to an already high population level of childhood obesity. As a growing number of children are exposed to hyperglycaemia in utero, understanding its role as an early developmental determinant can contribute to improving the health and development of the next generation.

Aim

The overarching aim of the study was to assess the impact of diabetes in pregnancy on childhood outcomes in an urban African setting. Objectives included investigating outcomes of pregnancies complicated by pre-gestational diabetes or HFDP, the impact of HIV-infection on these outcomes, and assessing the association between HFDP and childhood body composition and cognitive and motor development at preschool age.

Methods

Firstly, a retrospective analysis of the hospital records of 1071 women with hyperglycaemia in pregnancy at a large tertiary hospital in Soweto, Johannesburg was undertaken, with data of interest including pregnancy characteristics, comorbidities, and obstetric and neonatal outcomes. Secondly, a comparative study was performed in 3–6-year-old children born to mothers with HFDP, subdivided into gestational diabetes mellitus (GDM) and ‘overt’ diabetes in pregnancy (DIP), versus a group of HFDP-unexposed children, to examine the association with: 1) childhood overweight, adiposity and growth, and 2) cognitive and motor development. Inclusion criteria were met by 204 mother/child pairs. Children’s anthropometry was measured, and the deuterium dilution method was used to determine fat mass index (FMI). The Herbst Early Childhood Development Criteria test was performed to assess cognitive, fine

motor, and gross motor development, completed in 194 of the children. Hierarchical regression analyses were performed to assess the association between HFDP exposure and childhood outcomes. Generalized structural equation modelling (GSEM) was performed to explore the direct and indirect effects of maternal BMI on childhood BMI and adiposity in the context of HFDP.

Results

The rate of adverse obstetric and neonatal outcomes, including macrosomia (8.4%), caesarean section (72.2%), perinatal mortality (PNM) (3.7%), and prematurity (30.9%), were significant despite management of hyperglycaemia during pregnancy. HbA1c at the initial visit, maternal BMI, and glycaemic control during the third trimester were important predictors for neonatal outcomes. Moreover, PNM was significantly higher in pregnancies additionally complicated by maternal HIV infection (9.4% vs 1.8%).

At 3-6 years old, the prevalence of childhood overweight/obesity was 10.8% in HFDP-exposed children, and 3.9% in the HFDP-unexposed group. The prevalence of stunting was 13.7% in the HFDP exposed group and 9.8% in the HFDP-unexposed group. The association between HFDP and log-transformed FMI, significant in the DIP-exposed group (unstandardized B=0.12 [95% confidence interval (CI) =0.01-0.22], standardized β=0.17, p=0.026), was attenuated and no longer significant when adjusting for maternal pregnancy BMI (B maternal BMI=0.01 [95%CI=0.003-0.015], β=0.23, p=0.004). No significant association was found for childhood BMI. GSEM indicated a direct effect of maternal pregnancy BMI and birth weight z-score on both childhood BMI and FMI.

HFDP exposure was inversely associated with higher cognitive score categorisation, independent of maternal factors such as household socioeconomic status (OR 0.33 [95%CI 0.15-0.74], p=0.007). Differences in fine motor skills were attenuated when adjusting for maternal factors, particularly household socioeconomic score (OR 0.62 [95%CI 0.28-1.37], p=0.239), and no differences were found in gross motor skills between the groups.

Conclusions

Severity of hyperglycaemia, maternal BMI, and maternal HIV infection were associated with obstetric and neonatal outcomes. Maternal BMI was found to be associated with childhood

obesity and adiposity at preschool age, while stunting was present at a significant rate in the cohort. The identified association between HFDP and early cognitive development is concerning and requires further exploration and prevention efforts in our urban South African setting. These findings, relevant to other transitioning low-and middle-income countries, indicate the need for improved preconception care for maternal and child health, and highlight the need for additional research into early childhood risk factors as well as the intergenerational effects of maternal metabolic conditions.

Preface

During the completion of my medical degree in Utrecht, the Netherlands, I had the opportunity to develop my longstanding interest in global health through a research internship at the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU) in Soweto-Johannesburg, South Africa. Through this experience, non-communicable diseases (NCDs) were reframed for me as a health crisis that not only impacts low- and middle-income settings, but possibly has the direst consequences here due to existing constraints of health resources and limited population-specific research (1). With the guidance of my supervisors, Professor Shane Norris and Associate Professor Kerstin Klipstein-Grobusch, my budding interest in the intersection of NCDs, maternal, and child health developed into an aspiration to explore how increasingly prevalent conditions such as maternal diabetes may adversely impact childhood development, with its myriad of long-term individual, societal, and economic consequences.

The concept that environmental exposure at times of developmental plasticity, including the intrauterine period, can have consequences for offspring health and development has been described by the Developmental Origins of Health and Disease (DOHaD) theory (2). Understanding these long-term consequences and the role they play in an intergenerational cycle of disease could contribute to placing hyperglycaemia in pregnancy more firmly on the public health agenda.

A review of the existing literature and the development of a conceptual framework led to the consolidation of this concept into the overarching research question: "How does maternal hyperglycaemia in pregnancy impact childhood development and health in terms of birth outcomes, body composition, and early cognitive and motor development?"

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

Part I: Introduction

- **Chapter 1**: Background and literature review
- **Chapter 2**: Methods

Part II: Empirical chapters

- **Chapter 3**: Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV.
- **Chapter 4**: Obesity and adiposity of 3- to 6-year-old children born to mothers with hyperglycaemia detected in pregnancy in an urban South African setting.
- **Chapter 5**: Cognitive and motor development in 3- to 6-year-old children born to mothers with hyperglycaemia first detected in pregnancy in an urban African population.

Part III: Discussion and conclusion

- **Chapter 6**: Integrated discussion

Part 1: Introduction

Chapter 1: Introduction and literature review

1.1 Introduction and problem statement

Hyperglycaemia in pregnancy is an increasingly significant public health challenge and priority due to its growing prevalence worldwide (3). This is occurring in line with the rampantly increasing burden of non-communicable diseases (NCDs), including in sub-Saharan Africa (SSA), and South Africa more specifically, where the disease burden was traditionally associated with communicable diseases and undernutrition (4). However, there is a paucity of data on the consequences of a hyperglycaemic pregnancy for child outcomes in this setting. Consequently, while numerous short-term complications are associated with hyperglycaemia in pregnancy, the prevalence of these adverse obstetric and neonatal outcomes and associated maternal characteristics is not fully understood. Additionally, little is known about the long-term implications for the offspring, particularly for hyperglycaemia first detected in pregnancy (HFDP), the most common form of hyperglycaemia in pregnancy.

Evidence for the impact of prenatal factors on the health and development of the next generation is accumulating (5,6). Exposure to adverse environmental factors in pregnancy, such as hyperglycaemia (6,7), is thought to result in changes in fetal development leading to increased vulnerability to obesity and NCDs in later life. Similarly, cognitive and motor development may be impacted by the prenatal environment. These outcomes in childhood, in turn, have long-lasting implications for both the individual and for future generations, in a vicious cycle of NCDs and poverty.

However, this association is not fully understood, especially in the context of low- or middle-income countries (LMICs) such as South Africa, undergoing a nutritional and epidemiological transition. Understanding the health outcomes of the ever-growing group of children born to mothers with hyperglycaemia helps to create an informed platform for developing health interventions. Therefore, this thesis seeks to explore the short-term complications associated with pre-existing diabetes and HFDP, as well as the longer-term body composition, growth, and cognitive and motor development of children born to mothers with HFDP. To this end, a retrospective analysis of obstetric and neonatal outcomes was performed, and the development

of children born to mothers with HFDP in Soweto, South Africa, was evaluated at preschool age and compared to a group of HFDP-unexposed children.

1.2 Conceptual framework

Figure 1.1 shows the conceptual model that forms the basis of this study, which was conceptualised based on frameworks of early life programming and the theory of Developmental Origins of Health and Disease (8,9). In this chronological framework, pregnancy and early childhood represent periods of susceptibility to maternal and environmental exposures (marked in red) due to early life programming for development and future disease risk, specifically cognitive development and obesity/adiposity. Preconception and confounding factors play a role in determining which exposures are present from conception to early childhood, and a cyclical, intergenerational effect may be present.

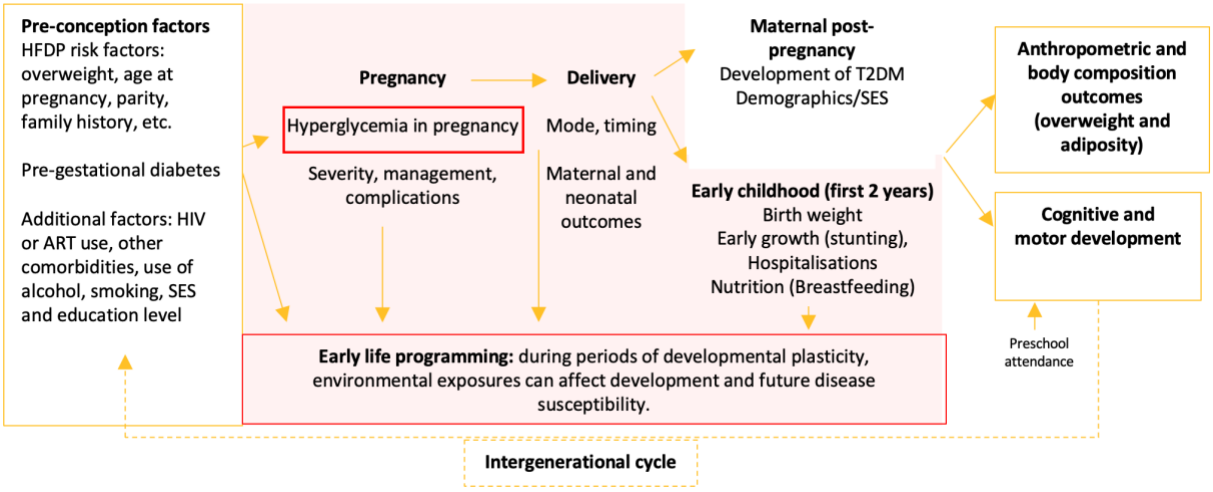


Figure 1.1: Conceptual framework used in this study.

1.2.1 Preconception factors

High pre-pregnancy body mass index (BMI), higher maternal age at pregnancy, and a family history of diabetes are pre-conception risk factors for developing HFDP, thereby impacting the intra-uterine environment. Moreover, maternal preconception factors themselves can have an independent effect on offspring development. These include maternal obesity/nutrition, presence of maternal comorbidities, and other environmental and behavioural factors such as alcohol and smoking.

Current evidence and theory suggest that these preconception factors are of great importance because they impact the periconception period (from oocyte and spermatozoa to blastocyst), in which environmental factors have an impact on the rapidly changing cell development, epigenetics, and metabolism (10). For example, both maternal overnutrition and undernutrition in the preconception period can impact the offspring through placental function (11), offspring development and long-term cardiometabolic outcomes (10). While the mechanistic pathways for this are not fully understood, maternal inflammation and epigenetic modifications likely play an important role (10,12).

1.2.2 Pregnancy, delivery, and early childhood

During the pregnancy, the presence of hyperglycaemia produces an environment of overnutrition, which leads to changes in the metabolome, hormone, and inflammatory exposure and induces epigenetic alterations (6,13). Disease-related factors that may influence this relationship include the severity of hyperglycaemia, and the presence and efficacy of management (14,15). The resulting fetal developmental changes can impact childhood and later life body composition, growth, and cognitive/motor development (Figure 1.1), particularly with the accumulation of additional risk in early childhood.

1.2.3 Early childhood

Furthermore, delivery and early childhood risk factors during critical perinatal and early childhood development periods, marked in red in Figure 1.1, may impact the relationship between HFDP and adverse childhood outcomes. These include timing of delivery (prematurity), neonatal outcomes such as low or high birthweight, stunting, early life hospitalizations, and breastfeeding practices. Together, these characteristics of the first 1000 days following conception impact early life programming for childhood development and, later, susceptibility to NCDs.

1.2.4 The intergenerational cycle

Childhood adiposity is associated with later obesity and higher cardiometabolic risk, including impaired glucose metabolism, increasing the likelihood of a diabetic pregnancy in female offspring (16). In addition, impaired cognitive and motor development are associated with

poorer health, school performance, and economic development, which in turn increase risk for poor development and fewer human capital opportunities (such as employment), and poorer health in the next generation (17). In this way, hyperglycaemia in pregnancy and the associated adverse childhood outcomes can contribute to an intergenerational cycle of disease and poor development (dotted arrow in Figure 1.1).

1.3 Study context: the burden of diabetes in LMICs and South Africa

The International Diabetes Foundation's (IDF) estimate of 415 million people worldwide with diabetes, is expected to increase to 642 million by 2040. Mortality from diabetes accounted for 10.7% of the global mortality among people aged 20 to 79 years in 2017 (18), and the prevalence of diabetes is rising faster in LMICs than in other regions (19).

Moreover, the burden of diabetes in LMICs is amplified by a lack of access to diabetes prevention, diagnosis, and management, with many remaining unaware of their diabetes status (20). An estimated 70% of people with diabetes in Africa are unaware of their diagnosis (18). The resulting impact of diabetes in LMICs is illustrated by the fact that diabetes is the fifth-leading cause of death in South Africa (21) (Figure 1.2).

This rising burden of diabetes, along with other NCDs, is often described using the frameworks of the epidemiological and nutrition transition. The epidemiological transition (22), describes a trend from infectious disease and malnutrition-related mortality to mortality at older ages due to non-communicable or degenerative diseases. The nutrition transition (23) focuses on the shift from traditional diets and problems such as undernutrition towards obesogenic lifestyles and high-caloric diets, resulting in a rise in obesity-associated NCDs. Drivers of the epidemiologic and nutrition transition in LMICs include urbanization, globalization, economic growth, and shifts in social or lifestyle behaviour patterns (4,23).

In practice, however, LMICs such as South Africa are found to display a double burden of disease, with NCDs increasingly occurring in combination with persistent infectious diseases and the introduction of new or re-emerging infectious diseases such as HIV and tuberculosis (4,24–27). Similarly, transitioning countries have been found to have a double burden of

malnutrition, where undernutrition and obesity coexist within the same community, household, or person (28–30).

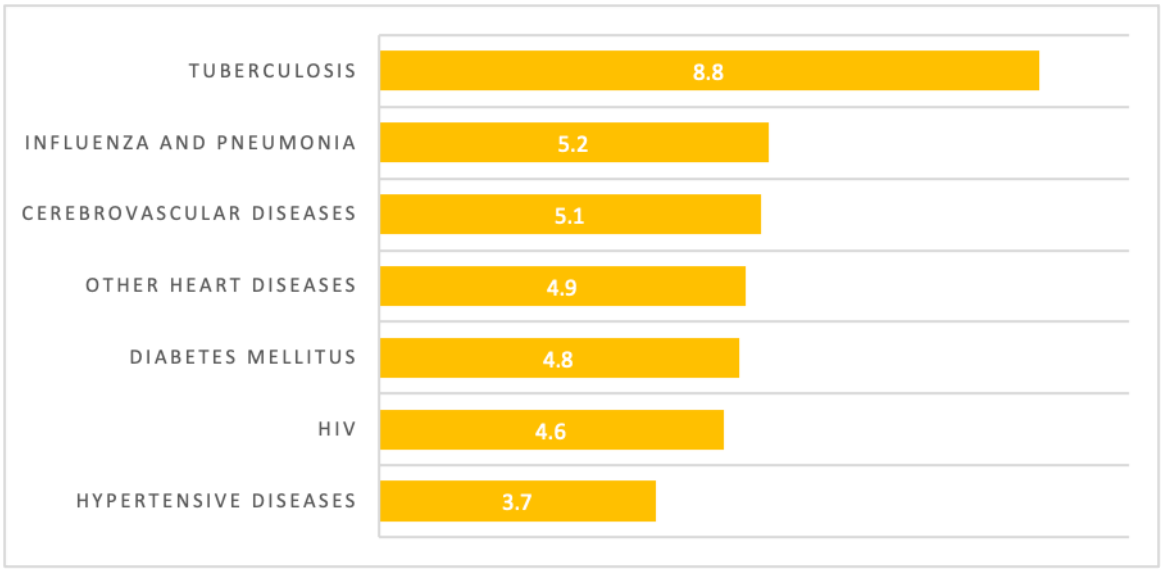


Figure 1.2: Leading causes of mortality by percentage in South Africa, with diabetes mellitus being the fifth (data from Statistics South Africa, 2018 (21))

1.4 Diabetes in pregnancy

1.4.1 Definition: A historical context

While hyperglycaemia in pregnancy has previously been divided into pre-gestational diabetes and the broad category of gestational diabetes mellitus (GDM), which in this context was defined as "any degree of hyperglycaemia with onset or first recognition during pregnancy" (3), the current definition used by the World Health Organisation (WHO) and the Federation of Gynaecology and Obstetrics (FIGO) is more precise in distinguishing between the less severe 'true' GDM and 'overt' diabetes in pregnancy (DIP), as two subcategories of HFDP (Figure 1.3) (3). The reasoning behind this is that women presenting with severe levels of hyperglycaemia, may in fact have previously undetected pre-gestational diabetes, requiring additional monitoring in terms of diabetic complications, such as microvascular complications. Consequently, the term "GDM" may refer to different concepts and levels of hyperglycaemia in pregnancy in the literature base, depending on the definition adopted by authors.

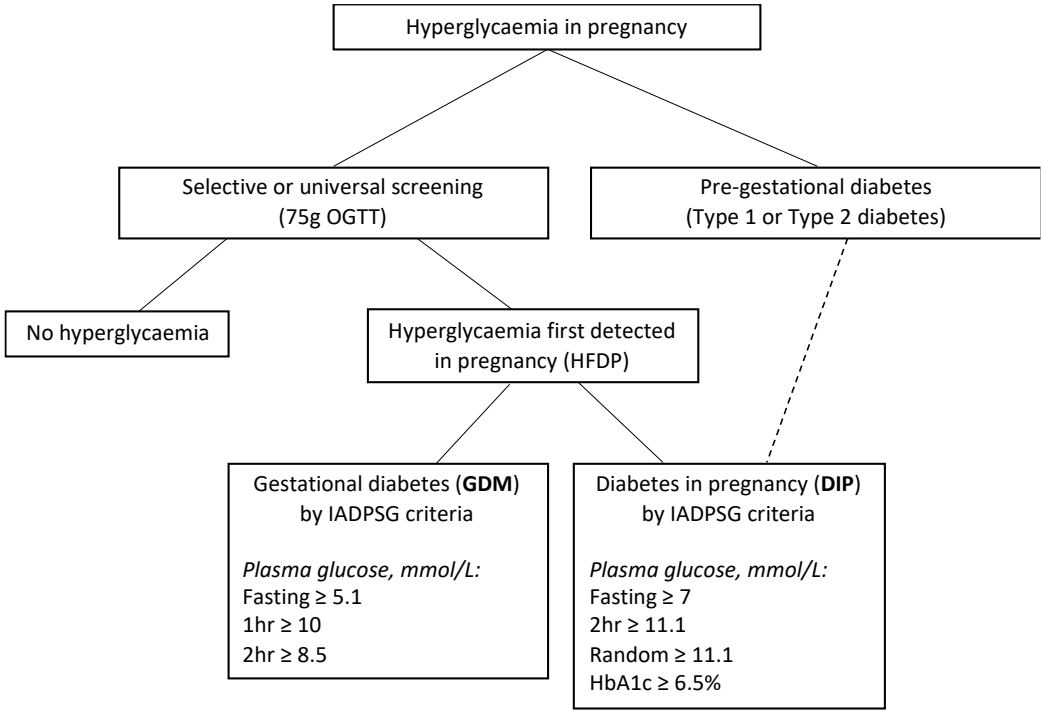


Figure 1.3: Graphical representation of the definition of hyperglycaemia in pregnancy, HFDP, GDM, and DIP.

These discrepancies in the theoretical definitions of GDM/HFDP are further compounded by a lack of international consensus over diagnostic criteria, both over time and between organisations. This is indicated by Figure 1.4, which presents a summarized timeline of the introduction of various diagnostic criteria and their endorsement by global and South African diabetic and gynaecological organisations. Criteria introduced in the 1960s (the O'Sullivan and Mahan criteria) for a 100g oral glucose tolerance test (OGTT) were based on taking two-standard deviations (SDs) above an established mean of blood glucose levels. These criteria were subsequently validated by determining an increased risk of postpartum development of diabetes in these women (31,32). These cut-offs were based on whole-blood analysis for glucose, and they have since been adapted to modern plasma analysis methods, resulting in the Carpenter and Coustan and the National Diabetes Data Group (NDDG) Criteria (32,33). In practice, these criteria for a 100g OGTT are commonly employed following a 50g OGTT screening test. Neither of these criteria were based on data establishing the risk of obstetric and neonatal complications as correlated to levels of maternal hyperglycaemia. Moreover, other criteria, such as the 1999 WHO criteria, were based on the diagnostic cut-offs for overt diabetes in non-pregnant women to diagnose "GDM".

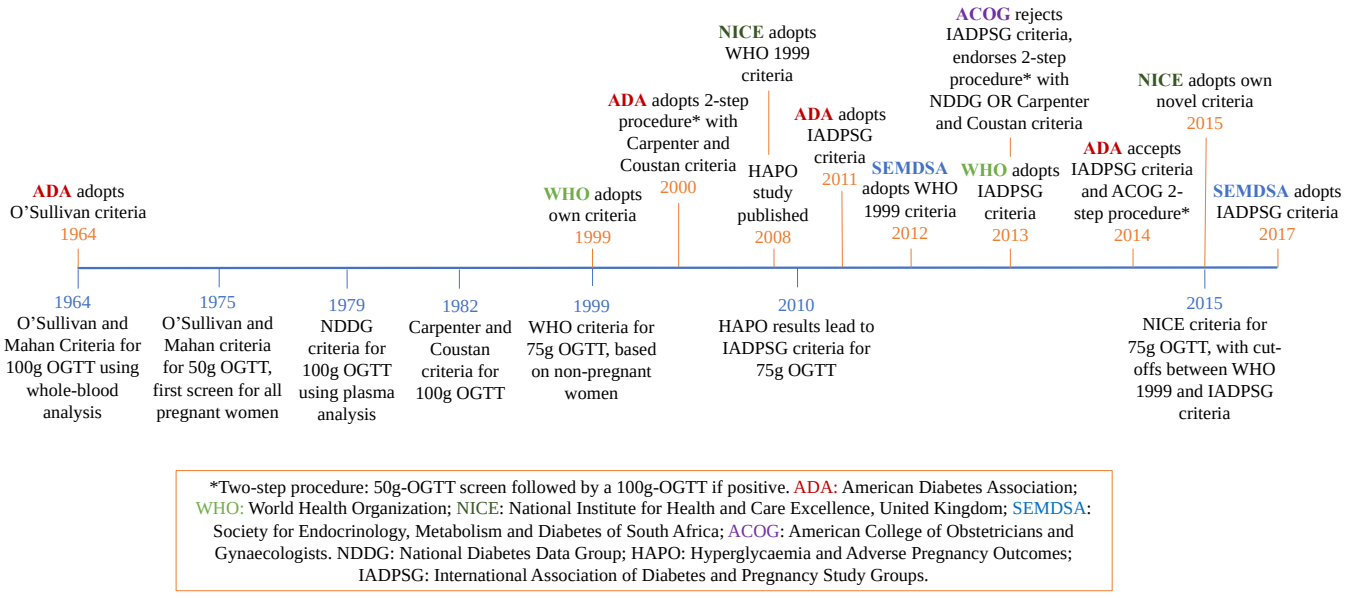


Figure 1.4: Timeline of introduction of diagnostic criteria for gestational diabetes and adoption by major international and South African organisations.

In an attempt to reach a global consensus for diagnostic criteria for GDM based on an increased risk of obstetric complications, the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) Study was carried out. This landmark study investigated the adverse maternal and neonatal outcomes associated with various degrees of hyperglycaemia following a 75-gram OGTT, in 25,505 pregnant women. Results indicated a strong relationship between maternal levels of hyperglycaemia lower than those diagnostic of overt diabetes, and adverse obstetric outcomes including birth weight, caesarean section, and neonatal hypoglycaemia. This resulted in the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria, shown in Table 1.1, which distinguishes between GDM and overt DIP. The diagnostic threshold for true GDM is lower than previous criteria such as the Carpenter and Coustan or NDDG criteria for a 100-gram OGTT. This has raised concerns that adopting IADPSG criteria would lead to a significant increase in the prevalence and burden of GDM on health systems. However, HAPO's evidence that these lower hyperglycaemic levels are associated with adverse obstetric outcomes has led to the endorsement of the IADPSG criteria by the WHO, International Diabetes Federation, and the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) (Figure 1.4).

Table 1.1: IADPSG criteria for diagnosis of GDM and DIP

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

Despite the recommendation of the IADPSG criteria by SEMDSA, the actual practice of healthcare institutions still varies widely within South Africa. Moreover, limited resources prohibits the recommended practice of universal screening in most settings. In addition, referral structures, as well as the diagnostic criteria, differ within the country (34,35). This results in underreporting and undermanagement of GDM, as well as limiting the generalizability of research results across the country. For additional information on the referral structures and clinical practice in South Africa, please see Chapter 2, Section 2.1.

For the purpose of this thesis, the terms pre-gestational diabetes and hyperglycaemia first detected in pregnancy (HFDP), consisting of gestational diabetes mellitus (GDM) and overt diabetes in pregnancy (DIP), will be used to distinguish the different types of hyperglycaemia in pregnancy, according to the 2013 WHO guidelines, which recommend using IADPSG criteria (36). When comparing to existing literature, the definition and diagnostic criteria used is important to consider. While in most cases "GDM" will have been used to describe any form HFDP, the cut-offs for diagnosing "GDM" were higher, so that the term was mostly used to describe more severe maternal hyperglycaemia than in our current definition of GDM.

1.4.2 Prevalence

Along with other demographic groups, women of reproductive age are increasingly at risk of developing diabetes, largely under the influence of rising presence of obesity and sedentary lifestyles. While the general age of onset of (pre-)diabetes and diabetes risk factors is decreasing, there is a trend of increasing childbearing age. This results in a greater overlap between these conditions. An estimated 1 in 6 live births (16.2%) are complicated by some form of hyperglycaemia in pregnancy globally (18), the majority of which consist of HFDP.

Data about diabetes in pregnancy in Africa is limited. A recent systematic review showed that merely 6 African countries (Ethiopia, Tanzania, Mozambique, South Africa, Morocco, and Nigeria; 11% of the countries on the continent) had published prevalence data of HFDP (37). The majority of studies were done in urban, low- or middle-income country (LMIC) settings and published prior to 2010, and varying diagnostic criteria were used. Reported HFDP prevalence rates fall between 8.8 and 9.4% (37–39). Within South Africa, varying prevalence rates could be due to differences in population, screening practices, and diagnostic methods (39,40).

1.4.3 Risk factors for HFDP

The known risk factors for HFDP are obesity, excessive gestational weight gain, older maternal age**,** glycosuria**,** South Asian descent, a family history of diabetes, high parity, past history of GDM, polycystic ovarian syndrome, a history of a macrosomic infant, a history of poor obstetric outcome, including miscarriages, stillbirths, or unexpected perinatal death, and polyhydramnios (35,41–43)

A recent study from South Africa identified BMI greater than 30kg/m, a family history of diabetes, and maternal age over 35 as risk factors that are of particular significance in this population, and also found GDM participants to have higher anaemia rates and higher socioeconomic score (SES) (39). A study from Ghana similarly found an increased risk of HFDP in the presence of overweight and obesity, although maternal age and education were not found to be significant risk factors (38). More studies to corroborate these findings and explore the relative importance of these risk factors are needed.

It is further important to recognise that not all women with HFDP have these identifiable risk factors, and selective screening for HFDP based on risk factors has been found to miss from 24%-48% of cases that would be identified by universal screening (44–46).

1.4.4 Pathophysiology of HFDP

Insulin requirements increase over the course of a healthy pregnancy due to a physiological increase in insulin resistance (47). This adaptation allows for plasma glucose to efficiently

cross the placenta and reach the foetus to accommodate fetal growth (48). This subsequently results in increased maternal insulin secretion during the pregnancy. The increased insulin resistance, resulting from changes in insulin-receptor signalling (49), is thought to be largely in response to placental products, in particular human placental growth hormone (hPGH) and tumour necrosis factor- α, and returns to normal following delivery (50,51).

Just like diabetes outside of pregnancy, HFDP is characterized by an imbalance between the body's insulin requirements and the level of insulin secretion from pancreatic β-cells (49), resulting in insufficient absorption of glucose into muscle and fat cells, and causing elevated plasma glucose concentrations.

In women with HFDP, the physiological insulin resistance described above is thought to compound an already existing, chronic level of insulin resistance. (49). Moreover, women who develop HFDP are unable to sufficiently increase insulin secretion to the level required, in most cases due to existing pancreatic β-cell dysfunction related to chronic insulin resistance (49). In some women, particularly those diagnosed with DIP, these mechanisms already resulted in pre-existing (Type 2) diabetes prior to it being detected in pregnancy. For the remaining women, the physiological changes of pregnancy are the tipping point resulting in a state of hyperglycaemia.

1.4.5 Management of hyperglycaemia in pregnancy

Management of hyperglycaemia in pregnancy includes diet and lifestyle interventions, regular assessment by a physician, self-monitoring of plasma glucose levels, and pharmaceutical treatment (including insulin and oral hypoglycaemic agents (OHAs)), if necessary. For pre-gestational diabetes, family planning and counselling to ensure optimal glycaemic control prior to conception is key.

Treatment of GDM has been found to be effective for preventing adverse pregnancy outcomes (3), as corroborated by data from a specialised clinic in South Africa, which found dramatically improved outcomes, including perinatal mortality (PNM), for women attending a specialised gestational endocrine clinic compared to a group presenting after 36-weeks' gestation or receiving less than 2 weeks medical intervention (52).

Aside from improving obstetric outcomes, health system contact during a diabetic pregnancy is also an opportunity to combat the rampant increase in obesity, diabetes, and cardiovascular disease (CVD) through lifestyle changes, education, and health monitoring in a population vulnerable to NCDs.

1.5 The consequences of hyperglycaemia in pregnancy

1.5.1 Maternal and neonatal consequences

Although it is not one of the four main causes of global maternal death, which are haemorrhage, hypertension, sepsis and obstruction of labour, hyperglycaemia in pregnancy contributes to maternal mortality and morbidity by increasing the risk of these direct causes (see Figure 1.5). The 2008 HAPO study provided robust evidence from more than 25,500 pregnancies that hyperglycaemia, on a continuous scale, is associated with adverse pregnancy outcomes, including birth weight above the 90th percentile, caesarean delivery, neonatal hypoglycaemia and hyperinsulinemia, preterm delivery, birth injury, stillbirths, preeclampsia, and neonatal intensive care admission.

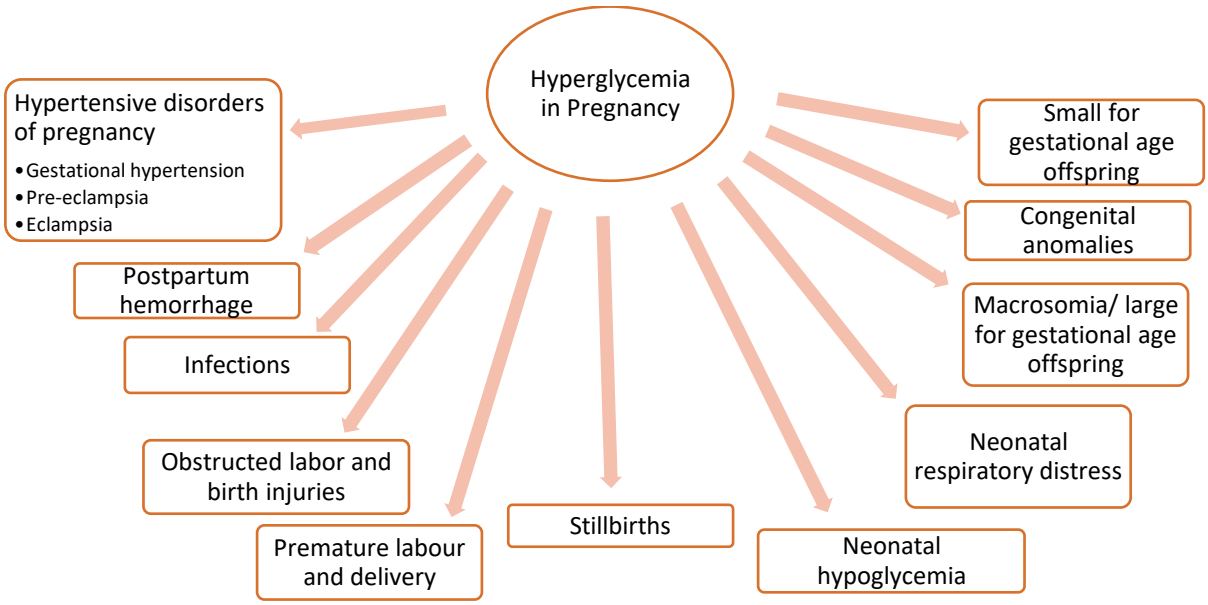


Figure 1.5: Obstetric and neonatal complications associated with hyperglycaemia in pregnancy.

The mechanism by which maternal hyperglycaemia impacts fetal growth is proposed by the Pedersen hypothesis, which states that an increased level of glucose crosses the placenta, resulting in increased fetal beta-cell insulin secretion and subsequently macrosomia, with its associated obstetric risks, such as birth injury, haemorrhage, respiratory distress, and prematurity. More recently, this theory has been extended to include the impact of other maternal metabolites in the context of hyperglycaemia, including lipids (53,54). Hyperglycaemia and hyperinsulinemia can result in fetal hypoxia (55), which adversely impacts fetal development, as is made evident by higher prevalence of miscarriages, stillbirths, and congenital anomalies in hyperglycaemic pregnancies. Moreover, a continued state of hyperinsulinemia after birth, when the maternal glucose supply is no longer available, can result in neonatal hypoglycaemia.

While some evidence on the outcomes of diabetes in pregnancy in SSA has arisen in recent years, which tends to show high rates of outcomes such as caesarean section, macrosomia and neonatal hypoglycaemia, most studies have relatively small sample sizes and heterogenous definitions for HFDP (46,56,57). Within South Africa, data on pregnancy outcomes of diabetes in pregnancy is specific to three specialised treatment centres. These previous studies suggest that adverse outcomes improved with treatment but were still higher than in the baseline non-diabetic population, particularly for Type 1 diabetes (52,58,59). One audit found few adverse outcomes in a mild hyperglycaemic group (defined as impaired glucose tolerance per the WHO 1999 guidelines) (58). This raises questions around the presence of complications and required level of management for women with true GDM as defined by the lower IADPSG cut-offs, requiring further research. Furthermore, the association of adverse outcomes with the use of oral hyperglycaemic agents in pregnancy was not consistent between studies (58,59). HIV infection and antiretroviral therapy during pregnancy are associated with adverse pregnancy outcomes, such as prematurity and low birth weight (60–62), but the role of HIV and antiretroviral therapy (ART) exposure in diabetic pregnancies has not, to our knowledge, been previously explored.

Since HFDP is the result of placental products that increase insulin resistance and glucose intolerance (49), particularly for GDM, glucose levels theoretically return to normal following delivery. However, the gestational disorder is indicative of future vulnerability to hyperglycaemia, with almost half developing type 2 diabetes. Postpartum follow-up is

therefore essential, but often not well-attended (63). This emphasizes the need to change our perspective of HFDP from a short-term condition to an early symptom of a long-term diabetes and CVD risk (64–66). Evidence for the long-term consequences for the offspring will be discussed in detail in the following sections.

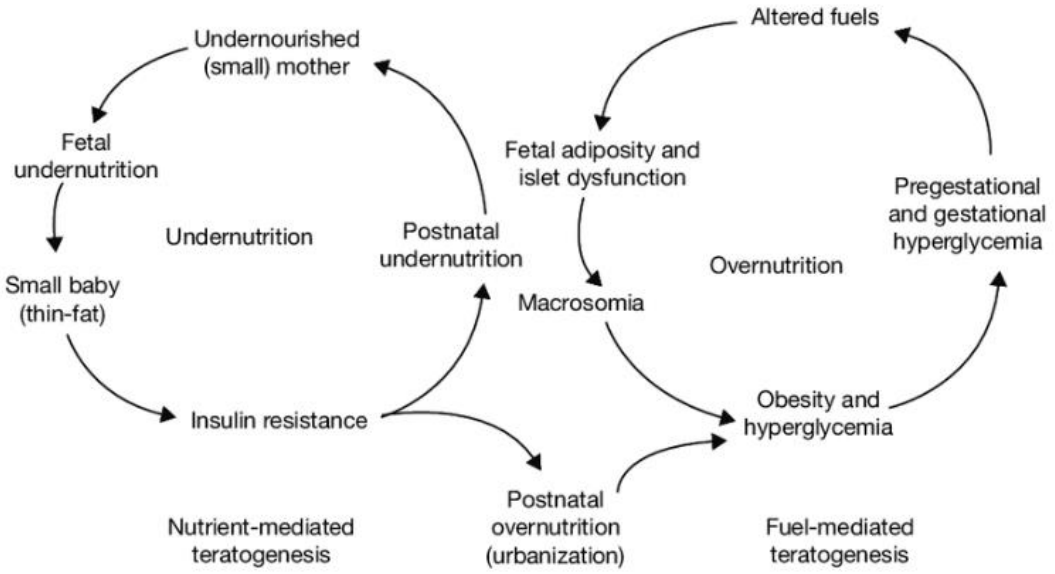
1.5.2 Hyperglycaemia in pregnancy and early life origins of disease

The 'Developmental Origins of Health and Disease' (DOHaD) theory recognizes that environmental exposures at times of developmental plasticity influence the development of future disease (2,67,68). The "first 1000 days" after conception (5,69) is a key time of developmental plasticity when rapid development occurs.

The DOHaD theory operates within a life-course approach to health and prevention, which explores biological, behavioural, and environmental determinants of NCDs from gestation to adult life and across generations. For instance, longitudinal cohort studies have shown an inverse relationship between birth weight and CVD in adulthood (70–72). Optimization of early growth and development is a cornerstone of the life-course approach to prevention.

Altered nutrient, inflammatory and hormone exposures in a diabetic pregnancy may result in epigenetic changes (73) and pre-programming for obesity and adverse cognitive development (6,13,74). Fuel-mediated teratogenesis, defined in 1980 by Freinkel and Metzger, describes altered intrauterine growth, with long-term health consequences associated with fetal hyperinsulinemia (31,75). The association of maternal undernutrition and low birth weight with increased NCD risk after a postnatal increase in nutrition has been described as "two sides of the same coin" (76), and both may be relevant in the context of hyperglycaemia in pregnancy in a transitioning country (figure 1.6).

In this framework, hyperglycaemia in pregnancy is not a transient disease with short-term obstetric consequences, as it has been approached in the past, but rather a complex risk profile for mother and child, raising questions regarding the health of future generations.



Fetal undernutrition results in insulin resistance, which, combined with postnatal overnutrition, increases the likelihood of obesity and hyperglycaemia, propagating the increase in obesity and NCDs in areas undergoing rapid transition.

Figure 1.6: The interrelationship of over-nutrition (fuel-mediated) and under-nutrition (nutrition-mediated) and the development of obesity and NCDs (hyperglycaemia); (Yajnik, 2009 (76))

1.5.3 HFDP and childhood obesity and overweight

Prevalence and implications

Childhood obesity and overweight, defined as "abnormal or excessive fat accumulation that presents a risk to health," was estimated to impact 41 million children under 5 years old in 2016 (77), and the burden continues to worsen in developed countries as well as LMICs (78–80). A meta-analysis showed that in SSA the trend towards higher obesity and overweight in school-aged children was more extreme in girls, those living in urban areas, and those with higher SES (78).

Childhood obesity is associated with socio-emotional consequences as well as health problems such as asthma, fatty liver disease, sleep apnoea, and type 2 diabetes, conditions that, in the past, were found predominantly in adults (81). Moreover, childhood obesity increases the risk for long-term health consequences, including CVD, type 2 diabetes, and certain cancers (82). An American study in 10,099 children found that up to 70% of obese 5- to 17-year-olds have at least one additional risk factor for CVD, including high blood pressure, poor lipid profile, or impaired glucose tolerance (16,83). Undeniably, adult adiposity is an important mediating factor in the relationship between childhood adiposity and future disease (84). Nevertheless, a

recent study in a black South African population showed that early-onset obesity or overweight in both girls and boys increased the likelihood of persistent overweight and obesity into adulthood (85).

For these reasons, children with an increased risk for obesity or overweight are a relevant target for early monitoring and lifestyle interventions in order to combat the non-communicable disease epidemic. Identifying such at-risk groups is therefore vital.

Aetiology of childhood obesity

Childhood obesity is a multi-faceted condition with an aetiology consisting of genetics and demographics (86,87), as well as modifiable environmental factors. These interrelated environmental factors include (87,88):

- Psychological factors, including body-image concepts and depression,
- Physical factors, including the home and neighbourhood,
- Socio-environmental factors, including family and peer behaviour and influence,
- Behavioural factors, such as dietary intake and physical activity,
- Biological factors, including metabolism and blood chemistry,
- Early life exposure factors.

A meta-analysis from 2016 explored early life exposure factors from conception to 2 years of age, associated with childhood obesity (89). Risk factors that were identified included maternal pre-pregnancy BMI, high infant weight gain, low SES, reduced infant sleep, attendance of childcare/preschool, premature cessation of breastfeeding, and possibly diabetes in pregnancy.

One additional consideration in SSA is the potential impact of HIV exposure on childhood body composition, particularly since there is a growing group of HIV-exposed, uninfected (HEU) children due to effective antiretroviral treatment during pregnancy. HEU children tend to be smaller than controls, with some reporting less overall body fat (90), but others report accelerated ‘catch-up’ growth in the first two years of life (91), and a South African study showed a high rate of 16% overweight in this group, similar to unexposed children (92). Moreover, evidence suggests that obese HEU children tend to have a worse cardiometabolic profile, possibly due to exposure to maternal ART (93). The impact of HIV-exposure in combination with other early life risk factors on childhood adiposity is not known.

Evidence for the role of HFDP

The association between diabetes in pregnancy and childhood obesity was first identified in the early 1980's in the Native American Pima population (94). Subsequently, results from studies have been contradictory, some supporting a positive association (15,95–100)**,** but others finding no evidence for an independent relationship between the two (101–105)**.** The role of the age of participants on the association is not entirely clear, due to the difficulty of comparing different methodologies and cohorts at different follow-up times, but there is some indication that the effect becomes more noticeable as participants age (106–108). While there is some evidence for a sex-specific association between HFDP and adiposity, these findings have been inconsistent across studies, with some only finding the association in boys (109), others in girls (who tend to have a higher fat mass in early childhood) (95,110), and others again showing similar findings in boys and girls (111).

Both individual studies and reviews have shown that correcting for maternal (pre-pregnancy) BMI has a strong attenuating effect on the association between maternal hyperglycaemia and childhood BMI (14,66,98–101,105,112–114), raising the question whether the effect is independent of maternal BMI. In a follow-up of the HAPO study, including 4832 children at a median of 11.4-years-old, obesity/overweight by BMI was not found to be significantly higher in the group exposed to untreated GDM when correcting for maternal BMI (66). However, other body composition measures, including body fat percentage by displacement plethysmography, skinfold thickness, and waist circumference remained statistically higher in the GDM-exposed group after correcting for maternal pregnancy BMI, and a significant independent association was found between childhood obesity and adiposity measures and continuous maternal glucose levels (111). This indicates that there may be independent and additive effects of GDM and maternal obesity on childhood adiposity.

BMI is not a fool-proof indicator of body composition or health outcomes during childhood, since it does not take into account varying body shapes during growth and the proportion of fat and lean body mass, which may differ between individuals and populations (115) (refer to Chapter 2, section 2.2.2). Aside from the HAPO-follow-up study, a number of other recent studies have explored the relationship between GDM and childhood adiposity with body composition measures other than BMI, with the majority finding an association between GDM

and adverse body composition, even if no independent association was found for BMI (Table 1.2).

Furthermore, evidence has arisen, from HAPO and other cohort studies, that children born to mothers with GDM may be predisposed to cardiometabolic risk factors, including impaired glucose tolerance, insulin resistance, higher blood pressure, heart rate, and lower height in various ethnic groups, not including Africans (116–119).

The mechanisms of these effects in terms of increased fetal growth/adiposity as well as long-term perinatal programming, are not fully understood. As described in the Pederson hypothesis, increased fetal glucose exposure results in hyperinsulinemia, which promotes growth and fat cell number and size. Fetal hyperinsulinemia may also influence hypothalamic feedback systems that regulate hunger and fat storage (120). However, fetal overexposure to various other fuels ('mixed nutrients') may also impact fetal growth and adiposity, including free fatty acids, triglycerides, and amino acids (54,120). For example, in an environment of insulin resistance, elevated maternal lipids may play an important role in increased fetal growth and obesity, although the exact molecular pathways are unclear (53). Recent metabolomics studies of participants of European, Asian, and Caribbean ancestry indicate that there are patterns of maternal lipid and sugar metabolites that are characteristic of hyperglycaemia, which may contribute to neonatal outcomes such as birth size (54,121). Additionally, medications used for hyperglycaemia may impact fetal and childhood growth. An example of this is metformin, which crosses the placenta and may impact foetal signalling pathways that affect nutrient uptake, requiring more research on possible adverse consequences (122).

Additionally, epigenetic modifications in response to the altered intrauterine environment are proposed to persistently alter expression of genes involved in "imprinting" the child's metabolism and energy homeostasis (120,123). Epigenetic changes may therefore be an important mechanism for the impact of GDM, not only on birth outcomes but on obesity and metabolic risk in later life. Although different forms of epigenetic modifications have been identified, DNA methylation, in which a methyl group is added to a gene site leading to gene silencing, is the most widely studied, including in the context of HFDP (120). In both human and animal studies, HFDP has been associated with changes in DNA methylation of various

genes, with changes detected at birth, during childhood, and in adult offspring (123–126), and this area is being actively researched.

Most of this existing evidence originates from high-income countries (HIC). A recent, as of yet unpublished, analysis of 5-6-year-old children born to mothers with HFDP in South Africa found an overweight/obesity prevalence of 26.5%, which may be higher than the background population (last national statistics are from 2012) (127), but no comparison to HFDP-unexposed children was made (T. Chivese, personal communication May 7, 2020). A multinational study showing a significant association at 7-11 years included data from two SSA countries, but only 14 hyperglycaemia exposed-cases from SSA were included, and context-specific trends or confounders were therefore unexplored (112). Therefore, the association of HFDP with childhood obesity within an African population, and within the context of high maternal HIV prevalence and the paradoxical trend of the double burden of malnutrition, remains to be thoroughly explored.

Table 1.2: Summary table of studies assessing the role of GDM/HFDP on body composition outcomes other than BMI.

Author, Year	Study design	Number of participants	Participant characteristics		Definition of exposure	Body composition measure	Summary of findings
			Age	Country of origin			
Chandler-Laney, 2012 (128)	Cross-sectional	51	5-10	United States of America (USA)	Participant-reported diagnosis of HFDP ("GDM"); criteria unclear.	Dual-energy X-ray Absorptiometry (DEXA)	Significantly higher fat percentage, trunk fat, and insulin secretion in exposed children, despite no difference being found in BMI-status. No adjustment for maternal BMI was done.
Gingras, 2013 (129)	Longitudinal	760 for DEXA (880 total)	13.2	USA	Impaired glucose tolerance and "GDM" by 2-step procedure with Carpenter & Coustan criteria.	DEXA	In early adolescence, no independent association was found between exposure ("GDM" or impaired glucose tolerance) and fat mass.
Grunnet, 2017 (116) *Danish Birth Cohort*	Longitudinal	637 for DEXA; (1158 total)	9-16	Denmark	HFDP ("GDM") as defined by 75-gram OGTT with Danish national criteria or WHO 1999 criteria.	DEXA	HFDP was associated with significantly higher fat mass and abdominal fat, significant after correcting for maternal BMI. Authors suggest that HFDP impacts childhood fat mass more significantly in the absence of maternal obesity.
Kaseva, 2018 (130)	Longitudinal	891	20-27	Finland	HFDP ("GDM") as defined by Finnish national criteria 1985-1989.	Bioelectric impedance analysis	Adverse body composition and higher fat percentage was found in children exposed to HFDP, in part explained through maternal obesity.

Kearney, 2017 (106)	Cross-sectional	86	3-12	Canada	HFDP ("GDM") by medical record and health registry review 2003-2013; criteria unclear.	DEXA	HFDP was associated with higher total and abdominal adiposity, and with an altered glycaemic profile.
Lowe, 2018 (66) *HAPO*	Longitudinal	4832	10-14	United Kingdom (UK), USA, China, Canada, West Indies, Israel, Thailand	GDM by IADPSG criteria.	Air-displacement plethysmography (BOD POD); skinfold thickness	Differences in body fat percentage, waist circumference and skinfold thickness, but not BMI, remained significant when adjusting for maternal BMI.
Patel, 2012 (105) *ALSPAC*	Longitudinal	4035 for DEXA (4198 total)	15	UK	HFDP ("GDM") by medical record review 1991-1992; criteria unclear.	DEXA	HFDP was associated with higher BMI, fat mass, and central adiposity, but this association was attenuated towards the null when adjusting for maternal BMI.
Zhao, 2016 (112) *ISCOLE*	Cross-sectional cohort	4740	9-11	Australia, Brazil, Canada, China, Colombia, Portugal Finland, India, Kenya, South Africa, UK, USA	Patient-reported HFDP ("GDM"); WHO 1999 and Carpenter & Coustan criteria.	Bioelectrical impendence analysis (portable device)	Central adiposity and body fat percentage were associated with GDM-exposure, but only central adiposity remained significant when adjusting for current maternal BMI.

A dual burden of stunting and obesity in childhood?

Stunting is defined as impaired growth and development in children due to poor nutrition, infection, and inadequate stimulation, and is quantified as height-for-age more than two SDs below a standardized median (131) (Figure 1.7). Data from South Africa suggests that, despite the increase in overweight and obesity, there is a persistent prevalence of stunting in South Africa (29). Stunting in early life is associated with far-reaching consequences, including adverse cognitive development (132) and a higher risk of NCDs (133,134), in particular if accompanied by later excessive weight gain.

Little is understood or researched about stunting in relation to HFDP, since the two do not traditionally occur in the same population groups. However, the growing prevalence of HFDP in a population group still contending with high rates of stunting has blurred these lines and created a situation where a stunting may follow an HFDP-pregnancy. Since stunting may be associated with development of childhood obesity (135–137), a compounded effect of both prenatal exposure to HFDP and stunting on obesity could be hypothesized.

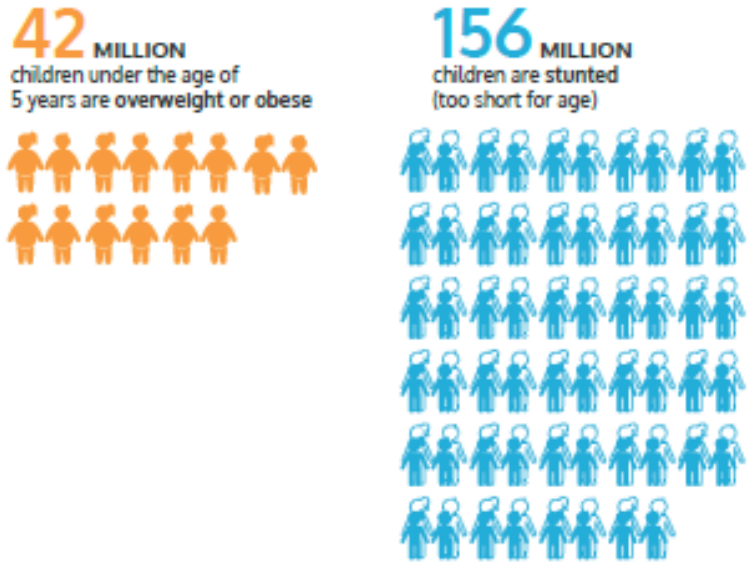


Figure 1.7: The double burden of both overweight and stunting in children is illustrated by these prevalence figures (WHO, 2016 (138)).

1.5.4 Childhood cognitive and motor development

Trends and implications of poor development

Since most LMICs don't have available data on childhood development, poor childhood development is largely an invisible one. It is estimated, however, that 61% of children in SSA

fail to reach their developmental potential (17,139), using global data for childhood growth retardation and absolute household poverty, which are important predictors for school achievement and cognition.

The consequences of the loss of developmental potential are relevant on both an individual and societal level. Studies in HIC and LMICs have shown that individuals with poorer early development have worse school performance, get fewer years of schooling, and go on to receive lower incomes, with one study estimating that poor development contributed to a decrease of 19.8% income per adult year (140,141). These factors, combined with higher fertility rates, contribute to the intergenerational cycle of poverty and developmental inequality (17). On a societal or national level, they impact economic development.

The importance of early child development at preschool age, also framed as 'school readiness', is supported by research showing that early life impacts brain development and neural connections (142,143), and a snag in the developmental process at an early age can lead to enduring changes in the brain's structure and function (144). Thus, cognitive performance in school, and thereafter, can be rooted in preschool exposures, environments, and stimulants.

However, vulnerability in early life can be offset by recovery through interventions, stimulation, and training. The earlier these interventions are initiated, the better the effect, as indicated by studies in Jamaica, Guatemala, and Africa (145–147). Therefore, interventions to improve developmental outcomes aimed at risk groups in LMICs at an early age may help to reduce persistent inequality.

Risk factors for poor cognitive and motor development

Early development is dependent on psychosocial, biological, and genetic factors. Figure 1.8 illustrates major risk factors for poor cognitive and motor development, adapted from those identified by the Lancet series on child health, 2007 (142,145).

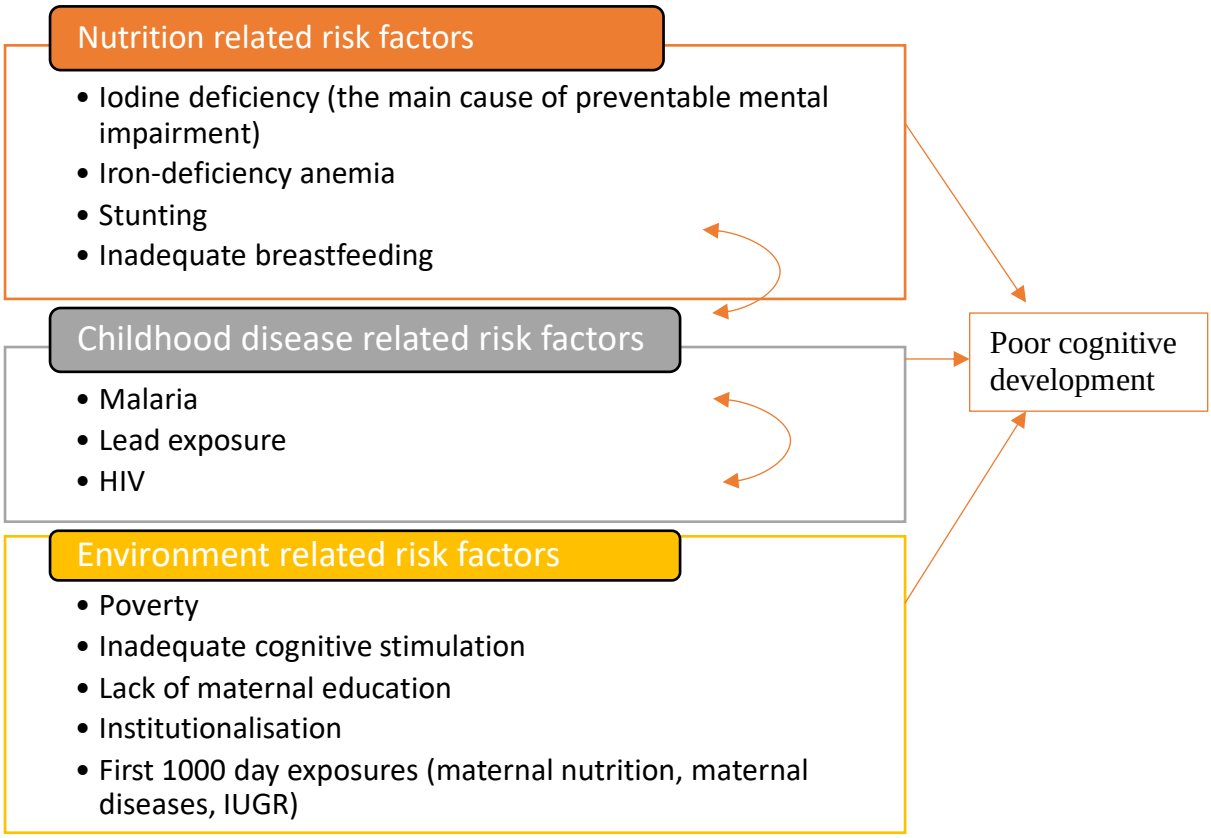


Figure 1.8: Risk factors for poor development, identified by the Lancet series on child health (142,145).

SSA carries a large burden of the risk factors identified in the model above, including a high prevalence of infectious diseases such as malaria and HIV, and a disproportionately high rate of stunting (142). A study in South Africa found a strong association between stunting at 2 years old and impaired cognitive development at 5 years old after controlling for child and caretaker characteristics and SES (132). This indicates an association between early life exposures and cognitive development and raises concerns about the impact of other intrauterine exposures, such as hyperglycaemia in pregnancy.

Evidence for the role of HFDP

Hyperglycaemia in pregnancy may lead to adverse developmental outcomes through alterations in brain development. A proposed mechanism for this is that fluctuating intrauterine glucose levels, as well as exposure to ketones (148) and potential neonatal periods of hypoglycaemia (149), lead to neuroinflammation (150), epigenetic modifications, and ultimately differential brain structure and/or function (6,151). The combination of these

mechanisms potentially results in changes to a range of cognitive functions, including memory, (visuospatial) executive function, language development, attention, as well as fine and gross motor outcomes (152,153). Genetic factors and environmental risk factors may attenuate or exacerbate this effect.

Two systematic reviews have shown evidence for the association between hyperglycaemia in pregnancy and adverse cognitive and motor development, although the included studies were heterogenous, did not always correct for confounders (154,155), and most had a small sample size (156,157) (Table 1.3).

Table 1.3: Summary of systematic reviews on the association of hyperglycaemia in pregnancy and cognitive development

Author, year	Number of studies included Age participants	Developmental area assessed	Limitations	Main findings
Adane, 2016 (156)	14; 6m-12y old	Cognitive development	Varying tools/ criteria; studies with low power; lack of adjustment for confounders; lack of randomised control trials; Variable outcomes	The majority of studies found a negative relationship between both GDM and pre-gestational diabetes and cognitive development, especially in language development, in studies with younger participants, and in studies with larger sample sizes.
Robles, 2015 (157)	12; 1-14y old	Cognitive development, Psychomotor development 1-2 years, IQ 3-12 years.	Heterogeneity between studies; low power; lack of randomised control trials; lack of adjustment for confounders; combined type of diabetes (pre-gestational and HFDP)	In a meta-analysis, infants of diabetic mothers (both pre-gestational and HFDP) had significantly lower scores of mental and psycho-motor development. There was some evidence for lower IQ score in older children (3-12 years) as well,

Studies published since these reviews continue to show contrasting results in terms of the impact on cognitive development, with differences attributed to the level of (control of) maternal hyperglycaemia (158–160), the age of participants (158), and small sample sizes (161,162). One large sibling study in 16-18-year-old males from Sweden showed differences associated with unspecified maternal hyperglycaemia and cognitive ability among non-siblings, but not within sibling pairs, and the authors suggest that a shared familial environment plays a more important role in the association over intrauterine mechanisms (155). The lack of an association within siblings could alternatively be due to undiagnosed hyperglycaemia in the mother during one of her pregnancies, but, nevertheless, other studies, including one from India, have stressed the importance of the postnatal environment and SES on neurodevelopmental outcomes (163,164). Additional evidence for a potential direct neurodevelopmental effect of HFDP was found in two studies showing an association with infant neuronal activity (165) and cortical visual evoked potentials, reflecting neuronal "maturity" (166).

Notably, a number of studies showed a stronger association between GDM and poor cognitive performance in younger participants (160,167,168), implying a role for mediating environmental factors over the course of childhood, likely in the form of adequate stimulation and learning (147). Under-stimulation of children in underprivileged families negatively impacts cognitive development (169), and since the vast majority of studies were performed in HICs, generalizing these results to LMIC settings is problematic. Exposure to maternal HIV-infection may also impact childhood development, although the evidence for this is inconclusive and may be attributable to SES and other early life exposures (170–172). This emphasizes the need for data from LMICs that explores the impact of hyperglycaemia in pregnancy on childhood development, but no existing evidence from SSA was identified.

1.6 Knowledge gaps and justification for the study

Hyperglycaemia in pregnancy is a growing problem worldwide, aided by an increase in obesity and sedentary lifestyles. Although it is clear that management of HFDP positively impacts maternal and neonatal outcomes (52), underdiagnosis, undermanagement, and a lack of research of hyperglycaemia in pregnancy (35,173,174) have contributed to a sparsity of data

on the prevalence of adverse outcomes following management and predictors for poor outcomes. Moreover, gaps in our knowledge regarding HFDP are exposed when the condition is placed in the wider context of long-term childhood health, rather than seeing it as a temporary obstetric condition. This is especially apparent and relevant in urbanizing LMICs, such as South Africa, where maternal metabolic conditions such as HFDP are on the rise, and thereby increasingly have the potential to adversely impact the health and development of the next generation.

The association of HFDP with offspring adiposity and growth in urban SSA is not well understood, as the consequences of HFDP have mostly been explored in populations from HIC, and these findings may not be generalizable to African populations. Prevalence of maternal obesity in women of reproductive age is high in South Africa, but the role that this maternal metabolic condition plays in the association between HFDP and childhood adiposity in our setting requires further exploration. Other factors of particular interest to SSA include the impact of the double-burden of malnutrition and an HIV (treatment)-exposure rate of up to 30%, which may impact childhood development (175–177). In addition, most existing studies use BMI as the main outcome marker for childhood obesity, even though other measures of adiposity have been found to be more reliable. Thus, the impact of HFDP on body composition measures beyond BMI remains a significant knowledge gap, specifically in SSA.

There is tentative evidence for the impact of HFDP on cognitive development, but no studies exploring this relationship in SSA were identified, despite the importance of childhood development in breaking the intergenerational cycle of poverty in LMICs. The impact of confounders such as attendance of pre-school, SES, and maternal morbidities remains to be explored.

The pre-school age (3-6 years old) is of special interest because of both the growing obesity rates in this age group (178), the importance of school readiness for future learning (142,179), and the effectiveness of interventions at this point in a child's development (145,180). Furthermore, investigating this age group allows for the exploration of environmental exposures in the first 1000 days and its impact on outcomes at pre-school age, addressing the

life-cycle approach for health and disease. Lastly, this sets the stage for future longitudinal research into the impact of HFDP on mid- to late childhood and adolescence in South Africa.

Thus, it is not known to what extent HFDP may be contributing to intergenerational cycles of obesity, NCDs, and poverty in the socioeconomic and clinical context of an urban, low-middle income setting in South Africa, as a case representing similar settings in SSA. Furthermore, with a lack of postpartum follow-up of diabetic pregnancies, monitoring of future risks in both mother and child is currently not accomplished.

Gaining a better understanding of the consequences of hyperglycaemia in pregnancy on offspring health can provide evidence for the importance of preconception health optimisation, improved screening for HFDP, adequate antenatal management, and potential postnatal interventions at critical developmental periods. This is of particular public health importance in the context of urban SSA due to the combined lack of current evidence and an ongoing disproportionate increase in non-communicable diseases.

1.7 Aim

By addressing the specific research questions outlined below, this study aims to increase understanding of the obstetric outcomes, and early childhood growth, body composition, and cognitive development of children born to mothers with hyperglycaemia in pregnancy in an urban SSA setting with a high burden of HIV, with a particular focus on HFDP.

1.7.1 Specific research questions and hypotheses

I. What are the rates of maternal and neonatal complications in pregnancies complicated with pre-gestational diabetes or HFDP in Soweto, and what pregnancy characteristics are associated with these outcomes?

> H_A = Pregnancies with pre-gestational diabetes and hyperglycaemia first detected in pregnancy are complicated by adverse maternal and neonatal outcomes, particularly those with more severe hyperglycaemia and poor control.

II. Is HIV-infection in a pregnancy with pre-gestational diabetes/HFDP associated with worse obstetric outcomes compared to the HIV-negative group?

H_A = HIV infection in pregnancy is associated with worse obstetric outcomes in diabetic pregnancies.

III. Is exposure to maternal HFDP associated with adverse body composition, measured by BMI and adiposity, in children aged 3-6 years old, independently of maternal pregnancy BMI?

H_A = Children born to mothers with HFDP have higher overweight and adiposity compared to a group of HFDP-unexposed children, independently of maternal pregnancy BMI.

IV. What are the direct and indirect effects of maternal pregnancy BMI on childhood obesity and adiposity, in the context of exposure to HFDP?

H_A = Maternal pregnancy BMI has a significant direct effect on childhood obesity (BMI) and adiposity (FMI).

V. Is exposure to maternal HFDP associated with adverse cognitive skill development in children aged 3-6 years, as compared to HFDP-unexposed children and an age-adjusted standard?

H_A = Children born to mothers with HFDP have poorer cognitive skill development compared to a group of HFDP-unexposed children.

VI. Is exposure to maternal HFDP associated with adverse fine and gross motor skill development in children aged 3-6 years, as compared to HFDP-unexposed children and an age-adjusted standard?

H_A = Children born to mothers with HFDP have poorer fine and gross motor skill development compared to a group of HFDP-unexposed children.

Chapter 2: Methods

While the specific methodology for each empirical paper can be found in the corresponding chapter (3 to 5), this chapter gives context in terms of the study setting, describes the retrospective data extraction, rationalizes and outlines the prospective measurement methods used in chapter 4 and 5, and provides a summary of the statistical methods used in each paper.

2.1 Study setting, Soweto and Chris Hani Baragwanath Academic Hospital

The study was conducted in Soweto, South Africa, a large urban township located roughly 15km from central Johannesburg. Soweto (an acronym of South West Township) came into existence in the early 1900s following the introduction of pass laws that prohibited black, coloured, and Indian people from living in the Johannesburg city centre under South Africa's Apartheid regime. Following rapid unplanned urban expansion throughout the latter half of the 20th century and after the end of Apartheid, Soweto has grown into a densely populated metropolis subdivided into 34 suburbs with more than 1,7 million inhabitants (181,182) (Figure 2.1). Although Soweto is developing economically and is heterogenous in terms of SES and living conditions, poverty is still pervasive, with high levels of unemployment and only 3% of households categorized as high-income (21). According to a 2011 census, 99% of the population is black African, and the most spoken languages are isiZulu, Sesotho, Setswana, Xitsonga, and isiXhosa (183). Due to the rapid urbanization occurring in Soweto, it is a setting that can illustrate the potential health consequences and challenges that developing countries undergoing nutritional and epidemiological transitions face. This has resulted in Soweto being the setting for, amongst others, the longest ongoing cohort study in Africa, the Birth to Twenty cohort.

In Soweto, first-line healthcare, including antenatal care and preventative child health services such as immunization, is provided by primary care clinics, lower-care level hospitals, maternity obstetric units, and community health centres across the township. For cases requiring escalation from primary care, Soweto and its surrounding areas are served by Chris Hani Baragwanath Academic Hospital (CHBAH), which is a large 3000-bed public secondary and tertiary hospital.

The antenatal clinic at CHBAH, which sees around 1400-1600 deliveries per month, has an antenatal HIV prevalence of 28% (184). In 2013, roughly 28% of deliveries at the antenatal CHBAH clinic resulted from self-referral to the hospital (185), indicating that the CHBAH antenatal unit does not function solely as a tertiary centre.

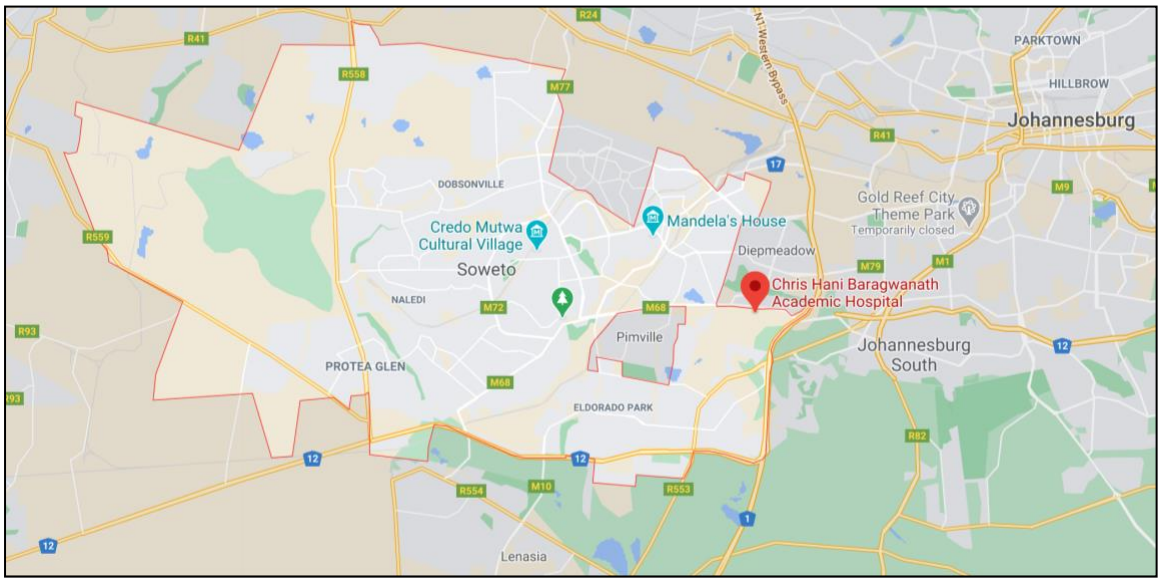


Figure 2.1: Map of Soweto, showing relative distance to Johannesburg and the location of Chris Hani Baragwanath Hospital (186).

For pregnant mothers in Soweto, screening for previously undiagnosed hyperglycaemia only occurs in routine antenatal care on the basis of risk-factors, including family, medical, and obstetric history, and dipstick urinalysis. Those that screen as high risk are referred to the gestational endocrine clinic of CHBAH for further diagnosis, although the rate of continuity of care in this process is not well known. The specialist gestational endocrine clinic was established in 1983 and employs a collaboration of physicians, obstetricians, paediatricians, and a diabetes nurse educator. Aside from women with HFDP, the clinic also manages women with pre-gestational diabetes and thyroid disorders during pregnancy. The clinic has used a 75-gram 2-hour OGTT for diagnosis of HFDP since 2014, using the IADPSG criteria for diagnosis of either GDM or DIP. Prior to 2014, women were diagnosed based on a 100-gram 3-hour OGTT with Carpenter and Coustan criteria (see the historical overview of GDM diagnostic methods, Chapter 1), but these cases were not included as participants in this thesis.

Clinical practices employed at the clinic for management of diabetes in pregnancy are summarized in Figure 2.2. For women with HFDP, treatment is initiated according to severity

of hyperglycaemia, as follows: metformin monotherapy is prescribed in the event of failure to correct hyperglycaemia through dietary measures. Subsequently, if necessary, therapy is escalated with either the addition of glibenclamide or a switch to insulin, depending on the severity of hyperglycaemia and a clinical assessment of the potential risk to the mother and child. Similarly, women with pre-gestational diabetes are switched to OHAs endorsed for use in pregnancy (metformin and glibenclamide) where necessary, and treatment is escalated if hyperglycaemic control is not achieved. All patients with HFDP not requiring ongoing therapy after delivery are asked to return for a 6-week postpartum 75-gram OGTT at the maternity clinic. However, rates of attendance at this postpartum follow-up were suboptimal in an audit study at CHBAH (around 48%), an issue that is not unique to South Africa but also contended with in international and high-income settings (52,63).

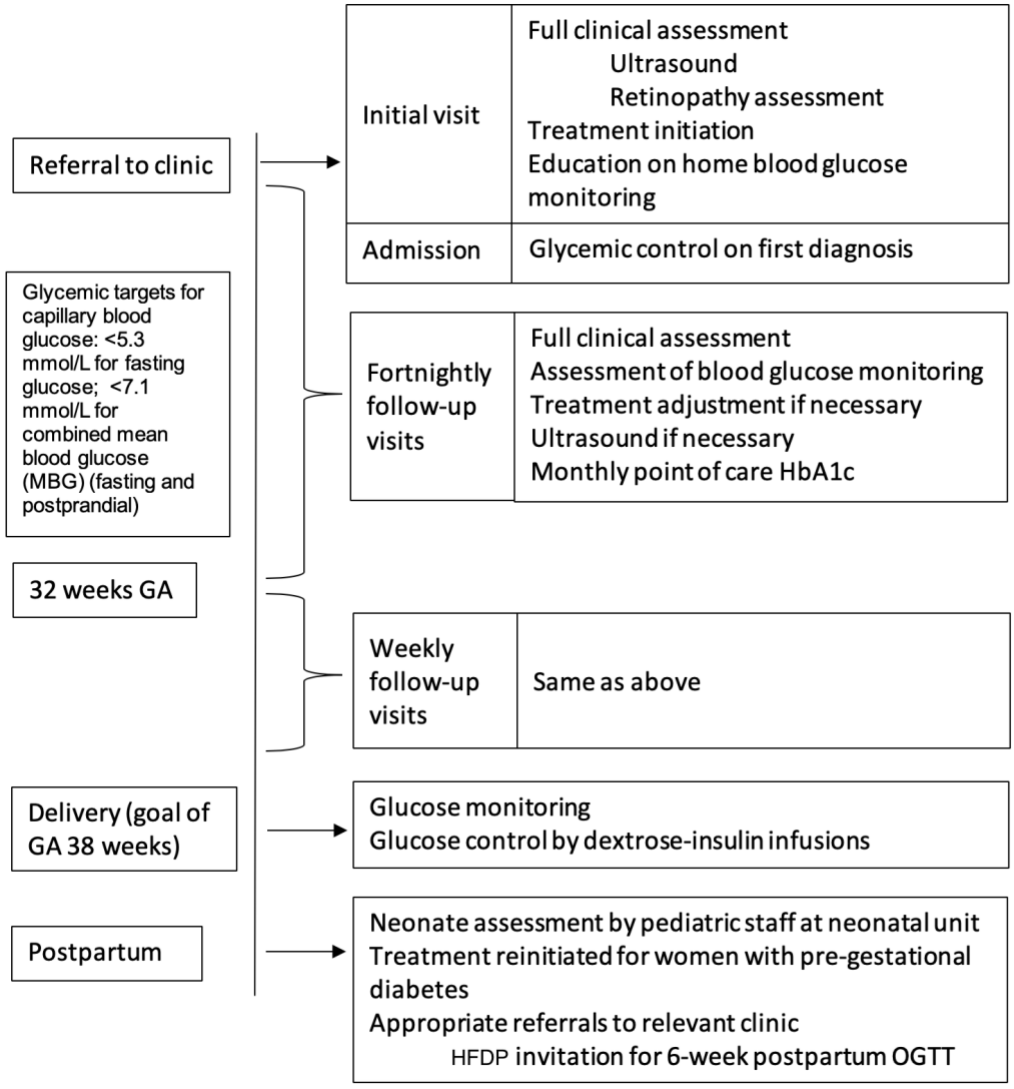


Figure 2.2: Flow diagram of clinical practice for women with diabetes in pregnancy at gestational endocrine clinic at CHBAH (187).

2.2 Study design and population

This project employed a retrospective analysis of hospital files of women who attended the gestational endocrine clinic for hyperglycaemia in pregnancy, including pre-gestational diabetes, between 2010 and 2016. Women with HFDP not diagnosed using the IADPSG criteria were excluded (2010-2013).

Subsequently, a comparative follow-up study was undertaken at the MRC/Wits Developmental Pathway for Health Research Unit (DPHRU), of 3- 6-year-old children born to women with or without HFDP during their index pregnancy between 2014 and 2016. (Figure 2.3). Participant selection and group allocation within the cohort was determined by exposure to maternal HFDP, giving rise to the terms ‘exposed group’ and ‘unexposed group’.

Women with HFDP were diagnosed in a clinical setting at the CHBAH gestational endocrine clinic by 75-gram OGTT and IADPGS criteria and identified from the hospital files analysed in the retrospective review. Their children constitute the ‘exposed’ group.

Women without HFDP had participated in a prevalence study of HFDP at the DPHRU between 2014 and 2016 while attending antenatal care at CHBAH and tested negative for HFDP according to a 75-gram OGTT and IADPSG criteria in this research setting. Since they were initially recruited from the antenatal clinic at CHBAH, these women had similar demographics to the mothers of the exposed group enrolled from the CHBAH gestational endocrine clinic. Participants born to women without HFDP, the HFDP-unexposed group, were recruited into our prospective follow-up study at 3-6 years old until there was a similar number per participant birth year as in the exposed group. Since all women were attending antenatal care at a tertiary hospital, they may represent higher risk pregnancies. However, as mentioned above, women can self-refer to CHBAH without classifying as a high-risk pregnancy.

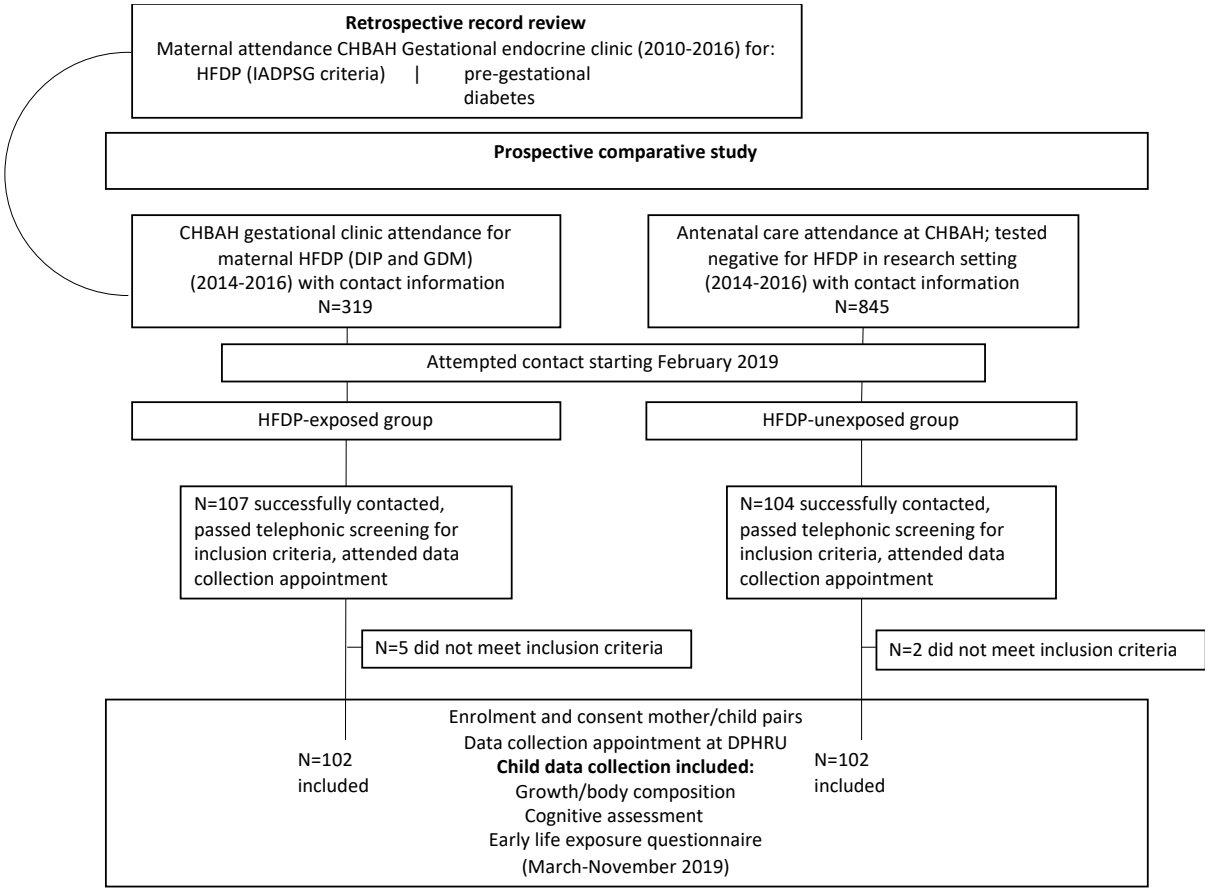


Figure 2.3: Overview of study design

2.2.1 Inclusion and exclusion criteria

This thesis employed separate inclusion and exclusion criteria for the retrospective and prospective aspects of this study, as described below.

For the retrospective study, pregnant women attending the gestational endocrine clinic between 2010 and 2016 with pre-gestational (Type 1 or Type 2 diabetes) or HFDP diagnosed according to the IADPSG criteria were included. The exclusion criteria were as follows:

- Twin pregnancy
- Women with HFDP diagnosed using different criteria
- Significant amount of missing information in medical files.
- For women with repeated pregnancies complicated by hyperglycaemia during the study period, only the first pregnancy was included.

Only children born to women with HFDP diagnosed by the IADPSG criteria, attending the gestational endocrine clinic between 2014 and 2016, were included in the prospective comparative study. Participants had to be able to attend a data collection appointment at DPHRU in Soweto, and the parent or guardian had to be able to give informed consent. Exclusion criteria were as follows:

- Twin pregnancies
- Inability to trace the parent/guardian
- Maternal or child death between the pregnancy and follow-up
- Major congenital disorders (e.g., Down Syndrome)
- Age <2.8 years old or >6 years old.

2.3 Data collection and measurement methods

2.3.1 Retrospective hospital record review

Relevant data was extracted by three medical doctors from paper-based medical files of women with pre-gestational diabetes or HFDP from the gestational endocrine clinic. A predetermined data extraction form based on REDCap (188) was used (Appendix E). In general terms, data of interest included OGTT results, gestational age at the first visit, maternal BMI at the time of OGTT mean blood glucose level and HbA1c at each visit and aggregated per trimester, the presence of any comorbidities (such as HIV or chronic hypertension), prescribed treatment, and delivery information (such as gestational age, birth weight, and the presence of any complications).

2.3.2 Prospective data collection

As indicated in Figure 2.3, the follow-up of women with HFDP at 3-6 years after the index pregnancy consisted of a single data collection appointment between March and November 2019. The data collection in children was one component of a study that also explored maternal outcomes, such as progression to Type 2 Diabetes and cardiovascular risk, in the mothers of the exposed and unexposed participants included in our prospective study. The main outcomes of the empirical papers presented in chapters 4 and 5 were body composition (overweight and adiposity) and cognitive and motor development. The rationale behind the chosen measurement method for these outcomes is described in more detail below.

Data on potential confounders and modifying factors were collected using a structured maternal questionnaire as the most practical way to gather reliable information about early life and current exposure variables (Appendix G). Where applicable, questions were adapted from existing instruments. This included a standardized household asset questionnaire used to estimate household socioeconomic score, adapting part of the National Income Dynamics Survey (189), and assessment of alcohol use and smoking currently and during pregnancy using an adaptation of the quantity/frequency method (QF) (190,191). Additionally, to gather information about the presence of early life hospitalization, (exclusive) breastfeeding, (exclusive) breastfeeding duration (192), preschool attendance, parity, and maternal HIV infection, maternal education questions were designed to be straightforward and informative, and were largely 'yes/no'-questions followed by questions to gather additional information, where necessary (for example, *Does the child attend preschool? If yes, since what age?*) (See Appendix G for the full questionnaire). Information was also gathered on maternal and child date of birth (age at study visit), and child sex. Negative HIV status was also confirmed using a point of care rapid HIV test (Homemed HIV1/2 rapid test kit).The questionnaire was completed by the participant's mother or guardian with the assistance of a trained research assistant.

For the exposed group, information on pregnancy and delivery was available from the retrospectively captured hospital files from the gestational endocrine clinic. For the HFDP-unexposed group this information was obtained from the participants' Road to Health Card (a patient-held medical document tracking childhood health check-ups from birth) and from the existing research database of the initial HFDP prevalence study at DPHRU in which they were enrolled during the index pregnancy (39).

Measures of body composition: overweight and adiposity

A number of measures have been developed to assess the body composition of children and to develop criteria for diagnosis of obesity or overweight. The WHO provides age and sex-adjusted BMI z-score growth charts for 0-5 year-olds and 5-19 year-olds (131), based on a database of measurements from various regions, including SSA, which are often used in African research.

Body fatness and fat distribution in African populations have been found to differ from Caucasian populations, including in children (193,194), and so have the associated differences in cardiometabolic risk (195,196), emphasizing the need for body composition studies specific to African children. In a recent study from Ghana, authors reported higher sensitivity for measuring adiposity using the WHO cut-offs in African children at 8-11 years, compared to other standards such as the International Obesity Task Force (IOTF) criteria (197). In children under 5 years old, which make up the large majority of our cohort, the WHO uses a conservative definition for overweight, in an attempt to balance out the potential damage of stringent diets and stigmatisation at this young age with the evidence for health consequences. This has resulted in the creation of a new categorisation in children under 5, namely those "at risk of being overweight," which corresponds with the standardised z-score cut-off value for "overweight" in children above 5 years old (198).

Throughout this thesis, the WHO growth standards/references were therefore used to determine anthropometric z-scores at 3-6 years old. On the other hand, throughout, birth weight z-scores were determined using the globally validated Intergrowth-21 standards (199), which adjust for gestational age at delivery in determining z-scores based on multinational data.

BMI, though widely used, is not as clear an indicator of health outcomes in children as it is in adults, due to varying body shapes throughout growth and the inability of BMI to distinguish fat and lean mass. A systematic review and meta-analysis that included younger children has shown a high specificity but low sensitivity of BMI compared to other measures of body fatness (200). Moreover, two recent studies in African 8-11 year-olds comparing how BMI related to body fatness measured by the deuterium dilution method, found that BMI-for-age z-scores underestimated the presence of excessive fatness, indicating that BMI may not accurately predict obesity in all situations (115,197).

Multi-component models for body composition measurement, which distinguish between fat, water, and remaining fat-free dry tissue (protein and mineral), are the most accurate. However, the cost, procedure, and equipment required for these methods are often impractical for the setting and scope of epidemiological research studies (115,201). Two-component techniques

of body composition measurement, including the deuterium dilution method, eliminate the inability of BMI to distinguish between fat and fat-free mass, and are more accessible than multi-component techniques. A comparison of the advantages and disadvantages of both simple and two-component methods can be found in Table 2.1.

The deuterium dilution method uses a measurement of total body water (TBW), which is found solely in fat-free mass, to estimate fat-free and fat mass. The participant is given a dose of 'heavy water', or deuterium oxide, which is water labelled with a non-radioactive isotope of hydrogen. After equilibration throughout the body water (2.5-3 hours after dosing in young children), the deuterium enrichment in saliva samples is measured using mass spectrometry (202). The method uses an assumed value for the hydration of fat-free tissue (201), which allows for accurate body composition measurement in healthy subjects, although it may be problematic in diseased states. The high reproducibility, accuracy, and ability to use the method in the field (203–206), has resulted in the deuterium dilution method being used as a reference method for body fatness measurements in research (115,207–210), and protocols for all age groups have been developed by the International Atomic Energy Agency (IAEA) (202). For this reason, the deuterium dilution method was chosen as the most accurate available measure of body composition to complement anthropometric measurements in the cohort of children investigated in this thesis. A description of the methods used and the coefficient of variance (CV) for both anthropometric measurement and the deuterium dilution method can be found in Chapter 4, Methods section, and the standard operating procedure for the deuterium dilution method is additionally provided in Appendix I.

Table 2.1: Comparison of advantages and disadvantages of different techniques for body composition measurement in children. Adapted from Wells, 2006 (201).

	Advantages	**Disadvantages**
Simple measurements (BMI; skinfold thickness; and waist circumference)	Simple, inexpensive, limited training required, non-invasive.	**BMI:** Measures nutritional status rather than body composition; not the most accurate reflection of health outcomes. **Skinfold thickness:** Population specific, only an estimate of regional fatness

		Waist circumference: Poor accuracy as a measurement of internal visceral fat
Bioelectric impedance analysis	Simple, non-invasive, not time-consuming, limited running costs	Based on prediction models and therefore population specific; inconsistent measure of body composition across groups and individuals; costs of device
DEXA	Accurate for limb and lean fat in 5+ years and infancy; non-invasive	Limited accuracy in younger children; radiation exposure (minimal); estimation of lean mass used in whole body analysis; higher running costs; trained radiographer required
Deuterium dilution method	Acceptable in all age groups, non-invasive, reproducible, appropriate for in-field use, limited running costs; limited training required for sample collection.	Inaccurate in disease states affecting hydration status; trained laboratory technician required; cost of spectrometry equipment.
Multicomponent models	Most accurate approach	Expensive, specialist, extensive training and resources required

Measuring cognitive and motor development

Assessment of early child development, including cognitive, sensory-motor, language, and social skills, is complicated by the rapid and sporadic nature of development, variations in child behaviour, the impact of context and surroundings on behaviour and development, and uneven development across domains (211). Since development is thought to be intertwined with social and cultural factors, as described by Vygotsky's theory, tools developed in Western HIC may not be appropriate for children in LMICs (169). The limited availability of translatable versions of tests with norms appropriate for global settings are a further barrier to their generalizability.

One approach to early child development is the concept of school readiness, which encompasses the cognitive, psychosocial, language and motor development, as well as physical wellbeing, that is required for children to succeed at future learning. While the term has been misinterpreted to exclude children deemed 'not ready' from school settings, when used appropriately, it is a useful tool evaluate early development, challenges faced in early life, and how to minimize disparities in school readiness (212). This study has focused on cognitive and motor development, two important aspects of school readiness.

In response to the lack of easy to use, readily available, and culturally adaptable assessment tools for the cognitive and motor skill aspects of school readiness, the Herbst Early Childhood Development Test (Herbst test) was developed for use in preschool-aged children from underprivileged backgrounds (169) (See Appendix K for a copy of the Herbst Test, and Table 2.2 for a summary). This cognitive and motor skill assessment tool was designed for and validated in South Africa for 3-6-year-old children, with the aim of being culturally neutral and child friendly. The test has two separate sections, taking a total of 60-90 minutes to complete. In evaluating the test-retest reliability, the coefficients of stability were 0.93 for the cognitive and 0.85 for the motor section (169). For a description of test application in our setting, please see chapter 5, Methods. Section A assesses the level of cognitive development, and is comprised of the following 10 subsections:

Table 2.2: Cognitive sections of the Herbst test.

1. Incomplete man	The child is asked to add body parts to an incomplete stick-figure drawing of a man, receiving points for the number of body parts added. (out of 8 marks) 
2.Visual-motor integration	The child uses a pencil to copy figures (e.g., a horizontal line or a triangle) presented to them on a total of 11 stimulus cards on to a blank piece of A4 paper using a pencil, starting with an exercise example. (out of 10 marks)
3.Block Building	To assess visuo-spatial and fine motor skills, the child is shown a total of 8 constructions built with 3-10 blocks by the examiner and is asked to build the same shape. (out of 8 marks)
4. Stick figures	Child uses small plastic sticks to copy successive forms presented to them on a total of 11 stimulus cards, starting with an exercise example, to assess spatial concepts and visual-motor integration. (out of 10 marks).

5. Direction and similarities	The child is shown 4 3D blocks with markings and is asked to identify the block that is oriented differently. Next, the child is a shown a sheet with rows of figures and asked to identify the figure that is oriented differently from the others. (out of 12 marks)
6. Form concept	Testing shape 2- and 3- dimensional shape recognition, the child is asked to place the correct shape on the correct form sheets according to shape, with 3 form sheets ranging from easier to difficult, within a time limit. Next, the child is asked to name 6 number or letters listed on a sheet and pointed at by the examiner. (out of 6 marks)
7. Colour concept	The child is asked to name the colour that the examiner is pointing at on a sheet. If not able, the child is shown 3 coloured beads and asked to match it to the correct colour on the sheet. (out of 6 marks)
8. Numerical and counting concepts	The child is asked to count up to 20 small plastic sticks, receiving extra points for moving the counted sticks to one side. (out of 6 marks)
9. Picture puzzles	The child is asked to correctly build picture puzzles successively from 2 to 6 pieces, in a time limit. (out of 7 marks)
10.Picture Perception	The child is asked to indicate the picture on a sheet, out of a row of similar pictures, that is the exact same as the picture on the stimulus card, testing visual discrimination. (out of 6 marks).

Section B consists of 1) the fine motor section: in which the child's ability to colour in a circle, cut out a circle, place small seeds in a small container, etc. are tested, and 2) the gross motor section in which the child is asked to walk on tiptoes and heels, jump on one leg, throw a ball, etc, and ability to do so is tested.

2.4 Sample size calculation

Sample size calculations were performed in Stata, version 13 (College Station, USA), assuming 80% power and a two-tailed significance level of 0.05. The main sample size calculation was conducted for detecting a difference in childhood BMI z-score. This expected difference to be detected between the exposed and unexposed group was estimated at 0.35 z-score based on existing evidence (ranging from 0.22-0.6, with different study designs (15,66,112,213)), and investigations into clinical relevance, which indicate that a difference in BMI z-score of around 0.6 and 0.25-5 correspond to meaningful changes in body fat percentage and metabolic sequelae in children, respectively (214,215). Using a population SD of 0.93 (for 5 years old) and 1.07 (for 3 years old), based on a cohort of Sowetan children (216), the calculated required

sample size was 130 participants per group. This is also sufficient to detect a difference in fat mass index (FMI) of 0.4 kg/m^2 ± 1 (n=100 per group) (109,217).

Due to the fact that the Herbst test, which was developed for underprivileged settings, has not been previously used to measure cognitive and motor outcomes in the context of a hyperglycaemic pregnancy, a reliable required sample size was difficult to estimate. However, based on results from a previous intervention study using the Herbst test, we determined that with a HFDP-unexposed group mean cognitive score of 37 ± 4, having 100 participants in each group would require a minimum difference of -1.59 (or, when rounded up, -2), to detect a significant difference between two groups (218). This is a smaller difference than found in the intervention study and was considered reasonable to expect as a minimum relevant mean difference.

2.5 Statistical methods

For all empirical papers, Stata 13 (StataCorp, Texas USA (219)), was used for statistical analysis. Descriptive statistics included number and percentage for categorical variables and mean and SD or median and interquartile range for non-parametric variables. Normality was tested using the skewness/kurtosis test for normality. Significance testing between the two groups was done using the chi^2 statistic or Fisher's exact test for categorical variables and student's t-test or Mann-Whitney U test for non-parametric variables for continuous variables. For differences between three groups in parametric and non-parametric variables, analysis of variance (ANOVA) and Kruskal Willis test was used, respectively. Throughout, significance was assumed at a two-sided p-value of <0.05.

Table 2.3 gives an overview of additional statistical methods used per empirical paper included in this thesis. For a more detailed description of the statistical methods used, please see the corresponding chapter.

Table 2.3: An overview of the statistical methods used per paper

Empirical paper	Title	Design	Additional Statistical Methods
I; Chapter 3	Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV.	Retrospective medical record review	- Logistic regression for adverse outcomes, using backwards selection, abiding by a positive outcome per variable ratio of 10.
III; Chapter 4	Obesity and adiposity of 3- to 6-year-old children born to mothers with hyperglycaemia detected in pregnancy in an urban South African setting.	Prospective comparative study	- Hierarchical linear regression analysis on basis of a priori determined conceptual pathways. - Path analysis using generalized structural equation modelling (GSEM).
IV; Chapter 5	Cognitive and motor development in 3- to 6-year-old children born to mothers with hyperglycaemia first detected in pregnancy in an urban African population.	Prospective comparative study	- Hierarchical ordinal regression analysis, on basis of a priori determined conceptual pathways, assessing proportional odds assumption by likelihood ratio test.

2.6 Ethical considerations

Ethical approval was obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand and granted for the retrospective study under clearance certificate M180204: “The impact of diabetes in pregnancy on maternal and fetal health.” Additionally, ethical approval for the prospective aspects of the study was obtained under clearance certificate M180317: “The development of children born to mothers with diabetes mellitus in pregnancy: growth, body composition, and cognitive and motor development”. Copies of the clearance certificates can be found in Appendix C and D. Parents and guardians gave informed consent for the study procedures (Appendix F) and accompanied their child wherever possible or when requested by the participants, and participants were given the option to stop procedures at any point. Confidentiality of participants was ensured through coding of all collected data.

Part 2: Empirical chapters

The following chapters, Chapters 3-5, consist of empirical findings presented as academic papers. Contributions to each paper have been described under "Publications and contributions", page ix-x. For each chapter, the main adaptations made from published versions are the following (please also see Appendix L):

- *Adaptations to general formatting, numbering of figures and sections, terminology, and previously defined abbreviations in order to fit into the formatting of this thesis;*
- *Additional text or details to improve the chapter's placement in the thesis*
- *Removal of individual abstracts to be replaced with this thesis' general abstract;*
- *Replacing figures and tables already presented in previous chapters with references to that chapter and figure/table, to avoid repetition within this thesis.*

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

Summary and context

The findings described in this chapter have been published as a peer-reviewed article:

Soepnel, L.M., Nicolaou, V., Huddle, K.R., Klipstein-Grobusch, K., Levitt, N.S. and Norris, S.A. Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV. *Int J Gynecol Obstet.* **2019**;147(3):404-412. https://doi.org/10.1002/ijgo.12956.

In terms of this thesis' study design, this chapter represents the retrospective record review of diabetic pregnancies from the gestational endocrine clinic at CHBAH. Of all 1071 included pregnancies, 43% had GDM, 19% T1DM, and 38% T2DM. Key findings include the high rate of maternal BMI across all diabetes types, which was an important predictor for maternal and neonatal outcomes along with initial glucose levels and glycaemic control. Furthermore, the impact of HIV infection on perinatal mortality requires further exploration. Of all cases, 94.9% had reached acceptable glycaemic control by the third trimester. This serves as important information to frame the follow-up studies described in Chapter 4 and 5.

3.1 Introduction

FIGO has called for hyperglycaemia in pregnancy to be a global health priority (3) due to its rising prevalence, including in LMICs, and the serious associated consequences for mother and child (23). These include pregnancy-induced hypertension (PIH), macrosomia, and stillbirth (31). Moreover, mothers with HFDP have a higher chance of developing type 2 diabetes (T2DM) and cardiovascular complications postpartum, and metabolic consequences for the child may contribute to an intergenerational cycle of disease (6,220).

Data about diabetes in pregnancy in SSA is limited. A recent review showed that merely 11% of countries had published prevalence data of HFDP (37). Most recent reported rates of HFDP prevalence for urban SSA are in the range of 9.1%–9.4% (38,39). Within South Africa, varying

rates of prevalence could be due to differences in population, screening practices, and diagnostic methods (39,40). Recent international recommendations for universal screening and the lower IADPSG diagnostic criteria, distinguishing between milder GDM and 'overt' DIP components of HFDP, are not uniformly implemented in South Africa, partly due to resource constraints (3,221). These discrepancies contribute to the underreporting and undermanaging of diabetes in pregnancy. A 20-year audit at a large urban hospital in South Africa between 1983 and 2002 showed the positive impact of management on maternal and neonatal outcomes for pregnancies complicated by hyperglycaemia (52). Since this publication, the same hospital has introduced OHAs, universal HIV monitoring and treatment, and the lower diagnostic criteria for HFDP and GDM.

OHAs are not currently licensed for use during pregnancy due to lack of long-term safety data, but use of metformin and glibenclamide is endorsed by major South African guidelines and is preferable to insulin in LMICs due to ease of use and affordability (34). Data from South Africa have shown a higher rate of macrosomia, neonatal hypoglycaemia, and PNM in patients treated solely with glibenclamide (59,222). However, it is not clear whether these findings are a direct drug effect, and generalizability across South Africa is problematic due to disparities in ethnicity, diagnostic tests and criteria, and definitions of glycaemic control.

Additionally, understanding the metabolic risk of HIV-infected patients remains critical, since the overall prevalence of glucose metabolism disorders in HIV-infected individuals on ART is as high as 25% (223); pregnancy is an additional provoking factor (177). Treatment with ART has dramatically improved outcomes in HIV-infected pregnancies. However, the combined effect of hyperglycaemia, HIV, and ART exposure on maternal and neonatal outcomes remains unexplored.

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

3.2 Methods

We retrospectively analysed medical records of women attending the Gestational Endocrine Clinic at CHBAH for diabetes in pregnancy between 2012 and 2018 (see Chapter 2, Figure 2.3: Overview of study design). CHBAH is a 3000-bed teaching hospital with 1400–1600 deliveries per month in the large urban township of Soweto, with an antenatal HIV prevalence of 30%. The population attending the clinic is predominantly black African.

Women with hospital files at the Gestational Endocrine Clinic with T1DM, T2DM, or HFDP were included in the study. Women were excluded in case of a twin pregnancy, if HFDP was diagnosed with any criteria aside from the IADPSG criteria, or if basic data was missing from the medical files, making them unusable. For women presenting more than once with a pregnancies complicated by hyperglycaemia during the study period, only the first pregnancy was included. The HFDP group was subdivided into those with GDM and more severe DIP (Chapter 1, Table 1.1). Women were screened for HFDP on the basis of risk factors, which included persistent glycosuria, (first degree) family history of diabetes, previous unexplained perinatal losses, previous HFDP, and history of a macrosomic baby.

A timeline of clinical practice after referral to the clinic was presented in Chapter 2, Figure 2.2. Participants with DIP were initiated on insulin based on a clinical decision where the degree of hyperglycaemia and presence of complications were taken into account. Excluding this, the first choice of pharmacological treatment for HFDP participants was metformin, which was escalated, if necessary, by either adding glibenclamide or transitioning to insulin, a decision based on levels of hyperglycaemia and the presence of diabetic complications. Those with T2DM on existing regimes were switched, where necessary, to either metformin and/or glibenclamide; otherwise, treatment was escalated as above. The HREC, University of the Witwatersrand approved this study (M180204).

3.2.1 Outcomes

Definitions of maternal and neonatal outcomes of interest are shown in Table 3.1.

Table 3.1: Definitions of maternal and neonatal outcomes.

Maternal outcomes	
Glycaemic control	Mean blood glucose <7.1 mmol/L for the 3rd trimester, measured by capillary blood glucose on a glucometer
Dietary Failure	Lack of glycaemic control after 2 weeks of dietary management
Maternal overweight	BMI ≥25
Maternal obesity	BMI ≥30
Nephropathy	Creatinine >71ummol/L and membrane attack complex (MAC) >30 mg/L
Anaemia	Haemoglobin levels < 11 g/dL
HIV-positive	Based on results from rapid or antibody ELISA tests before or at time of initial presentation
Hypertension	Blood pressure elevated >140/90mmHg for at least two measurements
Hypertensive disorders of pregnancy	Categorized as: Chronic hypertension: hypertension present before pregnancy Pregnancy induced hypertension (PIH): hypertension presenting >20 completed weeks Pre-eclampsia: hypertension with proteinuria Eclampsia: hypertension with proteinuria and seizures
Neonatal outcomes	
Prematurity	Delivery <37 completed weeks gestational age
Miscarriage	loss occurring at <28 completed weeks gestational age
Stillbirth	Loss occurring at ≥29 weeks gestational age
Early neonatal death	Neonatal demise occurring in the first week of life
Perinatal mortality (PNM)	The number of stillbirths and early neonatal deaths out of the total number of deliveries
Low birth weight (LBW)	Birth weight <2500 g
Large for gestational age	>90th percentile weight for gestational age
Macrosomia	Birth weight ≥ 4000 g
Major congenital anomaly	Spinal, cardiac, central nervous system, renal, or digestive system anomaly.

3.2.2 Data management and statistical analysis

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

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

3.3 Results

There were 1071 diabetic pregnancies during the study period: 461 (43.0%) HFDP; 408 (38.1%) T2DM; and 202 (18.9%) T1DM (Table 3.2). DIP constituted 235 (51.0%) of HFDP cases. The median gestational age at initial presentation was 15.6–16.1 weeks for those with pre-gestational diabetes and 28.0–29.5 weeks for those with HFDP. The T1DM group was younger (mean 26.5 ± 5.6), had a higher initial HbA1c (median 8.3, IQR 7–10.2), higher frequency of retinopathy and nephropathy 17 (8.4%) and 4 (4.7%), respectively, and a lower BMI (29.3 ± 6.6) compared to the HFDP and T2DM groups. For T2DM, the median initial HbA1c was 7.6 (IQR 6.4–9.1), for DIP it was 7.2 (IQR 6.5–8.8) and for GDM it was 5.8 (IQR 5.4–6.3). All four groups were overweight by mean BMI at presentation. Overall, 234 (21.8%)

women in the cohort had hypertension, with the T2DM group accounting for the highest prevalence at 136 (33.3%).

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

Table 3.2: Demographic, obstetric, comorbidity, and diabetes characteristics of participants with T1DM, T2DM, and HFDP.

	n=202	T1DM	n=408	T2DM	HFDP			
					n=235	DIP	n=226	GDM
Maternal characteristics at initial presentation								
Maternal age (mean, SD)	202	26.9 (5.6)	403	34.0 (5.2)	235	32.4(5.3)	226	32.9(6.1)
GA, weeks (median, IQR)	200	15.6 (9.8–23.1)	407	16.1 (11.0-24.3)	235	28.0 (21.7-32)	224	29.5 (26.6-33.0)
BMI (mean, SD)	112	29.3 (6.6)	207	35.1 (8.1)	116	36.6 (7.9)	105	35.5 (8.5)
Blood Pressure (mean, SD[†])	190	123/74.7 (16.0–11.5)	396	125.9/76.5 (15.0–11.6)	223	123.0/73.6 (14.0–10.9)	213	120.5/71.1 (14.2–9.6)
Obstetric history								
Primigravida (n, %)	200	69 (34.5)	407	21 (5.2)	235	20 (8.5)	226	22 (9.7)
Previous pregnancy losses (n, %)	200	49 (24.5)	407	147 (36.1)	235	94 (40.0)	226	99 (43.8)
Comorbidities at presentation								
Chronic hypertension (n, %)	202	27 (13.4)	408	136 (33.3)	234	35 (15.0)	225	36 (16.0)
HIV positive (n, %)	153	45 (29.4)	320	95 (29.7)	212	25 (11.8)	203	47 (23.1)
Anaemia (n, %)	199	46 (23.1)	403	56 (13.9)	455	25 (10.7)	455	34 (15.4)
Characteristics of diabetes								
Age at diagnosis (median, IQR)	196	21 (16–25)	383	31 (27–34)		NA		NA
Initial HbA1c, % (median, IQR)	191	8.3 (7–10.2)	393	7.6 (6.4–9.1)	215	7.2 (6.5–8.8)	207	5.8 (5.4–6.3)
Control at initial presentation (n, %)	86	48 (55.8)	133	63 (47.3)	136	NA	78	NA
Control in third trimester[§] (n, %)	147	141 (95.9)	351	335 (95.4)	207	187 (90.3)	196	191 (97.5)
Target organ damage (n, %)								
Retinopathy	202	17 (8.4)	407	15 (3.7)	234	3 (1.3)	226	2 (0.9)
Nephropathy	85	4 (4.7)	168	1 (0.6)	81	0	85	0
Therapy								
Exposure to metformin (n, %)	202	14 (6.9)	408	351 (86.0)	235	120 (52.8)	226	171 (75.7)
Exposure to glibenclamide (n, %)	202	1 (0.5)	408	151 (37.0)	235	80 (34.0)	226	76 (33.6)
Exposure to insulin (n, %)	202	202 (100.0)	408	270 (66.2)	235	134 (57.0)	226	25 (11.1)

Values are given as mean (standard deviation), median (interquartile range), or number (percentage). T1DM: Type 1 Diabetes; T2DM: Type 2 Diabetes; HFDP: hyperglycaemia first detected in pregnancy; GDM: gestational diabetes mellitus; DIP: diabetes in pregnancy; GA: gestational age; BMI: body mass index; HbA1c: glycated haemoglobin; MBG: mean blood glucose. [†]SD reported as (SD for systolic blood pressure, SD for diastolic blood pressure); [§]Control in third trimester: average MBG in third trimester <7.1.

In terms of maternal outcomes, loss to follow-up was highest in the T1DM (n=37, 18.3%) and GDM (n=47, 20.8%) groups (Table 3.3). The number of caesarean deliveries was 641 (72.2%) and the rate was highest in the T2DM group at 275 (78.4%). The rate of PIH was 9.2% (n=98) across all groups; the combined rate of pre-eclampsia and eclampsia was 2.7% (n=29). The rates of miscarriage and maternal mortality were highest in the T1DM group at 14 (6.9%) and 3 (1.5%), respectively. Initial HbA1c was the significant predictor for miscarriage when performing regression analyses (odds ratio (OR) 1.20, 95% CI 1.13–1.55, p=0.001). When investigating "any obstetric complication" (the combined rate of miscarriage, hypertensive disorders of pregnancy, urinary tract infection, polyhydramnios, oligohydramnios, maternal death), no significant differences were found between the groups.

Table 3.3: Neonatal and maternal outcomes for T1DM, T2DM, and HFDP, n (%).

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

Values are given as number (percentage). T1DM: Type 1 Diabetes; T2DM: Type 2 Diabetes; HFDP: hyperglycaemia first detected in pregnancy; GDM: gestational diabetes mellitus; DIP: diabetes in pregnancy; PIH: pregnancy induced hypertension; LBW: low birth weight; PNM: perinatal mortality. *p-value <0.05.

Of offspring, 747 (92.6%) had an APGAR score ≥7 at 1-minute postpartum (Table 3.3). The rate of prematurity (n=237, 30.9%) was significantly lower in the GDM group (n=41, 22.8%) than the other groups, but not when correcting for initial HbA1c (OR 1.17, 95%CI 1.08–1.26, p<0.001) and lack of glycaemic control in the third trimester (OR 2.36, 95%CI 1.24–4.47, p=0.009). Macrosomia, with a prevalence of 71 (8.4%) in the whole cohort, was highest in the DIP group (n=24, 12.3%) and significantly lower in the GDM group (n=7, 4.0%). For both low

birth weight (LBW), which was highest in the T1DM group, and macrosomia, the difference between the groups was no longer significant when adjusting for maternal BMI (OR 0.95, 95%CI 0.91–0.98, p=0.002; OR 1.05, 95%CI 1.00–1.09, p=0.027, respectively, for LBW and macrosomia), with multiparity (OR 0.61, 95%CI 0.37–0.99, p=0.047) and hypertension (OR 1.85, 95%CI 1.04–3.29, p=0.036) being the other two significant predictors for LBW. PNM was significantly higher in the T1DM group (n=10, 6.7%) when compared to T2DM and the HFDP group in its entirety (p=0.019), although HIV was the main statistical contributor in our model (OR 5.00, 95%CI 2.06–12.15, p<0.001). The T1DM group also had the most major congenital anomalies at 1.7% (n=2). Additional neonatal outcomes in the cohort included hypoglycaemia (n=10, 1.2%), respiratory distress syndrome (n=8, 1.0%), and jaundice (n=4, 0.5%), with no significant differences between groups.

Table 3.4: Overview of predictors for maternal and childhood outcomes following logistic regression analysis

Outcome	**Significant predictors**	**OR**	**95% CI**	**p-value**
Miscarriage	Initial HbA1c	1.20	1.13–1.55	0.001
Prematurity	Initial HbA1c	1.17	1.08-1.26	<0.001
	Lack of glycaemic control in 3rd trimester	2.36	1.24-4.47	0.009
Macrosomia	Maternal BMI	1.05	1.00-1.09	0.027
LBW	Maternal BMI	0.95	0.91-0.98	0.002
	Multiparity	0.61	0.37-0.99	0.047
	Hypertension	1.85	1.04-3.29	0.036
PNM	HIV-infection	5.00	2.06-12.15	<0.001

Logistic regression using stepwise method, considering the following variables: maternal age, initial BMI, weight gain over pregnancy, gestational age at initial presentation, HbA1c, chronic hypertension, HIV, anaemia, multiparity, glycaemic control by the third trimester, and exposure to OHAs. OR: Odds ratio; CI: Confidence interval; LBW: Low birth weight; PNM: Perinatal mortality.

When considering predictive factors for poor neonatal outcome (prematurity, LBW, macrosomia, or PNM) per type of diabetes**,** initial HbA1c was found to significantly predict poor neonatal outcome for T1DM. In the HFDP group, initial HbA1c (OR 1.17, 95%CI 1.00–1.37, p=0.044), gestational age at presentation (OR 0.95, 95%CI 0.91–0.98, p=0.005), and lack of glycaemic control by the third trimester (OR 3.12, 95%CI 1.21–9.08, p=0.019) were

significant predictors for poor neonatal outcome. Within the T2DM group, no significant predictors were found.

When comparing outcomes for the group exposed to OHAs (metformin monotherapy and glibenclamide exposure) to insulin monotherapy (Table 3.5), the characteristics with significant differences between groups were weight gain over pregnancy and initial HbA1c (both higher in the insulin group), and the number achieving glycaemic control by the third trimester (lower in the insulin group). In addition, BMI at first presentation in those with T2DM was significantly higher in the glibenclamide group versus the insulin monotherapy group (34.6 vs 31.6; IQR 31.6–41.8, 27.6–36.2). No significant differences were found in maternal outcomes between treatment groups.

T2DM: For the participants with T2DM, glibenclamide exposure was associated with a significantly higher prevalence of macrosomia in the T2DM group when compared to the insulin monotherapy group (n=10 [12.2%] vs 0, p=0.030) (Figure 3.1). Participants with T2DM who were on glibenclamide but were switched to insulin (n=51, 12.5%) also had higher rates of macrosomia compared to insulin monotherapy (n=9 [19.6%] vs 0, *P*=0.003). PNM did not significantly differ between the groups.

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

Table 3.5: Comparison of baseline characteristics in metformin monotherapy and glibenclamide-exposed groups versus insulin monotherapy.

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

Values are given as median (interquartile range) or number (percentage). T2DM: Type 2 Diabetes; HFDP: hyperglycaemia first detected in pregnancy; BMI: body mass index; HbA1c: glycated haemoglobin. [†]Control in third trimester: MBG in third trimester <7.1. [§]p-value for comparison between metformin monotherapy and insulin monotherapy groups; [‡]p-value for comparison between glibenclamide and insulin monotherapy groups; *p-value <0.05.

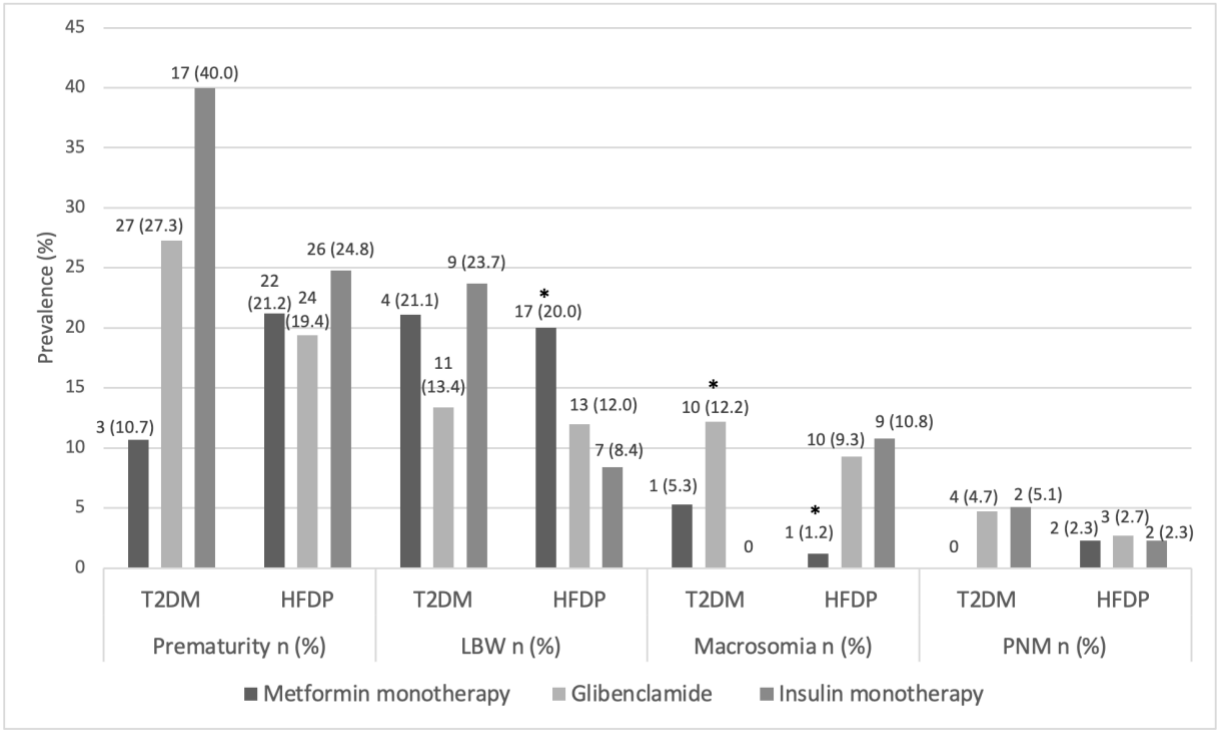


Figure 3.1: Neonatal outcomes in metformin monotherapy and glibenclamide-exposed groups versus insulin monotherapy in participants with T2DM and HFDP.

T2DM, type 2 diabetes; LBW, low birth weight; PNM, perinatal mortality.

HIV infection was found in 212 (24.0%) women in the cohort, the highest in the T1DM group with 45 (29.4%) participants and the lowest in the HFDP group with 72 (17.4%) participants. Maternal weight and BMI at first presentation, hypertension, gestational age at presentation, and glycaemic control in the third trimester were similar among HIV-infected and uninfected participants. However, the HIV-infected group had a significantly higher rate of anaemia (61 [29.2%] vs 65 [9.7%], p≤0.001) and a lower initial HbA1C (median 6.7, IQR 5.6–8.5 vs median 7.1, IQR 6.1–8.7, p=0.013). A lower rate of macrosomia was observed in the HIV-infected HFDP group (0 vs 28 [10.0%], p=0.012) (Figure 3.2). However, PNM was significantly higher in the HIV-infected T1DM (5 [14.7%] vs 0, p=0.002) and HFDP groups (4 [7.0%] vs 3 [1.1%], p=0.017).

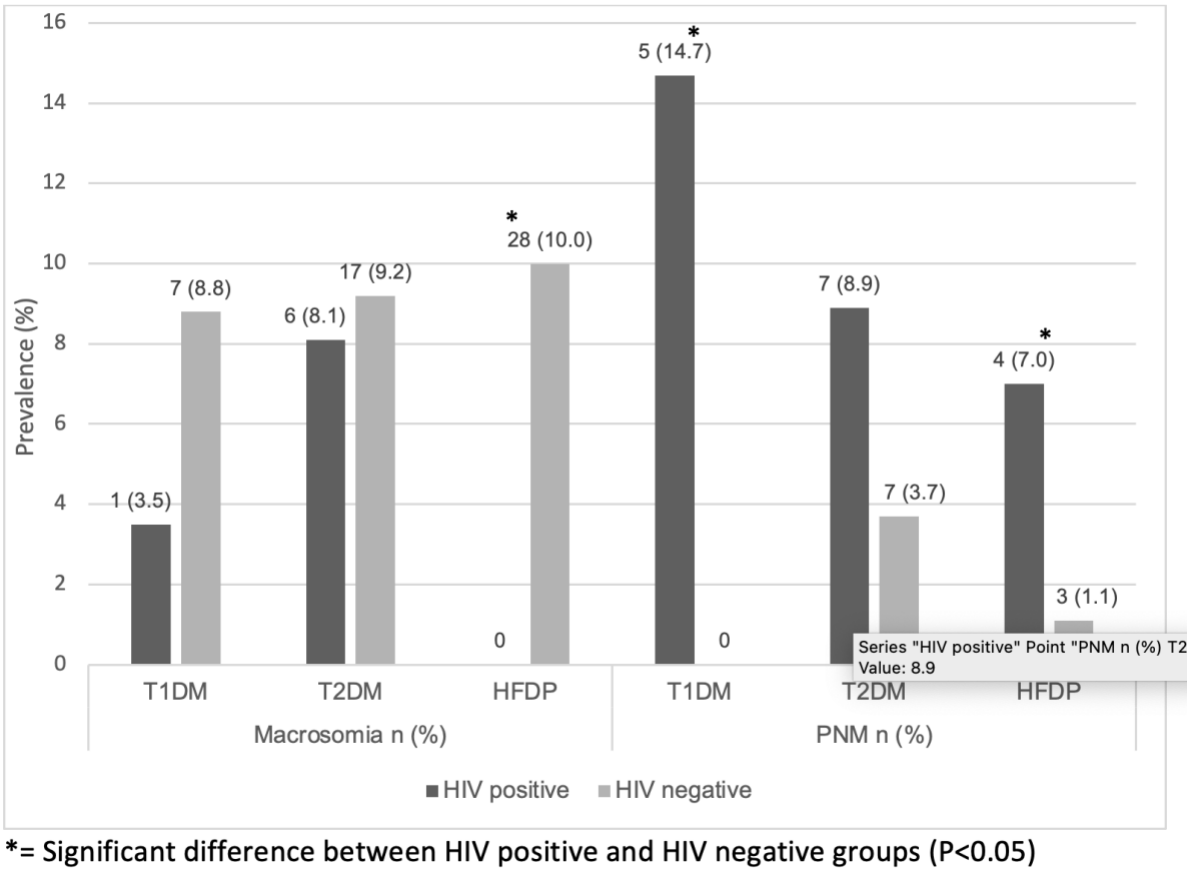


Figure 3.2: Neonatal outcomes in HIV-infected and uninfected participants with T1DM, T2DM, and HFDP.

T1DM, type 1 diabetes; T2DM, type 2 diabetes.

3.4 Discussion

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

The rates of key adverse outcome including birth weight (macrosomia and LBW), prematurity, fetal anomalies, hypertensive disorders of pregnancy, maternal mortality, and PNM were similar to a previous audit from 1992 to 2002 at our clinic (52) and to another recent South African audit (58). However, macrosomia, PNM, and prematurity remain worse compared to respective rates in South Africa for non-diabetic pregnancies (estimates for macrosomia: 3.4-4% (224,225), PNM: 2.9-3.3% (226–228), prematurity: <10-12.4% (229,230)). The rate of

caesarean delivery (n=641, 72.2%) was much higher in the present study than the estimated rate of caesarean delivery at CHBAH of 39.8% in non-diabetic pregnancies (231), but only moderately higher than the 1992–2002 audit of diabetic pregnancies (62%), a difference that could be due to an overall increase in rates of caesarean delivery over this period. When compared to two recent studies from the UK rates of caesarean delivery and PNM, which were in the range of 1.2–2.7%, were higher in our pre-gestational groups (232,233). However, comparisons to HIC as a whole are complicated by differences in population, study design, diagnosis, and management (234,235).

Obesity, so common in our cohort and in South African women in general, not only augments the metabolic burden of pregnancy, it also increases the risk of adverse outcomes (236,237). In our cohort, BMI was found to be a significant predictor for macrosomia. Even the T1DM group had a high BMI at presentation, in contrast to the traditional set of characteristics attributed to T1DM. This indicates that the rising public health burden of metabolic syndrome is impacting even unexpected population groups, a trend that is similarly being found in high-income settings (238,239).

The T1DM group showed higher rates of fetal anomaly, maternal mortality, miscarriage, and PNM than the other groups. This is most likely driven by the severity of hyperglycaemia, a trend that is consistent with the international literature (58,240). However, our high rates of PNM, compared to international studies (232,233) and South African background rates, reveals a critical area for improvement for women with pre-gestational diabetes, especially considering the unacceptably high initial HbA1c for both T1DM and T2DM and the lack of glycaemic control in 111 (49%) participants at initial presentation. This highlights the need for improved counselling and family planning for women of childbearing age with diabetes, for optimization of glycaemic control.

Notably, initial HbA1c and outcomes including prematurity and stillbirth were similar for the DIP and T2DM groups, but rates of macrosomia, PIH, and congenital anomalies were higher in the former, indicating that the DIP group most likely consisted of undetected T2DM. This highlights the importance of increased pre-conceptional detection of T2DM through strengthening of diabetic health systems in LMICs. The GDM group had fewer adverse

outcomes than the DIP group, which consisted of more than 50% of HFDP cases. This low rate of adverse outcomes and the 97.5% glycaemic control attained indicates the successful treatment of this group, predominantly with metformin (n=171, 75.7%). The discrepancy with DIP reiterates that severity of initial hyperglycaemia is an important predictor for worse outcomes and lack of glycaemic control.

The HIV prevalence rate in our cohort of 212 (24.0%), though high in an international context, is similar to regional estimates of 28% (184). The lower HbA1c in the HIV-infected population was unexpected, as this group had higher rates of anaemia, which results in higher HbA1c if caused by iron deficiency anaemia (241). However, this relationship is complex, since the cause of anaemia in our cohort was unknown and HbA1c has previously been found to underreport hyperglycaemia in HIV-positive patients (242). On the other hand, the observation that first visit mean blood glucose (MBG) was also lower in our HIV-infected group tends to affirm better control in this group. One possible explanation is that participants with known HIV infection may have increased contact with health services, improving lifestyle measures (243), although this was not reflected in earlier presentation for the HIV-infected group.

We found a higher PNM rate of 9.1% (16 cases) in HIV-infected participants. Although a background rate for PNM in HIV-infected patients on ART is not known, this was higher than the South African national PNM estimate for the general population (3.3%) (226). Previous studies, in both HICs and LMICs, have found an increased PNM in HIV-infected pregnancies (244,245). When considering the consequences of HIV infection, the contribution of chronic generalized inflammation, indirect HIV-associated conditions, and side-effects of ART are difficult to unravel (246). The higher rates of anaemia in the HIV-infected group may have contributed to the high PNM rates, given that anaemia is associated with higher neonatal morbidity and mortality (247). All of the HIV-infected participants would have likely been placed on a lifelong ART regimen, in adherence with “test and treat” guidelines (248). However, a lack of documented information on both severity of HIV and exact use of ART prohibited us from further analyses into these areas. Regardless, the high risk of PNM and higher rates of anaemia in this combined diabetic and HIV-infected population marks them as a group of patients that could benefit from close monitoring and further research.

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

For both T2DM and HFDP, maternal outcomes were not significantly different between patients exposed to glibenclamide and metformin compared to the gold standard of insulin. However, rates of macrosomia were higher among those exposed to glibenclamide (n=10 [12.2%] vs 0) despite a significantly lower initial HbA1c and better glycaemic control in the third trimester in this group. This was in line with results from a recent meta-analysis observing glibenclamide to be inferior to both metformin and insulin in terms of rates of macrosomia and neonatal hypoglycaemia (222). It has been suggested that the effect of glibenclamide may be due to a direct drug effect via placental transfer, but the relationship could also be due to the higher initial BMI in the glibenclamide group (224). Reassuringly, we found no significant relationship between use of OHAs and prevalence of congenital anomalies, but a limited number of patients with T2DM (n=115, 25.8%) and HFDP (n=9, 2.6%) were exposed to the drugs in the first trimester. Neither OHA negatively influenced PNM when compared to insulin. This contributes to the evidence that these agents may be a safe alternative to insulin in our setting (222,249).

Limitations of our study are related to the retrospective study design, resulting in missing data due to underreporting in files, including for BMI, HIV status and treatment details, possible rates of pre-eclampsia/eclampsia, and some neonatal outcomes (hypoglycaemia, jaundice, and respiratory distress syndrome). Moreover, we did not have data on pre-pregnancy BMI and the use of BMI at initial presentation unadjusted for gestational age could have resulted in an overestimation of rates of obesity and overweight. Likewise, we did not have extra information on causes of PNM, which could have given additional insight into the high rate of PNM in the HIV-infected group. It is important to note that the rate of miscarriage is likely an underestimation due to a lack of information on miscarriages in early gestation, since participants commonly presented during the second and third trimesters. Regression analyses could not be performed for all outcomes regarding HIV and treatment groups because of low

positive outcomes. Loss to follow-up of 166 (15.4%) participants may have resulted in bias, although the only significant differences in baseline characteristics were younger age at presentation and higher primiparity rates in the group lost to follow-up.

3.5 Conclusion

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

Chapter 4: Obesity and adiposity of 3- to 6-year-old children born to mothers with hyperglycaemia first detected in pregnancy in an urban South African setting.

Summary and context:

The findings described in this chapter have been published as the following peer-reviewed article:

Soepnel L.M., Nicolaou V., Slater C., Chidumwa G., Levitt N.S., Klipstein-Grobusch K., Norris S.A.. Obesity and adiposity of 3- to 6-year-old children born to mothers with hyperglycaemia detected in pregnancy in an urban South African setting. *Annals of Human Biology*. 2021; 48(2):81-92. doi: 10.1080/03014460.2021.1918245.

This is the first of two chapters that describes findings from the comparative follow-up study of children born to mothers with HFDP, compared to an HFDP-unexposed group, with this chapter focusing on adiposity, obesity, and growth as the outcomes of interest. As a key finding, maternal BMI was found to play an important role in the development of childhood adiposity. No independent association was found between HFDP-exposure and childhood adiposity at preschool age, identifying critical areas for future research.

4.1 Introduction

Nutritional changes and the increase of sedentary "modern" urban lifestyles are driving the obesity epidemic in LMICs (250). Globally, an estimated 40 million children under the age of 5 were impacted by overweight/obesity in 2018, with the majority living in LMICs (178,251). Aside from the negative physical and socio-emotional consequences of childhood obesity, it is also associated with a higher risk of persistent adulthood obesity, CVD, and T2DM (252). While overweight/obesity determined by BMI is the most widely recognized surrogate measure for childhood adiposity, measures that distinguish between fat and lean mass provide additional information, predictive of future disease risk (253). The search for early life obesity prevention strategies has prompted investigations into pregnancy exposures that contribute to childhood overweight and adiposity (254).

In urban SSA, the global obesity epidemic is also heavily impacting women of reproductive age, as exemplified by the overweight/obesity prevalence of around 62% in South African women aged 20-34 years (236,251,255). As a result, an increasing number of pregnancies are complicated by obesity-associated conditions, on top of existing communicable disease, maternal health, and (childhood) malnutrition challenges (28,256). One such increasingly prevalent condition is HFDP, which consists of less severe GDM and 'overt' DIP according to the 2013 WHO criteria (36). GDM alone has been found to impact up to 9.1% of pregnancies in urban SSA (38,39).

These conditions in pregnancy are thought to confer risk for childhood obesity and overweight through early developmental programming in response to altered intrauterine conditions, exacerbating the problem of childhood adiposity in the next generation (257). Maternal pre-pregnancy obesity itself was found to increase the odds of childhood obesity by 264% in a recent meta-analysis (258). In addition, evidence from middle- and high-income countries has indicated that maternal HFDP might be an independent risk for childhood obesity (15,98,112). However, contrasting findings have also arisen, possibly due to the use of varying diagnostic criteria, follow-up times, and measures of childhood adiposity (101–103). Most recently, evidence from a follow-up study of the HAPO study in 4832 children aged 10-14 years showed an independent association between a continuum of (untreated) maternal blood glucose levels and childhood adiposity (66,111). Despite the alarming rate of maternal obesity and the increase in hyperglycaemia in pregnancy in urban SSA, this association remains to be thoroughly explored in an African setting at any age. Preschool age is of interest in this relationship since SSA has a significant prevalence of preschool overweight/obesity (80) and interventions at this age have been found to be more effective than in later childhood (259,260).

Considering the growing number of children born to mothers with HFDP, the increasingly obesogenic environments children are exposed to in urban SSA and LMICs elsewhere, and the serious long-term consequences of childhood obesity, it is essential to build evidence to guide childhood obesity prevention efforts in SSA. We therefore aimed to assess the association between HFDP and childhood obesity and adiposity, hypothesizing that exposure is positively associated with these outcomes in preschool aged children from Soweto, South Africa,

independently of maternal BMI. Additionally, as a secondary objective, we explored the direct and indirect effects of maternal BMI on childhood obesity and adiposity within the context of HFDP.

4.2 Methods

4.2.1 Study population and hyperglycaemia first detected in pregnancy

Between March and November 2019, we conducted a study of 3-6-year-old children. The exposed and unexposed group were defined on the basis of maternal exposure to HFDP, as follows: children born to mothers who were diagnosed with HFDP (GDM or DIP) at CHBAH (exposed group) vs. to mothers who attended antenatal care at the same hospital but tested negative for HFDP (HFDP-unexposed group), between February 2014 and January 2017 (see Chapter 2, Figure 2.3, Overview of Study Design). CHBAH is an urban tertiary hospital with around 1400-1600 deliveries per month, servicing the region of Soweto, where the population consists primarily of Black South Africans (21).

The mothers of the exposed group attended the gestational endocrine clinic at CHBAH for GDM while pregnant with the participating child. Clinical characteristics and pregnancy outcomes of this group have been described in a previously publication (Chapter 3) (261). Diagnostic testing was based on selective screening of risk factors for GDM, except for some women universally screened in a research context (39). Women were diagnosed using a 75-gram 2-hour OGTT with IADPSG criteria (Chapter 1, Table 1.1), categorized into DIP and GDM.

The mothers of the HFDP-unexposed group attended CHBAH's antenatal clinic during the index pregnancy and tested negative for HFDP in a research setting, using the same diagnostic criteria as the clinic. Further information around diagnosis and participant characteristics has been previously published (39). Mother/child pairs qualifying as the HFDP-unexposed group were contacted in a random order and invited to attend the data collection appointment, until the number of mother/child pairs per the child's birth year was the same in the HFDP-unexposed group as in the exposed group.

Exclusion criteria included inability to contact the mother/guardian or attend the data collection appointment (e.g., due to relocation), being one of two twins, diagnosis of major congenital disorders or diabetes mellitus, maternal or child death since pregnancy, maternal diagnosis of pre-gestational diabetes, and age <2.8 years or >6 years old. The study was approved by the HREC of the University of the Witwatersrand (M180317).

4.2.2 Sample size calculation

Sample size calculation for means was performed in Stata, version 13 (College Station, USA). For 80% power at a 0.05 significance, the required minimum sample size was 130 participants per group to detect a difference in BMI z-score of 0.35 between the HFDP-unexposed and exposed group, based on existing evidence (ranging from 0.22-0.6 with varying study designs (15,66,112,213), calculated using population SD of 0.93 (5 year-old) and 1.07 (3 year-old) based on a cohort of Sowetan children (216). This would also be sufficient to detect a difference in FMI of 0.4 kg/m^2 with a SD of 1 (n=100 per group).

4.2.3 Outcomes

Primary outcomes were overweight (BMI) and adiposity (FMI). Secondary outcomes were linear growth (height) and stunting. Table 4.1 presents definitions for body composition outcomes used in this paper. Birth outcomes are the same as defined in Chapter 3, Figure 3.1.

Table 4.1: Definitions of maternal anthropometric characteristics at age 3-6 years.

Anthropometric characteristics at age 3-6 years	
Body mass index (BMI)	Weight (kg) divided by height (m) squared
Obesity	According to WHO definition: For children <5 years BMI z-score higher than 3SD from the mean; for children >5 years BMI z-score higher than 2SD from the mean.
Overweight	According to WHO definition: For children <5 years BMI z-score +2SD to 3SD from the mean; for children >5 years BMI z-score +1SD to 2SD from the mean.
At risk for overweight	According to WHO definition: For children <5 years BMI z-score +1SD to 2SD from the mean.
Stunting	Height < -2SD from the mean, according to WHO child growth standards per age
Fat mass index (FMI)	Estimated fat mass (kg) (by deuterium dilution method) divided by height (m) squared.

4.2.4 Anthropometric measurements and determining fat mass

Anthropometric measurements were done by a trained research assistant or nurse according to standardized procedures, after removing shoes and any outerwear. The mean of three measurements was used for analyses of weight, height, waist circumference, and mid-upper arm circumference (MUAC). Weight was assessed using a SECA digital scale (Hamburg, Germany), and height was measured using a fixed, mounted Holtain stadiometer (Crymych, UK). The mean weight and height per participant were used to calculate BMI (kg/m^2). Waist circumference was measured excluding clothing using a measuring tape placed halfway between the lower rib and the iliac crest. MUAC was measured halfway between the acromion and the olecranon process. The interobserver CV for anthropometric measures was <3%. Technical error measurement was <0.7cm, except for waist circumference where a technical error measurement of <1.5cm was considered acceptable.

Childhood adiposity was assessed using the deuterium dilution method, according to IAEA protocol adapted for 2-6-year-old children (202). The deuterium dilution method, a reference method for body composition assessment, measures the TBW following a dose of deuterium oxide. The deuterium equilibrates with hydrogen in body water and evenly distributes throughout the TBW after a few hours. Since water is found exclusively in fat-free mass, the level of enrichment in the saliva allows for a deduction of the fat-free mass and, subsequently, fat mass.

The procedure involved taking a baseline saliva sample using cotton wool according to the IAEA method (202), followed by giving the participant an oral 20-gram dose of 10% deuterium oxide diluted in water (2 grams of deuterium oxide). Participants were asked to empty their bladders prior to the dosing. After inverting the dose at least 10 times, participants drank through a straw, then 50mL of water was added to the dose bottle and consumed through the same straw. This was repeated with another 50mL of water. If any of the dose was lost in this process, the procedure was aborted. Subsequent saliva samples were taken at 2.5-3 hours after dosing. Food and drink were limited as follows: the last intake was at least 30 minutes prior to taking the baseline sample; a light meal and drink of <250mL was offered 30-60 minutes after dose consumption; no additional food or drink was consumed until after the final saliva sample. Saliva samples were stored in a -20-degree Celsius freezer until deuterium enrichment analysis

was performed by a qualified laboratory technician using a portable Fourier-transform infrared spectrometer (Agilent, 4500 Series, California, USA) (202). The criterion for acceptance of the deuterium analysis was a CV <2%, including the analysis of quality control standards at the start and end of the day, and saliva samples. TBW was accepted if the precision of TBW calculated separately from the first and second post-dose samples was <3%. The mean CV for deuterium analysis was 0.5% for the whole study. The mean CV for TBW was 1.1%.

4.2.5 Additional variables

For the exposed group, hospital files from the gestational endocrine clinic provided data regarding maternal OGTT results, gestational age at diagnostic OGTT, HbA1c at the first visit and in the 3rd trimester, treatment for HFDP, BMI at the first clinic visit, maternal HIV infection, and obstetric/birth outcomes (birth weight, gestational age at delivery, and obstetric/early neonatal complications). For the HFDP-unexposed group, the same information was collected from data collected in pregnancy (39) and the child's Road to Health Card, a patient-held medical record given to every child born in South Africa to assist with childhood health monitoring. Maternal BMI was calculated at the data collection appointment using the methods described above for children.

Each mother completed a questionnaire with the help of a research assistant. This provided information on household assets at the child's primary residence (household socioeconomic score) (189), child age and sex, maternal education level, maternal age, parity, maternal smoking and alcohol use in pregnancy (190), early life hospitalizations, breastfeeding (whether participant was breastfed, whether participant was breastfed exclusively, and the duration of any breastfeeding), and maternal HIV infection. The relevant questionnaire can be found in Appendix G, and are also described in Chapter 2, section 2.3.2.

4.2.6 Data management and statistical analysis

Data was captured and managed using REDCap (Vanderbilt University, Nashville, USA (188)), and imported into Stata 13 (College Station, USA). Outcomes at 3-6 years for weight-for-age, height-for-age, BMI-for-age, and MUAC-for-age were converted into standard scores (z-scores) using WHO growth standards or references (131,198). FMI (kg/m^2) was calculated using height and body fat mass from the deuterium dilution method. Birth weight was

converted into a z-score adjusted for gestational age using the Intergrowth-21 standards (199). Conditional weight gain was the male or female standardized residual of current weight regressed against birth weight. The participant's household assets were summed for a continuous household SES score (0-13). Breastfeeding was categorized dichotomously as any breastfeeding vs. no breastfeeding, and breastfeeding was categorized as exclusive if the mother indicated that she exclusively breastfed for at least 2 weeks (until any complimentary foods were introduced). Any statistical outliers were checked for (data capturing) errors but included for analysis if no error was found.

Student's unpaired t-test or the Mann-Whitney U test was used to test significance, depending on normality. Statistical differences between three groups (HFDP-unexposed, GDM and DIP), were tested using ANOVA or Kruskal-Wallis test. For categorical outcomes, a chi^2 goodness of fit or Fisher exact test were used. Throughout, significance was assumed at a two-sided p-value <0.05.

For the outcomes BMI z-score and FMI, multivariable hierarchical regression analysis was performed on an a priori basis, as follows: Model 1a) crude model for HFDP exposure, Model 1b) (for FMI) M1a + child sex and age, Model 2) Model 1b + maternal and household factors (maternal BMI in pregnancy, maternal HIV status in pregnancy, parity, alcohol and smoking during pregnancy, and socioeconomic household score.)

In order to explore the direct and indirect effects of maternal BMI in pregnancy on childhood FMI and BMI in the context of HFDP, generalised structural equation modelling (GSEM) was performed to explore the relationship between maternal BMI, HFDP (GDM and DIP), other significant maternal/household and mediating variables, and FMI/BMI, fitted with the multinomial family and logit link function. GSEM was performed because it allows for a pictographic representation of hypothesis-driven relationships between variables, including potential mediators, confounders, and composite latent variables (not applicable for this dataset). GSEM estimates path equations simultaneously, and allows for calculations of direct, indirect, and total effects (262). We started with a hypothesized model, based on theoretical meaning and results from regression analyses, as shown in Figure 4.2 (supplementary). Modifications to pathways and adding/removing variables were made iteratively and the

Aikake and Bayesian Information Criteria (IC) of each model was compared. The final model was selected for having a low IC and high theoretical relevance (Figure 4.1, Table 4.5 (supplementary)). Direct, indirect, and total effects were calculated using non-linear combination estimates.

4.3 Results

4.3.1 Baseline characteristics

Of 211 participants that attended the data collection appointment, 7 did not meet the inclusion criteria, resulting in 204 included participants.

The median age of participants was similar between the HFDP-unexposed and exposed groups at 3.5 years (IQR 3.1-4.1) and 3.4 years (IQR 3.1-4.1), respectively (Table 4.2). Of the participants exposed to HFDP, 45 (44.1%) were exposed to DIP, and 57 (55.9%) to GDM. The number of participants born via caesarean section was high in both groups at 58.8% (n=60) and 69.7% (n=69) in the HFDP-unexposed and exposed group, respectively. The rate of prematurity was higher in the HFDP-exposed group than the unexposed group (12.8% (n=13) vs 21.6% (n=21.6)), and highest in the DIP group at 24.4% (n=11). Macrosomia rates were notably similar between the HFDP-unexposed and exposed groups, but the birth weight z-score adjusted for gestational age was higher in the DIP group (Table 4.3). Breastfeeding was received in 80.4% (n=82) of the HFDP-unexposed group but only 71.6% (n=73) of the HFDP-exposed group.

At the time of the pregnancy OGTT, mothers with DIP had higher BMI, age, and fasting plasma glucose levels. The number of primiparous women was higher in the HFDP-unexposed group than in the HFDP-exposed group. In total, only 6 mothers reported smoking during pregnancy (2.9%), and 2 reported alcohol consumption during pregnancy (0.9%). The majority of mothers diagnosed with DIP or GDM were managed with medical therapy rather than diet (n=88, 86.3%). Upon measurement at the postpartum visit, maternal BMI was high across all three groups (median 31.4, IQR 26.7-36.1). The total maternal HIV infection rate at the follow-up visit was 43 (21.1%), with 86.0% diagnosed at or before the index pregnancy (n=37).

	HFDP-unexposed		HFDP-exposed					
	n		n	**Total group**	n	**GDM**	n	**DIP**
Number of participants	-	102	-	102	-	57	-	45
Age, year (median, IQR*)	102	3.5 (3.1-4.1)	102	3.4 (3.1-4.1)	57	3.3 (3.0-4.0)	45	3.7 (3.2-4.2)
Male (n, %)	102	49 (48.0)	102	56 (54.9)	57	30 (52.6)	45	26 (57.8)
Early life factors								
Received breastfeeding (n,%)	102	82 (80.4)	102	73 (71.6)	57	39 (68.4)	45	34 (75.6)
Exclusive breastfeeding	102	77 (75.5)	102	62 (60.8)	57	34 (59.7)	45	28 (62.2)
Hospitalized <2 years (n,%)	102	20 (19.6)	102	15 (14.7)	57	11 (19.3)	45	4 (8.9)
Pregnancy factors								
OGTT results (mean, SD†)	102		95		56		39	
Fasting		4.0 (0.5)		6.7 (1.9)		5.6 (0.7)		8.2 (2.0)
2-hour		5.4 (1.2)		10.5 (3.5)		8.6 (1.7)		13.1 (3.6)
HbA1c (median, IQR)		-	94					
First visit %				6.3 (5.6-7.1)	51	5.7 (5.3-6.1)	43	7.0 (6.6-8.2)
3rd trimester %				6.1 (5.6-6.6)	50	5.8 (5.5-6.3)	42	6.6 (6.1-7.3)
Exposure to: (n, %)		-	102		57		45	
Metformin				63 (61.8)		39 (68.42)		24 (53.3)
Glibenclamide				30 (29.4)		13 (22.8)		17 (37.8)
Insulin				32 (31.4)		7 (12.3)		25 (55.6)
Gestational age OGTT (median, IQR)	102	26 (25-27)	102	30.4 (25.6-33.6)	57	30.6 (27.3-34.1)	45	30.1 (23-32.6)
Parity (n,%): 0	102	36 (35.3)	102	12 (11.8)	57	8 (14.0)	45	4 (8.9)
1-2		59 (57.8)		78 (76.5)		43 (75.4)		35 (77.8)
>3		7 (6.9)		12 (11.8)		6 (10.5)		6 (13.3)
BMI at OGTT (median, IQR)	102	29.4 (24.8-33)	102	35.1 (30.8-40.0)	52	33.9 (30.5-37.5)	42	37.6 (32.6-41.3)
HIV-infection pregnancy (n,%)	102	20 (19.6)	102	17 (16.7)	57	13 (22.8)	45	4 (8.9)
Caesarean section (n, %)	102	60 (58.8)	99	69 (69.7)	56	40 (71.4)	43	29 (67.4)
Obstetric complication (n, %)	102	11 (10.8)	102	15 (14.7)	57	6 (10.5)	45	9 (20.0)
Macrosomia (n, %)	102	5 (4.9)	99	5 (5.1)	56	3 (5.4)	43	2 (4.7)
Large for gestational age (n,%)	102	11 (10.8)	99	21 (21.2)	56	6 (10.7)	43	15 (34.9)
Low birth weight (n,%)	102	17 (16.7)	99	16 (16.2)	56	10 (17.9)	43	6 (14.0)
Prematurity (n,%)	102	13 (12.8)	102	22 (21.6)	57	11 (19.3)		11 (24.4)
Maternal and household characteristics at visit 3-6 years postpartum								
Highest education level	101		102		57		45	
1 None/Primary school		0		3 (2.9)		2 (3.6)		1 (2.2)
2 Secondary school		73 (72.3)		66 (64.7)		40 (70.2)		26 (57.8)
3 Prof. training/university		28 (27.7)		33 (32.4)		15 (26.3)		18 (40.0)
Household SES (mean, SD)	102	8.6 (2.1)	102	8.0 (2.2)	57	7.8 (2.1)	45	8.3 (2.3)
Score ≤5 (n, %)		6 (5.9)		10 (9.8)		8 (14.0)		2 (4.4)
Maternal BMI (mean, SD)	98	30.1 (7.4)	95	33.8 (7.1)	54	33.2 (7.1)	41	34.6 (7.3)
Blood pressure >140/90 (n,%)	102	20 (19.6)	102	37 (36.3)	57	16 (28.1)	45	21 (46.7)
HIV-infection (n,%)	102	23 (22.6)	102	20 (19.6)	57	15 (26.3)	45	5 (11.1)
Taking FDC (n, %)	19	17 (89.4)	20	16 (80.0)	15	12 (80.0)	5	4 (80.0)

Table 4.2: Baseline characteristics of participants per group.

Values are given as mean (standard deviation), median (interquartile range), or number (percentage). HFDP: hyperglycaemia first detected in pregnancy; GDM: gestational diabetes mellitus; DIP: diabetes in pregnancy; OGTT: oral glucose tolerance test; HbA1c: glycated haemoglobin; BMI: body mass index; SES: socioeconomic score; FDC: fixed dose combination.

4.3.2 Childhood overweight and adiposity

In the HFDP-unexposed group, 4 (3.9%) children were overweight/obese by WHO definitions, compared to 6 (10.5%) and 5 (11.1%) in the GDM and DIP groups, respectively (Table 4.3). Additionally, 22.6% (n=22) of the HFDP-unexposed group and 17.7% (n=18) of the HFDP-exposed group were 'at risk of overweight'. BMI z-score, waist circumference by height, and height z-score were consistently higher in the DIP group. Although fat-free mass index was similar between the groups (12.2±0.7 and 12.1±1.1 in the HFDP-unexposed and exposed groups, respectively), fat mass index was highest in the DIP exposed group at 4.0 (IQR3.2-4.7), compared to 3.5 (IQR3.1-4.2) in the unexposed group. The highest rate of stunting at 9 (15.8%) was found in the true GDM group.

For multivariable regression analyses, none of the included variables showed multicollinearity, with variance inflation factors <2 for each model. Due to low rates of maternal alcohol consumption and smoking during pregnancy (n=2 and 6, respectively), these variables were not included in presented analyses. FMI was log-transformed to increase normality of residuals.

Log-FMI was higher in the children exposed to HFDP, even after correcting for age and sex, significantly so in the DIP group (unstandardized B 0.13 [95%CI 0.03-0.23], standardized β 0.18,p=0.014) (Table 4.4). In this model, being female was associated with having a higher FMI (B 0.10 [95%CI 0.02-0.18], β 0.17, p=0.014). In model 2, correcting for maternal/household factors, DIP-exposure was no longer significantly associated with log-FMI, and maternal BMI during pregnancy had the strongest association with log-FMI (B maternal BMI 0.01 [95%CI 0.003-0.015], β 0.23, p=0.004). Household socioeconomic score was positively associated with log-FMI (B 0.02 [95%CI -0.002-0.036], β 0.13, p = 0.072).

The (non-statistically significant) positive association between DIP/GDM and BMI z-score was attenuated in Model 2, with DIP exposure showing a slight inverse association with FMI (B -0.003 [95%CI -0.40-0.39], β -0.001, p=0.987). Maternal BMI was the main significant predictor of BMI z-score (B 0.04 [95%CI 0.02-0.06], β 0.29, p=<0.001). Maternal HIV infection was not significantly associated with either BMI or log-FMI. No significant interaction effect was found between maternal BMI and HFDP, for either BMI or log-FMI.

Table 4.3: Infant and childhood anthropometric and fat mass outcomes per group: HFDP-unexposed vs exposed, subdivided into GDM and DIP

		HFDP-unexposed		**HFDP-exposed**					**p-values**
	n		n	Total group	n	GDM	n	DIP	
Birth weight, g (median, IQR)	102	3040 (2800-3350)	99	3100 (2670-3100)	56	3042.5 (2667-3327)	43	3170 (2765-3685)	0.740[§], 0.409[‡]
Birth weight z-score[†] (mean, SD)	102	-0.1 (1.2)	99	0.3 (1.1)	56	-0.02 (1.1)	43	0.7 (1.1)	0.008*[§], <0.001*[‡]
Body composition by anthropometry									
Weight, kg (median, IQR)	102	14.8 (13.5-16)	102	15 (13.7-16.8)	57	14.7 (13.5-16.4)	45	15.2 (14.2-16.9)	0.589[§], 0.418[‡]
Weight z-score (mean, SD)	102	-0.3 (0.9)	102	-0.1 (1.1)	57	-0.1 (1.1)	45	-0.1 (1.1)	0.186[§], 0.417[‡]
Conditional weight gain (median, IQR)	102	-0.3 (-0.6-0.4)	99	-0.2 (-0.6-0.5)	56	-0.2 (-0.8-0.4)	43	-0.1 (-0.5-0.7)	0.455[§], 0.455[‡]
BMI, kg/m^2 (median, IQR)	102	15.6 (14.9-17.0)	102	16.0 (15.2-16.8)	57	15.9 (15.3-16.8)	45	16.0 (15.1-16.8)	0.145[§], 0.346[‡]
BMI z-score (mean, SD)	102	0.3 (0.9)	102	0.5 (1.2)	57	0.5 (1.1)	45	0.6 (1.2)	0.157[§], 0.345[‡]
At risk for overweight (n, %)		23 (22.6)		18 (17.7)		10 (17.5)		8 (17.8)	0.255[§], 0.273[‡]
Overweight		3 (2.9)		9 (8.8)		5 (8.8)		4 (8.9)	
Obese		1 (1.0)		2 (2.0)		1 (1.8)		1 (2.2)	
MUAC, cm (mean, SD)	102	16.5 (1.2)	101	16.7 (1.6)	56	16.5 (1.5)	45	17.0 (1.7)	0.199[§], 0.094[‡]
MUAC z-score	97	0.2 (-0.2-0.8)	98	0.4 (-0.3-1.0)	55	0.3 (-0.4-0.9)	43	0.6 (-0.0-1.2)	0.317[§], 0.259[‡]
WC (mean, SD)	102	49.8 (3.0)	100	50.9 (4.3)	55	50.5 (3.9)	44	51.4 (4.7)	0.037*[§], 0.057[‡]
WC by height index, cm/m	102	0.5 (0.04)	100	0.5 (0.04)	55	0.5 (0.04)	44	0.5 (0.05)	0.117[§], 0.268[‡]
Fat mass by deuterium method									
FFM, kg (mean, SD)	102	11.6 (1.6)	100	11.5 (1.8)	56	11.5 (1.9)	44	11.6 (1.5)	0.754[§], 0.924[‡]
% (median, IQR)		76.7 (74.4-79.6)		76.3 (72.4-78.8)		76.1 (73.1-78.8)		76.3 (70.1-78.8)	0.056[§], 0.137[‡]
FFMI, (mean, SD)	102	12.2 (0.7)	100	12.1 (1.1)	56	12.2 (1.2)	44	12.0 (1.0)	0.412[§], 0.428[‡]
FM, kg (median, IQR)	102	3.4 (2.9-4.1)	100	3.6 (3.0-4.5)	56	3.5 (2.9-4.4)	44	3.8 (3.1-4.6)	0.069[§], 0.101[‡]
% (median, IQR)		23.3 (20.4-25.6)		23.7 (21.2-27.6)		23.9 (21.2-26.9)		23.7 (21.2-29.9)	0.056[§], 0.137[‡]
FMI, (median, IQR)	102	3.5 (3.1-4.2)	100	3.9 (3.2-4.5)	56	3.8 (3.3-4.4)	44	4.0 (3.2-4.7)	0.052[§], 0.143[‡]
Height									
Height, cm (mean, SD)	102	97.3 (6.5)	102	97.3 (6.8)	57	96.8 (7.2)	45	97.9 (6.2)	0.974[§], 0.727[‡]
Height z-score (mean, SD)	102	-0.8 (0.9)	102	-0.8 (1.1)	57	-0.7 (1.2)	45	-0.8 (1.0)	0.563[§], 0.787[‡]
Stunted (n, %)	102	10 (9.8)	102	14 (13.7)	57	9 (15.8)	45	5 (11.1)	0.385[§], 0.526[‡]

Values are given as mean (standard deviation), median (interquartile range), or number (percentage). HFDP: hyperglycaemia first detected in pregnancy; GDM: gestational diabetes mellitus; DIP: diabetes in pregnancy; BMI: body mass index; MUAC: mid-upper arm circumference; WC: waist circumference; FFM: fat-free mass; FFMI: fat-free mass index; FM: fat mass; FMI: fat mass index. [†]Birth weight adjusted for sex and gestational age (Intergrowth-21). [§]p-value for HFDP-unexposed vs. exposed; [‡] p-value for HFDP-unexposed vs GDM and DIP; *p-value <0.05.

In an additional analysis of early life variables, birth weight z-score was significantly associated with both log-FMI and BMI z-score when additionally introduced to Model 2 (B 0.04 [95%CI 0.004-0.07], β 0.16, p=0.028; B 0.16 [95%CI 0.04-0.29], β 0.18, p=0.013, respectively), but breastfeeding was not.

GSEM showed a significant direct effect of maternal pregnancy BMI on log-FMI and BMI, and the total effect through DIP and birth weight was also significant (Figure 4.1). Birth weight z-score was significantly associated with both FMI and BMI (95%CI 0.01-0.71, p=0.025; B 0.14, 95%CI 0.02-0.26, p=0.020). The total effect of GDM-exposure was not significant for FMI or BMI, and there was no significant association between GDM and birth weight z-score. DIP-exposure had a significant positive effect on birth weight z-score, but the total DIP-effect on FMI/BMI was not significant (Figure 4.1, Table 4.5 (suppl.)). Household socioeconomic score had a positive total effect on log-FMI (B 0.02, 95%CI 0.002-0.04, p=0.032). In GSEM, parity had an indirect and total effect on BMI/log-FMI through the Maternal BMI, DIP-exposure, and birth weight z-score pathway, an association not seen for parity and childhood BMI/log-FMI in regression analysis, which adjusted for these pathway variables rather than calculating indirect effects as in GSEM.

Table 4.4: Linear regression model for BMI z-score and log-transformed FMI, reporting standardized regression coefficients (β), unstandardized regression coefficient (B) with 95% confidence interval, and p-value.

Outcome: BMI z-score	**M1a: crude model**			**M1b: N/A**			**M2: M1 + maternal factors[1]**		
	B (95% CI)	β	p-value				B (95% CI)	β	p-value
Hyperglycaemia first detected in pregnancy									
True GDM	0.176 (-0.167-0.518)	0.075	0.312				0.155 (-0.202-0.511)	0.067	0.393
DIP	0.250 (-0.121-0.621)	0.099	0.185				-0.003 (-0.402-0.395)	-0.001	0.987
Maternal and household factors									
Maternal pregnancy BMI							0.041 (0.019-0.063)	0.292	**<0.001***
Parity							-0.051 (-0.207-0.104)	-0.049	0.518
Socioeconomic household score							0.048 (-0.020-0.117)	0.100	0.163
HIV positive during pregnancy							0.037 (-0.246-0.421)	0.014	0.848
	N 204; adj. R^2 0.1%						N 196; adj. R^2 7.0%		
Outcome: Log-transformed FMI	**M1a: crude model**			**M1b: crude model + sex + age**			**M2: M1b + maternal factors[1]**		
	B (95% CI)	β	p-value	B (95% CI)	β	p-value	B (95% CI)	β	p-value
Hyperglycaemia first detected in pregnancy									
True GDM	0.066 (-0.028-0.159)	0.103	0.167	0.065 (-0.028-0.157)	0.101	0.169	0.064 (-0.034-0.161)	0.099	0.200
DIP	0.115 (0.014-0.217)	0.166	**0.026***	0.126 (0.026-0.226)	0.182	**0.014***	0.081 (-0.029-0.191)	0.117	0.148
Child age				-0.003 (-0.008-0.002)	-0.088	0.206	-0.005 (-0.010-0.000)	-0.133	0.059
Child sex (female)				0.099 (0.021-0.177)	0.172	**0.014***	0.075 (-0.002-0.152)	0.133	0.058
Maternal and household factors									
Maternal pregnancy BMI							0.009 (0.003-0.015)	0.226	**0.004***
Parity							-0.027 (-0.070-0.016)	-0.093	0.221
Socioeconomic household score							0.017 (-0.002-0.036)	0.129	0.072
HIV positive during pregnancy							-0.025 (-0.130-0.080)	-0.034	0.641
	N 202; adj. R^2 1.7%			N 202; adj, R^2 4.7%			N 194; adj. R^2 10.0%		

[1]Maternal factors included in M2: Maternal pregnancy BMI, parity, socioeconomic household score, and maternal HIV-status during pregnancy.

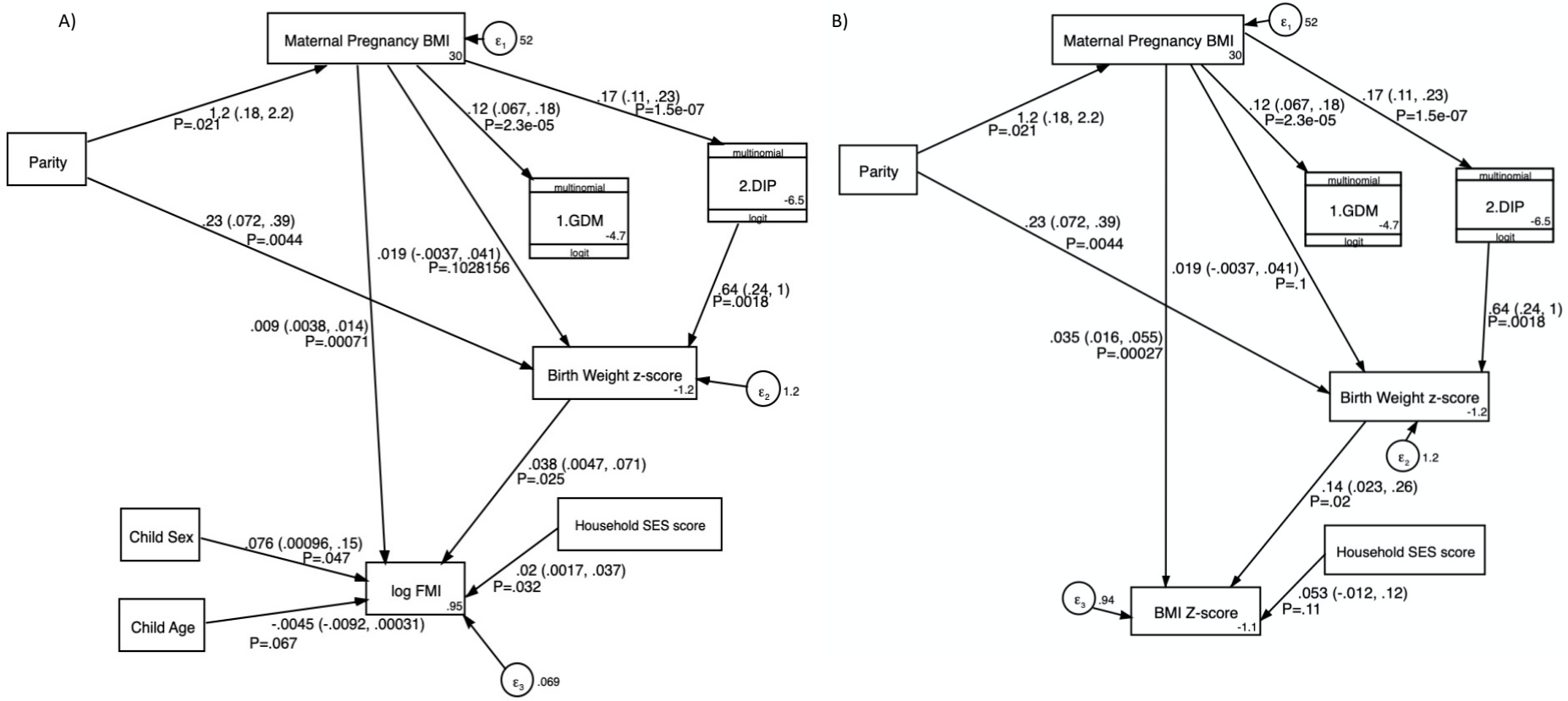


Figure 4.1: GSEM best-fit model of maternal and household variables, birth weight, and GDM/DIP for a) log-FMI, adjusting for sex and age, and b) BMI z-score.

Unstandardized coefficient (95%CI) and p-value are displayed.

4.4 Discussion

In our study, the association between exposure to HFDP and subsequent adiposity (FMI) in 3-6-year-old children was not independent of maternal BMI. The association between HFDP exposure and childhood BMI was not statistically significant. Maternal BMI, which was found to have a direct effect on childhood adiposity and BMI, may therefore be of greater concern than HFDP at preschool age in our urban African setting.

Previous literature from high- and middle-income countries presents a heterogeneous picture of the impact of HFDP and GDM on childhood BMI. While some studies have found an independent effect of HFDP on BMI and overweight/obesity (15,95,96,116,213,263,264), others, similar to our study, showed no significant association (66,99,101–103,106,130). Interestingly, some studies found a significant association in school-aged children and adolescents that was not evident in earlier childhood (15,103,107), possibly due to the accumulation of environmental and behavioural risks for obesity throughout childhood (107,108). The overweight/obesity prevalence in our cohort did not exceed national estimates of 11-13%, but 26% of the HFDP-unexposed and 28-29% of the exposed group were at least 'at-risk of overweight', raising concerns for progression to overweight/obesity in later childhood (255). Since exposure to additional obesogenic factors might not occur in the same pattern in high vs low-middle income countries, a longitudinal prospective analysis in our setting would provide valuable additional insight.

A number of recent studies exploring adiposity, as opposed to BMI or overweight status alone, found a positive association with HFDP (66,106,112,128,130). This was mirrored in our results by the significant unadjusted association between DIP-exposure and childhood FMI. Exposure may thus impact fat rather than lean mass, increasing adiposity ahead of a detectable difference in weight or BMI (66,128). This has particular clinical relevance because adiposity is associated with increased metabolic risk (265), potentially contributing to the rise in early onset of metabolic conditions, including Type 2 diabetes (266,267).

However, we did not find the association with FMI to be significant in the less severely hyperglycaemic GDM group. This finding should be interpreted in the context of changing

diagnostic criteria, since existing evidence is largely based on definitions of "GDM" that align more closely with our DIP group. A significant impact of GDM by the lower IADPSG criteria used in our study has only been previously shown in an untreated context by the HAPO follow-up studies, in 10-14-year-old children. In preschool-aged children of mothers with treated GDM, we could not corroborate their findings that an effect of maternal glucose levels on childhood adiposity is evident, even at levels lower than those diagnostic of GDM (111).

The majority of existing studies show an attenuating effect of maternal BMI on the association between HFDP and childhood adiposity and overweight (66,105,106,112,116,129), and a number of these, including two systematic reviews (220,268), also found no remaining significant independent effect of maternal hyperglycaemia. It has been suggested that the effect of hyperglycaemia might be more pronounced in normal weight maternal populations, based on a stratified analysis of the impact of hyperglycaemia in normal weight, overweight, and obese mothers within the Danish Birth Cohort (15,116). The consequences of high maternal BMI in cohorts such as ours might thus override consequences of HFDP, although more evidence is needed to corroborate this. The significant impact of maternal pregnancy BMI on both childhood overweight and adiposity (80,258) is likely due in part to shared genetics and lifestyle. However, fetal programming in response to intrauterine exposures, such as to the maternal metabolome (269), has also been shown to play a role (252). In particular, based on both animal (270) and human studies (271,272) programming may occur through DNA methylation, while changes to hypothalamic development and functioning have recently also suggested as an additional mechanism (273). As a modifiable risk factor with alarming prevalence in our participants and South African women of reproductive age in general (256), our findings highlight the importance of maternal obesity as a major target for (preconception) prevention for both maternal and child health (274).

In GSEM analysis, birth weight was shown to significantly impact BMI and FMI. While exposure to DIP significantly increased birth weight, exposure to GDM did not. Treatment of HFDP, including early induction of labour to reduce the risk of macrosomia (261), could have restricted birth weight and thereby minimized differences in FMI/BMI, particularly in the group exposed to GDM (275). Although changes to an array of nutrients during a pregnancy complicated by HFDP, as opposed to glucose dysregulation and subsequent fetal

hyperinsulinemia alone, are thought to be responsible for excess fetal growth, these results highlight the potential importance of good glycaemic control in HFDP pregnancies to curtail the consequences in childhood (75,276). However, this does not rule out the possibility of a programming effect manifesting in later childhood in our population, especially since fetal changes have been measured prior to treatment initiation, at 16-18 weeks' gestation, in a population similar to our GDM group (275). The lack of a statistically significant association found for GDM-exposure and birth weight in GSEM may also be a result of our limited sample size. Further research into the timing and biological mechanisms of such programming is needed in our setting.

A higher household socioeconomic score was positively associated with children's FMI in GSEM and linear regression, a relationship typically described in LMICs as opposed to the inverse association found in HICs (277). The early life risks involved in this relationship requires further exploration, especially in light of recent findings suggesting an association between GDM, parent-reported nutritional risk score at 2 years, and metabolic risk at 5 years (278). Of early childhood nutritional exposures, breastfeeding has been shown to decrease childhood adiposity, including in rural South Africa (279), but the role in hyperglycaemic pregnancies is less clear (280), and we found no association with breastfeeding and FMI/BMI in our study.

We did not find an association between HIV infection and childhood BMI/FMI. Although body composition trends of HIV-exposed, uninfected children (HEU) remain unclear, previous evidence has indicated that HEU children who are obese may have inferior cardiovascular outcomes compared to unexposed obese children, and maternal ART may play a role (93), possibly mirroring the metabolic effect of certain ART regimens on mothers (177). In our study, the majority of HIV-infected women were likely on ART during pregnancy. However, the impact of maternal HIV-exposure and -treatment on such childhood outcomes requires exploration in a larger sample of HEU children with more detailed data on maternal ART use.

A strength of this study was the use of a reference method for determining fat mass, which allowed us to explore both obesity and adiposity. Another strength is the findings' relevance to African settings, where data on the topic is scarce but increasingly important. This includes

exploring the rate of stunting, which was similar to regional averages of 11.9% in HFDP-unexposed and DIP groups, and slightly higher in the GDM group at 15.8% (127). Stunting remains a large public health problem in South Africa (281), and, as an example of the double burden of malnutrition, children exposed to HFDP do not seem to be less vulnerable to stunting at 3-6 years old.

Difficulties tracing participants 3-6 years after delivery, mostly due to relocation or change of contact details, resulted in a lower sample size and hence less power for between-group comparisons, particularly for the GDM and DIP subgroups. As a result, the study was also not powered to assess between-sex differences or the role of HFDP treatments on the outcomes. Another limitation is that, since the study design was not a prospective pregnancy cohort, the pregnancy and early childhood data was retrospective, and we were consequently unable to explore the role of early life nutrition (except breastfeeding), physical activity/sedentary behaviour, or paternal factors. Furthermore, the higher gestational age at OGTT in the exposed group opens up the possibility that a small number of participants might have been sorted into a different group if the timing of OGTT's was the same between the two groups (whether later in the HFDP-unexposed, or earlier in the exposed group). This may have minimized differences between the groups and resulting outcomes. Lastly, we did not have data for maternal pre-pregnancy BMI or gestational weight gain. By using BMI measured during pregnancy, weight gain during pregnancy may have resulted in an overestimation of maternal BMI, but there was no significant interaction between pregnancy BMI and the gestational age at measurement.

4.5 Conclusion

This study is, to our knowledge, the first in an SSA population to compare BMI and adiposity at preschool age in children exposed and unexposed to HFDP. The relevance of our findings extends to LMICs similarly experiencing an increase in HFDP and obesity. We did not find a significant difference in FMI or BMI at 3-6 years old when correcting for maternal BMI. Limiting birth weight by clinical management may have minimized differences between groups in our study, and additional longitudinal data is required to further explore associations into later childhood. Our results also indicate that changes in children's adiposity may reflect before a difference in BMI is detectable at preschool age. The significant association between maternal BMI and childhood adiposity raises concerns for an intergenerational cycle of obesity

in this urban African population and calls for increased efforts to curb maternal obesity for the promotion of maternal and child health.

4.6 Supplementary tables and figures

Table 4.5 (suppl.): indirect, direct, and total effect for GSEM best-fit model for log-transformed FMI and for BMI z-score where different from FMI model.

	Log-transformed FMI				**BMI z-score**			
Variable	**Outcome**	**Direct effect**	**Indirect effect**	**Total effect**	**Outcome**	**Direct effect**	**Indirect effect**	**Total effect**
Maternal BMI	Log-FMI	0.01 (0.004-0.01)*	0.01 (-0.001-0.01) *Via DIP; BW z-score*	0.01 (0.01-0.02)*	BMI z-score	0.04 (0.02-0.05)*	0.02 (-0.01-0.04) *Via DIP; BW z-score*	0.05 (-0.03-0.08)*
	BW z-score	0.02 (-0.004-0.04)	0.11 (0.03-0.19)* *Via DIP*	0.13 (0.05-0.20)*				
	GDM	0.12 (0.07-0.18)*	-	0.12 (0.07-0.18)*				
	DIP	0.17 (0.11-0.23)*	-	0.17 (0.11-0.23)*				
DIP	Log-FMI	-	0.02 (-0.002-0.05) *Via BW z-score*	0.02 (-0.002-0.05)	BMI z-score	-	0.09 (-0.01-0.19) *Via BW z-score*	0.09 (-0.01-0.19)
	BW z-score	0.64 (0.24-1.04)*	-	0.64 (0.24-1.04)*				
Parity	Mat. BMI	1.22 (0.18-2.25)*	-	1.22 (0.18-2.25)*				
	BW z-score	0.230 (0.07-0.39)*	0.15 (-0.01-0.32) *Via Mat. BMI; DIP*	0.39 (0.17-0.60)*				
	Log-FMI	-	0.04 (0.01-0.05)* *Via Mat. BMI, DIP, BW z-score*	0.04 (0.01-0.05)*	BMI z-score	-	0.10 (0.02-0.18)* *Via Mat. BMI, DIP, BW z-score*	0.10 (0.02-0.18)*
BW z-score	Log-FMI	0.04 (0.01-0.07)*	-	0.04 (0.01-0.07)*	BMI z-score	0.14 (0.02-0.26)*	-	0.14 (0.02-0.26)*
Household SES[3]	Log-FMI	0.02 (0.002-0.04)*	-	0.02 (0.002-0.04)*	BMI z-score	0.05 (-0.01-.12)	-	0.05 (-0.01-0.12)

Direct effects refer solely to the effect of the variable of interest (first column of table, *for example, maternal BMI*) on the outcome of interest (second column, for example, *birth weight z-score*); indirect effects refer to effects through other 'intermediate' variables in the effect pathway (in italics under the indirect effect, *for example, DIP-exposure*); the total effects are a combination of the direct and indirect effects. Results are displayed as unstandardized coefficient (95%CI). *indicates significance <0.05. [1] Mat. BMI: Maternal Pregnancy BMI; [2]BW: birth weight. [3]Household SES: household socioeconomic score.

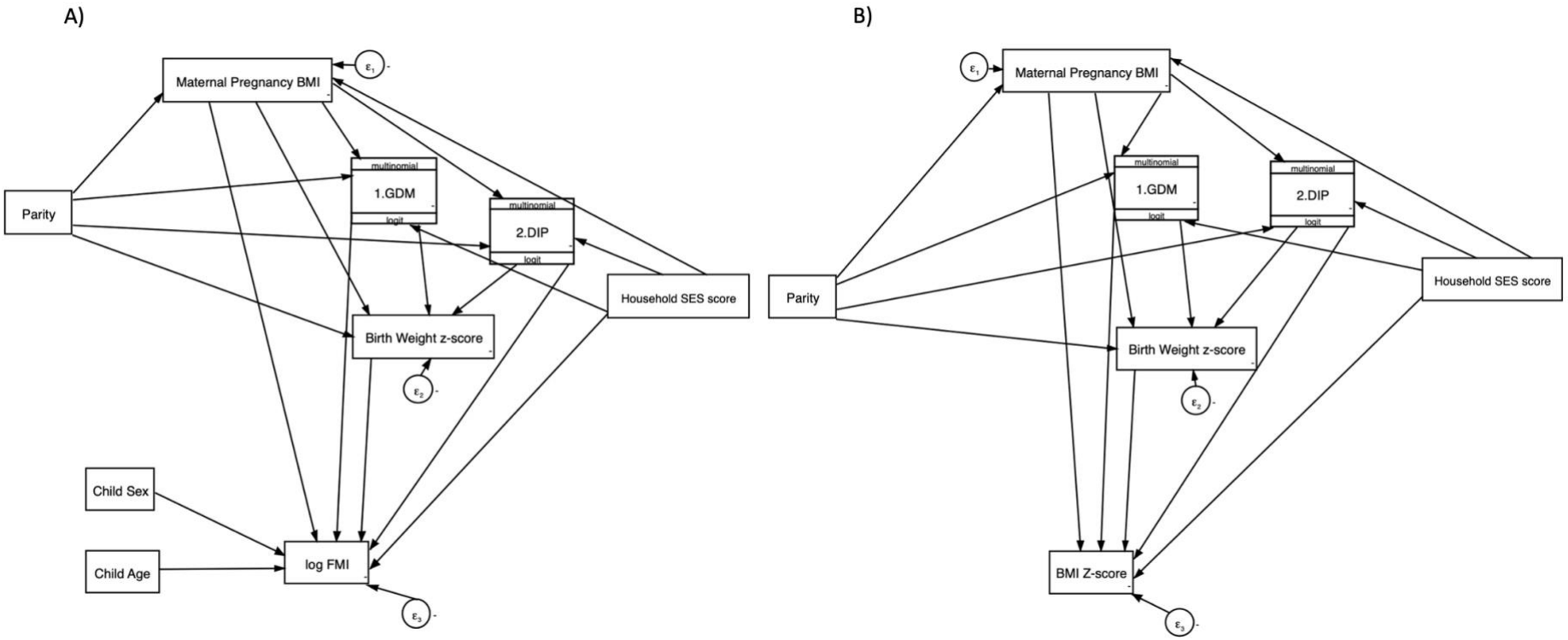


Figure 4.2 (suppl.): Hypothetical GSEM models based on existing literature, bivariate analyses, and regression modelling, for a) log-transformed FMI, and b) BMI z-score.

Chapter 5: Cognitive and motor development in 3- to 6-year-old children born to mothers with hyperglycaemia first detected in pregnancy in an urban African population.

Summary and context:

The work described in this final empirical chapter has been submitted for publication as a peer-reviewed article:

Soepnel LM, Nicolaou V, Draper CE, Levitt NS, Klipstein-Grobusch K, Norris SA. Cognitive and motor development in 3- to 6-year-old children born to mothers with hyperglycaemia first detected in pregnancy in an urban African population. *Maternal and Child Health Journal (Submitted 5 November 2020).*

Just like the previous chapter, this chapter presents results from our comparative follow-up study, assessing cognitive and motor development in preschool aged children exposed and unexposed to HFDP. The main findings included that exposure to HFDP was negatively associated with cognitive development at this age after correcting for potential confounders, suggesting the importance of both maternal (preconception) health and early childhood stimulation for early childhood development in this setting.

5.1 Introduction

Worldwide, an estimated 219 to 279 million children do not reach their developmental potential, and a large proportion of these children are in SSA (17). Cognitive setbacks at preschool age (3-6 years) can have lasting consequences, from poorer school performance to lower future incomes (144). Risk factors for poorer cognitive development often co-occur, and include childhood malnutrition, disease exposure, lack of cognitive stimulation, and other early environmental exposures, including exposures during pregnancy (145).

Due to the epidemiological and nutritional transitions, LMICs with such risk factors for poorer child development are also increasingly contending with maternal metabolic conditions, such

as hyperglycaemia first detected in pregnancy (HFDP) (28,39). The consequences of this condition are increasingly recognised to extend past the immediate pregnancy period for both mother and child (282).

Exposure to HFDP has been suggested to adversely impact childhood cognitive and motor development, through altered brain structure and/or function due to neuroinflammation, altered cell differentiation, and epigenetic modifications (283). However, existing epidemiological studies of this association in HIC have found contradictory evidence (158,284,285), and the heterogeneity of study designs and findings was confirmed in two recent systematic reviews on cognitive and psychomotor development, although both authors suggest that larger studies tended to show a more significant effect of hyperglycaemia on cognitive development (156,157). In addition, studies with younger participants showed a stronger association with cognitive development, which might imply that mediating environmental factors, such as cognitive stimulation, have the potential to alleviate early life vulnerabilities (156,167).

Despite the growing prevalence of HFDP in urban SSA, we did not identify any existing studies investigating the association with early childhood cognitive development in Africa, at any age. Lower SES has been found to compound the effect of hyperglycaemia on neurodevelopment in preschool children (163), and urban SSA is affected by additional risk factors for poor cognitive development, including childhood stunting and socioeconomic inequalities (142). Therefore, on top of being largely inconclusive, findings from HICs may also not accurately represent the issue in SSA and LMICs elsewhere.

Improved understanding of this association at preschool age, a period of rapid neurodevelopment that affects the child's later performance (286), can help inform life-course strategies for improving child development. Our main objective was therefore to assess the association between exposure to maternal HFDP and early childhood cognitive development in 3-to 6-year-old children in Soweto, South Africa. Fine and motor skill development were assessed as secondary outcomes.

5.2 Methods

5.2.1 Design and setting

We conducted a comparative study from March to November 2019 at CHBAH, a large tertiary hospital servicing the urban region of Soweto, which is populated by around 1.3 million people living in both formal and informal housing. In Soweto, an estimated 42% of households are categorised as low-income, and only around 3% as high-income (21). Around 38.3% of the adult population has attained a high school degree (21).

Participants were 3-to 6-year-old children born to mothers managed for HFDP at a specialised endocrine clinic at CHBAH between 2014 and 2016 (exposed group); or born to mothers who attended antenatal care visits at CHBAH but tested negative for hyperglycaemia in a research setting (HFDP-unexposed group) in the same time period (see Chapter 2, Figure 2.3: Overview of study design) (39). HFDP, comprised of GDM and DIP, was diagnosed using the 75-gram 2-hour OGTT with IADPSG criteria (36) (Chapter 1, Table 1.1).

Children were excluded in case of inability to trace their parent/guardian, inability to attend the data collection appointment (for example due to relocation), presence of a major congenital disorder such as Down Syndrome, ages <3 years or >6 years old, maternal diagnosis of type 1, type 2, steroid-induced, or other pancreatic diabetes during the index pregnancy, or incomplete cognitive, fine, or gross motor sections of the assessment.

Ethical approval for this study was obtained from the HREC of the University of the Witwatersrand (M180317).

5.2.2 Outcomes

The primary outcome of this study was cognitive development, measured using the Herbst Early Childhood Development Criteria test. Secondary outcomes were fine and gross motor skill development.

5.2.3 Cognitive and motor assessment

The Herbst Early Childhood Development Criteria test (169) was used to assess cognitive and motor skill development. Developed in South Africa to assess cognitive and motor tasks that

represent important aspects of school readiness in 3- to 6-year-old children from varying socioeconomic backgrounds, the Herbst test is specifically geared towards culturally unbiased evaluation of cognitive and motor skill development. The test consists of 10 cognitive subsections, one fine motor subsection (including, for example, colouring within lines, cutting out a circular shape), and one gross motor subsection (including, for example, walking on heels and tiptoes, catching and throwing a ball) (Appendix K). The complete assessment takes around 1 hour to complete. Results are evaluated against age-adjusted norms based on a South African reference population of 40-72-month-old children. In evaluating the test-retest reliability, the coefficients of stability were 0.93 for the cognitive and 0.85 for the motor section (169). An alpha coefficient between 0.81 to 0.84 was previously reported (218). The tester (LS) was trained by an experienced administrator of the Herbst test (CD) and assisted by a research assistant fluent in the home language of the participant. If the participant requested the presence of the mother in the testing room, the mother was asked to join but to refrain from participating (e.g., prompting the child). The participants received a light meal and refreshment prior to the testing.

5.2.4 Collection of data on additional variables

Information on participants' age and sex were recorded and the mother of each child completed a validated questionnaire with the help of a research assistant. This provided information on the following: preschool attendance; presence of breastfeeding; hospitalizations in the first two years of life; maternal highest level of education; socioeconomic score based on household assets; maternal smoking and alcohol during pregnancy; and maternal HIV infection.

Anthropometric measures of the child included height, measured using a Holtain mounted stadiometer (Crymych, UK), and weight, measured using an electronic SECA scale (Hamburg, Germany). Both weight and height were measured three times, recorded to the nearest 0.1 kg or cm, respectively, and the average of the three measurements was used, as described in more detail in Chapter 4.2, Methods.

For the HFDP exposed group, information on the index pregnancy, including their OGTT results, obstetric complications, birth weight, gestational age at delivery, and early neonatal complications, was obtained from hospital files from the gestational endocrine clinic. For the

HFDP-unexposed group, OGTT results collected in research setting (39) and the child's Road to Health Card, a patient-held medical record used to monitor health from birth, were used to extract the same information. Early neonatal complications included prematurity, LBW, macrosomia, respiratory distress syndrome, ICU admission, jaundice, seizures, and hypoglycaemia. The definitions used for prematurity, LBW, and macrosomia are described in Chapter 3, Figure 3.1.

5.2.5 Data management

Data was collected and managed electronically using REDCap (Vanderbilt University, Nashville, USA (188). The cognitive and motor skill scores from the Herbst test were converted into an age-adjusted z-score using the Herbst test norm data, rounding up to 40 months for children aged 36-39.9 months, to categorize the results into very low/low (≤-1SD), normal (0SD), or high/very high (≥+1SD). BMI was calculated as the average weight divided by average height, squared (kg/m^2). Height and BMI were converted into z-scores using the WHO growth standards and references, and stunting was defined as being below -2 from the standard median (131). Birth weight was converted into a z-score adjusted for gestational age using the Intergrowth-21 standards (199). A continuous household SES score was determined by summing the participant's current household assets. Maternal education level was categorised into having primary/secondary education vs further education (professional training or university).

5.2.6 Statistical analysis

Data analysis was performed using Stata 13 (College Station, USA) (219). For baseline characteristics of children and their mothers, a skewness-kurtosis test was performed to test for normality. Continuous variables were described using mean and SD or median and interquartile range, whereas categorical variables were described as a number and percentage. For testing of significant differences between groups per continuous outcome variable, a student's t-test or Mann-Whitney U test was used, depending on normality. Significance was assumed at a two-sided P-value of <0.05.

For analysis of the norm-adjusted categorical cognitive and motor outcomes, children born prematurely (before 37 weeks completed gestation) were excluded to minimize the impact of

prematurity, associated with poorer early development (287), on the relationship between HFDP and cognitive and motor performance. A sub-analysis of the premature vs at-term baseline characteristics and outcomes was performed. Due to the smaller resulting sample size, a further breakdown into GDM vs DIP subgroups was not included for subsequent analyses.

Ordinal regression analysis was performed for norm-adjusted categorical cognitive and motor outcomes. For each model, the proportional odd's assumption was tested using a likelihood ratio test, and only interpreted if this assumption was met. Age was included in the models to additionally adjust scores for age-related differences in children <40 months old. Models were built according to the conceptual pathways illustrated in Figure 5.1, as follows: M1: exposure to HFDP; M2: M1 + age, sex, preschool attendance, not being the first-born child; M3: M2 + maternal and household factors: maternal age and BMI at pregnancy OGTT, maternal HIV infection in pregnancy, alcohol use in pregnancy maternal education, and household socioeconomic score; Additional models: M3 + potential early life intermediates (individually and simultaneously): low birth weight, having any neonatal complication; breastfeeding, hospitalisation <2years old, and child stunting and BMI-age z-score.

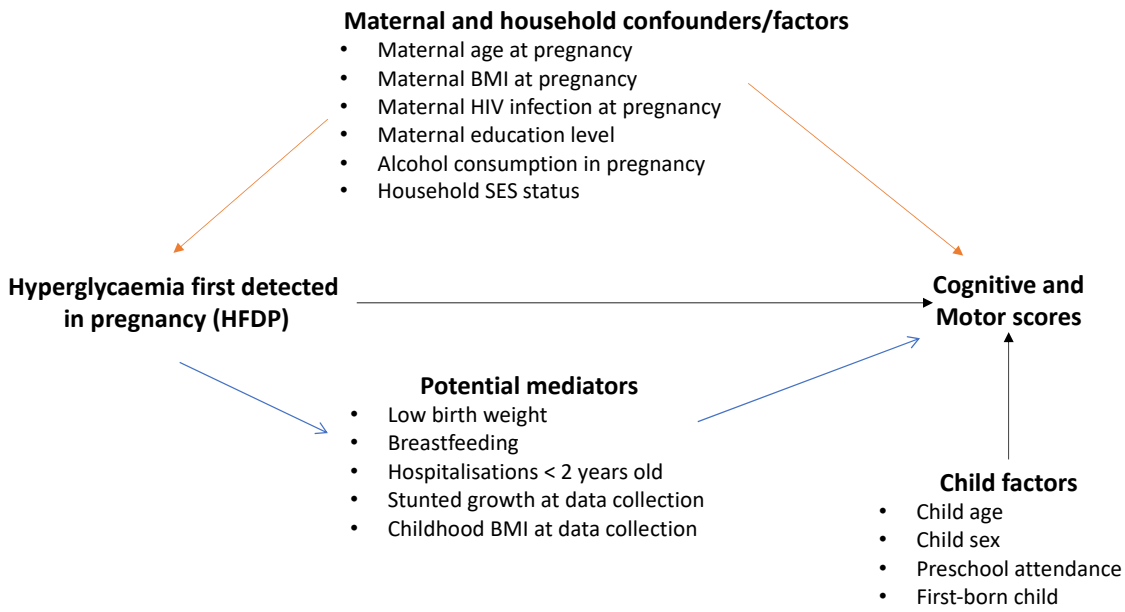


Figure 5.1: A priori conceptual pathways between variables included in ordinal regression analysis.

5.3 Results

Of 211 recruited participants, 9 did not meet the inclusion criteria, and 8 children did not complete any part of the Herbst test, resulting in a total of 194 participants. Additionally, three of these participants only completed the cognitive and fine motor subsection, while one participant only completed the motor subsections of the Herbst test. The standardized Cronbach-alpha coefficient for the raw subsection scores of the cognitive assessment was 0.90.

Participants exposed to HFDP had lower reported rates of (exclusive) breastfeeding (Table 5.1). More children in the exposed group attended preschool (82 [86.3%] vs 79 [79.8%]), although the median duration of preschool attendance was slightly lower in this group (17.6 IQR 9.2-30.1 vs 21.8 IQR 12.5-33.8). The proportion of children that were their mother's first-born was similar between the exposed and unexposed groups, at 27 (27.3%) and 25 (26.3%), respectively.

Mothers with HFDP were older, had higher BMI in pregnancy, and had lower household SES score compared to the HFDP-unexposed group, but HIV infection rates were similar between the groups. In pregnancy, 35 (18.0%) of mothers were HIV-positive, with 29 (85.3%) diagnosed prior to the index pregnancy, and 31(91%) on fixed-dose combination (FDC) treatment (Table 5.1).

Table 5.1: Baseline characteristics and cognitive and motor score outcome of participants in the HFDP-unexposed and HFDP-exposed group.

	n	HFDP-unexposed	n	HFDP-exposed
Number	-	99	-	95
Age, years (median, IQR)	99	3.5 (3.1-4.1)	95	3.5 (3.1-4.1)
Male (n, %)	99	48 (48.5)	95	49 (51.6)
Early childhood				
Received breastfeeding (n, %)	99	81 (81.8)	95	68 (71.6)
Month's breastfeeding (median, IQR)	81	15 (6-24)	65	15 (5-24)
Exclusive breastfeeding (n,%)	99	76 (76.8)	95	57 (60.0)*
Hospitalized in first 2 years (n, %)	99	19 (19.2)	95	14 (14.7)
BMI at data collection (median, IQR)	99	15.6 (14.9-17.0)	95	16.0 (15.2-16.9)
Stunted at data collection (n, %)	99	9 (9.1)	95	13 (13.7)
Attending preschool (n, %)	99	79 (79.8)	95	82 (86.3)
Obstetric factors				
OGTT (mean, SD)				
Fasting	99	4.0 (0.53)	88	6.7 (1.9)*

2-hour		5.4 (1.2)		10.5 (3.6)*
Maternal age at OGTT (mean, SD)	99	30.0 (5.8)	95	32.3 (5.5)*
Maternal BMI (median, IQR)	99	29.6 (24.8-33.0)	88	35.4 (30.8-40.2)*
HIV infection in pregnancy (n,%)	99	19 (19.2)	95	16 (16.8)
Caesarean section (n, %)	99	60 (60.6)	92	65 (70.7)
Birth weight, g (mean, SD)	99	3027 (640)	92	3032 (640)
Macrosomia (n, %)	99	5 (5.1)	92	4 (4.4)
LBW (n, %)	99	16 (16.2)	92	16 (17.4)
Prematurity (n, %)	99	13 (13.1)	95	21 (22.1)
Maternal and household characteristics				
Highest education (n, %)	98		53	
1 none/primary school		0 (0)		3 (3.2)
3 secondary school		70 (71.4)		61 (64.2)
4 professional training/university		28 (28.6)		31 (32.6)
Household SES score (mean, SD)	99	8.6 (2.1)		8.0 (2.2)*
Mother is primary caregiver (n, %)	99	93 (93.9)	95	90 (94.7)
Cognitive and motor score outcome				
Cognitive score (median, IQR)	98	24.5 (19-34)	95	22 (18-32)
Percentile cognitive subtests:				
Incomplete man		69 (50-82)		69 (50-82)
Visual-motor integration		75 (56-99)		75 (56-92)
Block building		47 (19-81)		47 (19-78)
Stick figures		85.5 (56-94)		78 (56-88)
Direction and similarities		88 (62-94)		76 (50-88)*
Form concept		58 (56-81)		58 (56-81)
Colour concept		94 (89-100)		94 (84-99)
Counting concepts		53 (25-82)		44 (25-82)
Picture puzzle		78 (56-100)		72 (56-100)
Picture perception		88 (87-88)		88 (71-88)
Fine motor score (median, IQR)	99	7 (6-8)	95	6 (5-7)*
Gross motor score (median, IQR)	97	10 (9-13)	94	10 (9-12)

Values are given as mean (standard deviation), median (interquartile range), or number (percentage). HFDP: hyperglycaemia first detected in pregnancy; BMI: body mass index; OGTT: oral glucose tolerance test; LBW: low birth weight; SES: socioeconomic score. *p-value <0.05

Children born prematurely, who were excluded for analysis of categorical z-score outcomes, did not have any significant differences in baseline characteristics compared to at-term participants, except having a higher rate of low birth weight. Participants born prematurely and at-term did not have significantly different median cognitive (23, IQR:17-33 vs. 22.5, IQR:19-32, p=0.979), fine (6, IQR:5-8 for both, p=0.731), and gross motor scores (10, IQR:9-12 for both, p=0.761).

5.3.1 Cognitive and motor assessment

Overall raw cognitive scores and most subtest percentiles were higher in the HFDP-unexposed group than in the group exposed to HFDP, but this difference was only statistically significant for one cognitive subtest ("Directions and similarities") (Table 5.1). The raw fine motor score, but not gross motor score, was significantly higher in the group of HFDP-unexposed participants.

Figure 5.2 illustrates that the proportion of participants born at-term that scored 'low' according to age-adjusted z-scores was higher in the group exposed to HFDP for cognitive, fine motor, and gross motor skills, and a higher percentage of HFDP-unexposed children scored 'high'. The difference between groups was larger for cognitive and fine motor than for gross motor scores.

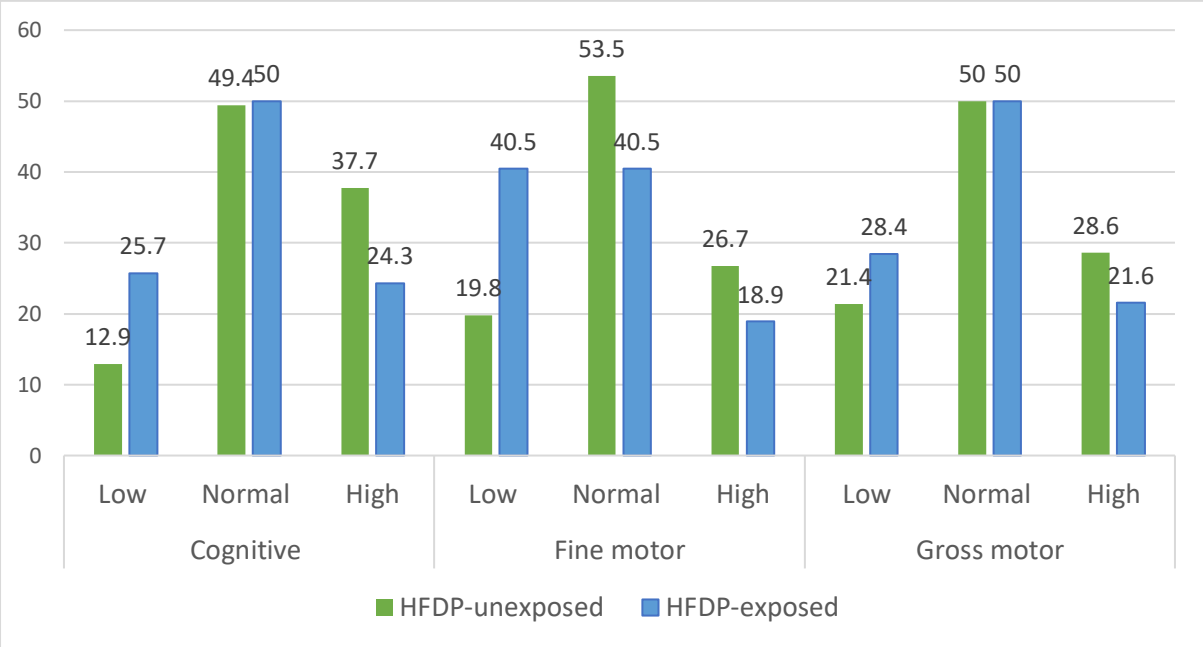


Figure 5.2: Norm-based categorical results for at-term participants by exposure group for cognitive (n=159), fine motor (n=160) and gross motor score (n=158) by percent (%) scoring low, normal, or high.

In ordinal regression, results indicate that participants exposed to HFDP had a significantly lower odds of scoring in a higher cognitive category even when adjusting for age, sex, preschool-attendance, and being first-born (OR 0.37, 95%CI 0.19-0.71, p=0.003) (Table 5.2), and when adjusting for potential maternal confounders. Maternal HIV in pregnancy (OR 0.39,

95%CI 0.16-0.98, p=0.045) and household SES score (OR 1.18, 95%CI 1.01-1.38, p=0.034) were significant in this model. Since there were only 2 cases of reported maternal alcohol use in pregnancy, this variable was not included in analysis. When adding potential mediators to the model, none were significant associated with cognitive skills, and they did not notably attenuate the relationship between HFDP and cognitive score, even when all included in the model simultaneously (OR HFDP=0.36, 95%CI 0.16-0.82, p=0.014) (Table 5.3 (supplementary)).

Ordinal regression for fine motor results showed significantly lower odds of scoring in a higher z-score category in children exposed to HFDP, but this association was not significant when correcting for maternal factors in Model 3 (OR 0.62, 95%CI 0.28-1.37, p=0.239; Table 5.2). Of these maternal factors, household SES was significantly associated with the outcome (OR 1.33, 95%CI 1.10-1.22, p=0.001). In the model including all potential mediators, two significantly impacted the fine motor category: low birth weight (OR 0.19, 95%CI 0.05-0.73, p=0.015) and childhood BMI (OR 0.66, 95%CI 0.45-0.97, p=0.033). However, these did not largely change the effect size or significance level of HFDP-exposure (Table 5.4 (supplementary)). No significant association was found for gross motor score category and HFDP in ordinal regression analysis (Table 5.5 (supplementary)).

Table 5.2: Ordinal logistic regression for A) cognitive and B) fine motor score category (low, normal, or high) in participants born at-term.

A) Cognitive score	**M1: crude model**		**M2: M1+ age, sex, preschool, first-born**		**M3: M2 + maternal factors**	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
HFDP	0.49 (0.27-0.90)	0.021*	0.37 (0.19-0.71)	0.003*	0.33 (0.15-0.74)	0.007*
Child age			1.11 (1.06-1.16)	<0.001*	1.11 (1.05-1.16)	<0.001*
Child sex			1.28 (0.68-2.40)	0.444	1.16 (0.60-2.25)	0.669
Preschool attendance			5.23 (2.08-13.18)	<0.001*	6.53 (2.31-18.42)	<0.001*
First-born child			0.55 (0.28-1.10)	0.091	0.49 (0.23-1.04)	0.062
Maternal and household factors						
Maternal age at pregnancy					1.05 (0.99-1.12)	0.130
Maternal BMI at pregnancy					1.01 (0.96-1.06)	0.785
Maternal HIV in pregnancy					0.39 (0.16-0.98)	0.045*
Maternal education level					0.79 (0.38-1.67)	0.541
Household socioeconomic score					1.18 (1.01-1.38)	0.034*
	N=159; R^2=0.02		N=159; R^2=0.15		N=152; R^2=0.18	
B) Fine motor score	**M1: crude model**		**M2: M1+ age, sex, preschool, first-born**		**M3: M2 + maternal factors**	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
HFDP	0.46 (0.25-0.84)	0.011*	0.44 (0.23-0.85)	0.015*	0.62 (0.28-1.37)	0.239
Child age			1.14 (1.09-1.20)	<0.001*	1.16 (1.10-1.22)	<0.001*
Child sex			1.36 (0.73-2.52)	0.334	1.37 (0.70-2.67)	0.359
Preschool attendance			1.89 (0.79-4.52)	0.154	1.73 (0.65-5.63)	0.276
First-born child			0.88 (0.45-1.73)	0.709	0.85 (0.41-1.79)	0.674
Maternal and household factors						
Maternal age at pregnancy					1.02 (0.96-1.08)	0.592
Maternal BMI at pregnancy					0.96 (0.91-1.01)	0.146
Maternal HIV in pregnancy					0.95 (0.39-2.32)	0.910
Maternal education level					0.67 (0.32-1.40)	0.289
Household socioeconomic score					1.33 (1.13-1.57)	0.001*
	N=160, R^2=0.02		N=160, R^2=0.15		N=153, R^2=0.20	

Values given are Odd's Ratio (95% confidence interval) and p-value; BMI: body mass index; R^2= pseudo-R^2. *p-value <0.05.

5.4 Discussion

In this study, preschool aged children exposed to HFDP and born at-term had lower scores for cognitive and fine motor skills. The difference in cognitive skills was independent of maternal and pregnancy factors such as household SES. No differences were found in gross motor skills between the two groups. To our knowledge, this is the first study to explore this relationship in Africa.

Our findings of poorer cognitive performance corroborate findings from a number of HIC studies that early neurodevelopment may be directly impacted by intrauterine exposure to maternal hyperglycaemia (156,157,285), rather than through confounding or intermediate factors. Potential mechanisms, while far from fully understood, include the deleterious effects of increased inflammation, oxidative stress, and altered brain insulin-signalling on brain function and structure, as well as hyperglycaemia-induced epigenetic changes (288). Additionally, maternal and/or neonatal complications of HFDP (such as ketoacidosis or hypoglycaemic events) may adversely impact fetal brain development (156). HFDP-specific risks, including the level of hyperglycaemic control attained, seem to impact child neurodevelopment more than, for example, type of diabetes (pre-gestational vs detected in pregnancy) (157,284), which needs to be further explored in our setting. Interestingly, HFDP may also have neurobehavioral consequences on offspring, such as attention-deficit disorders (163), with one study finding an impact on inattention levels, but not cognition (153). In the present study, underlying neurobehavioral symptoms may have impacted cognitive performance, requiring further clarification.

Our results highlight the impact of early childhood environmental exposures on cognitive and fine motor aspects of school readiness, including stimulation in the form of preschool attendance (169) and household SES (163,218). The impact of lower SES has been attributed to decreased stimulation and exposure to task-related tools (such as scissors and pencils), and associated factors including increased psychosocial stress or compromised nutrition and health care (289). The importance of environmental stimulation is further emphasized by the theory that poorer development in children exposed to HFDP may not persist into later childhood in the case of adequate stimulation, as has been suggested based on evidence from later childhood and early adulthood in HICs (156,157). Though reassuring, it is unclear whether this same

"catch-up" of cognitive skills would also occur spontaneously in a setting such as ours, in which children are impacted by socioeconomic and educational inequalities. The evidence on early learning skills in South Africa suggests that children who start behind, stay behind (290).

In contrast to existing research from HICs (291), we did not find evidence for an impact of HFDP on gross motor development at preschool age. Previous research in South African preschool aged children has shown a high level of (unstructured) physical activity (292). This likely contributes to good motor skill development, even in socioeconomically disadvantaged children (218,293), which may have moderated any differences between HFDP-unexposed and exposed groups in the present study. Alternatively, a more refined tool to measure gross motor skill than the succinct subsection of the Herbst test may have been necessary to detect differences in motor performance between the two groups.

Of our participants, 18% were HIV-exposed uninfected children, and this exposure was significantly associated with poorer cognitive scores. While some studies have similarly found an impact of HIV and ARV exposure on childhood development (170), others have found no evidence for an adverse effect (172), and differences may be due to other developmental risks such as increased poverty and high caregiver burden in this population, instead. Our findings could similarly be due to residual socioeconomic confounding, or reduced breastfeeding behaviours in HIV positive mothers (171), since breastfeeding, while not significantly associated with cognitive performance, did attenuate the association of HIV with cognitive score (attenuated OR 0.44, 95%CI 0.17-1.19, p=0.107) (Table 5.3 (supplementary)). This requires further exploration in a cohort with a greater number of HIV-exposed uninfected children.

A strength of this study was using the Herbst test, which was developed to be culturally neutral and uses a South African reference population. Our ability to adjust for a variety of known confounders in regression analysis was another strength.

However, one limitation is the retrospective nature of the pregnancy and infancy data, for which we relied on hospital records and maternal recall. For example, neonatal hypoglycaemia may have been under-recorded in the medical records and therefore inadequately adjusted for

as a potential mediator. Furthermore, we were not able to include data on paternal education level, preventing us from adjusting fully for genetic and family environmental factors, possibly contributing to the lower pseudo-R^2 in our final regression models. Additionally, by excluding children who did not or were unable to perform or complete the assessment, we may have introduced selection bias in favour of higher-scoring children, but, since 6 of the 8 excluded children were in the exposed group and their baseline characteristics did not differ significantly from those included, this likely would not have altered the direction of our findings. Another limitation is the fact that the age of participants <40 months was rounded up for the Herbst test norms, possibly affecting the reliability of their categorization into 'low', 'normal', or 'high' scores for these younger children. However, ages were similar between the HFDP-unexposed and exposed groups, and we additionally adjusted for age in the ordinal regression models. Lastly, our sample size was limited by the ability to trace participants 3-6 years after the index pregnancy, which may have resulted in larger confidence intervals for factors included in regression analysis.

5.5 Conclusion

In conclusion, our results suggest that at 3-6 years old, children born to mothers with HFDP score lower on cognitive development than those unexposed to maternal HFDP. Differences in fine motor score were largely attributable to maternal confounders and household SES. As the prevalence of HFDP increases in urban SSA, the implications of these findings stand to impact a growing number of children. Understanding the consequences for early child development and subsequently optimising maternal (preconception) health and early childhood stimulation could help more children reach their developmental potential.

5.6 Supplementary tables

Table 5.3 (suppl): adjusted odds of HFDP with categorical cognitive score for Model 3 plus potential mediators.

	Odds ratio, HFDP	Standard error	P-value	Lower 95% CI	Upper 95% CI
Model A) M3+ childhood BMI	0.34	0.14	0.007*	0.15	0.75
Model B) M3 + stunting	0.36	0.15	0.011*	0.16	0.79
Model C) M3 + child hospitalization <2 years	0.33	0.13	0.006*	0.15	0.73
Model D) M3 + breastfeeding	0.34	0.14	0.008*	0.15	0.76
Model E) M3 + LBW	0.33	0.14	0.007*	0.15	0.74
Model F) M3 + any neonatal complication	0.34	0.14	0.01*	0.15	0.76
Model G) M3 + mediators A-E.	0.36	0.15	0.014*	0.16	0.82

M3: Model 3, adjusting for child factors and maternal and household factors; HFDP: hyperglycaemia first detected in pregnancy; BMI: body mass index; LBW: low birth weight. * p-value <0.05.

Table 5.4 (suppl): adjusted odds of HFDP with categorical fine motor score for Model 3 plus potential mediators.

	Odds ratio, HFDP	Standard error	P-value	Lower 95% CI	Upper 95% CI
Model A) M3+ childhood BMI	0.63	0.25	0.252	0.29	1.39
Model B) M3 + stunting	0.61	0.25	0.223	0.27	1.36
Model C) M3 + child hospitalization <2 years	0.61	0.25	0.225	0.27	1.36
Model D) M3 + breastfeeding	0.64	0.26	0.269	0.29	1.42
Model E) M3 + low birth weight	0.61	0.25	0.235	0.27	1.38
Model F) M3 + any neonatal complication	0.61	0.26	0.24	0.27	1.39
Model G) M3 + mediators A-E.	0.61	0.26	0.243	0.27	1.40

M3: Model 3, adjusting for child factors and maternal and household factors; HFDP: hyperglycaemia first detected in pregnancy; BMI: body mass index; LBW: low birth weight

Table 5.5 (suppl): ordinal logistic regression models of categorical gross motor score (low, normal, or high).

A)	**Odds ratio**	**Standard error**	**P-value**	**Lower 95% CI**	**Upper 95% CI**
HFDP	0.69	0.21	0.218	0.38	1.25
B)	**Odds ratio**	**Standard error**	**P-value**	**Lower 95% CI**	**Upper 95% CI**
HFDP	0.62	0.20	0.13	0.33	1.15
Child age	1.04	0.02	0.031*	1.00	1.09
Female sex	0.95	0.29	0.871	0.52	1.73
Preschool	2.09	0.94	0.102	0.86	5.07
First born child	1.24	0.42	0.529	0.63	2.42

A) Model 1: exposure to HFDP; B) Model 2: Model 1 + child age, sex, preschool attendance, being the first-born child. HFDP: hyperglycaemia first detected in pregnancy. *p-value <0.05.

Part 3: Discussion

Chapter 6: Integrated discussion

In this chapter, findings from the empirical studies will be summarized according to the key objectives of this thesis. One methodological theme, defining research outcomes in children, and one empirical theme, maternal preconception care, emerging from these findings will be discussed in greater detail. This is followed by a re-examination of the conceptual framework underpinning this thesis, which was first presented in Chapter 1. Lastly, the implications of the findings, key limitations, and opportunities for future research will be considered.

6.1 Summary of empirical findings

This thesis aimed to, firstly, describe the characteristics and outcome of diabetic pregnancies in South Africa, and secondly to evaluate development outcomes in children exposed to pregnancies complicated by HFDP. This was achieved through the retrospective analysis of the maternal and neonatal outcomes in a cohort of women with pre-gestational diabetes and HFDP, and a subsequent study investigating the association of HFDP with child development and body composition in a subgroup of children at 3-6 years after the index pregnancy. Table 6.1 presents the main findings according to key objectives for each empirical paper.

Table 6.1: Summary of the research objectives and findings for each empirical study presented in this thesis.

Objective	Chapter	Findings
Objective 1: To retrospectively investigate pregnancy characteristics and prevalence of maternal and neonatal outcomes of pregnancies complicated by pre-gestational diabetes or HFDP in Soweto, South Africa **Hypothesis**: Pregnancies with pre-gestational diabetes and HFDP are complicated by adverse maternal and neonatal outcomes, particularly those with more severe hyperglycaemia or comorbidities.	3	• **The hypothesis was accepted** • Rates of obstetric outcomes including macrosomia, PNM and prematurity were higher compared to rates in South African non-diabetic pregnancies. • Pregnancy characteristics associated with obstetric outcomes included type of hyperglycaemia (with DIP and type 1 diabetes showing worse outcomes), severity of hyperglycaemia (HbA1c), hyperglycaemic control in the third trimester, and maternal BMI. • Maternal BMI was high in all diabetic groups, including type 1 diabetes.
Objective 2: To retrospectively assess the association between maternal HIV-infection in pregnancies complicated by pre-gestational diabetes/ HFDP and obstetric outcomes. **Hypothesis:** HIV infection in pregnancy is associated with worse obstetric outcomes in diabetic pregnancies.	3	• **The hypothesis was accepted** • HIV exposure, present in 24% of the cohort, was associated with higher PNM. • Other obstetric outcomes were similar or, in the case of macrosomia, lower in the HIV-exposed group • Participants with HIV had lower HbA1c and mean blood glucose at initial presentation during pregnancy
Objective 3: To assess the association between exposure to maternal HFDP and offspring overweight and adiposity in children aged 3-6 years old, independently of maternal pregnancy BMI. **Hypothesis:** Children born to mothers with HFDP have higher overweight and adiposity compared to a group of HFDP-unexposed children, independently of maternal pregnancy BMI.	4	• **Our hypothesis was rejected** • Exposure to HFDP was not significantly associated with childhood BMI or fat mass at 3-6 years old, when correcting for maternal pregnancy BMI. • Maternal pregnancy BMI was identified as a s modifiable preconception risk factor significantly associated with childhood adiposity outcomes. • Around 27% of all children were ‘at risk of becoming overweight’, ‘overweight’, or ‘obese.’

		• Childhood stunting was highest in the GDM group at 15.8%. • Birth weight, which obstetric management may have limited in our group, had a significant direct effect on childhood BMI and FMI at preschool age, and DIP-exposure significantly impacted birth weight
Objective 4: To assess the direct and indirect effects of maternal pregnancy BMI on childhood obesity and adiposity in the context of HFDP. **Hypothesis:** Maternal pregnancy BMI has a significant direct effect on childhood obesity (BMI) and adiposity (FMI).	4	• **The hypothesis was accepted** • Maternal pregnancy BMI had a significant direct effect on childhood BMI and FMI. • Although the indirect effect of maternal pregnancy BMI through birth weight and HFDP-exposure was not significant for childhood BMI/FMI, the total effect including these pathways was.
Objective 5: To assess the association between exposure to HFDP and offspring cognitive skill development at age 3-6 years old. **Hypothesis:** Children born to mothers with HFDP have poorer cognitive skills at 3-6 years, compared to a group of HFDP-unexposed children.	5	• **The hypothesis was accepted** • HFDP was negatively associated with cognitive development skills at 3-6 years old after adjustment for confounders. • Household preschool attendance, socioeconomic score, child age, and exposure to maternal HIV in pregnancy were additional significant predictors of cognitive development.
Objective 6: To assess the association between exposure to HFDP and offspring motor skill development at age 3-6 years old. **Hypothesis:** Children born to mothers with HFDP have poorer fine and gross motor skills at 3-6 years, compared to a group of HFDP-unexposed children.	5	• **The hypothesis was rejected** • The difference in fine motor skill between the groups seemed to be attributable to maternal and household factors such as SES • No difference was found for gross motor development

6.2 Emerging research themes

In this section, two emerging themes from the consolidated findings will be explored in more detail: 1) defining research outcome measures in children and 2) maternal preconception care related to child health.

6.2.1 Methodological theme*:* Defining research outcomes in children

Assessing variations in health and development within a cohort of 'healthy' children, in the absence of defining disease characteristics or markers, requires multidisciplinary decisions to determine which outcomes are of interest and how to appropriately measure these outcomes. In the scope of this thesis, two areas of health and development linked to hyperglycaemia in pregnancy were the focus, namely body composition and cognitive and motor development. However, the careful conceptualisation, findings, and challenges of this thesis gave rise to broader questions on how best to choose and measure relevant aspects of childhood health, particularly in an LMIC setting.

The concept of childhood health has evolved from a bygone definition focused solely on the lack of disease. Aside from the ability to function optimally, another recognised aspect of childhood health is adaptability vs susceptibility to future adverse circumstances (294). Measuring this is complex, as exemplified by the National Child Study's attempt to get a full picture of childhood well-being in the United States, identifying the broad categories of "performance, potential, experience, and adaptability" (295).

In considering this wide range of outcomes relevant to a child's health, a study's setting, including cultural or socioeconomic context, impacts the relevance of particular outcomes, and how best to measure these. Moreover, since children grow and change, a study's chosen age range may determine outcome measurement. Successfully navigating these complexities leads to more meaningful research output. Therefore, nine criteria for selecting relevant, suitable, and ethical outcome measures in LMIC child health research settings are suggested here, as shown in Table 6.2, with accompanying examples and/or challenges faced in this thesis.

Table 6.2: Suggested criteria for selecting outcomes measures in LMIC child health research

Consideration	The outcome measure should:	Examples and challenges from the thesis
I. What is measured? (relevant)	i. Measure an outcome relevant for children's future health or development	Cognitive development and childhood overweight/obesity are linked to adult health and potential reached. These outcomes are therefore undeniably relevant to the child's future. However, it would be beneficial to be able to quantify adult clinical risks associated with degrees of variation in body composition (BMI/FMI).
	ii. Measure an outcome that is relevant for population health in the (LMIC) setting	The rising prevalence of childhood obesity in South Africa calls for a better understanding of the aetiology in this context. Moreover, in this setting, findings around overnutrition are more fully interpreted when also measuring childhood stunting levels, since this remains an extremely relevant health concern. Similarly, an estimated 38% of children under 5 years old are at risk of poor development, and a large proportion of these are found in sub-Saharan Africa.
	iii. Measure an outcome hypothesized to be related to the exposure(s) of interest, where applicable.	If there is a specific exposure of interest, such as hyperglycaemia in pregnancy in this study, a logical link between the exposure and outcome should be established.
II. How is it measured? (suitable)	iv. Be age appropriate	The deuterium method was chosen over other body composition methods because of greater age-appropriateness, leading to more accurate measure of fat mass. Furthermore, the test for cognitive development was made to be fun, colourful, and short enough to remain appealing to young children.
	v. Be culturally appropriate	For cognitive and motor skill testing, cultural and language differences can impact outcomes, for example, by using pictures of instructions of unfamiliar objects in the testing criteria. The Herbst test was designed to be culturally unbiased. Additional study methods were also adjusted to accommodate culture or norms, such as some children being accustomed to drinking juice rather than water during the deuterium method.
	vi. Have local or global standards or reference population for standardization, where possible	The Herbst reference standards were based on South African children. Additionally, the WHO growth standards and references for BMI and other anthropometrics are global standards. However, one challenge was the lack of available global or South Africa fat mass (index) standards in children

		of preschool age. This would have allowed for a more reliable comparison across sex and age and could also help provide a cut-off for a normal or acceptable fat mass for our setting.
	vii. Be accessible and feasible in the setting	The deuterium dilution method used in this thesis has been adapted to be suitable for most field work settings, to optimise accessibility across settings, but access to the equipment necessary for analysis is more costly. If, unlike in our setting, this is not feasible, conducting simple anthropometrics may be the best available option for determining body composition estimates in resource-limited settings. For this reason, it is important to explore more fully under which circumstances (including participant age, medical comorbidities, and presence of stunting) anthropometric measures such as BMI z-score can be reliably interpreted as a surrogate measure for adiposity.
III. Is it in child's best interest? (ethical)	viii. Avoid stigmatizing the child, while signalling important health risks	We referred children to a health care professional in the case of overweight/ obesity, or any other significant clinical concerns. However, we acknowledge the importance of not stigmatizing children with a 'diagnosis' or medical visits at such a young age unless necessary. In line with this, the WHO cut-offs for obesity/overweight in children under five are less stringent in order to avoid rigorous diets and the psychological effects of a diagnosis, unless supported by robust evidence of adverse health risks.
	ix. Be minimally invasive and conducted in a safe space with trusted people	Invasive outcome measures were avoided, but it was occasionally still challenging to overcome the child's fear of the "doctor" (researcher). We intended to build trust with the child over the visit, for example by building play into the various data collection activities. Furthermore, we aimed to keep the mother and child together for the majority of the data collection appointment.

6.2.2 Empirical theme: maternal preconception care

Although the data evaluated for this thesis was centred on pregnancy, early childhood, and preschool age, findings from all three empirical papers suggest that maternal preconception health, including nutritional status, is key for childhood health and development, and that this has yet to be adequately leveraged by healthcare professionals and policy makers to improve childhood outcomes in South Africa and similar settings.

The impact of maternal preconception overnutrition on childhood health and development is not only due to subsequent increased risk-exposure over the course of the pregnancy, for example in the form of hyperglycaemia in pregnancy adversely impacting neurodevelopment, but also due to consequences of developments around conception. These developments (the periconception period, from the maturation of oocytes through embryo implantation) represent a 'critical period' highly susceptible to environmental exposures (10,296). Mechanisms involved include increased inflammation, disturbed mitochondrial function, endoplasmic reticulum stress, and epigenetics (10). Evidence from animal models as well as epidemiological studies have shown how this kind of programming impacts susceptibility to a myriad of childhood outcomes, including obesity and cardiovascular risk, which may track into adulthood (254,297).

In the South African setting, the importance of the preconception period is reinforced by the presence of a double burden of malnutrition. On individual and population level, maternal conditions associated with overnutrition coexist with enduring trends of undernutrition in urban SSA (30), and more specifically South Africa (298). In the current study, 12% of participants were stunted at 3-6 years old. This has been associated with adverse cognitive development (139,141), and increased risk for obesity and cardiometabolic risk in later life if combined with subsequent weight gain (250). During the periconception period, maternal undernutrition, including micronutrient deficiencies, impacts epigenetic and metabolic programming, influencing fetal development and growth (10). Figure 6.1 shows a complex conceptualization of the interconnected intergenerational cycles of overnutrition and undernutrition (250), and while I will not discuss the intricacies of this figure in great detail, it effectively illustrates how maternal obesity/hyperglycaemia is part of a larger biological puzzle linking maternal nutrition,

accumulated exposures, and the health of the next generation. This highlights the importance of interventions that can address both over- and undernutrition in urban SSA, relevant even in a cohort largely characterized by maternal obesity and hyperglycaemia in pregnancy.

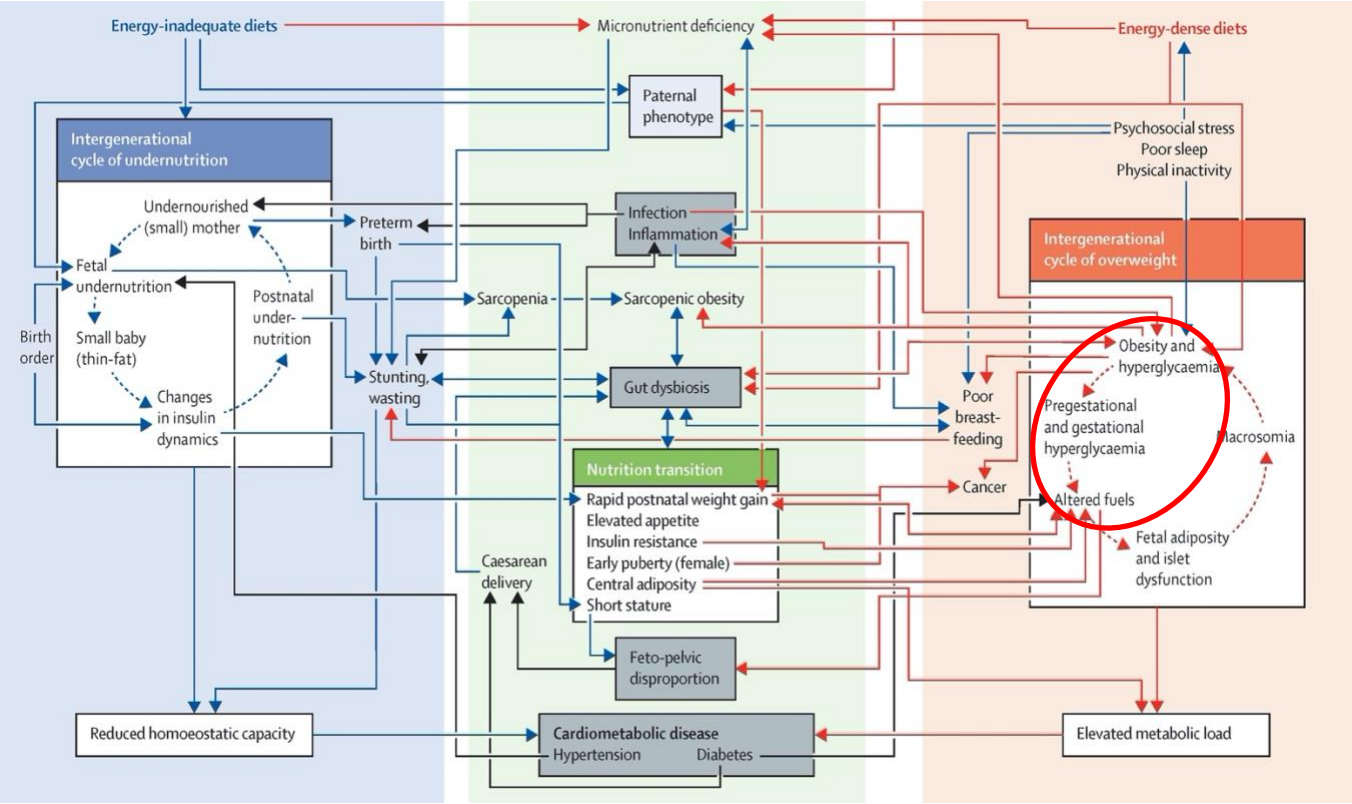


Figure 6.1: Conceptual framework of the interconnecting pathways between the intergenerational cycles of undernutrition and overweight, and associated health outcomes, from the 2020 Lancet Series on the Double Burden of Malnutrition (250).

While the biological phase of conception is one way to define the preconception period, the 2018 Lancet series on preconception health proposes two additional definitions in terms of levels of intervention (299). Firstly, on an individual level, for people planning to get pregnant, and secondly, on a population level, encompassing relevant public health goals over the life course. These represent separate but not mutually exclusive approaches for strengthening South Africa's child health through preconception care (Figure 6.2).

One important benefit of individual preconception care delivery to those aiming to get pregnant is the increased motivation of potential parents to optimise pregnancy outcomes and the health of future children (300), but dedicated preconception care is currently not standard in South

Africa (301). Although not much is known about the feasibility or cost-effectiveness of such care in an LMIC setting, the international knowledge base is fast expanding (300,302). Recently, FIGO published international recommendations for preconception prevention of NCDs by clinicians, including BMI screening, assessment of nutritional deficiencies, adequate management of pre-existing diabetes, and education and counselling around physical activity and nutrition (303). This kind of screening, management, and referral could be delivered at clinics dedicated to preconception care or in primary and reproductive healthcare settings (304).

This model of care is, however, contingent upon adequate pregnancy planning prior to conception, and the rate of unplanned pregnancies is estimated to be between 33-64% in South Africa (305,306), clearly limiting this method's reach. Therefore, population-based interventions are important to optimise health (behaviours) in childhood, adolescence, and adulthood. Within the clinical domain, this could involve an emphasis on healthy nutrition at children's routine health check-ups, and integrating risk identification, screening for pregnancy intent, and FIGO's management recommendations into women's regular healthcare visits (303). These approaches require training of healthcare providers, time allotment, patient awareness, and resource accessibility across medical disciplines, barriers which remain relevant globally (304,307). In South Africa, and SSA generally, one important delivery avenue would be HIV care services, since the majority of HIV positive patients are of reproductive age (308,309). However, contact with healthcare for 'healthy' women of reproductive age in resource-strapped settings is likely to be even lower than rates reported in high income countries such as the UK and USA (304,310).

Additional public health measures outside of the traditional clinical domain will therefore be required to tackle over- and undernutrition regardless of age or intent, and community healthcare workers may be a promising way to deliver community care (311), if adequately supported. Possible strategies include improved education and public health messaging on healthy lifestyles and the importance of the preconception period, and systems level policy efforts to prevent obesity and undernutrition, including regulating the marketing and labelling of food, particularly aimed at children, and fiscal measures on unhealthy foods (312). Moreover, community risk screening for conditions such as hypertension and diabetes could

increase early identification and control. For example, the high proportion of undiagnosed pre-existing diabetes (the DIP-group) identified in the current study supports the need for increased opportunity and implementation of diabetes screening. However, ensuring continuity of care by establishing a strong infrastructure between community healthcare and primary care facilities is necessary for community screening efforts to be effective (313).

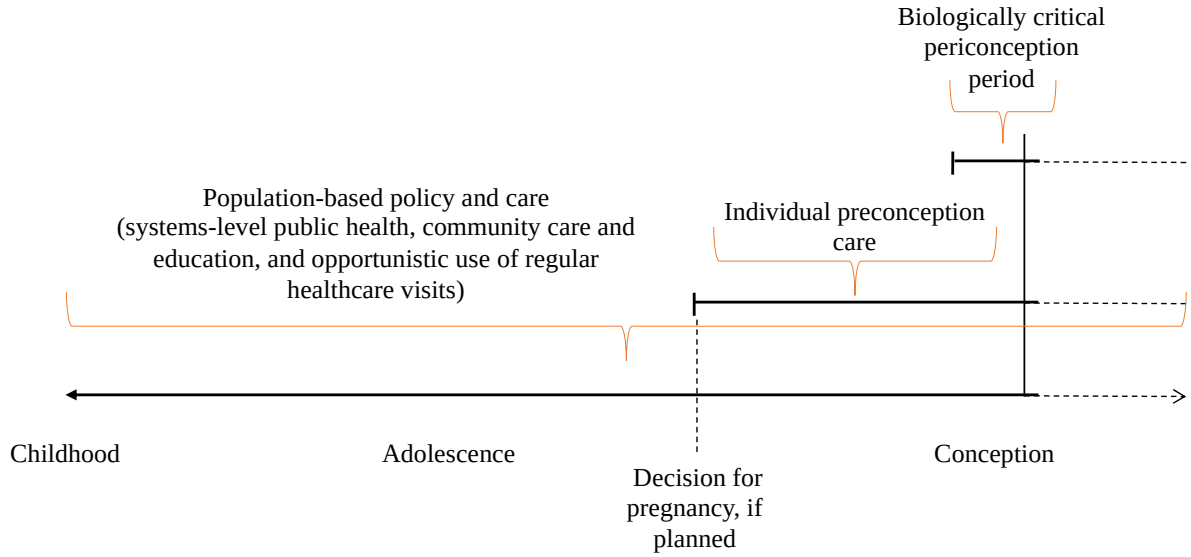


Figure 6.2: Framework for interpretation of Lancet Preconception Health Series of definitions of the preconception period, with opportunities for intervention at population and individual levels, adapted from Stephenson et al, 2018 (299).

Studies with cost-effectiveness analyses of preconception care are mostly from high income countries (314) and focused on short-term outcomes of maternal and neonatal morbidity/mortality, and on women with known pre-gestational diabetes (315,316). Nevertheless, these results indicate that the costs saved by prevention of, for example, hospital admissions, outweigh the cost of various kinds of preconception care (314,317). This includes LMIC settings, where there is more to gain both for short- and long-term healthcare.

Aside from challenges around the setting and implementation, there are also important ethical considerations associated with preconception care. It is important to emphasise that women's health is important in its own right irrespective of reproductive intent or potential. Moreover, focusing on the lifestyle, behaviour, and bodies of women of reproductive age for the health of their children can place undue blame and responsibility on individual women (318). At its

extreme, this can be detrimental to maternal mental health, limit freedoms by vilifying particular behaviours, and reduce reproductive autonomy (319). To prevent these unintended reverberations of maternal preconception care for child health, it is important to frame interventions in the context of structural change, community opportunities, and health system improvement. Furthermore, while this thesis' question focused on maternal hyperglycaemia, paternal preconception health should not be neglected as an important factor for childhood health (320).

Knowledge around the best implementation strategy, targets, and cost-effectiveness of preconception care in SSA is currently still largely lacking. Many of the proposed interventions are complex and need to take into account barriers to care, socioeconomic challenges, and motivations for change specific to different target groups (300,311). It is clear, however, that strengthening of both primary healthcare and community-based preconception care aimed at maternal nutritional status and risk of hyperglycaemia is warranted. This will require the multidisciplinary involvement of healthcare professionals and policy makers and should be synchronised with antenatal care and strengthening of the nurturing care framework in early childhood.

6.3 Revisiting the conceptual framework

In the light of the findings of this thesis, the conceptual framework explored in Chapter 1 is revisited in this section. The conceptual framework uses a chronological approach starting with preconception maternal (risk) factors, moving into the pregnancy, delivery, and early childhood phases of early life programming, and resulting in an impact on childhood development outcomes. It thereby emphasizes that the role of intra-uterine exposures, such as HFDP, do not stand alone, but interact with other existing factors (such as maternal BMI) and subsequent exposures (Figure 6.3). This concept carried through the analyses and results of all three empirical papers.

Results from our series of studies confirm some aspects of this conceptual framework, while others were unconfirmed or require further research. Early fetal development in a hyperglycaemic environment may result in poorer cognition, although we could not

conclusively specify the role of all neonatal or delivery outcomes based on our data. The role of maternal HIV infection and treatment during the index pregnancy may be associated with cognitive development, but no link was found for childhood adiposity. The independent role of programming through HFDP could not be confirmed at preschool age. We would need more comprehensive data in larger populations to evaluate the role of other early childhood factors such as nutrition and physical activity as presented in the framework.

According to these findings, the conceptual framework can be adapted as follows (Figure 6.3, B). A greater emphasis should be placed on preconception factors such as maternal nutritional status, and their effect on childhood outcomes, such as adiposity, at 3-6 years old. A shared postnatal environment is also relevant to add here, as is the role of birth weight. Highlighting these factors that can serve as targets for prevention, is another addition to the conceptual framework.

Additionally, the impact of early childhood environmental factors, including socioeconomic circumstances and exposure to preschool, on cognitive and fine motor development outcomes should be highlighted. This is especially applicable since increased educational stimulation could help promote a 'catch-up' in a cognitive setback associated with hyperglycaemia-exposure.

The framework should also be expanded to show the temporal effects of early life programming, with outcomes manifesting at varying ages. While our research focused on preschool age, the context provided by existing literature suggests that body composition may affect later in childhood with the accumulation of further risk, while cognitive delay can possibly be caught up with adequate stimulation.

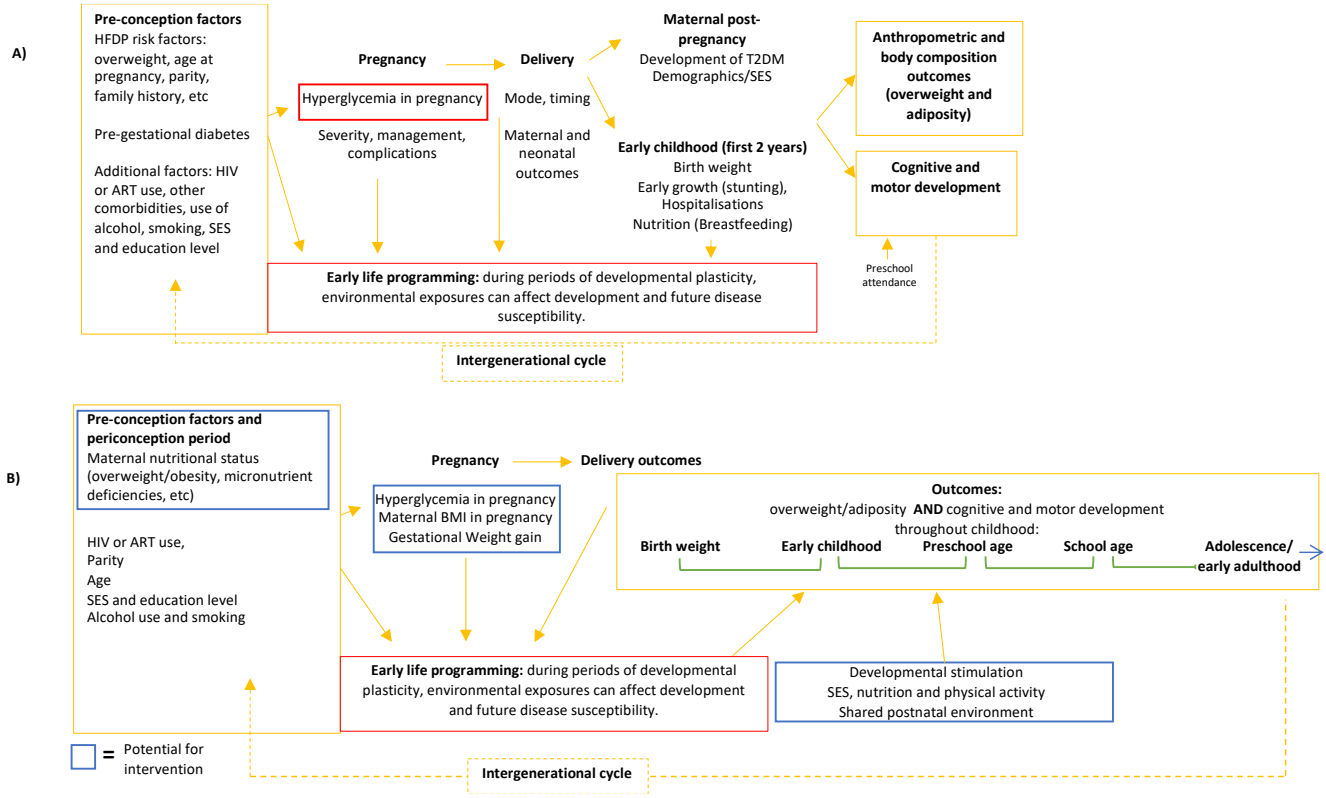


Figure 6.3: A. The conceptual framework for this thesis as presented in Chapter 1; B. Modified conceptual framework based on the findings in this thesis, with a focus on temporal effects of early exposures (green) and potential avenues for intervention (blue).

6.4 Implications

To the best of our knowledge, this is the first study in an African setting to evaluate body composition and cognitive development in offspring exposed to HFDP compared to an HFDP-unexposed group, and the retrospective analysis of obstetric and neonatal outcomes is the largest of its kind in SSA, to date. The findings have important implications for preconception, pregnancy, and early childhood health policy and research in South Africa. However, their relevance also extends to other LMICs experiencing an increase in diabetes and associated risk factors at reproductive age, and where poor early development and, increasingly, childhood obesity are significant concerns.

This thesis has highlighted **the importance of preventing and reducing maternal overweight** in South Africa, which could contribute to reducing both HFDP (and associated cognitive effects) and childhood adiposity, in order to break the current intergenerational cycle

of obesity. As recommended in this thesis, preconception care and health education have untapped potential for achieving this goal.

The suggested association between maternal HFDP and poorer cognitive development at preschool age, affecting around 10% of pregnancies and only increasing in prevalence, could have significant **consequences for later childhood cognitive development and adult outcomes,** both socioeconomic and health related. This thesis has provided evidence that, aside from efforts to reduce hyperglycaemic pregnancies, **early life exposures could mitigate these consequences**. Encouraging early educational stimulation on a community and household level, alongside adequate support and education moving into later childhood, could therefore promote 'catch-up' cognitive development in this group of children. This is unlikely to succeed without also addressing poverty and (educational) structural inequalities that play a role in early child development in LMIC settings.

Almost one third of children in our cohort were 'at risk of overweight', 'overweight', or 'obese' by WHO criteria, indicating **a need to combat childhood overweight/adiposity** in our setting. Educating the whole family on healthy physical activity and nutrition behaviours in preschool aged children to prevent and reduce obesity in later childhood, for example through community programs in a preschool setting, could have lifelong and intergenerational benefits. South Africa's patient-held childhood health and development record, the Road to Health booklet, has a new iteration since 2018, with nutrition as one of its main pillars (321). This can function as both a screening tool (by measuring anthropometrics at standard health visits), and a tool for health promotion. Evaluation of the use, impact, and barriers of using this tool in terms of childhood overweight could provide useful insight (322). As with childhood cognitive development, tackling issues around socioeconomic inequalities, safety, and access to healthy food options on a national and community level is crucial.

Improved screening and earlier detection of HFDP could also help improve obstetric and neonatal outcomes of diabetic pregnancies. In our cohort of women attending a gestational endocrine clinic, adequate management may have helped to mitigate the impact of HFDP on childhood adiposity at preschool age by limiting birth weight. However, specialised gestational endocrine clinics are uncommon in South Africa, with only three specialised centres in the

country. Moreover, although FIGO recommends universal screening for HFDP (3), selective risk-factor based screening is widely used in South Africa, due to resource constraints. Due to the low reported sensitivity and specificity of this approach, including in a South African population (3,35), pregnancies complicated by HFDP may go undiagnosed and/or may not receive the specialist treatment of our participants. Potentially, lack of treatment may increase the impact of HFDP on birth weight and subsequent body composition, as well as cognitive development, but the extent of these impacts are unknown and remain undetected. Since universal screening, while ideal, may overwhelm lower-resource healthcare systems, research into effective selective screening methods is needed (323).

A method for selecting childhood outcome measures in LMICs, developed as a series of criteria in this thesis, could contribute to **more (cost)-effective, meaningful life-course research in lower-resource settings** and increase the ability for comparisons across settings. Additionally, establishing uniform terminology and diagnostic cut-offs for GDM and HFDP, both nationally and internationally, would be beneficial for improving national screening and management practices and guidelines, as well as increasing comparability and quality of research in the field.

The findings around the potential impact of maternal HIV infection on both PNM rates and cognitive development, require further exploration of underlying mechanisms in order to begin tackling these poorer outcomes. It is possible that women with metabolic conditions in pregnancy as well as HIV infection may need additional monitoring, for example prior to delivery and into early childhood, but more information from a larger study sample is needed to inform healthcare providers. In a broader sense, these findings serve as a reminder of the **need to consider noncommunicable disease burden in South Africa in the context of the ongoing communicable disease challenges.** This includes exploring possibilities for leveraging learnings and healthcare infrastructure from the HIV/AIDS epidemic in South Africa in the fight against NCDs.

6.5 Limitations

While the limitations of the empirical studies in this thesis have been addressed in their respective chapters, this section addresses two general limitations of this thesis as a whole.

6.5.1 Limited age range

One part of the study conceptualisation and planning involved deciding on a particular age group for our participants. We considered existing evidence on the importance of the preschool age period for both child development, in terms of school readiness and childhood overweight, and the numerous opportunities for interventions at preschool age. Moreover, to create a robust data set within existing time and resource constraints, considerations were made around the availability of a verified HFDP-unexposed group, and an exposed group diagnosed with homogenous criteria.

As a result, and due to ongoing development and growth during childhood, we can only speculate on how these findings might progress in later childhood. The association we found between hyperglycaemia and cognitive development, may be diluted in later childhood due to the onset of schooling. Conversely, an impact on body composition may only present in later childhood, and various body composition trajectories may even arise due to additional accumulated risk exposure in both the HFDP-unexposed and exposed group. Therefore, caution is necessary when concluding how exposure to HFDP impacts overall childhood growth and development.

6.5.2 Lack of prospective, longitudinal data

Using existing, real world data, such as hospital records, is a cost- and time-effective method for gathering information outside of a single data collection appointment, which also reflects patient care practices. In this thesis, we recruited participants through historical hospital or research records, and these records also allowed us to contextualise the exposure to hyperglycaemia in pregnancy with pregnancy information and obstetric outcomes relevant to the conceptual framework.

However, since this data was retrospective, information on potential confounders and mediators from pregnancy to delivery was dependent on the detail and accuracy of record-keeping during pregnancy for each variable of interest. Moreover, there was unfortunately no preconception data available and early childhood information was limited to information gathered through the questionnaire at 3-6 years postpartum, possibly introducing recall bias.

As a result, some data was unavailable, such as the child's early life physical activity and gestational weight gain; or collected in a less controlled setting than the prospective data stringently collected during the study's data collection appointment.

This method for the study set-up also had consequences for the study's sample size, which was dependent on a finite list of potential participants who had attended the gestational endocrine clinic or tested negative 3-6 years previously, and their willingness to participate in the research. Together with resource constraints, this resulted in a smaller sample than initially intended for the study. This prevented us from carrying out further sub-group analysis for the cognitive outcome. For analysis of childhood BMI z-score, about 80% of the calculated sample size was reached. Consequently, results must be cautiously interpreted. The level of significance found for more subtle differences between groups and for subgroup analyses may be underestimated, although from a clinical perspective the relevance of more subtle differences in BMI z-score at preschool age remains unclear (214,324). Furthermore, results from different childhood adiposity outcomes supported each other, with FMI having a lower calculated required sample size, for example. Nevertheless, as discussed in the next section, these factors open the door for more research studies to answer follow-up questions in urban SSA.

6.6 Future research

In providing some of the first evidence for an impact of the increase in maternal obesity and hyperglycaemia on the health and development of the next generation in SSA, this thesis has raised a number of questions for future research, pertinent to the international evidence base as well as specific to an urban South African context.

6.6.1 Impact on childhood outcomes in later childhood and adolescence

A question that remains is how childhood body composition (BMI and fat mass index) and cognitive development in children exposed and unexposed to HFDP progress into later childhood (for example up to 18 years) in urban African populations, and whether different fat mass trajectories can be identified from birth to childhood, possibly differentiated by the child's sex. This would ideally be addressed in the form of a prospective, longitudinal study, and

additional outcomes of interest include childhood cardiovascular health, as measured by surrogate markers such as arterial stiffness. Arterial stiffness meets criteria for selecting childhood research outcomes as presented in section 6.2, such as being age appropriate, relevant for future adult cardiovascular health (325,326), and having minimally invasive measurement techniques available (pulse wave velocity).

The most cost-efficient method would be a longer-term follow-up of the cohort described in this study, but a larger sample and more detailed information on early pregnancy and childhood could be garnered if women are recruited in early pregnancy and consequently screened for gestational diabetes. This could be done at CHBAH, antenatal clinics in Soweto, and other urban South African sites, using a standard diagnostic method for HFDP, to increase generalisability and sample size. Recruitment and screening by OGTT for hyperglycaemia would be followed by data collection during each trimester of pregnancy, with collaboration from gestational endocrine clinics in case of a diagnosis of HFDP. Data collection would include delivery, followed by postnatal visits in the first two years of life, and extending to (for example, biennial) visits in childhood and adolescence. By inviting the father to a data appointment during pregnancy and subsequently during early childhood, the impact of paternal health (including BMI, nutrition, and physical activity) on childhood development outcomes could be investigated, since global research in this area is sparse. Analysis of childhood outcomes could include using multilevel modelling and, potentially, trajectory modelling to determine if individual adiposity trajectories can be identified.

The universal screening employed in the study would also allow for exploration of the sensitivity and specificity of different hypothetical selective screening methods (risk factor based, fasting or random blood glucose based, as some examples), to contribute to the evidence that may inform a uniform recommendation for screening in South Africa and SSA. In particular, the sensitivity, specificity, and cost-effectiveness of an initial screening by fasting glucose followed by a full OGTT if indicated, as has been previously suggested in our setting, could be assessed (3,39).

An additional objective that could be more adequately assessed within this set-up, in a larger sample of children exposed to HFDP, is the impact of OHAs (metformin and glibenclamide)

vs insulin, on childhood outcomes, since OHAs are endorsed by South African guidelines but remain somewhat controversial for use in pregnancy (34,222), and research on the long-term effects on childhood body composition is sparse (122,327,328).

Lastly, whether women with both HFDP and HIV infection have additional risk of adverse short-term outcomes (such as PNM) and longer-term outcomes, such as cognitive development, should be more thoroughly explored, which would require a considerably larger sample of HIV-infected women screened for HFDP. Questions around the role of the severity of HIV (for example, CD4 count), exposure to different ARVs, which effectively reduce vertical transmission and adverse perinatal outcomes but are also associated with some obstetric risks (61), and associated comorbidities (such as anaemia) can be explored. This could help identify targets for preventing an accumulation of risks.

6.6.2 The impact of childhood stunting

An additional area that requires further exploration is the cumulative effect of maternal overnutrition (obesity and/or HFDP) and childhood stunting in the first 1000 days on later childhood development. Women with obesity in early pregnancy (or, alternatively, preconceptionally) and a group of women without obesity could be recruited, and these mother/child pairs followed into early childhood. During early pregnancy, gestational weight gain and maternal micronutrient status could be assessed across exposure groups, since obese women are thought to be vulnerable to micronutrient deficiencies (250). An estimate of the prevalence of childhood stunting in children born to mothers with obesity could be determined, to establish the extent of this problem in South Africa. Subsequently, data from later childhood could be analysed according to different exposure groups: 1) an unexposed group; 2) those exposed to overnutrition in pregnancy, 3) those unexposed to maternal obesity but stunted; and 4) those exposed to maternal overnutrition detected in pregnancy with subsequent stunting (Figure 6.4). Specific outcomes of interest, at 2 years and subsequently in later childhood, include fat mass, hypertension, and cardiovascular surrogate markers such as arterial stiffness, and cognitive development or school performance.

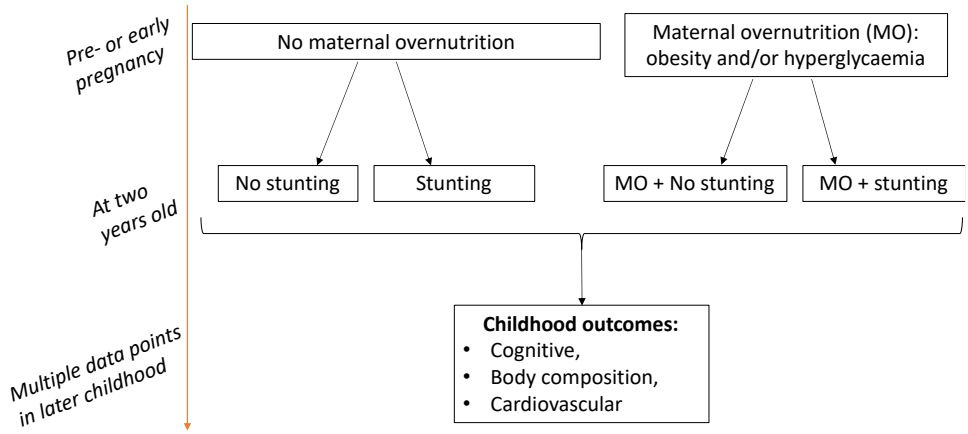


Figure 6.4: Example of a study design to explore the cumulative effects of maternal overnutrition and childhood stunting on later childhood development and health.

6.6.3 Intervention-based studies

A number of possible interventions arising from this thesis remain to be explored in future research. In terms of preconception healthcare, a multidisciplinary approach to explore interventions and associated challenges in an urban LMIC setting is necessary, for both individual-based as well as system-level interventions. Bukhali, the South African arm of the Healthy Life Trajectories Initiative (HeLTI), is a currently ongoing trial exploring methods for optimising young women's preconception health, including nutritional status with mind to the double burden of malnutrition. The results relating to both women's health during pregnancy, including the onset of HFDP, and childhood health are eagerly anticipated in view of our current study's findings (311,329). Health promotion in early childhood also requires further exploration in SSA settings (330). Here, outcome measures for success could be chosen with the aid of the criteria for relevance, suitability, and ethical outcome measures previously described in this chapter.

Lastly, future research aimed at exploring the feasibility and impact of an education stimulation intervention to promote adequate cognitive development in children exposed to HFDP in urban South Africa is valuable. This could be done using a mixed-methods approach. Firstly, qualitative interviews and focus groups could be performed in a representative urban South African population to explore acceptance of and the most preferrable setting for such an intervention (for example, by giving tools and advice at existing health visits in early childhood, or alternatively, community educational stimulation sessions from birth to

preschool age with community healthcare workers). Subsequently, a prospective quantitative analysis of cognitive scores, or more general school readiness measures, in children exposed and unexposed to HFDP with and without the planned intervention could be assessed.

6.7 Conclusion

In conclusion, hyperglycaemia in pregnancy and associated maternal preconception conditions, such as obesity, impact child development and health, warranting further research to guide prevention efforts in an urban African context.

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Appendices

Appendix A: Plagiarism Declaration

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Larske Marit Soepnel (Student number: 1562760) am a student registered for the degree of Doctor of Philosophy in the academic year 2021.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 25 June, 2021

1

Appendix B: Co-author's agreement

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

I, Larske Marit Soepnel, student number 1562760, declare that this Thesis is my own work and that I contributed adequately towards research findings published in the articles stated below which are included in my Thesis.

Signature of Student 

Date: 11/1/2021

Name of Primary Supervisor: Professor Shane Norris

Signature of Primary Supervisor: 

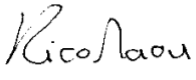
Date: 12/1/2021

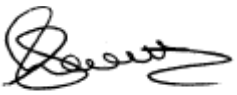
Agreement by co-authors:

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

Article 1:

Soepnel LM, Nicolaou V, Huddle KRL, Klipstein-Grobusch K, Levitt NS, Norris SA. Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV. *International Journal of Gynecology and Obstetrics*, 147(3): . https://doi.org/10.1002/ijgo.12956. (*Published online 3 September 2019).*

Authors	**Name**	**Signature**	**Date**
1st author	Dr. Larske M Soepnel		11 January 2021
2nd author	Dr. Veronique Nicolaou		11 January 2021

3rd author	Professor Kenneth Huddle		15 January 2021
4th author	Dr. Kerstin Klipstein-Grobusch		11 January 2021
5th author	Professor Naomi Levitt		11 January 2021
6th author	Professor Shane Norris		11 January 2021

Comments by primary supervisor:

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Article 2:

Soepnel LM, Nicolaou V, Slater C, Chidumwa G, Levitt NS, Klipstein-Grobusch K, Norris SA. Obesity and adiposity of 3- to 6-year-old children born to mothers with hyperglycaemia detected in pregnancy in an urban South African setting. *Annals of Human Biology (Accepted 6 April 2021).*

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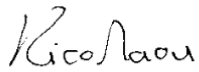
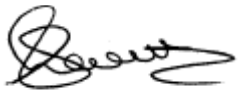
1st author	Dr. Larske M Soepnel		11 January 2021
2nd author	Dr. Veronique Nicolaou		11 January 2021
3rd author	Dr. Christine Slater		11 January 2021
4th author	Mr. Glory Chidumwa		15 January 2021

5th author	Professor Naomi Levitt		11 January 2021
6th author	Dr. Kerstin Klipstein-Grobusch		11 January 2021
7th author	Professor Shane Norris		11 January 2021

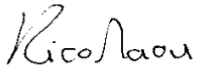
Comments by primary supervisor:

……

……

Article 3:

Soepnel LM, Nicolaou V, Draper CE, Levitt NS, Klipstein-Grobusch K, Norris SA. Cognitive and motor development in 3- to 6-year-old children born to mothers with hyperglycaemia first detected in pregnancy in an urban African population. *Maternal and Child Health Journal (In Review, Submitted November 2020).*

Authors	**Name**	**Signature**	**Date**
1st author	Dr. Larske M Soepnel		11 January 2021
2nd author	Dr. Veronique Nicolaou		11 January 2021
3rd author	Dr. Catherine Draper		11 January 2021
4th author	Professor Naomi Levitt		11 January 2021

5th author	Dr. Kerstin Klipstein-Grobusch		11 January 2021
6th author	Professor Shane Norris		11 January 2021

Comments by primary supervisor:

……

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Appendix C: Clearance Certificate from Health Research Ethics Committee, Larske Soepnel (M180317)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Larske Soepnel

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180317

NAME: (Principal Investigator)	Dr Larske Soepnel
DEPARTMENT:	School of Clinical Medicine Paediatrics MRC/Wits Developmental Pathways for Health Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	The Development of Children Born to Mothers with Diabetes Mellitus in Pregnancy: Growth, Obesity, and Cognitive and Motor Development
DATE CONSIDERED:	06/04/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shane Norris
APPROVED BY:	C B Penny Prof C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	15/05/2018

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix D: Clearance Certificate from Health Research Ethics Committee (M180204)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Veronique Nicolaou

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180204

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

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix E: Retrospective Data Collection Form

Initial Patient Assessment

Demographics	
Date of First Visit	_____ (D/M/Y)
Mothers Age:	_____ (years)
Mother's Age	_____ (if dob is not known)
Parity:	_____
Gravidity:	_____
Previous Losses	_____ (number)
Specify Previous Losses	☐ 1. Termination ☐ 2. Miscarriage ☐ 3. Stillbirth ☐ 4. Early neonatal death (first week post delivery) ☐ 5. Not specified ☐ 6. Ectopic pregnancy ☐ 7. Molar pregnancy ☐ 8. Late neonatal death (1 week-1 month) ☐ 9. Infant death (1month- 2 year)

Diagnosis	
Diagnosis	◯ 1. Pre-gestational T1DM ◯ 2. Pre-gestational T2DM ◯ 3. Gestational diabetes (GDM)
Duration of Pregestation Diabetes	_____ (years)
If 3, specify	◯ 1. Overt GDM ◯ 2. True GDM ◯ 3. Not specified
Date of Diagnosis of GDM	_____ (D/M/Y)

Diagnostic Method Used	◯ 1. 75-gram OGTT ◯ 2. 100-gram OGTT ◯ 3. Random plasma glucose >11.1 ◯ 4 HbA1C
Result FPG__	_____ (mmol/L)
Result 1 hr___	_____ (mmol/L)
Result 2 hr	_____ (mmol/L)
Result 3 hr___	_____ (mmol/L)
Result Random blood glucose	_____ (mmol/L)
Risk factors for GDM	◯ Yes ◯ No
If yes, specify	☐ 1. Repeated glycosuria ☐ 2. Previous GDM ☐ 3. Family history (first degree relative) ☐ 4. Polyhydramnios ☐ 5. High birth weight >4.5kg ☐ 6. Obesity >30kg/m2 ☐ 7. Women of South Asian descent ☐ 8. Poor obstetric history
Current Therapy	☐ 1. Insulin ☐ 2. Oral agent ((if pre-GDM))
Initiated treatment	☐ 1. Diet alone ☐ 2. Insulin ☐ 3. Oral agent ((if GDM))
Insulin Type	☐ 1. Human ☐ 2. Analog
Total Daily Dose Insulin	_____ (units/day)
Oral Agent Type	☐ 1. Metformin ☐ 2. Glibenclamide ☐ 3. Other

If other, specify	_____
Dose Metformin	_____ (mg/day)
Dose Glibenclamide	_____ (mg/day)

Initial Clinical Assessment

HIV Status	◯ 1. Positive ◯ 2. Negative ◯ 3. Unknown
CD4 Range >0	_____ ((If HIV Positive))
Anti-retroviral therapy (ARVs)	◯ Yes ◯ No ((If HIV positive))
If yes, describe	_____
Weight	_____ (kg (1 decimal point))
Height	_____ (cm)
BMI	_____
Systolic blood pressure	_____ (mmHg)
Diastolic Blood Pressure	_____ (mmHg)
Retinal Exam	◯ 1. Pre-proliferative changes ◯ 2. Proliferative changes ◯ 3. No diabetic retinopathy ◯ 4. Not Done
Other Medical Illnesses?	◯ Yes ◯ No
If yes, specify:	_____ (specify)

Initial Baseline Investigations

Haemoglobin(Hb)

_____ (g/dL)

Creatinine

_____ (umol/L)

Urine microalbumin/creatinine ratio

_____

HbA1C

_____ (mmol/mol, %)

Total blood glucose

_____ (mmol/L)

Number

_____ (n)

Mean Blood Glucose

_____ (mmol/L)

Foetal ultrasounds

Baseline Ultrasound Date

_____ (D/M/Y)

Gestational age at baseline ultrasound

_____ (weeks + days)

Expected Date of Delivery from Ultrasound

_____ (EDD)

Days to EDD at first visit

_____

Weeks to EDD

_____

Gestational Age in days

_____

Gestational age at first visit (weeks)

_____

Gestational age at first visit (days)

_____

Follow-up Visit 1 to 4

Date of Follow-up Visit 1	_____ (D/M/Y)
Days to EDD	_____
Weeks to EDD	_____
Gestational age in days	_____
Gestational age (weeks)	_____
Gestational age (days)	_____
Gestational age at first follow-up visit is [ga_weeks_fu1] weeks and [ga_days_fu1] days.	
Trimester	_____ (1, 2, or 3)
Measurements	
Systolic Blood Pressure	_____ (mmHg)
Diastolic Blood Pressure	_____ (mmHg)
Weight	_____ (kg)
Blood Investigation	
HbA1c	_____ (%)
Total Blood Glucose	_____
Number	_____
Mean Blood Glucose	_____

Treatment	
Treatment	☐ 1. Diet alone ☐ 2. Insulin ☐ 3. Oral Agent
If Insulin, Specify	☐ 1. Human ☐ 2. Analog
Total Daily Dose Insulin	_____ (units/day)
If Oral Agent, Specify	☐ 1. Metformin ☐ 2. Glibenclamide
Dose Metformin	_____ (mg/day)
Dose Glibenclamide	_____ (mg/day)

Aggregate data trimester 1-3 (trimester 1 provided as example)

Number of Visits in 1st Trimester	_____ (Not including FU visits 1-4, if these fall in 1st trimester)
Blood pressure	
No of BP measurements	_____ (Not including FU visits 1-4, if these fall in 1st trimester)
Systolic BP initial pt assessment (if in trimester 1)	_____
Systolic BP follow-up visit 1 (if in trimester 1)	_____
Systolic BP follow-up visit 2 (if in trimester 1)	_____
Systolic BP follow-up visit 3 (if in trimester 1)	_____
Systolic BP follow-up visit 4 (if in trimester 1)	_____
BP systolic 1	_____ (mmHg)
BP systolic 2	_____ (mmHg)
BP systolic 3	_____ (mmHg)
BP systolic 4	_____ (mmHg)
BP systolic 5	_____ (mmHg)
BP systolic 6	_____ (mmHg)

Mean Systolic Blood Pressure	_____ (mmHg)
Diastolic BP initial pt assessment (if in trimester 1)	_____
Diastolic BP follow-up visit 1 (if in trimester 1)	_____
Diastolic BP follow-up visit 2 (if in trimester 1)	_____
Diastolic BP follow-up visit 3 (if in trimester 1)	_____
Diastolic BP follow-up visit 4 (if in trimester 1)	_____
BP diastolic 1	_____ (mmHg)
BP diastolic 2	_____ (mmHg)
BP diastolic 3	_____ (mmHg)
BP diastolic 4	_____ (mmHg)
BP diastolic 5	_____ (mmHg)
BP diastolic 6	_____ (mmHg)
Mean Diastolic Blood Pressure	_____ (mmHg)

Weight	
Number of weight measurments	_____ (Not including FU visits 1-4, if these fall in 1st trimester)
Weight initial pt assessment (if in trimester 1)	_____
Weight follow-up visit 1 (if in trimester 1)	_____
Weight follow-up visit 2 (if in trimester 1)	_____
Weight follow-up visit 3 (if in trimester 1)	_____
Weight follow-up visit 4 (if in trimester 1)	_____
Weight 1	_____ (kg)
Weight 2	_____ (kg)
Weight 3	_____ (kg)
Weight 4	_____ (kg)
Weight 5	_____ (kg)
Weight 6	_____ (kg)
Mean weight	_____ (kg)

Glucose control	
Number of HbA1c Measurements	_____ (Not including FU visits 1-4, if these fall in 1st trimester)
HbA1c initial pt assessment (if in trimester 1)	_____
HbA1c follow-up visit 1 (if in trimester 1)	_____
HbA1c follow-up visit 2 (if in trimester 1)	_____
HbA1c follow-up visit 3 (if in trimester 1)	_____
HbA1c follow-up visit 4 (if in trimester 1)	_____
HbA1c 1	_____ (%)
HbA1c 2	_____ (%)
HbA1c 3	_____ (%)
HbA1c 4	_____ (%)
HbA1c 5	_____ (%)
HbA1c 6	_____ (%)
Mean HbA1c	_____ (%)
Number of Mean Glucose Measurements	_____ (Not including FU visits 1-4, if these fall in 1st trimester)
MBG initial pt assessment (if in trimester 1)	_____

MBG follow-up visit 1 (if in trimester 1)	_____
MBG follow-up visit 2 (if in trimester 1)	_____
MBG follow-up visit 3 (if in trimester 1)	_____
MBG follow-up visit 4 (if in trimester 1)	_____
Mbg 1	_____ (mmol/L)
Mbg 2	_____ (mmol/L)
Mbg 3	_____ (mmol/L)
Mbg 4	_____ (mmol/L)
Mbg 5	_____ (mmol/L)
Mbg 6	_____ (mmol/L)
Mean 'Mean Blood Glucose'	_____

Treatment

Treatment during trimester	☐ 1. Diet Only ☐ 2. Insulin ☐ 3. Oral Agent
Insulin dose initial pt assessment (if in trimester 1)	_____
Insulin dose fu1 (if in trimester 1)	_____
Insulin dose fu2 (if in trimester 1)	_____
Insulin dose fu3 (if in trimester 1)	_____

Insulin dose fu4 (if in trimester 1)	_____
Insulin dose 1	_____
Insulin dose 2	_____
Insulin dose 3	_____
Insulin dose 4	_____
Insulin dose 5	_____
Insulin dose 6	_____
Mean Insulin Dose per Day	_____ (units/day)
If Oral, Specify	☐ 1. Metformin ☐ 2. Glibenclamide
Any comments for trimester?	_____

Delivery Details

MATERNAL OUTCOME	
Date of Delivery	_____ (D/M/Y)
Days to EDD	_____
Weeks to EDD	_____
Gestational age in days	_____
Gestational age (Weeks)	_____
Gestational age (days)	_____
Gestational age at delivery is [ga_weeks_delivery] weeks and [ga_days_delivery] days.	
Gestational Age (controls)	_____ (only fill in for control group)
Mode of Delivery	◯ 1. NVD ◯ 2. Caesarian Section ◯ 3. Lost to follow-up/delivered elsewhere
Indication for Caesarian Section	☐ 1. Previous Caesarian Section ☐ 2. Foetal distress ☐ 3. Macrosomia ☐ 4. Other
If other, specify:	_____
Obstetric complications	◯ Yes ◯ No

If yes, specify	☐ 1. Premature labour ☐ 2. Spontaneous abortion (miscarriage) ☐ 3. Chronic hypertension ☐ 4. Gestational hypertension ☐ 5. UTI ☐ 6. Pre-eclampsia/eclampsia ☐ 7. Polyhydramnios ☐ 8. Oligohydramnios ☐ 9. Maternal death ☐ 10. Other (miscarriage occurs at < 20 weeks gestation)
If other, specify	_____
Treatment?	_____

Transition to Insulin

Was insulin started during pregnancy?	◯ Yes ◯ No
What date was insulin started?	_____
Days to EDD at insulin initation	_____
Weeks to EDD	_____
GA in days	_____
Gestational Age at Insulin Initiation (weeks)	_____
Gestational Age at insulin initiation (days)	_____

Gestational age at insulin initiation is [ga_in_weeks_ins_init] weeks and [ga_days_ins_init] days.

NEONATAL OUTCOME

Outcome	◯ 1. Live infant ◯ 2. Stillbirth (IUFD) (stillbirth occurs at >20 weeks gestation)
Twin?	◯ Yes ◯ No
Gender	◯ Male ◯ Female

Birth Weight	_____ (g)
Apgar score: 1 minute	_____ (0-10)
Apgar score: 5 minutes	_____ (0-10)
Early neonatal complications	◯ Yes ◯ No
If yes, early neonatal death?	◯ Yes ◯ No
If yes, describe:	_____
Foetal Anomalies	◯ Yes ◯ No
If yes, describe:	_____
Gender 2	◯ Male ◯ Female
Birth Weight 2	_____ (g)
Apgar score 2: 1 minute	_____ (0-10)
Apgar score 2: 5 minutes	_____ (0-10)
Early neonatal complications 2	◯ Yes ◯ No
If yes, describe:	_____
If yes, early neonatal death?	◯ Yes ◯ No
Foetal Anomalies 2	◯ Yes ◯ No
If yes, describe:	_____
Postpartum OGTT done?	◯ Yes ◯ No

Postpartum OGTT used:	◯ 1. 75-g OGTT ◯ 2. 100-g OGTT
FPG	_____ (Fasting Plasma Glucose)
2 hour PG	_____ (2 hour Plasma Glucose)

Appendix F: Information, parental/guardian consent, and assent form

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

Information Sheet

The development of children born to mothers with diabetes mellitus in pregnancy: growth, body composition, and cognitive and motor development.

My name is Dr. Larske Soepnel, and I am a researcher at the Developmental Pathways for Health Research Unit (DPHRU). We want to understand better whether a mother's diabetes during pregnancy has an impact on how a child grows and develops. For this reason, my colleagues and I are inviting women who attended the Gestational Endocrine Clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) during pregnancy to bring their children to an appointment at our research unit in the hospital. There, we will use a variety of measurements and activities to collect information about your child's growth, physical development, nutritional status, and cognitive development.

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

Study procedures

If you agree to take part in this study, we will ask you and your child to attend a single data collection appointment at DPHRU on the CHBAH premises. The visit will take approximately 3 to 4 hours.

During this appointment, we will ask you to fill in a questionnaire with questions concerning your demographics, household, illnesses you or your child have or have had, your usual and past lifestyle habits and diet, and whether your child attends a facility such as a crèche or pre-primary.

We will measure your child's weight using scales, their height using a height board, the circumference of their upper arm, and the circumference of their waist using a measuring tape. We will also estimate body composition, or how much of your child's body is made up of fat, as opposed to bone or lean tissue. We do this using a machine called DEXA, which uses x-rays to determine body composition. We also measure body composition using a technique called Deuterium Dilution Technique, which involves giving your child 20 grams of a water-like solution called Deuterium. We will ask you to not feed your child anything 30 minutes before the appointment for this technique to be effective. This solution is completely safe and tastes like water. Before the solution is given, 2.5 hours after it is given, and again 3 hours after it is given, we will collect about 1mL of your child's saliva using a cotton swab. The solution that we give your children will help us determine their body composition. After the three hours is over, you can feed your child.

During the time we are waiting for the Deuterium Dilution Technique, we will also measure your child's heart rate (how often their heart beats in one minute), and blood pressure (the pressure with which the heart pumps blood through the body). This will be done by placing a cuff around your child's arm attached to a machine, which then automatically takes the measurements. An ultrasound machine will be used to determine the carotid intima-media thickness, which is how thick the lining of the blood vessel is, and a monitor will be used to assess pulse-wave velocity, which is how quickly each pulse pushes blood through the body. Using a small needle to collect a single drop of blood from your child's finger, we will also test for the amount of sugar in your child's blood.

Finally, if your child is older than 3 years, we will ask them to perform a series of activities, such as completing a stick-figure drawing, copying a shape using blocks, cutting along a line, or walking in a straight line. This will help us to establish your child's cognitive and motor development (ability to think, understand, and move around in response to their surroundings).

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

Risks and benefits

There are no significant medical risks for you, or your child associated with participation in this study. Certain measurements taken, including the finger stick for blood sugar, drinking the deuterium solution, and waiting for a saliva sample to be taken may cause slight discomfort or restlessness for your child. There will be some games and activities at the research unit. The use of x-rays in DEXA exposes your child to a very small dose of radiation. During the Deuterium Dilution method, we will ask your child to drink a small dose of heavy water, formally called deuterium oxide or D20. This is a form of water that contains a larger than normal amount of the hydrogen isotope deuterium than normal water. Heavy water is not radioactive at all and drinking the deuterium solution will not harm your child. The body will treat the heavy water just as it treats normal water, so that it spreads throughout the body water and then leaves the body through sweat, urine, or saliva. We can then measure the level of deuterium in the saliva and determine how much water, fat, and lean mass your child has.

If you or your child are feeling unwell or are unable to continue at any point, the data collection will be stopped or resume another time.

There are no direct benefits to participating in this study. Indirect benefits of participation include contributing to important research and access to basic development and health assessments for your child during data collection.

Reimbursement

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

Referral

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

Voluntary participation in research

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

Confidentiality

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

Ethical approval

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

Do you have any questions?

The investigator listed on the first page of this form is available to answer any of your questions about this research study. You may contact the investigator at any time on the following number: (011)933-1122. You can also contact Professor Shane Norris, the Director of DPHRU, by e-mail at Shane.Norris@wits.ac.za. If you require any further information or have any questions, comments, or complaints about eh study, please contact the Human Research Ethics Committee of the University of the Witwatersrand: Chairperson Prof. P Cleaton-Jones, at (011)7172301, or the secretariat: Zanele Ndlovu at (011)7171252/1234 or through e-mail at zanele.ndlovu@wits.ac.za.

YOU WILL HAVE A COPY OF THIS INFORMATION SHEET TO KEEP

If you are willing to participate in this study with your child, please read and sign the attached consent form on behalf of your child. Your signature on the consent form certifies that:

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

Study Number

_____

Consent Form

The development of children born to mothers with diabetes mellitus in pregnancy: growth, obesity, and cognitive and motor development.

I understand that the study will involve a data collection appointment where my child's growth, obesity, body composition, and cognitive and motor development will be determined. All the details and aims of the study have been explained to me. I understand that I have the right to refuse participation in this study, and that I and my child will not benefit directly from research done with information collected at the appointment.

I agree to my child being a participant in this study, on the condition that:

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

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

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

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

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

PARTICIPANT'S MOTHER/GUARDIAN

Participant's Mother/Guardian Printed Full Name

_____

Participant's Mother/Guardian Signature

_____

Date and Time

_____

RESEARCH ASSISTANT
Research Assistant Printed Full Name _____
Research Assistant Signature _____
Date and Time _____

Assent form

My name is Dr. Larske Soepnel.

I am starting an exciting research project.

Will you help us with our project?

What is research?

Research is a way to learn about what makes people and children healthy or unhealthy. Research is important because it helps us find better ways of helping people and children who are sick or at risk of getting sick.

Why me?

We are asking you and your mother to join our research, because when she was pregnant with you, she came to our clinic.

This is because she had "**Diabetes Mellitus"**, which means that she had high levels of sugar in her blood.

What will happen during the study?

There are FOUR main steps:

1 Your mother will answer some questions about you and herself

2

How much do you weigh?

How tall are you?

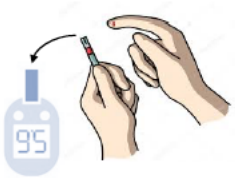
How thick is your arm?

Scan: what is your body made up of?

3

How fast does your heart beat?

How does your heart pump blood through your vessels?



How much sugar do you have in your blood?

We will ask you to do some fun activities:

4

How do you think and understand?

How well can you follow instructions?

Can you do movements and tricks we ask you to?

Is it dangerous?

No, it is not dangerous. Some of the steps may be a bit uncomfortable, for example when we ask you to lie still for a long time or quickly prick your finger.

If you want to stop at any point, you can **always** say so!

What will I get from this?

You will learn about your body and what we can measure.
You will also help us with our important research!

Who will know I am in the study?

We will not tell anyone except your mother or guardian that you are part of this study.
We will keep your information safe and secret.

Can I ask questions?

Always! You can ask your mother or guardian, or the researchers:

- Dr Larske Soepnel at (011)933-1122
- Professor Shane Norris, the Director of DPHRU, by e-mail at Shane.Norris@wits.ac.za

Do you understand what will happen if you take part?

YES ☐ NO ☐

Do you agree to take part?

YES ☐ NO ☐

Do you understand that you can stop at any point?

YES ☐ NO ☐

Signature or Mark _____ Date _____
(From 7 years or if deemed to have sufficient insight and understanding)

Appendix G: Maternal questionnaire

Maternal Questionnaire

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

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

Maternal Education

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

Socioeconomic Status	

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

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

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

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

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

Current maternal morbidities

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

Diabetes status

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

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

Have you been diagnosed with any of these conditions?

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

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

Mother -CVD event

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

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

Maternal and family lifestyle factors

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

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

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

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

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

_____

If yes, how many units at a time?

_____

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

How many times in a week?

_____

How many glasses at a time?

_____

Do you smoke cigarettes
- ○ Yes
- ○ No

If yes to previous, how many cigarettes per day?

_____

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

What was the reason for hospitalization?	_____

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

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

Appendix H: Data collection sheet anthropometry

Anthropometry Child

Study Number	_____
CHILD MEASUREMENTS	
Date of Visit	_____
Data Collector	_____
Child Date of Birth	_____
Current age	_____
Age (years)	_____
Age (months)	_____
Age is [gd_cm_age_yr] years and [gd_cm_age_mo] months.	
Sex of child	◯ Male ◯ Female
1.1) Weight(kg)	_____
1.2) Weight(kg)	_____
1.3) Weight(kg)	_____
Average weight	_____
2.1) Height (cm)	_____
2.2) Height (cm)	_____
2.3) Height (cm)	_____
Average height	_____

3) BMI	_____
4.1) Mid-upper arm circumference	_____
4.2) Mid-upper arm circumference	_____
4.3) Mid-upper arm circumference	_____
Average Arm Circumference	_____
5.1) Waist circumference	_____
5.2) Waist circumference	_____
5.3) Waist circumference	_____
Average Waist Circumference	_____
All anthropometry complete?	◯ Yes ◯ No
If no, why not?	_____

Appendix I: Standard operating procedure deuterium dilution method

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

Standard Operating Procedure for Deuterium Dilution Method in 3- to 6-year-old children

This document describes the procedures personnel will use in conducting the Deuterium Dilution Method as an assessment body composition in 3-6-year-old children.

Study: The Development of Children Born to Mothers with Diabetes Mellitus in Pregnancy
Version Number1.0

	NAME	TITLE	SIGNATURE
Author	Larske Soepnel	Project Coordinator	
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PURPOSE AND SCOPE

To describe the procedures for performing the Deuterium dilution method in 3- to 5-year-old children, including the necessary equipment, preparation of the deuterium dilution, administration of the dose, collection of saliva samples, and storage/analysis of saliva samples.

This SOP is based on the **latest IAEA protocol for the Deuterium Dilution Method for children and adults**, authored by Christine Slater and reviewed by Pernille Kaestel, Grace Munthali, Tom Preston and Christine Slater in September 2018, and the **IAEA/Multi-Center Body Composition Reference Study (MBCRS) Handbook**, authored by Christine Slater, Nadine Coulibaly, Tarik Becic, and Najat Mokhtar. However, this document is solely intended to describe the procedures used in the present study (The Development of Children Born to Mothers with Diabetes Mellitus in Pregnancy).

RESPONSIBILITIES AND SAFETY

- It is the responsibility of all staff involved to adhere to the SOP as outlined in this document.
- Since the study depends on voluntary participants, and children, every effort must be made to make the procedure as easy and painless as possible for them. If the participant asks for the test to end, or asks for their mother/guardian, these requests should be complied with immediately. The procedures should be explained in language and gestures understandable to a 3 to 6-year-old child.
- The study team must remain calm and professional, even when faced with the most nervous or inquiring participant.

BACKGROUND AND SUMMARY OF PROCEDURE

i. Body Composition

On its own, body weight is a relatively poor indicator of health and nutritional status. A more important indicator is the components that comprise an individual's body weight, including fat mass (FM) and fat free mass (FFM).

ii. Total Body Water (TBW)

Water is found exclusively within the FFM and includes both intracellular and extracellular fluid. The FFM contains approximately 73.2% water in adults, but the hydration of FFM is higher in infants and children. If TBW is measured, the amount of FFM can be estimated. Body FM is calculated as the difference between body weight and FFM [1-5].

iii. Deuterium oxide

The Deuterium Dilution Method is a stable isotope technique used in human nutrition studies for the past 50 years. It uses "heavy water", which is formally called deuterium oxide or 2H2O or D2O. This is a form of water that contains a larger than normal amount of the hydrogen isotope deuterium. Deuterium is given orally as deuterium oxide. Once deuterium is in the body, it behaves in the same way as hydrogen, and is dispersed throughout the body water within a few hours (equilibrated). After mixing with the body water, deuterium is eliminated from the body in urine, saliva, sweat and human milk. The small amount of deuterium naturally present is known as 'natural abundance.' The amount of deuterium in excess of this naturally occurring amount is known as 'deuterium enrichment.'

In general, the dose of deuterium oxide needed to estimate TBW is 0.1g per kg body weight. In this study, an accurately weighed dose of 2 grams will be given to all children in the form of 20g of a 1 in 10 dilution of 99.8% deuterium oxide.

iv. Sampling and Assessing TBW by deuterium dilution

After collecting a baseline saliva sample, the deuterium oxide (D2O) dose is consumed orally by the child. The deuterium will reach a plateau of equilibration after 2-3 h. Two post-dose saliva samples should be collected and the TBW is calculated from the enrichment of deuterium in the body water and the amount of deuterium oxide consumed by the child.

PREPARATION OF DEUTERIUM DILUTION

Procedure:

a. Tare a 1L bottle and its cap on the balance capable of weighing 2kg to 0.01g.
b. Using a 500 mL beaker and a funnel, add 900 g of drinking water to the bottle.

c. Replace the cap, record the weight to 0.01 g, and tare the bottle.
d. Using the 100 mL measuring cylinder pour 90 mL of deuterium oxide (approximately 100 g).
e. Replace the cap and record the weight to 0.01 g.
f. Check that the cap on the 1L bottle is tightly closed.
g. Swirl the bottle to mix the contents and invert the bottle at least 10 times

PREPARATION OF INDIVIDUAL DOSES

a. In a notebook, keep a record of the batch number of the stock solution of deuterium oxide used to make the doses, the date the doses were prepared, the dose number, the weight of the dose in the bottle (to 0.001g), and the weight of the dose bottle. The weight of the deuterium oxide dose consumed by the participant is required for the calculation of total body water.
b. Label dose bottles with individual dose bottle numbers
c. Zero the balance
d. Weigh the empty dose bottle with its lid and label on the analytical balance with a precision of 0.0001 g. Record the weight.
e. Pour some of the dose stock into a dry, clean 250 mL beaker
f. Transfer the required amount (20, 40 or 60 mL) of the dose stock (10% D_2O) to a 100 mL glass measuring cylinder.
g. Cover the beaker with aluminium foil to prevent fractionation.
h. Add the contents of the measuring cylinder to the pre-weighed bottle and replace the cap. Record the weight (bottle and dose stock) to 0.0001 g in the dose notebook.
i. Repeat step 2 to 6 for the remaining dose bottles, using the same beaker and measuring cylinder.
j. Make sure that all dose bottles are tightly sealed.
k. The last dose bottle will be incomplete (residual dose). This should be labelled and kept to make the dose dilution for analysis with the saliva samples.
l. From the noted dose weights, calculate the weights of the dose stock in each dose bottle, and note the dose bottle numbers.
m. Write the weight on the dose bottle label.
n. The doses can be stored in a refrigerator if they will be used within one week, or in a freezer at -20 degrees for a maximum of 6 months.
o. Doses should not be stored in the same place as saliva or other clinical specimens.

PROCEDURE FOR PREPARATION OF A DILUTED DOSE FOR ANALYSIS WITH THE SAMPLES

An aliquot (1-2 mL) of the dose stock solution (10% D_2O) should be sent to the analytical laboratory for analysis with the saliva specimens.

a. Tare a 100 mL volumetric flask with its stopper on the balance weighing to 0.0001 g.
b. Pour approximately 50 mL of drinking water into the volumetric flask. Replace the stopper.
c. Weigh the half full volumetric flask with its stopper to 0.0001 g and record the weight in the dose record book.
d. Tare the balance.
e. Pipette 200 µL of the residual dose into the half full volumetric flask. Replace the stopper and record the weight to 0.0001g in the dose record book. Tare the balance.
f. Fill to the mark and replace the stopper. *Don't worry if it is not exactly on the mark as the content will be weighed. Don't remove any contents!*
g. Record the weight to 0.0001 g in the dose record book.
h. Make sure the stopper is firmly on and invert the volumetric flask at least 5 times to ensure that the contents are thoroughly mixed.
i. Transfer the content of the volumetric flask to 10 cryovials with red caps labelled with "dose dilution", the batch number, and the date
j. Transfer the water from the wash bottle to 10 labelled cryovials with green caps labelled with "drinking water" and the date
k. Store cryotubes in a cool, dark, place until required for analysis with the saliva samples from the participants whose doses were prepared from this batch.

PROCEDURE FOR ADMINISTRATION OF DOSE

Equipment

a. Dose bottle labelled with bottle number, weight of dose, and the date it was prepared
b. Straw
c. Drinking water (or juice if necessary)

Procedure

a. **Food and drink:** The dose should be consumed at least 30 minutes after the last meal. A small meal may be consumed 30 minutes to 1 hour after the dose was taken. No additional food or drink consumed until after the final saliva sample.
b. Baseline saliva samples must be obtained before the dose is consumed.
c. If the dose has been frozen, it should be thawed before use
d. Invert the bottle 10 times to mix any condensation on the cap into the bulk of the liquid. This should be done immediately before the dose is consumed.
e. Do not open the bottle until it is time for the dose to be consumed.
f. Note the bottle number, the time and date the dose was taken on the data sheet and REDCap
g. Ask the participant to drink the dose through the straw to avoid spillage
h. After the bottle is emptied, add about 50mL of drinking water to the dose bottle, invert 10 times, and ask the participant to drink this through the straw.
i. Repeat step 'h' one last time.

PROCEDURE FOR SALIVA SAMPLING

Equipment

a. Cool box with ice pack and cryobox/rack
b. REDCap or report form for saliva collection
c. Doses (prepared in the laboratory)
d. Drinking water
e. Cotton wool swabs
f. Disposable 20mL syringe
g. Storage tubes with screw top (white, yellow, or red top)
h. Disposable gloves
i. Label for storage tubes

Procedure

a. Saliva sampling should take place in a calm environment, with all the necessary equipment in a pre-prepared kit. Explain the procedure to the mother and child before sampling.
b. Use pre-labelled tubes (with participant number and date); use a white cap tubes for baseline sample, the yellow top tube for the 2.5-hour sample, and the red top tube for the 3-hour sample.
c. Ask when the last time was that the child ate anything (needs to be at least 15-30 minutes before collection)
d. Place the cotton ball in the hollow of the cheeks, wait for about 1 to 2 minutes, and remove the cotton balls. Watch the child carefully while they have the cotton in their mouth, so they don't swallow it.
e. Remove the plunger from the 20mL syringe, and place the cotton in the barrel of the syringe, then replace the plunger into the barrel of the syringe
f. Remove the lid from the cryotube and push down the syringe plunger to extract saliva from the cotton wool into the sample storage vial. Replace the lid and make sure it is properly closed.
g. If there is not at least 1mL of saliva, repeat the above steps with a new cotton wool ball.
h. Discard the syringe, cotton wool, and gloves.
j. Record all information on the REDCap database or the data entry form, including dates and times of data collection.

STORAGE OF SALIVA SAMPLES

Equipment

a. Cryotubes (containing the saliva samples) with correct labels
b. Freezer (-20C)

Procedure

a. To avoid contamination, never store samples and doses together.
b. Close containers firmly to prevent loss of water by evaporation, and cross-contamination between samples
c. To minimize bacterial growth, saliva samples should be stored in a cool box or fridge until they can be transferred to a freezer at approximately -20C for storage, until analysis
d. Note on the GDM Sample Tracking Document in which cryobox and slot each sample is stored and update this daily.

ANALYSIS

Analysis of deuterium enrichment performed by a qualified laboratory technician using a portable Fourier-transform infrared spectrometer (Agilent, 4500 Series, California, USA). The calculation of body fat was done according to IAEA procedure for 2-6-year-old children, using the level of enrichment and body weight.

Appendix J: Data collection sheet deuterium dilution method

Child Deuterium Method

Study Number	_____
Dose information	
Dose bottle number	_____
Child weight	_____ (kg (0.1))
Height	_____
BMI	_____
Time of child's last meal/drink	_____
Stock and dose measures	
Stock (batch) number	_____
Weight of 99% D20 in stock	_____
Weight of drinking water added to stock	_____
Weight of 10% deut in dose bottle	_____
Baseline sample and dosing	
Data collector taking baseline sample and administering dose	_____
Baseline sample collection time	_____
Dose administration time	_____
Was the dose consumed in full?	◯ Yes ◯ No

Discontinue the test if the dose was not consumed in full, or measure how much was not consumed!

Post-dosing saliva samples	
Data collector for 1st post-dose saliva sample	_____
First post-dose samples collection time	_____
Data collector for 2nd post-dose saliva sample	_____
Second post-dose samples collection time	_____

Post Sampling	
Did the child have anything to eat or drink between the dose and the end of the test?	◯ Yes ◯ No
If yes above, at what time?	_____
If yes, how much did the child drink between the dose and the end of the test?	_____
Did the child go to the bathroom between dosing and the end of the test?	◯ Yes ◯ No
If yes above, what did the child do?	☐ Urine ☐ Faeces ☐ unknown
Comments	_____

Outputs	
Total body water	_____ (kg (0.1))
Percent body weight water	_____ (%)
Fat-free mass	_____ (kg (0.1))
Percent fat free mass	_____ (%)

Body fat mass	_____ (kg (0.1))
Percent body fat	_____ (%)

Appendix K: Herbst Early Childhood Development Criteria Test

EARLY CHILDHOOD DEVELOPMENT CRITERIA

FOR COGNITIVE AND MOTOR SCHOOL READINESS RISK AREAS

CHILD'S NAME: ..... SEX: .....

REFERRED BY/FOR:..... NO.: .....

Y M D

Date of test: .....

Date of birth: .....

Age: .....

EXAMINER: .....

SECTION A: SRRA UNDERLYING COGNITIVE CONCEPTS

1. INCOMPLETE MAN

Child uses a pencil/crayon to draw body parts on an unfinished man

Mark correct as appropriate

	Correctly point out 5 body parts on self	*0*
1	Adds 1 body part to the incomplete figure	1
2	Adds 2 body parts to the incomplete figure	1
3	Adds 3-4 body parts to the incomplete figure	1
4	Adds 5-6 body parts to the incomplete figure	1
5	Adds 7 body parts to the incomplete figure	1
6	Adds 8 body parts – correctly attached	1
7	Adds more than 8 body parts – correctly attached	1
8	Spontaneously draws clothes, buttons or other details not on picture	1

Highest possible total: 8

TOTAL:

2/16/2009

Test A.1 ***Name:*** .....

2. VISUAL MOTOR INTEGRATION

Child uses a pencil to copy from stimulus cards, on to a blank sheet of paper (A4).

1	*(exercise example)*	*0*
2		1
3	 (reasonably round)	1
4		1
5		1
6	 **(true corners)**	1
7		1
8		1
9		1
10		1
11		1

Highest possible total: 10

TOTAL:

3. BLOCK BUILDING

Child uses green blocks to copy the construction built by the examiner (behind a screen)

Time: Maximum of 60 seconds per item

1	Copy train with 3 blocks		1
2	Copy train with 5 blocks		1
3	Copy bridge with 3 blocks		1
4	Copy bridge with rotated blocks		1
5	Copy gate with rotated blocks		1
6	Copy vertical pyramid		1
7	Copy stairs with 10 blocks		1
8	Build flat on table		1

Highest possible total: 8

TOTAL:

4. STICK FIGURES

Child uses red plastic sticks to copy successive figures from a set of stimulus cards (red)

Mark right/wrong

1	*(Exercise example)*	*0*
2		1
3		1
4		1
5		1
6		1
7		1
8		1
9		1
10		1
11		1

Highest possible total: 10

TOTAL:

5. 3D & 2D PERCEPTION OF DIRECTION AND SIMILARITIES

Child indicates the block that differs from other in the row, and then does the same with 3D figures.

3D: Blocks (*60 seconds per item*)

0	*Blue – exercise example* 	*0*
1	Yellow rectangular 	1
2	Blue rectangular 	1

2D: Figures (*30 seconds per item*)

3		1
4		1
5		1
6		1
7		1
8		1
9		1
10		1
11		1
12		1

Highest possible total: 12

TOTAL:

6. 3D & 2D FORM TEST

Child sorts and matches various 3D & 2D forms

	Matches 1-3 red forms correctly on form card	*0*
1	Matches 4 red forms correctly on form card	1
2	Matches 5 red forms correctly on form card (30 sec.)	1
3	Places 8 of the 12 Perspex forms correctly (60 sec.) ⏲	1
4	Places all 12 Perspex forms correctly (90 sec.)	1
5	Sorts <u>all</u> 12 cards with coloured forms correctly according to shape (90 sec.)	1
6	Names 6 numbers or 6 letters pointed to by tester	1

Highest possible total: 6

TOTAL:

7. COLOUR CONCEPT

Child matches and names colours

	Matches less than 3 basic colours	*0*
1	Matches 3 basic colours	1
2	Names 2 basic colours indicated by tester	1
3	Names 3 basic colours indicated by tester	1
4	Names 4 - 5 colours on the plastic colours sheet	1
5	Names 8 colours on the plastic colours sheet	1
6	Grades colour intensity of 5 plastic strips by placing them from dark to light	1

Highest possible total: 6

TOTAL:

8. NUMBER AND COUNTING CONCEPTS

The child counts red plastic sticks with and without showing I I I I I

	Counts to 3 without showing	*0*
1	counts to 5 without showing	1
2	Shows and counts 2 sticks	1
3	Counts to 10 without showing	1
4	Shows and counts 4 sticks correctly	1
5	Shows and counts 10 sticks correctly	1
6	Shows and counts 13 – 20 sticks correctly	1

Highest possible total: 6

TOTAL:

9. PICTURE PUZZLES

Child correctly builds puzzle with the indicated number of pieces in the allotted time – ***use picture & ⏲***

1	2 pieces - 60 seconds 	1
2	3 pieces - 60 seconds 	1
3	4 pieces - 60 seconds 	1
4	4 pieces - 90 seconds 	1
5	5 pieces - 120 seconds 	1
6	5 pieces - 120 seconds 	1
7	6 pieces - 120 seconds 	1

Highest possible total: 7

TOTAL:

10. PICTURE PERCEPTION

Child correctly indicates the picture identical to that on the stimulus card

(Time limit of 30 seconds applies)

	Practice question: ***Match separate frog picture to same on row of frogs*** *Look carefully at this picture. Which one of the pictures in this row looks exactly the same?*	*0*
1	Pencil	1
2	Beads	1
3	Flower	1
4	Match	1
5	Tree	1
6	Car	1
7	Leaf	1
8	Umbrella	1
9	Candle	1
10	Clown	1
11	Chicken	1
12	Doll	1

Highest possible total: 12 /2 = 6

TOTAL:

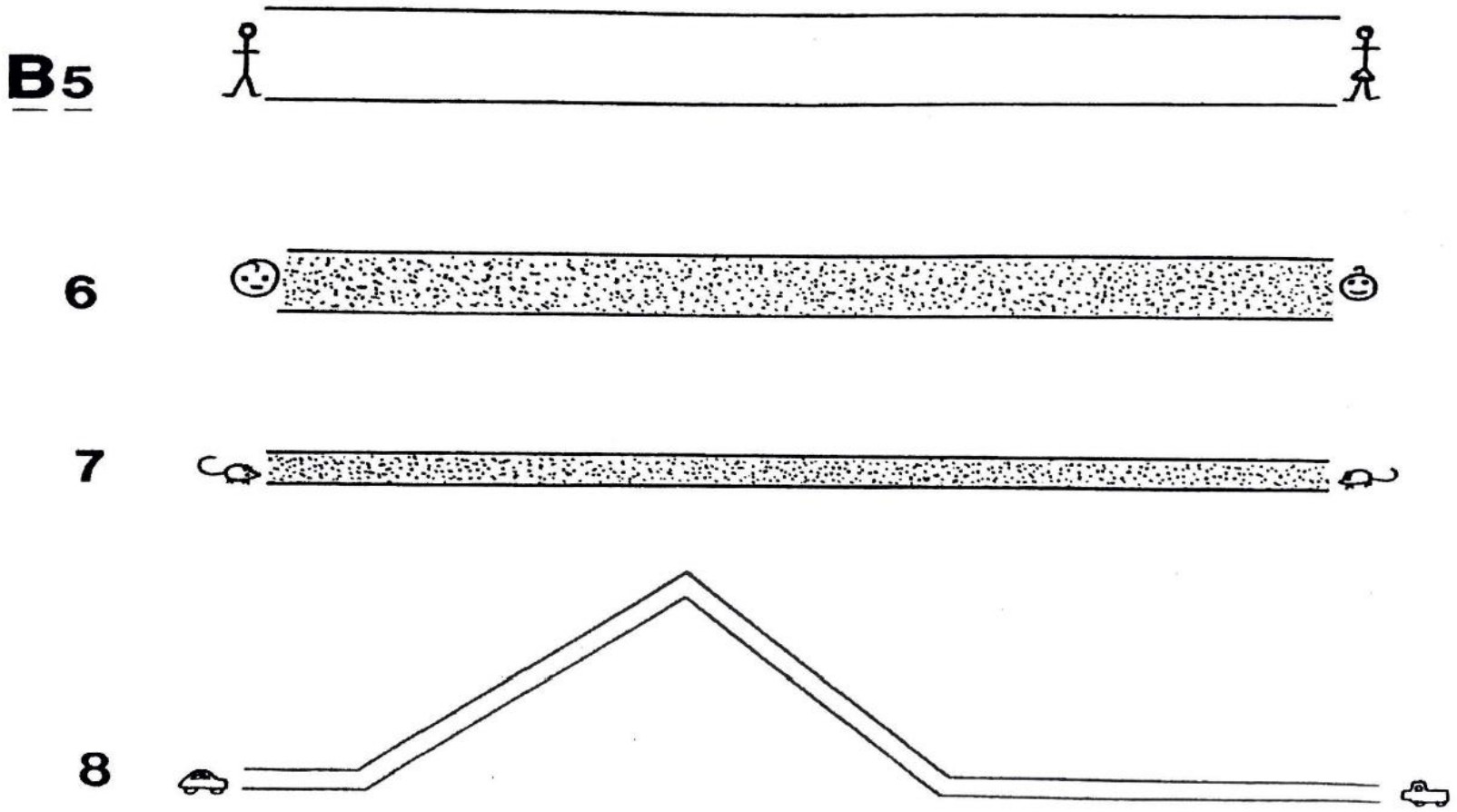
SECTION B: FINE MOTOR CO-ORDINATION

Child does the following:

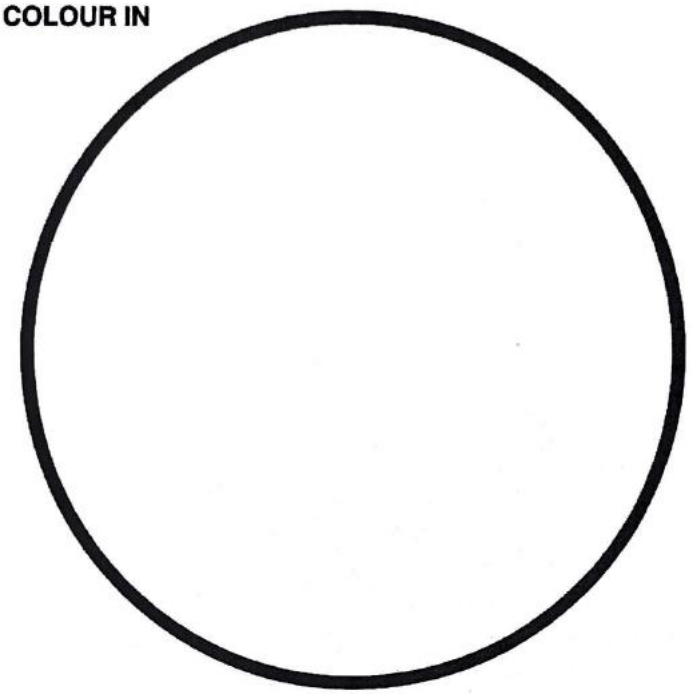
1	Threads 4 large beads in 2 minutes	1
2	Puts 10 pellets in container < 30 seconds	1
3	Puts 10 pellets in container < 20 seconds	1
4	Puts 10 pellets in container <18 seconds	1
5	Draws between parallel lines without touching	1
6	Draws between parallel lines without touching	1
7	Draws between parallel lines without touching	1
8	Draws between parallel lines without touching	1
9	Colours in the circle, keeping within lines	1
10	Colours the circle in neatly and evenly	1
11	Cuts circle out: >1 cm deviation or unevenly	1
12	Cuts circle out: <1 cm deviation with good co-ordination	1

Highest possible total: 12

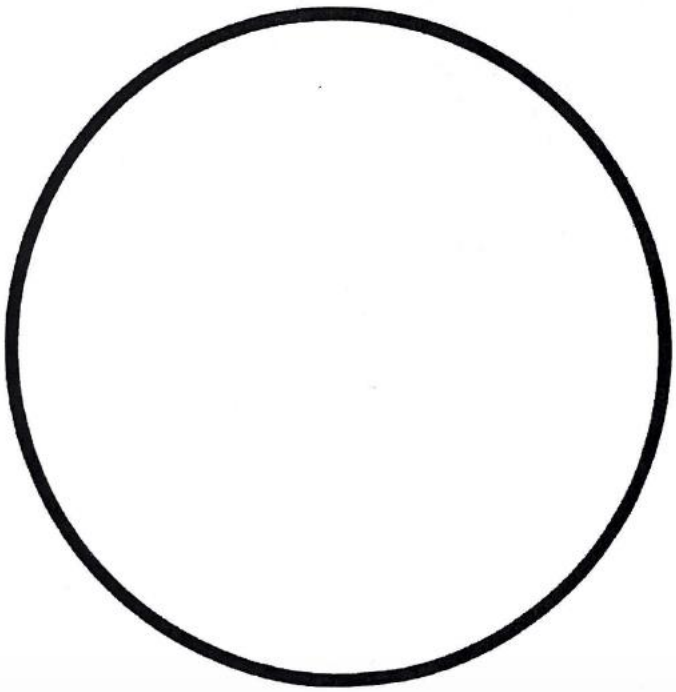
TOTAL:


B5
6
7
8

B 9 & 10: COLOUR IN



B 11 & 12: CUT OUT



SECTION C: GROSS MOTOR CO-ORDINATION

WALK

Walks for 10 steps on a straight line (gait):

Forward (heel-toe) .....
Backward (toe-heel) .....
On toes .....
On heels .....

BALANCE

Balance by standing on one leg:

For 2 seconds .....
For 4 to 8 seconds .....
For 10 seconds .....

HOP

On one leg

4 - 6 times .....

JUMP

Jump from standing position

50 cm. .....
65 cm. .....
75 cm. .....
90 cm. .....

THROW BEAN BAG

Underhand .....
Overhand .....

CATCH BEAN BAG

Arms against chest .....
Hands against chest .....
Only with hands .....

Highest possible total: 17

TOTAL:

Appendix L: Published papers

Chapter 3 (Paper I): Accepted Manuscript

Please note that the terms used to describe HFDP in the published version of this manuscript align with those used by the Society for Endocrinology, Metabolism, and Diabetes of South Africa (SEMDSA), whereas terminology aligning more closely with the international literature and World Health Organization guidelines was used throughout this thesis (please also refer to Chapter 1, Section 1.4.1 for an overview and explanation of the terminology used throughout this PhD thesis).

The terms used for this PhD thesis, and the corresponding terms for the manuscript version published in the International Journal of Gynaecology and Obstetrics (IJGO) are outlined below:

PhD thesis	**Published Manuscript Paper I (IJGO)**
Hyperglycaemia first detected in pregnancy (HFDP)	Gestational diabetes mellitus (GDM)
Diabetes in pregnancy (DIP)	Overt GDM
Gestational diabetes mellitus (GDM)	True GDM

Below is the accepted manuscript version of the following published article: Soepnel, L.M., Nicolaou, V., Huddle, K.R., Klipstein-Grobusch, K., Levitt, N.S. and Norris, S.A. Maternal and neonatal outcomes following a diabetic pregnancy within the context of HIV. Int J Gynecol Obstet. 2019;147(3):404-412. https://doi.org/10.1002/ijgo.12956.

CLINICAL ARTICLE

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

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

Synopsis
Outcomes following diabetic pregnancies in South Africa show the need for improved pre-conception care, and high perinatal mortality in HIV-infected group requires further research.

ABSTRACT

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

Methods: A review of medical records of 1071 diabetic pregnancies (between 2012 and 2018)

at a tertiary hospital in South Africa.

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

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

1 INTRODUCTION

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

Data about diabetes in pregnancy in sub-Saharan Africa (SSA) is limited. A recent review showed that merely 11% of countries had published prevalence data of GDM (6). Reported rates of GDM prevalence for Africa are in the range of 8.8%–9.3% (6–8). Within South Africa, varying rates of prevalence could be due to differences in population, screening practices, and diagnostic methods (8,9). Recent international recommendations for universal screening and the lower International Association of Diabetes and Pregnancy Study Groups (IADPSG) diagnostic criteria, distinguishing between overt and true GDM, are not uniformly implemented in South Africa, partly due to resource constraints (1,10). These discrepancies contribute to the underreporting and undermanaging of diabetes in pregnancy. A 20-year audit at a large urban hospital in South Africa between 1983 and 2002 showed the positive impact of management on maternal and neonatal outcomes for pre-gestational and GDM (11). Since

this publication, the same hospital has introduced oral hypoglycemic agents (OHAs), universal HIV monitoring and treatment, and lower diagnostic criteria for GDM.

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

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

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

2 MATERIALS AND METHODS

We retrospectively analyzed medical records of women attending the Gestational Endocrine Clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) for diabetes in pregnancy between 2012 and 2018. CHBAH is a 3000-bed teaching hospital with 1400–1600 deliveries per month in the large urban township of Soweto, with an antenatal HIV prevalence of 30%. The population attending the clinic is predominantly black African. Figure S1 shows a timeline of clinical practice after referral to the clinic. Participants with overt GDM were initiated on insulin based on a clinical decision where the degree of hyperglycemia and presence of complications were taken into account. Excluding this, the first choice of pharmacological treatment for GDM participants was metformin, which was escalated, if necessary, by either adding glibenclamide or transitioning to insulin, a decision based on levels of hyperglycemia

and the presence of diabetic complications. Those with T2DM on existing regimes were switched, where necessary, to either metformin and/or glibenclamide; otherwise, treatment was escalated as above. Only women with singleton pregnancies treated for T1DM, T2DM, and GDM diagnosed according to the definitions in Table S1a were included in the present study. See Table S1b for definitions of maternal and neonatal outcomes. The Human Research Ethics Committee, University of the Witwatersrand (M180204) approved this study.

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

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

3 RESULTS

There were 1071 diabetic pregnancies during the study period: 461 (43.0%) GDM; 408 (38.1%) T2DM; and 202 (18.9%) T1DM (Table 1). Overt GDM constituted 235 (51.0%) of GDM cases. The median gestational age at initial presentation was 15.6–16.1 weeks for those with pre-gestational diabetes and 28.0–29.5 weeks for those with GDM. The T1DM group was

younger (mean 26.5 ± 5.6), had a higher initial HbA1c (median 8.3, IQR 7–10.2), higher frequency of retinopathy and nephropathy 17 (8.4%) and 4 (4.7%), respectively, and a lower BMI (29.3 ± 6.6) compared to the GDM and T2DM groups. For T2DM, the median initial HbA1c was 7.6 (IQR 6.4–9.1), for overt GDM it was 7.2 (IQR 6.5–8.8) and for true GDM it was 5.8 (IQR 5.4–6.3). All four groups were overweight by mean BMI at presentation. Overall, 234 (21.8%) women in the cohort had hypertension, with the T2DM group accounting for the highest prevalence at 136 (33.3%).

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

In terms of maternal outcomes, loss to follow-up was highest in the T1DM (n=37, 18.3%) and true GDM (n=47, 20.8%) groups (Table 2). The number of cesarean deliveries was 641 (72.2%) and the rate was highest in the T2DM group at 275 (78.4%). The rate of PIH was 9.2% (n=98) across all groups; the combined rate of pre-eclampsia and eclampsia was 2.7% (n=29). The rates of miscarriage and maternal mortality were highest in the T1DM group at 14 (6.9%) and 3 (1.5%), respectively. Initial HbA1c was the significant predictor for miscarriage when performing regression analyses (odds ratio [OR] 1.2, 95% confidence interval [CI] 1.13–1.55, *P*=0.001). When investigating “any obstetric complication” (the combined rate of miscarriage, hypertensive disorders of pregnancy, urinary tract infection, polyhydramnios, oligohydramnios, maternal death), no significant differences were found between the groups. Of offspring, 747 (92.6%) had an APGAR score ≥7 at 1-minute postpartum (Table 2). The rate of prematurity (n=237, 30.9%) was significantly lower in the true GDM group (n=41, 22.8%) than the other groups, but not when correcting for initial HbA1c (OR 1.17, 95% CI 1.08–1.26, *P*<0.001) and lack of glycemic control in the third trimester (OR 2.36, 95% CI 1.24–4.47, *P*=0.009). Macrosomia, with a prevalence of 71 (8.4%) in the whole cohort, was highest in the overt GDM group (n=24, 12.3%) and significantly lower in the true GDM group (n=7, 4.0%). For both low birth weight (LBW), which was highest in the T1DM group, and macrosomia, the difference between the groups was no longer significant when adjusting for maternal BMI (OR 0.95, 95% CI 0.91–0.98, *P*=0.002; OR 1.05, 95% CI 1.00–1.09, *P*=0.027, respectively, for LBW and macrosomia), with multiparity (OR 0.61, 95% CI 0.37–0.99, *P*=0.047) and hypertension (OR 1.85, 95% CI 1.04–3.29, *P*=0.036) being the other two significant predictors

for LBW. Perinatal mortality (PNM) was significantly higher in the T1DM group (n=10, 6.7%) when compared to T2DM and the GDM group in its entirety (*P*=0.019), although HIV was the main statistical contributor in our model (OR 5.00, 95% CI 2.06–12.15, *P*<0.001). The T1DM group also had the most major congenital anomalies at 1.7% (n=2). Additional neonatal outcomes in the cohort included hypoglycemia (n=10, 1.2%), respiratory distress syndrome (n=8, 1.0%), and jaundice (n=4, 0.5%), with no significant differences between groups.

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

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

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

GDM: In the GDM metformin-exposed group, a higher number of offspring had LBW (17 [20.0%] vs 7 [8.4%], *P*=0.032) and a lower number presented with macrosomia than in the insulin monotherapy group (1 [1.2%] vs 9 [10.8%], *P*=0.009) (Fig. 1). There were too few cases

of LBW and macrosomia in the GDM group to pursue regression analysis. As with T2DM, PNM was not significantly different between groups.

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

4 DISCUSSION

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

The rates of key adverse outcome including birth weight (macrosomia and LBW), prematurity, fetal anomalies, hypertensive disorders of pregnancy, maternal mortality, and PNM were similar to a previous audit from 1992 to 2002 at our clinic (11) and to another recent South African audit (17). However, macrosomia, PNM, and prematurity remain inferior to respective rates in South Africa for non-diabetic pregnancies (18–22). The rate of cesarean delivery (n=641, 72.2%) was much higher in the present study than the estimated rate of cesarean delivery at CHBAH of 39.8% in non-diabetic pregnancies (23), but only moderately higher than the 1992–2002 audit of diabetic pregnancies (62%), a difference that could be due to an overall increase in rates of cesarean delivery over this period.

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

Obesity, so common in our cohort and in South African women in general, not only augments

the metabolic burden of pregnancy, it also increases the risk of adverse outcomes (28,29). In our cohort, BMI was found to be a significant predictor for macrosomia. Even the T1DM group had a high BMI at presentation, in contrast to the traditional set of characteristics attributed to T1DM. This indicates that the rising public health burden of metabolic syndrome is impacting even unexpected population groups.

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

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

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

may have increased contact with health services, improving lifestyle measures (34), although this was not reflected in earlier presentation for the HIV-infected group.

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

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

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

For both T2DM and GDM, maternal outcomes were not significantly different between patients exposed to glibenclamide and metformin compared to the gold standard of insulin. However, rates of macrosomia were higher among those exposed to glibenclamide (n=10 [12.2%] vs 0) despite a significantly lower initial HbA1c and better glycemic control in the third trimester in this group. This was in line with results from a recent meta-analysis observing glibenclamide to be inferior to both metformin and insulin in terms of rates of macrosomia and neonatal hypoglycemia (13). It has been suggested that the effect of glibenclamide may be due to a direct drug effect via placental transfer, but the relationship could also be due to the higher initial BMI in the glibenclamide group (21).

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

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

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

Author contributions

All authors contributed to the conceptualization of the study. LMS and VN contributed equally

to planning, data capturing and analysis, and manuscript writing and editing. KKG, NSL, and SAN contributed to planning, interpretation of data, and manuscript writing and editing. KH contributed to the database, and manuscript writing and editing.

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Conflicts of interest

The authors have no conflicts of interest.

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FIGURE LEGENDS

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

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

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

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

	n=202	**T1DM**	**n=408**	**T2DM**	**GDM**			
					n=235	**Overt**	**n=226**	**True**
Maternal characteristics at initial presentation								
Maternal age (mean, SD)	202	26.9 (5.6)	403	34.0 (5.2)	235	32.4(5.3)	226	32.9(6.1)
GA, weeks (median, IQR)	200	15.6 (9.8–23.1)	407	16.1 (11.0-24.3)	235	28.0 (21.7-32)	224	29.5 (26.6-33.0)
BMI (mean, SD)	112	29.3 (6.6)	207	35.1 (8.1)	116	36.6 (7.9)	105	35.5 (8.5)
BP (mean, SD)	190	123/74.7 (16.0–11.5)	396	125.9/76.5 (15.0–11.6)	223	123.0/73.6 (14.0–10.9)	213	120.5/71.1 (14.2–9.6)
Obstetric history								
Primigravida (n, %)	200	69 (34.5)	407	21 (5.2)	235	20 (8.5)	226	22 (9.7)
Previous pregnancy losses (n, %)	200	49 (24.5)	407	147 (36.1)	235	94 (40.0)	226	99 (43.8)
Comorbidities at presentation								
Chronic hypertension (n, %)	202	27 (13.4)	408	136 (33.3)	234	35 (15.0)	225	36 (16.0)
HIV positive (n, %)	153	45 (29.4)	320	95 (29.7)	212	25 (11.8)	203	47 (23.1)
Anemia (n, %)	199	46 (23.1)	403	56 (13.9)	455	25 (10.7)	455	34 (15.4)
Characteristics of diabetes								
Age at diagnosis, y (median, IQR)	196	21 (16–25)	383	31 (27–34)		NA		N/A
Initial Hba1c, % (median, IQR)	191	8.3 (7–10.2)	393	7.6 (6.4–9.1)	215	7.2 (6.5–8.8)	207	5.8 (5.4–6.3)

Control at initial presentation (n, %)	86	48 (55.8)	133	63 (47.3)	136	NA	78	NA
Control at third trimester [a] (n, %)	147	141 (95.9)	351	335 (95.4)	207	187 (90.3)	196	191 (97.5)
Target organ damage (n, %) Retinopathy Nephropathy	202 85	17 (8.4) 4 (4.7)	407 168	15 (3.7) 1 (0.6)	234 81	3 (1.3) 0	226 85	2 (0.9) 0
Therapy								
Exposure to metformin (n, %)	202	14 (6.9)	408	351 (86.0)	235	120 (52.8)	226	171 (75.7)
Exposure to glibenclamide (n, %)	202	1 (0.5)	408	151 (37.0)	235	80 (34.0)	226	76 (33.6)
Exposure to insulin (n, %)	202	202 (100.0)	408	270 (66.2)	235	134 (57.0)	226	25 (11.1)

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; GDM, gestational diabetes; SD, standard deviation; GA, gestational age; IQR, interquartile range; BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; MBG, mean blood glucose.

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

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

	n	**T1DM**	**n**	**T2DM**	**GDM**				***P* value**
					n	**Overt**	**n**	**True**	
Lost to follow-up	202	37 (18.3)	408	51 (12.5)	235	31 (13.2)	226	47 (20.8)	0.020 [b]
Maternal outcomes									
CD	155	104 (67.1)	351	275 (78.4)	199	142 (71.4)	177	120 (67.8)	0.015 [b]
Miscarriage	202	14 (6.9)	408	12 (2.9)	235	4 (1.7)	226	2 (0.9)	0.001 [b]
PIH	202	23 (11.4)	408	31 (7.6)	235	27 (11.5)	226	17 (7.5)	0.198

Pre-eclampsia/ Eclampsia	202	5 (2.5)	408	13 (3.2)	235	2 (0.9)	226	9 (4.0)	0.749
Maternal mortality	202	3 (1.5)	408	0 (0)	235	1 (0.4)	226	0 (0)	0.017 [b,c]
Neonatal outcomes									
Prematurity	165	57 (34.6)	358	117 (32.9)	212	65 (31.9)	195	41 (22.8)	0.065
Macrosomia	141	12 (8.5)	333	28 (8.4)	195	24 (12.3)	174	7 (4.0)	0.042 [b]
LBW	141	41 (29.1)	333	59 (17.7)	195	26 (13.3)	174	23 (13.2)	0.001 [b]
Major congenital anomaly	115	2 (1.7)	282	2 (0.7)	172	2 (1.2)	147	0 (0)	0.450 [c]
PNM	149	10 (6.7)	346	15 (4.3)	201	4 (2.0)	178	3 (1.7)	0.053 [c]
Stillbirth	149	8 (5.4)	346	11 (3.2)	201	4 (2.0)	178	3 (1.7)	0.225

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; GDM, gestational diabetes; CD, cesarean delivery; PIH, pregnancy-induced hypertension; LBW, low birth weight; PNM, perinatal mortality.

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

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

[c] Fisher exact test.

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

	n	**Exposed to metformin monotherapy [a] (n=106)**	**n**	**Exposed to glibenclamide [b] (n=296)**	**n**	**Exposed to insulin monotherapy (n=332)**	***P* value**
T2DM GDM	408 461	29 (7.1) 106 (23.0)		100 (24.5) 124 (26.9)		46 (11.3) 106 (23.0)	<0.001 [a], <0.001 [b]
Age at presentation (median, IQR) T2DM GDM	2610 6	35 (28–38) 33 (39–37)	9912 4	34 (30–37) 33 (29–37)	4610 6	35 (31–38) 34 (29–38)	0.518 [a], 0.391 [b] 0.660 [a], 0.593 [b]
BMI at first presentation (median, IQR) T2DM GDM	1251	32.95 (27.3–42.1) 33.8 (30.3–40)	5555	34.6 (31.6–41.8) 36.7 (31.6–41.5)	2950	31.6 (27.6–36.2) 35.35 (31.2–41.1)	0.626 [a], 0.021 [b,c] 0.250 [a], 0.674
Weight gain over pregnancy (median, IQR) T2DM GDM	2810 0	3.5 (0.45–6.4) 0.95 (-0.2 to 2.5)	9711 7	3.3 (1–7) 2.5 (0–6)	4310 1	7.1 (2.5–11) 5 (1.9–9.8)	0.008 [a,c], 0.003 [b,c] <0.001 [a,c], <0.001 [c]
Initial HbA1c (median, IQR) T2DM GDM	2799	6.2 (5.5–7.5) 5.8 (5.4–6.2)	9711 9	6.7 (5.9–7.6) 6.2 (5.8–6.9)	4594	7.9 (6.6–9.5) 8.55 (7–10.1)	0.002 [a,c], <0.001 [b,c], <0.001 [a,c], <0.001 [b,c]
Number controlled at third trimester [d] (n, %) T2DM	2396	22 (95.7) 96 (100)	9211 9	92 (100) 117 (98.3)	4096	36 (90.0) 86 (89.6)	0.644 [a], 0.008 [b,c]

GDM							0.002 [a,c], 0.007 [b,c]

Abbreviations: T1DM, type 1 diabetes; T2DM, type 2 diabetes; IQR, interquartile range; HbA1c, glycated hemoglobin; MBG, mean blood glucose.

[a] *P* value for comparison between metformin monotherapy and insulin monotherapy groups.

[b] *P* value for comparison between glibenclamide and insulin monotherapy groups.

[c] Statistical significance (*P*<0.05).

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

Table S1. a. Definition and criteria for diagnosis of diabetes in pregnancy, including T1DM, T2DM, and GDM. b. Definition of maternal and neonatal outcomes.

a.	
GDM	Diabetes first discovered in pregnancy, diagnosed using a 2-h 75-g OGTT and the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, or a random plasma glucose ≥11.1 mmol/L. This method distinguished between true GDM and overt GDM, diagnosed when fasting plasma glucose ≥7.0 mmol/L, or 2-h or random plasma glucose ≥11.1 mmol/L. For the purposes of this study, cases of GDM diagnosed pre-2014 by a 100-g 3-h oral glucose tolerance test were excluded. Diagnostic testing based on selective screening of risk factors for GDM, except for women universally screened in the context of a research study between 2014 and 2016.
Risk factors for GDM	Persistent glycosuria, (first degree) family history of diabetes, previous unexplained perinatal losses, previous GDM, history of a macrosomic baby.
Pre-gestational diabetes	Diabetes diagnosed previous to conception, categorized as T1DM or T2DM according to the clinical diagnosis made on the hospital file.
b.	
Maternal outcomes	
Glycemic control	Mean blood glucose <7.1 mmol/L for the 3rd trimester, measured by capillary blood glucose on a glucometer
Dietary Failure	Lack of glycemic control after 2 weeks of dietary management
Maternal overweight	BMI ≥25
Maternal obesity	BMI ≥30
Nephropathy	Creatinine >71 ummol/L and MAC >30 mg/L
Anemia	Hemoglobin levels < 11 g/dL
HIV-positive	Based on results from rapid or antibody ELISA tests before or at time of initial presentation
Hypertension	Blood pressure elevated > 140/90 mmHg for at least two measurements
Hypertensive disorders of pregnancy	Categorized as: Chronic hypertension: hypertension present before pregnancy

	PIH: hypertension presenting >20 completed weeks Pre-eclampsia: hypertension with proteinuria Eclampsia: hypertension with proteinuria and seizures
Neonatal outcomes	
Prematurity	Delivery <37 completed weeks gestational age
Miscarriage	loss occurring at <28 completed weeks gestational age
Stillbirth	Loss occurring at ≥29 weeks gestational age
END	Neonatal demise occurring in the first week of life
PNM	The number of stillbirths and early neonatal deaths out of the total number of deliveries
LBW	Birth weight <2500 g
Macrosomia	Birth weight ≥ 4000 g
Major congenital anomaly	Spinal, cardiac, central nervous system, renal, or digestive system anomaly.

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

Chapter 4 (Paper II): Published manuscript

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RESEARCH PAPER

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Obesity and adiposity of 3- to 6-year-old children born to mothers with hyperglycaemia first detected in pregnancy in an urban South African setting

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ABSTRACT

Background: Understanding the association between maternal metabolic conditions in pregnancy and the risk of childhood overweight, a growing concern in sub-Saharan Africa (SSA), helps to identify opportunities for childhood obesity prevention.

Aim: To assess the association between hyperglycaemia first detected in pregnancy (HFDP) (gestational diabetes mellitus [GDM] and diabetes in pregnancy [DIP]) and child obesity and adiposity in pre-school-aged children in South Africa, independently of maternal BMI.

Subjects and methods: Measurement of anthropometry and fat mass index (FMI) by the deuterium dilution method was done for 102 3–6-year-old children born to mothers with HFDP and 102 HFDP-unexposed children. Hierarchical regression analysis and generalised structural equation modelling (GSEM) were performed.

Results: The prevalence of overweight/obesity was 10.5% and 11.1% in children exposed to GDM and DIP, respectively, and 3.9% in the HFDP-unexposed group. Log-transformed FMI was significantly higher in the DIP-exposed group ($\beta = 0.166$, 95% CI = 0.014–0.217 $p = .026$), but not when adjusting for maternal pregnancy BMI ($\beta = 0.226$, 95% CI = 0.003–0.015, $p = .004$). GSEM showed significant total effects of maternal BMI and birth weight on FMI/BMI.

Conclusions: Maternal pregnancy BMI seems to play a greater role in the development of childhood adiposity than maternal hyperglycaemia, requiring further research and identifying maternal BMI as a relevant prevention target in our setting.

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Gestational hyperglycaemia; childhood obesity; childhood adiposity; South Africa; maternal BMI

Introduction

Nutritional changes and the increase of sedentary "modern" urban lifestyles are driving the obesity epidemic in low- and middle-income countries (LMICs) (Wells et al. 2020). Globally, an estimated 40 million children under the age of 5 were impacted by overweight/obesity in 2018, with the majority living in LMICs (Black et al. 2013; UNICEF, WHO, World Bank Group 2018). Aside from the negative physical and socio-emotional consequences of childhood obesity, it is also associated with a higher risk of persistent adulthood obesity, cardiovascular disease, and type 2 diabetes (Lakshman et al. 2012). While overweight/obesity determined by BMI is the most widely recognised surrogate measure for childhood adiposity, measures that distinguish between fat and lean mass provide additional information predictive of future disease risk (Samadi et al. 2013). The search for early life obesity prevention strategies has prompted investigations into pregnancy exposures that contribute to childhood overweight and adiposity (Gillman and Ludwig 2013).

In urban sub-Saharan Africa (SSA), the global obesity epidemic is also heavily impacting women of reproductive age, as exemplified by the overweight/obesity prevalence of around 62% in South African women aged 20–34 years (Black et al. 2013; Onubi et al. 2016; South African National Department of Health et al. 2019). As a result, a rising number of pregnancies is complicated by obesity-associated conditions, on top of existing communicable disease, maternal health, and (childhood) malnutrition challenges (Steyn and Mchiza 2014; Kruger 2018). One such increasingly prevalent condition is hyperglycaemia first detected in pregnancy (HFDP), which consists of less severe gestational diabetes mellitus (GDM) and "overt" diabetes first diagnosed in pregnancy (DIP), according to the 2013 WHO criteria (World Health Organization [WHO] 2013). GDM alone has been

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found to impact up to 9.3% of pregnancies in urban SSA (Oppong et al. 2015; Macaulay, Munthali, et al. 2018; Macaulay, Ngobeni, et al. 2018).

These conditions in pregnancy are thought to confer risk for childhood obesity and overweight through early developmental programming in response to altered intrauterine conditions, exacerbating the problem of childhood adiposity in the next generation (Catalano 2010). Maternal pre-pregnancy obesity itself was found to increase the odds of childhood obesity by 264% in a recent meta-analysis (Heslehurst et al. 2019). In addition, evidence from middle and high-income countries has indicated that maternal HFDP might be an independent risk for childhood obesity (Zhao et al. 2016; Zhu et al. 2016; Logan et al. 2017). However, contrasting findings have also arisen, possibly due to the use of varying diagnostic criteria, follow-up times, and measures of childhood adiposity (Pettitt et al. 2010; Pham et al. 2013; Bider-Canfield et al. 2017). Most recently, evidence from a follow-up study of the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study in 4832 children aged 10–14 years showed an independent association between a continuum of (untreated) maternal blood glucose levels and childhood adiposity (Lowe et al. 2018, 2019). Despite the alarming rate of maternal obesity and the increase in hyperglycaemia in pregnancy in urban SSA, this association remains to be thoroughly explored in an African setting at any age. Pre-school age is of interest in this relationship since SSA has a significant prevalence of pre-school overweight/obesity (Gebremedhin 2015) and interventions at this age have been found to be more effective than in later childhood (Goldfield et al. 2012; Draper et al. 2017).

Considering the growing number of children born to mothers with HFDP, the increasingly obesogenic environments children are exposed to in urban SSA and LMICs elsewhere, and the serious long-term consequences of childhood obesity, it is essential to build evidence to guide childhood obesity prevention efforts in SSA. We therefore aimed to assess the association between HFDP and childhood obesity and adiposity, hypothesising that exposure is positively associated with these outcomes in pre-school aged children from Soweto, South Africa, independently of maternal BMI. Additionally, as a secondary objective, we explored the direct and indirect effects of maternal BMI on childhood obesity and adiposity within the context of HFDP.

Subjects and methods

Study population and hyperglycaemia first detected in pregnancy

Between March and November 2019, we conducted a study of 3–6-year-old children. The exposed and HFDP-unexposed group were defined on the basis of maternal exposure to HFDP, as follows: children were born to mothers who were diagnosed with HFDP (GDM or DIP) at Chris Hani Baragwanath Academic Hospital (CHBAH) (exposed) *vs.* to mothers who attended antenatal care at the same hospital but tested negative for HFDP (HFDP-unexposed), between February 2014 and January 2017 (see Figure 1 for study flow diagram). CHBAH is a tertiary hospital with around 1400–1600 deliveries per month, servicing the urban region

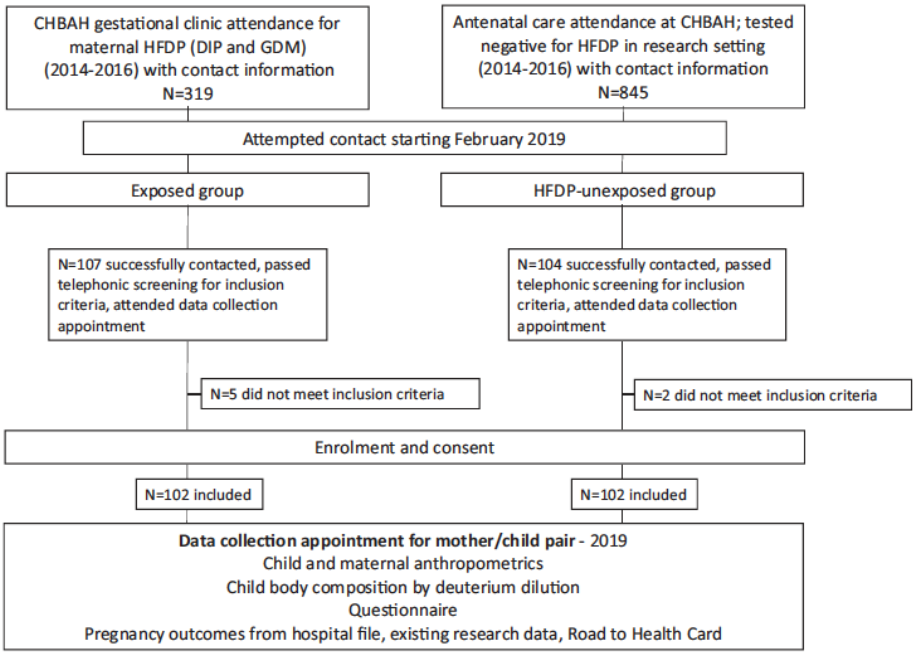

Figure 1. Flow chart depicting study design.

Table 1. Definitions of HFDP, birth outcomes, and anthropometric characteristics at age 3–6 years.

Maternal diagnosis	
HFDP	Diagnosed using a 2-h 75-g OGTT and IADPSG criteria, distinguishing between GDM and overt diabetes in pregnancy (DIP).
GDM	One of the following: fasting plasma glucose ≥5.1 mmol/L, 1-h plasma glucose ≥10 mmol/L, two-hour plasma glucose ≥8.5 mmol/L
DIP	One of the following: fasting plasma glucose ≥7.0 mmol/l, 2-h plasma glucose ≥11.1 mmol/L, random plasma glucose ≥11.1 mmol/l, HbA1c ≥6.5%
Birth outcomes	
Prematurity	Delivery <37 completed weeks gestational age
Low birth weight (LBW)	Birth weight <2500 g
Macrosomia	Birth weight ≥ 4000 g
Large for gestational age	Birth weight >90th percentile, by Intergrowth-21 standards
Anthropometric characteristics at age 3–6 years	
Body mass index (BMI)	Weight (kg) divided by height (m) squared
Obesity	According to WHO definition: For children <5 years BMI z-score higher than 3SD from the mean; for children >5 years BMI z-score higher than 2SD from the mean.
Overweight	According to WHO definition: For children <5 years BMI z-score +2SD to 3SD from the mean; for children >5 years BMI z-score +1SD to 2SD from the mean.
At risk for overweight	According to WHO child growth standards: For children <5 years BMI z-score +1SD to 2SD from the mean.
Stunting	Height lower than −2SD from the mean, according to WHO child growth standards per age.
Fat mass index (FMI)	Estimated fat mass (kg) (by deuterium dilution method) divided by height (m) squared.

of Soweto, where the population consists primarily of Black South Africans (Statistics South Africa 2016).

The mothers of the exposed group attended a gestational endocrine clinic at CHBAH for HFDP while pregnant with the participating child. Clinical characteristics and pregnancy outcomes of this group have previously been published (Soepnel et al. 2019). Diagnostic testing was based on selective screening of risk factors for GDM, except for some women universally screened in a research context (Macaulay, Munthali, et al. 2018; Macaulay, Ngobeni, et al. 2018). Women were diagnosed using a 75-g 2-h oral glucose tolerance test (OGTT) with IADPSG criteria (Table 1).

The HFDP-unexposed group was selected from children born to mothers who attended CHBAH's antenatal clinic during the index pregnancy and tested negative for HFDP in a research setting, using the same diagnostic criteria as the clinic. Further information around diagnosis and participant characteristics has been previously published (Macaulay, Munthali, et al. 2018; Macaulay, Ngobeni, et al. 2018). Mother/child pairs qualifying as the HFDP-unexposed group were contacted in a random order and invited to attend the data collection appointment until the number of mother/child pairs per the child's birth year in the HFDP-unexposed group was the same as in the exposed group.

Exclusion criteria included inability to contact the mother/guardian or attend the data collection appointment (e.g. due to relocation), being one of two twins, diagnosis of major congenital disorders or diabetes mellitus, maternal or child death since pregnancy, maternal diagnosis of pre-gestational diabetes, and age <2.8 years or >6 years old.

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M180317). Written consent was obtained from parents before participation in the study.

Sample size calculation

Sample size calculation for means was performed in STATA version 13 (STATA Corp, College Station, TX). For 80% power at 0.05 significance, the required minimum sample size was 130 participants per group to detect a difference in BMI z-score of 0.35 between the HFDP-unexposed and exposed group, based on existing evidence (ranging from 0.22 to 0.6 with varying study designs (Zhao et al. 2016; Zhu et al. 2016; Lowe et al. 2018; Gu et al. 2019), calculated using population standard deviations (SDs) of 0.93 (5-year-old) and 1.07 (3-year-old) based on a cohort of Sowetan children (Richter et al. 2007). This would also be sufficient to detect a difference in FMI of 0.4 kg/m^2 with a SD of 1 (n=100 per group).

Outcomes

Primary outcomes were overweight (BMI) and adiposity (fat mass index [FMI]). Secondary outcomes were linear growth (height) and stunting (Table 1).

Anthropometric measurements and determining fat mass

Anthropometric measurements were done by a trained research assistant or nurse according to standardised procedures, after removing shoes and any outerwear. The mean of three measurements was used for analyses of weight, height, waist circumference, and mid-upper arm circumference (MUAC). Weight was assessed using a SECA digital scale (Hamburg, Germany), and height was measured using a fixed, mounted Holtain stadiometer (Crymych, UK). The mean weight and height per participant were used to calculate BMI (kg/m^2). Waist circumference was measured excluding clothing using a measuring tape placed halfway between the lower rib and the iliac crest. MUAC was measured halfway between the acromion and the olecranon process. The inter-observer coefficient of variance (CV) for anthropometric measures was <3%. Technical error measurement was <0.7 cm, except for waist circumference where a technical error measurement of <1.5 cm was considered acceptable.

Childhood adiposity was assessed using the deuterium dilution method, according to International Atomic Energy Agency (IAEA) protocol adapted for 2–6-year-old children (IAEA 2010). The deuterium dilution method, a reference method for body composition assessment, measures the total body water (TBW) following a dose of deuterium oxide. The deuterium equilibrates with hydrogen in body water and evenly distributes throughout the TBW after a few hours. Since water is found exclusively in fat-free mass, the level of enrichment in the saliva allows for a deduction of the fat-free mass and, subsequently, fat mass.

The procedure involved taking a baseline saliva sample using cotton wool according to the IAEA method (IAEA 2010), followed by giving the participant an oral 20-g dose of 10% deuterium oxide diluted in water (2 g of deuterium oxide). If any of the dose was lost in this process, the procedure was aborted. Subsequent saliva samples were taken at 2.5–3 h after dosing. Food and drink were limited as follows: the last intake was at least 30 min prior to taking the baseline sample; a light meal and drink of <250 mL was offered 30–60 min after dose consumption; no additional food or drink was consumed until after the final saliva sample. Saliva samples were stored in a −20 °C freezer until deuterium enrichment analysis was performed by a qualified laboratory technician using a portable Fourier-transform infra-red spectrometer (Agilent, 4500 Series, Santa Clara, CA) (IAEA 2010). The criterion for acceptance of the deuterium analysis was a CV <2%, including the analysis of quality control standards at the start and end of the day, and saliva samples. TBW was accepted if the precision of TBW calculated separately from the first and second post-dose samples was <3%. The mean CV for deuterium analysis was 0.5% for the whole study. The mean CV for TBW was 1.1%.

Additional variables

For the exposed group, hospital files from the gestational endocrine clinic provided data regarding maternal OGTT results, BMI at the first clinic visit, maternal HIV infection, and obstetric/birth outcomes (birth weight, gestational age at delivery, and obstetric/early neonatal complications). For the HFDP-unexposed group, the same information was collected from data collected in pregnancy (Macaulay, Munthali, et al. 2018; Macaulay, Ngobeni, et al. 2018) and the child's Road to Health Card, a patient-held medical record given to every child born in South Africa to assist with childhood health monitoring. Maternal BMI was calculated at the data collection appointment using the methods described above for children.

Each mother completed a questionnaire with the help of a research assistant. This provided information on household assets at the child's primary residence (household socioeconomic score), maternal education level, maternal smoking and alcohol use in pregnancy, early life hospitalisations, breastfeeding (whether participant was breastfed, whether participant was breastfed exclusively, and the duration of any breastfeeding), and maternal HIV infection.

Data management and statistical analysis

Data was captured and managed using REDCap (Vanderbilt University, Nashville, TN; (Harris et al. 2009)), and imported into STATA version 13 (College Station, TX). Outcomes for weight-for-age, height-for-age, BMI-for-age, and MUAC-for-age were converted into standard scores (*z*-scores) using WHO growth standards or references (WHO 2006; de Onis et al. 2007). FMI (kg/m^2) was calculated using height and body fat mass from the deuterium dilution method. Birth weight was converted into a *z*-score adjusted for gestational age using the Intergrowth-21 standards (Chatfield et al. 2013). Conditional weight gain was the male or female standardised residual of current weight regressed against birth weight. The participant's household assets were summed for a continuous household socioeconomic status score. Breastfeeding was categorised dichotomously as any breastfeeding *vs.* no breastfeeding. The occurrence of any childhood hospitalisation prior to 2 years old, maternal HIV infection during pregnancy and at the follow-up visit and use of fixed-dose-combination antiretroviral therapy (ART) at follow-up were treated as dichotomous variables. Any statistical outliers were checked for (data capturing) errors but included for analysis if no error was found. Throughout, significance was assumed at a two-sided p value $< .05$.

For the outcomes BMI *z*-score and FMI, multivariable hierarchical regression analysis was performed on an *a priori* basis, as follows: Model (1a) crude model for HFDP exposure; Model (1b) (for FMI) M1a + child sex and age; Model (2) Model 1b + maternal and household factors (maternal BMI in pregnancy, maternal HIV status in pregnancy, parity, alcohol, and smoking during pregnancy, and socioeconomic household score).

In order to explore the direct and indirect effects of maternal BMI in pregnancy on childhood FMI and BMI in the context of HFDP, generalised structural equation modelling (GSEM) was performed to explore the relationship between maternal BMI, HFDP (GDM and DIP), other significant maternal/household and mediating variables, and FMI/BMI, fitted with the multinomial family and logit link function. GSEM was performed because it allows for a pictographic representation of hypothesis-driven relationships between variables, including potential mediators, confounders, and composite latent variables (not applicable for this dataset). GSEM estimates path equations simultaneously, and allows for calculations of direct, indirect, and total effects (Lei and Wu 2007). We started with a hypothesised model, based on theoretical meaning and results from regression analyses, as shown in Figure S1. Modifications to pathways and adding/removing variables were made iteratively and the Aikake and Bayesian Information Criteria (IC) of each model was compared. The final model was selected for having a low IC and high theoretical relevance (Figure 2, Table S1). Direct, indirect, and total effects were calculated using non-linear combination estimates.

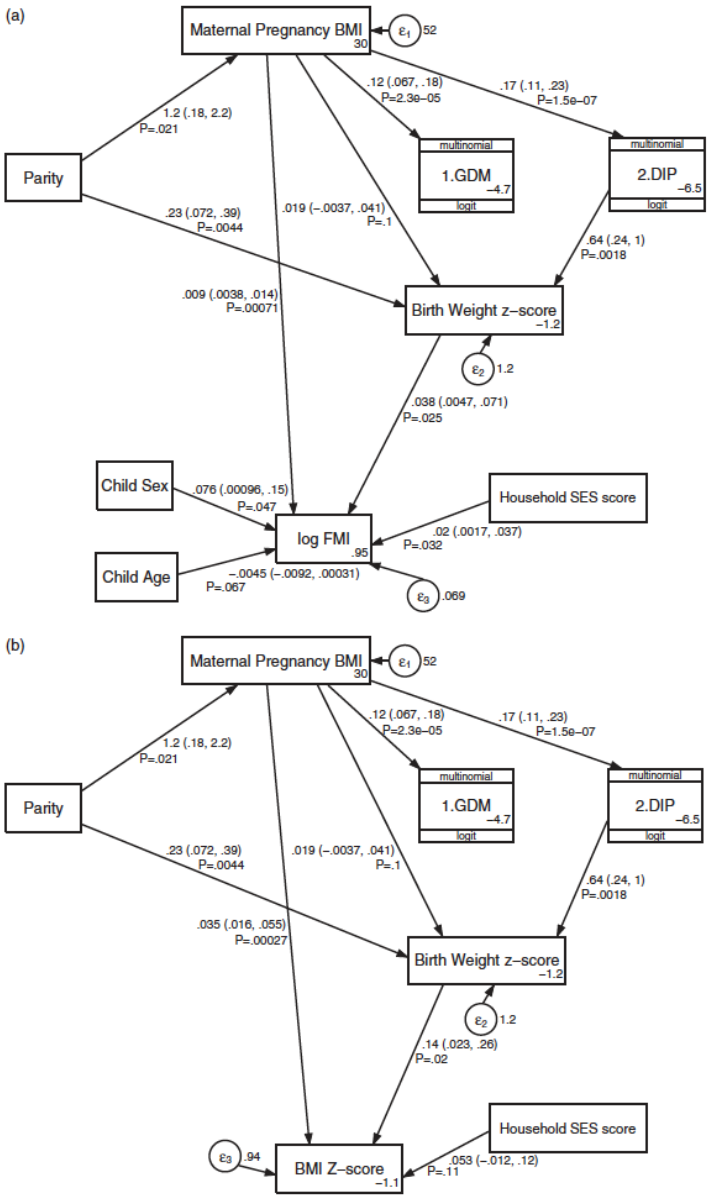

Figure 2. GSEM best-fit model of maternal and household variables, birth weight, and GDM/DIP for (a) log-FMI, adjusting for sex and age, and (b) BMI z-score. Unstandardised coefficient (95% CI) and *p* values are displayed.

Results

Baseline characteristics

Of 211 participants that attended the data collection appointment, 7 did not meet the inclusion criteria, resulting in 204 included participants.

The median age of participants was similar between the HFDP-unexposed and exposed groups at 3.5 years (IQR 3.1–4.1) and 3.4 years (IQR 3.1–4.1), respectively (Table 2). Of the participants exposed to HFDP, 45 (44.1%) were exposed to DIP, and 57 (55.9%) to GDM. The number of participants born *via* caesarean section was high in both groups at 58.8%

Table 2. Baseline characteristics of participants per group.

				Hyperglycaemia first detected in pregnancy				
	n	HFDP-unexposed	*n*	Total group	*n*	GDM	*n*	DIP
Number of participants	–	102	–	102	–	57	–	45
Age, year (median, IQR[a])	102	3.5 (3.1–4.1)	102	3.4 (3.1-4.1)	57	3.3 (3.0-4.0)	45	3.7 (3.2–4.2)
Male (*n* %)	102	49 (48.0)	102	56 (54.9)	57	30 (52.6)	45	26 (57.8)
Early life factors								
Received breastfeeding (*n*%)	102	82 (80.4)	102	73 (71.6)	57	39 (68.4)	45	34 (75.6)
Exclusive breastfeeding	102	77 (75.5)	102	62 (60.8)	57	34 (59.7)	45	28 (62.2)
Hospitalised <2 years (*n*%)	102	20 (19.6)	102	15 (14.7)	57	11 (19.3)	45	4 (8.9)
Pregnancy factors								
OGTT[b] results (mean, SD[c])	102		95		56		39	
Fasting		4.0 (0.5)		6.7 (1.9)		5.6 (0.7)		8.2 (2.0)
2-hour		5.4 (1.2)		10.5 (3.5)		8.6 (1.7)		13.1 (3.6)
HbA1c (median, IQR)		–	94					
First visit %				6.3 (5.6-7.1)	51	5.7 (5.3–6.1)	43	7.0 (6.6-8.2)
3rd trimester %				6.1 (5.6-6.6)	50	5.8 (5.5–6.3)	42	6.6 (6.1–7.3)
Exposure to: (*n*, %)		–	102		57		45	
Metformin				63 (61.8)		39 (68.42)		24 (53.3)
Glibenclamide				30 (29.4)		13 (22.8)		17 (37.8)
Insulin				32 (31.4)		7 (12.3)		25 (55.6)
Gestational age OGTT (median, IQR)	102	26 (25–27)	102	30.4 (25.6–33.6)	57	30.6 (27.3-34.1)	45	30.1 (2–32.6)
Parity (*n*, %): 0	102	36 (35.3)	102	12 (11.8)	57	8 (14.0)	45	4 (8.9)
1–2		59 (57.8)		78 (76.5)		43 (75.4)		35 (77.8)
>3		7 (6.9)		12 (11.8)		6 (10.5)		6 (13.3)
BMI at OGTT (median, IQR)	102	29.4 (24.8-33)	102	35.1 (30.8–40.0)	52	33.9 (30.5-37.5)	42	37.6 (32.6–41.3)
HIV-infection pregnancy (*n*, %)	102	20 (19.6)	102	17 (16.7)	57	13 (22.8)	45	4 (8.9)
Caesarean section (*n*, %)	102	60 (58.8)	99	69 (69.7)	56	40 (71.4)	43	29 (67.4)
Obstetric complication (*n*, %)	102	11 (10.8)	102	15 (14.7)	57	6 (10.5)	45	9 (20.0)
Macrosomia (*n*, %)	102	5 (4.9)	99	5 (5.1)	56	3 (5.4)	43	2 (4.7)
Large for gestational age (*n*, %)	102	11 (10.8)	99	21 (21.2)	56	6 (10.7)	43	15 (34.9)
Low birth weight (*n*, %)	102	17 (16.7)	99	16 (16.2)	56	10 (17.9)	43	6 (14.0)
Prematurity (*n*, %)	102	13 (12.8)	102	22 (21.6)	57	11 (19.3)		11 (24.4)
Maternal and household characteristics at visit 3–6 years postpartum								
Highest education level	101		102		57		45	
1 None/primary school		0		3 (2.9)		2 (3.6)		1 (2.2)
2 Secondary school		73 (72.3)		66 (64.7)		40 (70.2)		26 (57.8)
3 Prof. training/university		28 (27.7)		33 (32.4)		15 (26.3)		18 (40.0)
Household SES[d] (mean, SD)	102	8.6 (2.1)	102	8.0 (2.2)	57	7.8 (2.1)	45	8.3 (2.3)
Maternal BMI (mean, SD)	98	30.1 (7.4)	95	33.8 (7.1)	54	33.2 (7.1)	41	34.6 (7.3)
Blood pressure >140/90 (*n*, %)	102	20 (19.6)	102	37 (36.3)	57	16 (28.1)	45	21 (46.7)
HIV-infection (*n*, %)	102	23 (22.6)	102	20 (19.6)	57	15 (26.3)	45	5 (11.1)
Taking FDC[e] (*n*, %)	19	17 (89.4)	20	16 (80.0)	15	12 (80.0)	5	4 (80.0)

[a]IQR: interquartile range; [b]OGTT: oral glucose tolerance test; [c]SD: standard deviation; [d]SES: socioeconomic score; [e]FDC: fixed dose combination antiretroviral medication.

(*n*=60) and 69.7% (*n*=69) in the HFDP-unexposed and HFDP-exposed group, respectively. The rate of prematurity was higher in the HFDP-exposed group than the HFDP-unexposed group (12.8% (*n*=13) *vs.* 21.6% (*n*=21.6), and highest in the DIP group at 24.4% (*n*=11). Macrosomia rates were notably similar between the HFDP-unexposed and HFDP exposed groups, but the birth weight *z*-score adjusted for gestational age was higher in the DIP group (Table 3). Breastfeeding was received in 80.4% (*n*=82) of the HFDP-unexposed group but only 71.6% (*n*=73) of the HFDP-exposed group.

At the time of the pregnancy OGTT, mothers with DIP had higher BMI, age, and fasting plasma glucose levels. The number of primiparous women was higher in the HFDP-unexposed group than in the HFDP-exposed group (35.3 *vs.* 11.8%). In total, only six mothers reported smoking during pregnancy (2.9%), and two reported alcohol consumption during pregnancy (0.9%). The majority of mothers diagnosed with DIP or GDM were managed with medical therapy, including metformin or glibenclamide, rather than diet (*n*=88, 86.3%). Upon measurement at the postpartum visit, maternal BMI was high across all three groups (median 31.4, IQR 26.7–36.1). The total maternal HIV infection rate at the follow-up visit was 43 (21.1%), with 86.0% diagnosed at or before the index pregnancy (*n*=37).

Childhood overweight and adiposity

In the HFDP-unexposed group, four (3.9%) children were overweight/obese by WHO definitions, compared to six (10.5%) and five (11.1%) in the GDM and DIP groups, respectively (Table 3). Additionally, 22.6% (*n*=23) of the HFDP-unexposed group and 17.7% (*n*=18) were "at risk of overweight." BMI z-score, waist circumference by height, and height z-score were higher in the DIP group. The mean weight *z*-score was lower in the HFDP-unexposed group (−0.3±0.9) than in the HFDP-exposed groups (−0.1± 1.1). Although fat-free mass index was similar between the groups (12.2±0.7 and 12.1± 1.1 in the HFDP-unexposed and HFDP-exposed groups, respectively), FMI was highest in the DIP exposed group at 4.0 (IQR 3.2–4.7), compared to 3.5 (IQR 3.1–4.2) in the HFDP-unexposed group. The highest rate of stunting at 9 (15.8%) was found in the GDM group.

For multivariable regression analyses, none of the included variables showed multicollinearity, with variance

Table 3. Infant and childhood anthropometric and fat mass outcomes per group: HFDP-unexposed *vs.* HFDP, subdivided into GDM and DIP.

			HFDP-exposed					
	n	HFDP-unexposed	*n*	Total group	*n*	GDM	*n*	DIP
Birth weight, g (median, IQR)	102	3040 (2800–3350)	99	3100 (2670–3100)	56	3042.5 (2667–3327)	43	3170 (2765–3685)
Birth weight *z*-score[a] (mean, SD)	102	−0.1 (1.2)	99	0.3 (1.1)	56	−0.02 (1.1)	43	0.7 (1.1)
Body composition by anthropometry								
Weight, kg (median, IQR)	102	14.8 (13.5–16)	102	15 (13.7–16.8)	57	14.7 (13.5–16.4)	45	15.2 (14.2–16.9)
Weight *z*-score (mean, SD)	102	−0.3 (0.9)	102	−0.1 (1.1)	57	−0.1 (1.1)	45	−0.1 (1.1)
Conditional weight gain (median, IQR)	102	−0.3 (−0.6–0.4)	99	−0.2 (−0.6–0.5)	56	−0.2 (−0.8–0.4)	43	−0.1 (−0.5–0.7)
BMI, kg/m^2 (median, IQR)	102	15.6 (14.9–17.0)	102	16.0 (15.2–16.8)	57	15.9 (15.3–16.8)	45	16.0 (15.1–16.8)
BMI z-score (mean, SD)	102	0.3 (0.9)	102	0.5 (1.2)	57	0.5 (1.1)	45	0.6 (1.2)
At risk for overweight		23 (22.6)		18 (17.7)		10 (17.5)		8 (17.8)
Overweight		3 (2.9)		9 (8.8)		5 (8.8)		4 (8.9)
Obese		1 (1.0)		2 (2.0)		1 (1.8)		1 (2.2)
MUAC[b], cm (mean, SD)	102	16.5 (1.22)	101	16.7 (1.6)	56	16.5 (1.5)	45	17.0 (1.7)
MUAC *z*-score	97	0.2 (−0.2–0.8)	98	0.4 (−0.3–1.0)	55	0.3 (−0.4–0.9)	43	0.6 (−0.0–1.2)
WC[c] (mean, SD)	102	49.8 (3.0)	100	50.9 (4.3)	55	50.5 (3.9)	44	51.4 (4.7)
WC by height index, cm/m	102	0.5 (0.04)	100	0.5 (0.04)	55	0.5 (0.04)	44	0.5 (0.05)
Fat mass by deuterium method								
FFM[d], kg (mean, SD)	102	11.6 (1.6)	100	11.5 (1.8)	56	11.5 (1.9)	44	11.6 (1.5)
% (median, IQR)		76.7 (74.4–79.6)		76.3 (72.4–78.8)		76.1 (73.1–78.8)		76.3 (70.1–78.8)
FFMI (mean, SD)	102	12.2 (0.7)	100	12.1 (1.1)	56	12.2 (1.2)	44	12.0 (1.0)
FM, kg (median, IQR)	102	3.4 (2.9–4.1)	100	3.6 (3.0–4.5)	56	3.5 (2.9–4.4)	44	3.8 (3.1–4.6)
% (Median, IQR)		23.3 (20.4–25.6)		23.7 (21.2–27.6)		23.9 (21.2–26.9)		23.7 (21.2–29.9)
FMI (median, IQR)	102	3.5 (3.1–4.2)	100	3.9 (3.2–4.5)	56	3.8 (3.3–4.4)	44	4.0 (3.2–4.7)
Height								
Height, cm (mean, SD)	102	97.3 (6.5)	102	97.3 (6.8)	57	96.8 (7.2)	45	97.9 (6.2)
Height *z*-score (mean, SD)	102	−0.8 (0.9)	102	−0.8 (1.1)	57	−0.7 (1.2)	45	−0.8 (1.0)
Stunted (*n*, %)	102	10 (9.8)	102	14 (13.7)	57	9 (15.8)	45	5 (11.1)

All z-scores and cut-offs according to WHO standards. [a]Birth weight z-score adjusted for sex and gestational age (Intergrowth 21); [b]MUAC: mid-upper arm circumference; [c]WC: waist circumference; [d]FFM: fat-free mass; FFMI: fat-free mass index; FM: fat mass; FMI: fat mass index.

Table 4. Linear regression model for BMI z-score and log-transformed FMI, reporting standardised regression coefficients (β) with 95% confidence interval, and *p* values.

	M1a: crude model		M1b: N/A		M2: M1 + maternal factors	
Outcome: BMI *z*-score	β (95% CI)	*p* Value			β (95% CI)	*p* Value
Hyperglycaemia first detected in pregnancy						
True GDM	0.075 (−0.167–0.518)	.312			0.067 (−0.202–0.511)	.393
DIP	0.099 (−0.121–0.621)	.185			−0.001 (−0.402–0.395)	.987
Maternal and household factors						
Maternal pregnancy BMI					0.292 (0.019-0.063)	<.001*
Parity					−0.049 (−0.207–0.104)	.518
Socioeconomic household score					0.100 (−0.020–0.117)	.163
HIV positive during pregnancy					−0.014 (−0.246–0.421)	.848
	N 204; adj. R^2 0.1%				*N* 196; adj. R^2 7.0%	
Outcome: log-transformed FMI	M1a: crude model		M1b: crude model + sex + age		M2: M1b + maternal factors	
	β (95% CI)	*p* Value	β (95% CI)	*p* Value	β (95% CI)	*p* Value
Hyperglycaemia first detected in pregnancy						
True GDM	0.103 (−0.028–0.159)	.167	0.101 (−0.028–0.157)	.169	0.099 (−0.034–0.161)	.200
DIP	0.166 (0.014–0.217)	.026*	0.182 (0.026–0.226)	.014*	0.117 (−0.029–0.191)	.148
Child age			−0.088 (−0.008–0.002)	.206	−0.133 (−0.010–0.000)	.059
Child sex (female)			0.172 (0.021–0.177)	.014*	0.133 (−0.002–0.152)	.058
Maternal and household factors						
Maternal pregnancy BMI					0.226 (0.003–0.015)	.004*
Parity					−0.093 (−0.070–0.016)	.221
Socioeconomic household score					0.129 (−0.002–0.036)	.072
HIV positive during pregnancy					−0.034 (−0.130–0.080)	.641
	N 202; adj. R^2 1.7%	*N* 202; adj, R^2 4.7%			*N* 194; adj. R^2 10.0%	

*Indicates *p* value <.05.

inflation factors <2 for each model. Due to low rates of maternal alcohol consumption and smoking during pregnancy (n=2 and 6, respectively), these variables were not included in presented analyses. FMI was log-transformed to increase normality of residuals.

Log-FMI was higher in the children exposed to HFDP, even after correcting for age and sex, significantly so in the DIP group (β = 0.18 , 95% CI 0.03–0.23, p= .014) (Table 4). In this model, being female was associated with having a higher FMI (β = 0.17, 95% CI 0.02–0.18, p= .014). In model 2, correcting for maternal/household factors, DIP-exposure was no longer significantly associated with log-FMI, and maternal BMI during pregnancy had the strongest association with log-FMI (β = maternal BMI = 0.23, 95% CI 0.003–0.015,

$p = .004$). Household socioeconomic score was positively associated with log-FMI ($\beta = 0.13$, 95% CI −0.002–0.036, $p = .072$).

The (non-statistically significant) positive association between DIP/GDM and BMI *z*-score was attenuated in Model 2, with DIP exposure showing a slight inverse association with BMI ($\beta = -0.001$, 95% CI −0.402–0.395, $p = .987$). Maternal BMI was the main significant predictor of BMI *z*-score ($\beta = 0.29$, 95% CI 0.02–0.06, $p = <.001$). Maternal HIV infection was not significantly associated with either BMI or log-FMI. No significant interaction effect was found between maternal BMI and HFDP, for either BMI or log-FMI.

In an additional analysis of early life variables, birth weight *z*-score was significantly associated with both log-FMI and BMI z-score when additionally introduced to Model 2 ($\beta = 0.16$, 95% CI 0.004–0.074, $p = .028$, $\beta = 0.18$, 95% CI 0.035–0.291, $p = .013$, respectively), but breastfeeding was not.

GSEM showed a significant direct effect of maternal pregnancy BMI on log-FMI and BMI, and the total effect through DIP and birth weight was also significant (Figure 2). Birth weight *z*-score was significantly associated with both FMI and BMI (Unstandardized coefficient B = 0.04 95% CI 0.005–0.07, $p = .025$; B = 0.14, 95% CI 0.02–0.26, $p = .020$). The total effect of GDM-exposure was not significant for FMI or BMI, and there was no significant association between GDM and birth weight *z*-score. DIP-exposure had a significant positive effect on birth weight z-score, but the total DIP-effect on FMI/BMI was not significant (Figure 2 and Table S1). Household socioeconomic score had a positive total effect on log-FMI (B = 0.02, 95% CI 0.002–0.04, $p = .032$). In GSEM, parity had an indirect and total effect on BMI/log-FMI through the maternal BMI, DIP-exposure, and birth weight z-score pathway. No significant association was found between parity and childhood BMI/log-FMI in regression analysis (Table 4), which adjusts for the pathway variables rather than calculating indirect effects through them, as in GSEM.

Discussion

In our study, the association between exposure to HFDP and subsequent adiposity (FMI) in 3–6-year-old children was not independent of maternal BMI. The association between HFDP exposure and childhood BMI was not statistically significant. Maternal BMI, which was found to have a direct effect on childhood adiposity and BMI, may therefore be of greater concern than HFDP at pre-school age in our setting in SSA.

Previous literature from high- and middle-income countries presents a heterogeneous picture of the impact of HFDP on childhood BMI. While some studies have found an independent effect of HFDP on BMI and overweight/obesity (Nehring et al. 2013; Hillier et al. 2016; Zhu et al. 2016; Grunnet et al. 2017; Tam et al. 2017; Wang et al. 2018; Gu et al. 2019), others showed no significant association (Pettitt et al. 2010; Pham et al. 2013; Bider-Canfield et al. 2017; Kaseva et al. 2018; Kearney et al. 2018; Lowe et al. 2018; Pitchika et al. 2018). Interestingly, some studies found a significant association in school-aged children and adolescents that was not evident in earlier childhood (Pettitt et al. 2010; Crume et al. 2011; Zhu et al. 2016), possibly due to the accumulation of environmental and behavioural risks for obesity throughout childhood (Silverman et al. 1991; Crume et al. 2011). The overweight/obesity prevalence in our cohort did not exceed national estimates of 11–13%, but 26% of the HFDP-unexposed and 28–29% of the exposed group were at least "at-risk of overweight," raising concerns for progression to overweight/obesity in later childhood (South African National Department of Health et al. 2019). Since exposure to additional obesogenic factors might not occur in the same pattern in high *vs.* low-middle income countries, a longitudinal prospective analysis in our setting would provide valuable additional insight.

A number of recent studies exploring adiposity, as opposed to BMI or overweight status alone, found a positive association with HFDP (Chandler-Laney et al. 2012; Zhao et al. 2016; Kaseva et al. 2018; Kearney et al. 2018; Lowe et al. 2018). This was mirrored in our results by the significant unadjusted association between DIP-exposure and childhood FMI. Exposure may thus impact fat rather than lean mass, increasing adiposity ahead of a detectable difference in weight or BMI (Chandler-Laney et al. 2012; Lowe et al. 2018). This has particular clinical relevance because adiposity is associated with increased metabolic risk (Shah et al. 2014), potentially contributing to the rise in early onset of metabolic conditions, including Type 2 diabetes (Jensen and Dabelea 2018; Twig et al. 2020).

However, we did not find the association with FMI to be significant in the less severely hyperglycaemic GDM group. This finding should be interpreted in the context of changing diagnostic criteria, since existing evidence is largely based on definitions of "GDM" that align more closely with our DIP group. A significant impact of GDM by the lower IADPSG criteria used in our study has only been previously shown in an untreated context by the HAPO follow-up studies, in 10–14-year-old children. In pre-school-aged children of mothers with treated GDM, we could not corroborate their findings that an effect of maternal glucose levels on childhood adiposity is evident even at levels lower than those diagnostic of GDM (Lowe et al. 2019).

The majority of existing studies show an attenuating effect of maternal BMI on the association between HFDP and childhood adiposity and overweight (Patel et al. 2012; Zhao et al. 2016; Grunnet et al. 2017; Gingras et al. 2018; Kearney et al. 2018; Lowe et al. 2018), and a number of these, including two systematic reviews (Kim et al. 2002; Kawasaki et al. 2018), also found no remaining significant independent effect of maternal hyperglycaemia. It has been suggested that the effect of HFDP might be more pronounced in normal weight maternal populations, based on a stratified analysis of the impact of HFDP in normal weight, overweight, and obese mothers within the Danish Birth Cohort (Zhu et al. 2016; Grunnet et al. 2017). The consequences of high maternal BMI in cohorts such as ours might thus override consequences of HFDP, although more evidence is needed to corroborate this. The significant impact of maternal pregnancy BMI on childhood overweight and adiposity

(Gebremedhin 2015; Heslehurst et al. 2019) is likely due, in part, to shared genetics and lifestyle. However, foetal programming in response to intrauterine exposures, such as the maternal metabolome (Kadakia et al. 2019), has also been shown to play a role (Lakshman et al. 2012). In particular, based on both animal (Ren et al. 2018) and human studies (Boyle et al. 2017; Hjort et al. 2018), programming may occur through DNA methylation, while changes to hypothalamic development and functioning have additionally been suggested as a possible mechanism (Page et al. 2019). As a modifiable risk factor with alarming prevalence in our participants and South African women of reproductive age in general (Kruger 2018), our findings highlight the importance of maternal obesity as a major target for (preconception) prevention to improve both maternal and child health (Ware et al. 2019).

In GSEM analysis, birth weight was shown to significantly impact BMI and FMI. Changes to an array of nutrients during a hyperglycaemic pregnancy, as opposed to glucose dysregulation and subsequent foetal hyperinsulinaemia alone, are thought to be responsible for excess foetal growth through intrauterine programming (Freinkel 1980; Barbour and Hernandez 2018). While exposure to DIP significantly increased birth weight, exposure to GDM did not. Treatment of hyperglycaemia in pregnancy, including early induction of labour to reduce the risk of macrosomia (Soepnel et al. 2019), could have restricted birth weight and thereby minimised differences in FMI/BMI, particularly in the group exposed to GDM (Macaulay, Munthali, et al. 2018; Macaulay, Ngobeni, et al. 2018). However, this does not rule out the possibility of a programming effect manifesting in later childhood in our population, especially since foetal changes have been measured prior to treatment initiation, at 16–18 weeks' gestation, in a population similar to our GDM group (Macaulay, Munthali, et al. 2018; Macaulay, Ngobeni, et al. 2018). Further research into the timing and biological mechanisms of such programming is needed in our setting.

A higher household socioeconomic score was positively associated with children's FMI in GSEM and linear regression, a relationship typically described in LMICs as opposed to the inverse association found in high-income countries (Wang 2001). The early life exposures involved in this require further exploration, especially in light of recent findings suggesting an association between GDM, parent-reported nutritional risk score at 2 years, and metabolic risk at 5 years (Patel et al. 2020). Of early childhood nutritional exposures, breastfeeding has been shown to decrease childhood adiposity, including in rural South Africa (Houle et al. 2014), but the role in hyperglycaemic pregnancies is less clear (Kaul et al. 2019), and we found no association with breastfeeding and FMI/BMI in our study.

We did not find an association between HIV infection and childhood BMI/FMI. Although body composition trends of HIV-exposed, uninfected children (HEU) remain unclear, previous evidence has indicated that HEU children who are obese may have inferior cardiovascular outcomes compared to unexposed obese children, and maternal ART may play a role (Jao et al. 2019), possibly mirroring the metabolic effect of certain ART regimens on mothers (Soepnel et al. 2017). In our study, the majority of HIV-infected women were likely on ART during pregnancy. However, the impact of maternal HIV-exposure and -treatment on such childhood outcomes requires exploration in a larger sample of HEU children with more detailed data on maternal ART use.

A strength of this study was the use of a reference method for determining fat mass, which allowed us to explore both obesity and adiposity. Another strength is the findings' relevance to African settings, where data on the topic are scarce but increasingly important. This includes exploring the rate of stunting, which was similar to regional averages of 11.9% in HFDP-unexposed and DIP groups, and slightly higher in the GDM group at 15.8% (Shisana et al. 2013). Stunting remains a large public health problem in South Africa (Said-Mohamed et al. 2015), and, as an example of the double burden of malnutrition, children exposed to maternal hyperglycaemia do not seem to be less vulnerable to stunting at 3–6 years old.

Difficulties tracing participants 3–6 years after delivery, mostly due to relocation or change of contact details, resulted in a lower sample size and hence less power for between-group comparisons, particularly for the GDM and DIP subgroups. As a result, the study was not powered to assess between-sex differences or the role of HFDP treatments on the outcomes. Another limitation is that, since the study design was not a prospective pregnancy cohort, the pregnancy and early childhood data was retrospective, and we were consequently unable to explore the role of childhood nutrition, physical activity/sedentary behaviour, or paternal factors. Furthermore, the higher gestational age at OGTT in the exposed group opens up the possibility that a small number of participants might have been sorted into a different group if the timing of OGTT's was the same between the two groups (whether later in the HFDP-unexposed, or earlier in the exposed group). This may have minimised differences between the groups and resulting outcomes. Lastly, we did not have data for maternal pre-pregnancy BMI or gestational weight gain. By using BMI measured during pregnancy, weight gain during pregnancy may have resulted in an overestimation of maternal BMI, but there was no significant interaction between pregnancy BMI and the gestational age at measurement.

This study is, to our knowledge, the first in an SSA population to compare BMI and adiposity at pre-school age in children exposed and unexposed to HFDP. The relevance of our findings extends to LMICs similarly experiencing an increase in maternal hyperglycaemia and obesity. We did not find a significant difference in FMI or BMI at 3–6 years old when correcting for maternal BMI. Birth weight, which was limited in the HFDP group by clinical management, likely impacts adiposity and BMI at this age, and additional longitudinal data is required to further explore these associations into later childhood in our setting. Our results also indicate that changes in children's adiposity may reflect before a difference in BMI is detectable at pre-school age. The association of maternal BMI with childhood adiposity raises concerns for an intergenerational cycle of obesity in this

urban African population and calls for increased efforts to curb maternal obesity for promotion of both maternal and child health.

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Ethical standards

The authors assert that all procedures contributing to this work comply with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by the Human Research Ethics Committee of the University of the Witwatersrand (ref: M180317). The parent and/or legal guardian of each participant gave written informed consent for participation in the study prior to the start of any study procedures. Participant data was fully anonymised to protect participants' privacy.

Author contributions

SAN, KKG, NL, VN, and LMS contributed to the research conceptualisation. VN and LMS contributed to the study project coordination and data collection. CS advised on the deuterium dilution method and assisted with calculations of body composition. GC contributed to statistical methodology. LMS contributed to data analysis and wrote the initial manuscript version, and all the authors contributed to interpretation of data, editing, and revising the manuscript. All authors had final approval of this submitted version.

Disclosure statement

The authors have no conflict of interest to declare.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, LMS, upon reasonable request.

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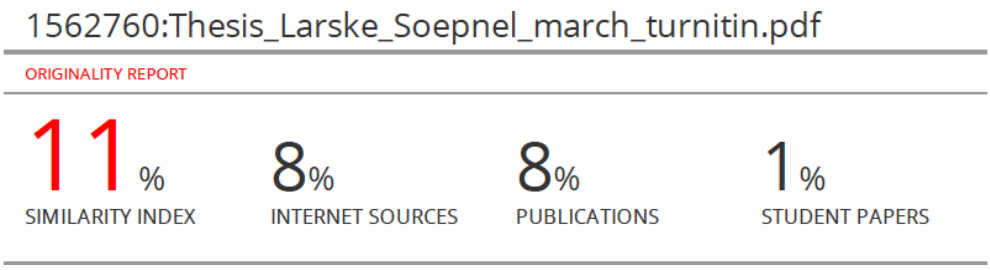
Appendix M: Turnitin report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