MATERNAL NUTRITION DURING PREGNANCY AND ITS ASSOCIATION WITH BIRTH OUTCOMES AND NEONATAL BODY COMPOSITION IN THE CONTEXT OF HIV IN URBAN BLACK SOUTH AFRICANS

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A THESIS

Submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in the fulfilment of the requirements for the degree of

Doctor of Philosophy

JOHANNESBURG, SOUTH AFRICA

2018

DECLARATION

I, Stephanie Victoria Wrottesley, 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.

Signed: 

On the 2nd day of June 2018, in Johannesburg

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Chapter Four (Article published in Eur J Clin Nutr. 2016)

Wrottesley SV, Pisa PT, Micklesfield LK, Pettifor JM, Norris SA. A comparison of body composition estimates using dual-energy x-ray absorptiometry and air-displacement plethysmography in South African neonates. Eur J Clin Nutr. 2016 Nov;70(11):1254-1258

Chapter Five (Article published in Nutrients. 2017)

Wrottesley SV, Pisa PT, Norris SA. The influence of maternal dietary patterns on body mass index and gestational weight gain in urban black South African women. Nutrients. 2017 Jul 11;9(7).

Chapter Six (Article accepted in Br J Nutr. 2018)

Wrottesley SV, Ong KK, Pisa PT, Norris SA. Maternal traditional dietary pattern and antiretroviral treatment exposure are associated with neonatal size and adiposity in urban, black South Africans. Br J Nutr (accepted: 30/05/2018).

OTHER RELATED PEER REVIEWED PUBLICATIONS

Prioreschi A, Wrottesley S, Draper CE, Tomaz SA, Cook CJ, Watson ED, et al. Maternal and early life nutrition and physical activity: setting the research and intervention agenda for

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PRESENTATIONS BY PhD CANDIDATE

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The importance of nutrition in the first 1000 days. *Postgraduate lunchtime talks, Department of Paediatrics, University of the Witwatersrand Faculty of Health Sciences, Chris Hani Baragwanath Academic Hospital (2016)*

Importance of the first 1000 days. *South African Medical Research Council (SAMRC) quinquennial review (QQR) of the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU), Chris Hani Baragwanath Academic Hospital (2015)*

HONOURS AND GRANTS AWARDED TO PhD CANDIDATE

DST-NRF Centre of Excellence in Human Development Doctoral Bursary. *University of the Witwatersrand. (2015-2017)*

RCUK Newton Fund PhD Partnering Scheme: non-communicable disease epidemiology and public health. *MRC Epidemiology Unit, University of Cambridge and Developmental Pathways for Health Research Unit (DPHRU), University of the Witwatersrand. (2017)*

African Nutrition Leadership Programme (ANLP) Course Fee and Travel Grant. *DST-NRF Centre of Excellence in Nutrition, North-West University. (2017)*

DST-NRF Centre of Excellence in Food Security scholarship to attend the 2nd International Congress of the World Public Health Nutrition Association. *University of the Western Cape. (2016)*

ACRONYMS AND ABBREVIATIONS

ADP	Air displacement plethysmography
AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral treatment
BMC	Bone mineral content
BMI	Body mass index
CHBH	Chris Hani Baragwanath Academic Hospital
CoE	Centre of Excellence
CVD	Cardiovascular disease
DA	Dietary assessment
DOHaD	Developmental origins of health and disease
DPHRU	Developmental Pathways for Health Research Unit
DXA	Dual-energy x-ray absorptiometry
FFQ	Food frequency questionnaire
FMI	Fat mass index
GDM	Gestational diabetes mellitus
GDP	Gross domestic product
GWG	Gestational weight gain
Hb	Haemoglobin
HICs	High income countries
HIV	Human immunodeficiency virus
IDA	Iron deficiency anaemia
IoM	Institute of Medicine
IUGR	Intrauterine growth restriction
LBW	Low birth weight
LGA	Large-for-gestational age

LMICs	Low-or middle-income countries
MDGs	Millennium Development Goals
MNS	Maternal nutritional status
MRC	Medical Research Council
MUAC	Mid-upper arm circumference
NCDs	Non-communicable diseases
PCA	Principal component analysis
PMTCT	Prevention of Mother-to-Child Transmission
QFFQ	Quantitative food-frequency questionnaire
RCT	Randomised controlled trials
RDA	Recommended daily allowance
S1000	Soweto First 1000-Day Study
SADHS	South African Demographic and Health Survey
SBGS	Soweto Baby Growth Study
SDGs	Sustainable Development Goals
SES	Socioeconomic status
SFGS	Soweto Fetal Growth Study
SGA	Small-for-gestational-age
SMART	Specific, measurable, achievable, relevant and time-bound
T2DM	Type 2 diabetes mellitus
TB	Tuberculosis
UN	United Nations
UNICEF	United Nations Children's Fund
WHO	World Health Organisation
WLR	Weight-to-length ratio

ACKNOWLEDGEMENTS

There are numerous people who have made this PhD possible over the last three years, but I would specifically like to extend my thanks and appreciation to the following:

To my family who, although being in a different city, have been my most constant foundation and support through this process. Firstly, to my sister, Alex, who is always armed with positivity when I need it. Secondly, to my mom and stepdad who have travelled this path with me every step of the way; making endless sacrifices and providing the unwavering support that has allowed me to take this journey. It is impossible to thank my mom enough for the unlimited patience, reassurance and encouragement she gives me, as well as for always believing in me and my capabilities.

To my supervisors, Dr Pedro Pisa and Prof Shane Norris, for making this PhD possible and for sharing valuable guidance and expertise that helped to shape my PhD work. A special thank you to Shane in particular for all of the opportunities he has given me to learn and to gain experience in field of research.

To Prof Ken Ong and the Growth and Development Team at the MRC Epidemiology Unit (University of Cambridge) for hosting me during a stimulating and inspiring exchange programme in Cambridge. My PhD experience was all the richer for the time spent at the unit and many thanks to Ken in particular for providing valuable supervision, guidance and feedback during this time.

To A/Prof Lisa Micklesfield for her committed support during my early days with the Developmental Pathways for Health Research Unit (DPHRU) and for the enthusiasm she had for me to pursue a PhD, as well as her assistance in shaping my PhD path. In addition, thank

you to Lisa, Prof John Pettifor, Prof Ken Ong and Cynthia Lamper for the valuable contributions made as co-authors on my PhD publications.

To the Soweto First 1000-Day Study (S1000) team for all of their hard work and for sharing expertise and experience with me along the way; particular thanks to Thokozile Lephoto and Thabile Sibiya for their assistance as co-ordinators of the pregnancy and neonatal projects respectively. Additionally to Martha Manonga for being my office companion during long days of data collection and capturing, as well as Dr Alessandra Prioreschi for always being willing to provide input and advice when I needed it and for being a constant ear and support throughout this process.

Finally, to the funders who made this PhD possible: Linda Richter and the Centre of Excellence (CoE) in Human Development who funded my PhD bursary and provided opportunities to attend conferences and to network with other students and researchers across the three year period; Prof Shane Norris and DPHRU for additional support during the PhD, including opportunities to attend training programmes and conferences; RCUK Newton Fund PhD Partnering Scheme (joint award to MRC Epidemiology Unit, University of Cambridge and DPHRU) for funding my exchange trip to Cambridge.

PREFACE

"The food you eat can either be the safest and most powerful form of medicine…or the slowest form of poison." ~Ann Wigmore

My interest in nutrition started at an early age when I was encouraged to pursue a career in a field that was constantly growing and from which I could always learn. While gaining a BSc in nutrition at the University of Nottingham I was struck by the extent to which nutritional status underpinned health across the life course and how it could be a vehicle for optimising the health of nations; particularly in vulnerable settings. I was determined to understand how my learning in the field of nutrition could have relevance across populations and enrolled for an MSc in Public Health Nutrition at the London School of Hygiene and Tropical Medicine. Here I was exposed to some of the leading academics in the field and was inspired by their expertise, as well as the opportunities that academia provided to learn and grow across one's career while potentially influencing policy and population health. With every intention of returning to South Africa after my MSc I seized the opportunity to conduct my research project at the Developmental Pathways for Health Research Unit (DPHRU) in Johannesburg. This, as well as the subsequent year spent as a research assistant with the unit in Cape Town, exposed me to the amazing research being done on home soil and the vast opportunities for nutrition research in this setting.

I took a brief break from academia in 2013/2014 in order to gain exposure to the South African health system through an Analyst position at Broadreach Healthcare. My main role was to assess key health indicators (predominantly human immunodeficiency virus (HIV)/tuberculosis (TB) and maternal and child health focused) across specified health districts and to analyse the potential root causes of poor performance in these indicators at primary healthcare facilities. This provided me with unique insight into the South African

health system and the various challenges being faced at facility level in improving health outcomes. I was amazed by the lack of focus on nutrition, particularly in optimising health of pregnant women who were exposed to multiple morbidities such as obesity and HIV that greatly increased the risk of adverse outcomes for themselves and their infants. However, I was pleasantly surprised by the motivation that these pregnant women had to improving their health behaviours during pregnancy – for example via adherence to antiretroviral treatment - and the window of opportunity for optimising maternal and child health during this time; thereby establishing improved health trajectories in the long term. This is really where my interest in maternal and child nutrition was established and I decided that a PhD in this area was the perfect next step. Through discussions with A/Prof Lisa Micklesfied and Prof Shane Norris at DPHRU, I discovered that my research aspirations were perfectly aligned with those of the unit and that the opportunity to explore maternal dietary intake and its association with infant outcomes were available within their wider Soweto First 1000-Day Study (S1000). I was lucky enough to be awarded a PhD Scholarship by the Centre of Excellence (CoE) in Human Development at the beginning of 2015; following which I relocated to Johannesburg to begin this PhD journey.

This is a PhD with publications; with each paper filling a key research gap in the field of maternal and child nutrition and providing the formative work for my academic career in nutritional epidemiology. Three of the four articles have been published in peer reviewed journals; with the fourth currently under review with the British Journal of Nutrition (submitted: 20/12/2017). Section 1 provides the background to my PhD study as well as its participants and methods (Chapters 1-3). Chapter 1 outlines the background and motivation for the study and includes the problem statement, conceptual frameworks and global context, as well as the overall aim and specific objectives of the PhD. As a result of the research gaps identified in Chapter 1 - particularly with regard to the African setting - Chapter 2 presents

the results of objective one of my study; using a systematic approach to report on the available data from Africa. An outline of the overall study methods – including the study context, participants and ethical clearance are presented in Chapter 3. Section 2 contains the empirical chapters, with Chapters 4-6 presenting the results for study objectives 2-4. Lastly, Section 3 provides an integrated discussion of the thesis as a whole, as well as its final conclusion.

ABSTRACT

Background: Maternal pre-pregnancy overweight and obesity and excessive gestational weight gain (GWG) are established predictors of fetal growth, which substantially increase the risk of adverse birth outcomes, such as high birth weight and large-for-gestational age deliveries. While sub-optimal growth in utero has serious implications for infant health in the short term, nutritional insults during this critical period of plasticity may additionally impair growth and development of body tissues and thereby, increase long-term risk of obesity and non-communicable diseases in later life. This double burden of malnutrition (maternal overweight coupled with micronutrient deficiencies) is of particular relevance to low-or middle-income countries, such as South Africa, where rapid urbanisation and a transition towards diets high in saturated fat, sugar, salt and processed foods and decreased levels of physical activity has resulted in substantial increases in obesity. Although the implications of anthropometrically defined maternal nutritional status (MNS) on birth size have been well established, the role of dietary patterns within these relationships has not been thoroughly examined. In addition, the use of birth weight as a proxy for fetal growth does not distinguish between the components of body composition (i.e. fat mass and fat-free mass), which may be more indicative of metabolic risk. Lastly, the influence of other maternal factors such as human immunodeficiency virus (HIV) on the associations between maternal nutrition and infant outcomes and metabolic risk is yet to be explored.

Aim: The overall aim of this thesis was to examine maternal nutrition (nutritional status; dietary patterns) of urban, black South African women and explore the relationship between maternal dietary patterns during pregnancy and birth outcomes (including neonatal body composition). Furthermore, the extent to which other maternal factors – i.e. HIV/antiretroviral treatment (ART) status, body mass index (BMI) at recruitment, GWG,

demographics, socioeconomic status etc. - act as confounders or effect modifiers to these associations was explored. The following four specific study components addressed this aim: 1) To review and report on MNS in African women and its associations with fetal, birth, neonatal and infant outcomes in the first 1000 days; 2) to compare body composition measurements using two methods, namely (i) dual-energy x-ray absorptiometry (DXA) and (ii) air displacement plethysmography (ADP; Peapod), in black South African neonates; 3) to characterise, depict and report on maternal dietary patterns during pregnancy using multivariate dimension-reduction techniques in urban black South African women and to examine the association between dietary patterns and GWG in the context of other maternal lifestyle and socioeconomic factors; and 4) to examine the associations between maternal dietary patterns and birth size and neonatal body composition and explore how specific maternal factors – i.e. HIV/ART status, maternal BMI and GWG – may influence these associations.

Methods: Comprehensive literature searches were independently performed by two researchers in May 2015 in order to identify all relevant studies conducted in Africa. The review used a systematic approach to search the following databases: Medline, EMBASE, Web of Science, Google Scholar, ScienceDirect, SciSearch and Cochrane Library. Full-text articles were obtained and reviewed and data were extracted from relevant publications into tables appropriately.

Within a wider longitudinal cohort study taking place in Soweto, Johannesburg (the Soweto First 1000-Day Study; S1000), habitual dietary intake of 538 pregnant women was assessed using a quantitative food-frequency questionnaire and dietary patterns were depicted via principal component analysis. Associations between dietary patterns and BMI-specific GWG were analysed using linear and multinomial logistic regression. "Traditional" diet pattern adherence (pattern score) was used to classify maternal diet for the final study objective

(objective 4) and multiple linear regression models were used to examine associations between maternal "traditional" diet pattern score, HIV/treatment status [three groups: HIV negative, HIV positive (antenatal ART initiation), HIV positive (pre-pregnancy ART initiation)], BMI and GWG (kg/week) and: newborn (1) weight-to-length ratio (WLR, kg/m) in 393 mother-neonate pairs; (2) Peapod estimated fat mass index (FMI, kg/m^3) in a 171-pair subsample.

Results: Twenty-six studies met the inclusion criteria for the literature review (objective 1). Overall, MNS in Africa showed features typical of the epidemiological transition; including higher overweight and obesity and lower underweight prevalences, alongside high anaemia prevalences' and poor-quality diets. Maternal BMI and GWG were positively associated with birth weight; however, maternal overweight and obesity were associated with both increased macrosomia (birth weight >4kgs) and intrauterine growth restriction risk. In addition, maternal anaemia was associated with lower birth weight and both macro- and micronutrient supplementation during pregnancy was associated with improvements in GWG, birth weight and mortality risk.

During the comparison of body composition assessment techniques (objective 2), significant correlations were observed between ADP and DXA measurements of fat mass ($r = 0.766$; $p<0.001$), fat-free mass ($r = 0.942$; $p<0.001$) and %fat ($r = 0.630$; $p<0.001$). However, fat mass (408 ± 172 g vs. 337 ± 165 g; $p<0.001$) and body fat percentage ($12.9 \pm 4.4\%$ vs. $9.9 \pm 4\%$; $p<0.001$) were significantly higher and fat-free mass (2681 ± 348 g vs. 2969 ± 375 g; $p<0.001$) significantly lower when estimated by ADP than by DXA. There was greater consistency in the estimation of fat-free mass between the methods when compared to estimates of fat mass and body fat percentage.

Longitudinal assessment (objectives 3 and 4) identified three dietary patterns in urban black South African women during pregnancy: namely "western", "traditional" and "mixed". "Western" and "mixed" diet patterns were associated with 35 g/week (p=0.021) and 24 g/week (p=0.041) higher GWG in normal weight and obese women respectively. High intakes of the "traditional" diet pattern were associated with a reduced odds of excessive weight gain in the total sample (OR: 0.81; p=0.006) and in normal weight women (OR: 0.68; p=0.003). In the final, fully adjusted study models, maternal obesity and GWG were associated with 0.25 kg/m (P=0.008) and 0.48 kg/m (P=0.002) higher newborn WLR, while "traditional" diet pattern score was associated with lower newborn WLR (-0.04 per +1 SD; P=0.033). Additionally, "traditional" pattern score was associated with 0.13 kg/m^3 (P=0.027) and 0.32 kg/m^3 (P=0.005) lower FMI in the total sample and in newborns of normal weight women, respectively. HIV positive (pre-pregnancy ART) vs. HIV negative (ref) status was associated with 1.11 kg/m^3 (P=0.002) higher newborn FMI in a fully adjusted model.

Conclusion: This thesis confirms the rapid transition in MNS across urban African populations and demonstrates the implications that the rise in maternal overweight and obesity alongside poor dietary patterns and micronutrient deficiencies may have on birth outcomes, as well as potentially on longer term health trajectories. However, it also highlights a lack of data on infant outcomes beyond birth, and therefore, a need for longitudinal data that examines longer-term implications in the African setting.

In South Africa in particular, the thesis indicates that promotion of a traditional-style diet pattern - high in whole grains, legumes, vegetables and traditional meats and low in processed foods - alongside a healthy preconception weight in urban, black women would significantly improve both maternal and infant adiposity profiles. This may have substantial benefits in reducing long-term risk of non-communicable diseases in both current and future generations. However, the need for a holistic approach which incorporates other health and

lifestyle determinants of growth and adiposity in the infant is critical in optimising metabolic health trajectories. In HIV-positive women for example, development of targeted monitoring and management strategies is necessary in order to limit the treatment-specific effects on adiposity in the newborn.

SECTION 1: BACKGROUND AND LITERATURE REVIEW

CHAPTER 1: Introduction

1.1 Problem statement

In low-or middle-income countries (LMICs), rapid economic development and continued inequality in education, employment and food security have shifted the focus of the maternal and child nutrition agenda from undernutrition to a double burden of disease, where both stunting and obesity occur in parallel (1). In South Africa, although chronic undernutrition persists in childhood, the substantial increase in overweight and obesity – particularly prevalent in adult women – is coupled with increasing burdens of non-communicable diseases (NCDs) such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (2–4). These dual burdens not only increase the risk of poor educational attainment and productivity, morbidity and mortality at individual levels, but have serious implications for economic growth and development at a national level, while placing enormous pressure on the health system (1,5,6).

Maternal obesity, adiposity and excessive weight gain, as well as, poor dietary diversity and micronutrient deficiencies, prior to and during pregnancy, are associated with increased risk of developing gestational diabetes mellitus (GDM) and pre-eclampsia during pregnancy, adverse birth outcomes such as preterm birth, neonatal death, low birth weight (LBW) and macrosomia (birth weight >4kgs), sub-optimal infant growth and development, and an increased risk of future NCDs for both mother and infant (1,7–11). However, the influence of maternal diet on adiposity (i.e. fat vs. fat-free mass) in the newborn within the obesogenic context of urbanising African settings is not known. An understanding of nutritional status and dietary patterns in pregnant South African women and the associations with gestational weight gain (GWG), fetal growth and neonatal adiposity are therefore critical in order to develop interventions designed to improve health outcomes and metabolic profiles – both during pregnancy and infancy, as well as in the longer term.

In addition, the associations between maternal nutritional status (MNS) and infant growth and development may be further complicated by other maternal factors, such as demographics, lifestyle factors and socioeconomic status (SES) (12–14). In particular, the high burden of human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) in this setting must be considered; with both HIV infection and antiretroviral treatment (ART) exposure being associated with changes in weight, fat distribution and glucose and fat metabolism, as well as with pre-term birth and LBW (15–19). Without a holistic approach which examines the relationships between MNS, dietary patterns and infant growth and body composition in this broader context, substantial and sustained improvements in maternal and child nutrition and health will not be possible.

1.2 Conceptual frameworks

In 2008, The Lancet recognised maternal and child undernutrition as a key global issue, due to a high prevalence in LMICs and evidence that they make a significant contribution to morbidity and mortality in these settings. The Maternal and Child Undernutrition Series (20–24) was based on a conceptual framework developed by UNICEF in 1990 (Figure 1), which described maternal and child undernutrition as a consequence of multisectoral development inadequacies and elucidated the immediate (individual), underlying (household and community level) and basic (societal structures and processes) causes of undernutrition. Additionally, the series outlined the consequences of maternal and child undernutrition – namely, morbidity, mortality and disability in the short term, and reduced adult size, intellectual ability and economic productivity, plus an increased risk of metabolic and cardiovascular disease, in the long term.

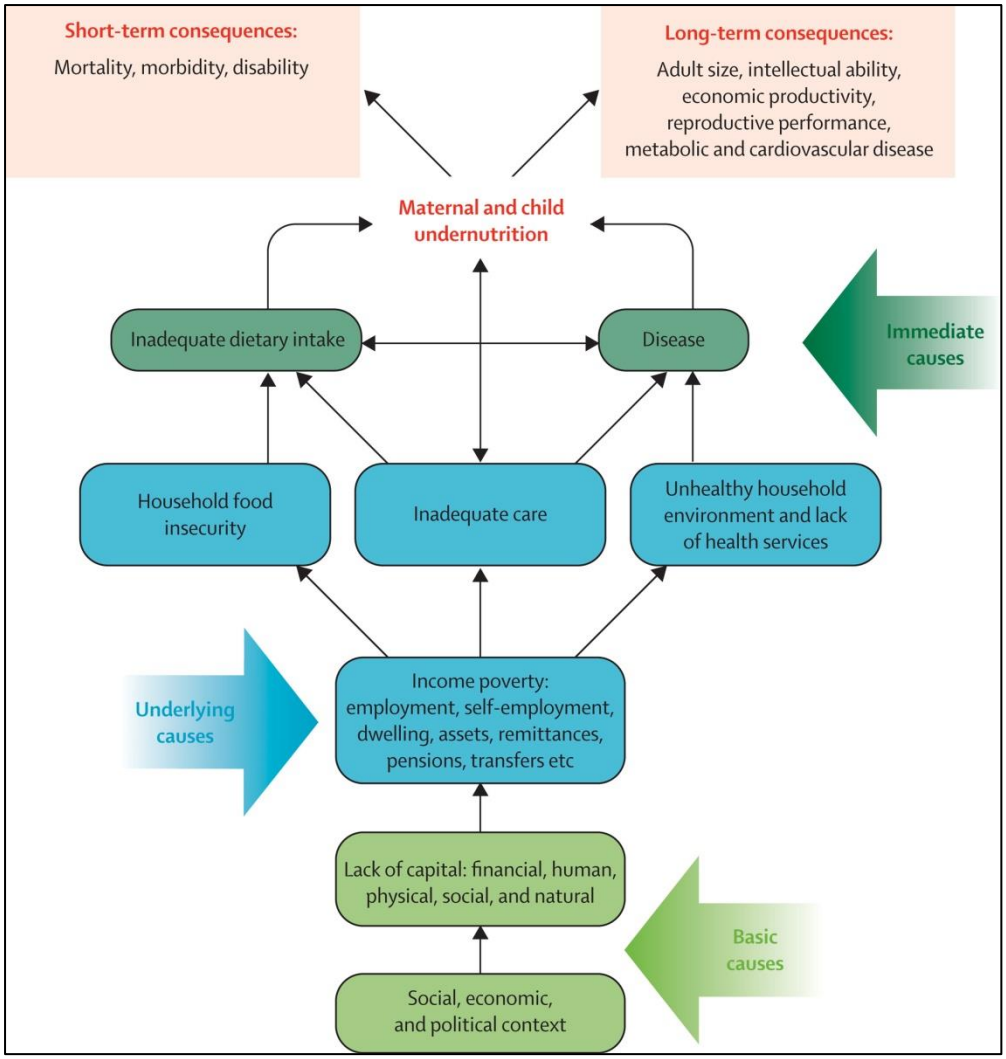

Figure 1: United Nations Children's Fund (UNICEF) conceptual framework of the relations between poverty, food insecurity, and other underlying and immediate causes to maternal and child undernutrition and its short-term and long-term consequences (20)

Although this series and framework confronted a critical component of the challenges facing maternal and child health, while emphasising the need to focus on multifaceted influences of nutrition and subsequent health outcomes across sectors, the rapid effects of urbanisation on maternal and child nutrition in LMICs in recent years encouraged a re-evaluation of global priorities and strategies in this area. While undernutrition remains a prominent issue, with

stunting affecting approximately 165 million children under five in 2011 worldwide, overweight affected approximately 43 million under-fives in 2011 (the majority in LMICs) and the number continues to rise (1).

As a result, The Lancet Series on Maternal and Child Nutrition (second series) was published in 2013, identifying the need to focus on both under- and overnutrition, due to the increasing double burden of malnutrition being experienced in LMICs. This series introduced a new conceptual framework (Figure 2) that illustrates the means to optimum fetal and child growth and development by tackling malnutrition from both sides of the disease spectrum. This framework outlines the dietary, behavioural, and health determinants of optimum nutrition, growth and development, and how they are affected by various underlying conditions (food security, care-giving resources, health services, etc.), which are in turn shaped by economic and social conditions, national and global contexts, capacity, resources and governance. In addition, the framework outlines the different levels at which determinants can be changed to enhance maternal and childhood outcomes, including nutrition-specific interventions that address both the immediate and underlying causes of malnutrition.

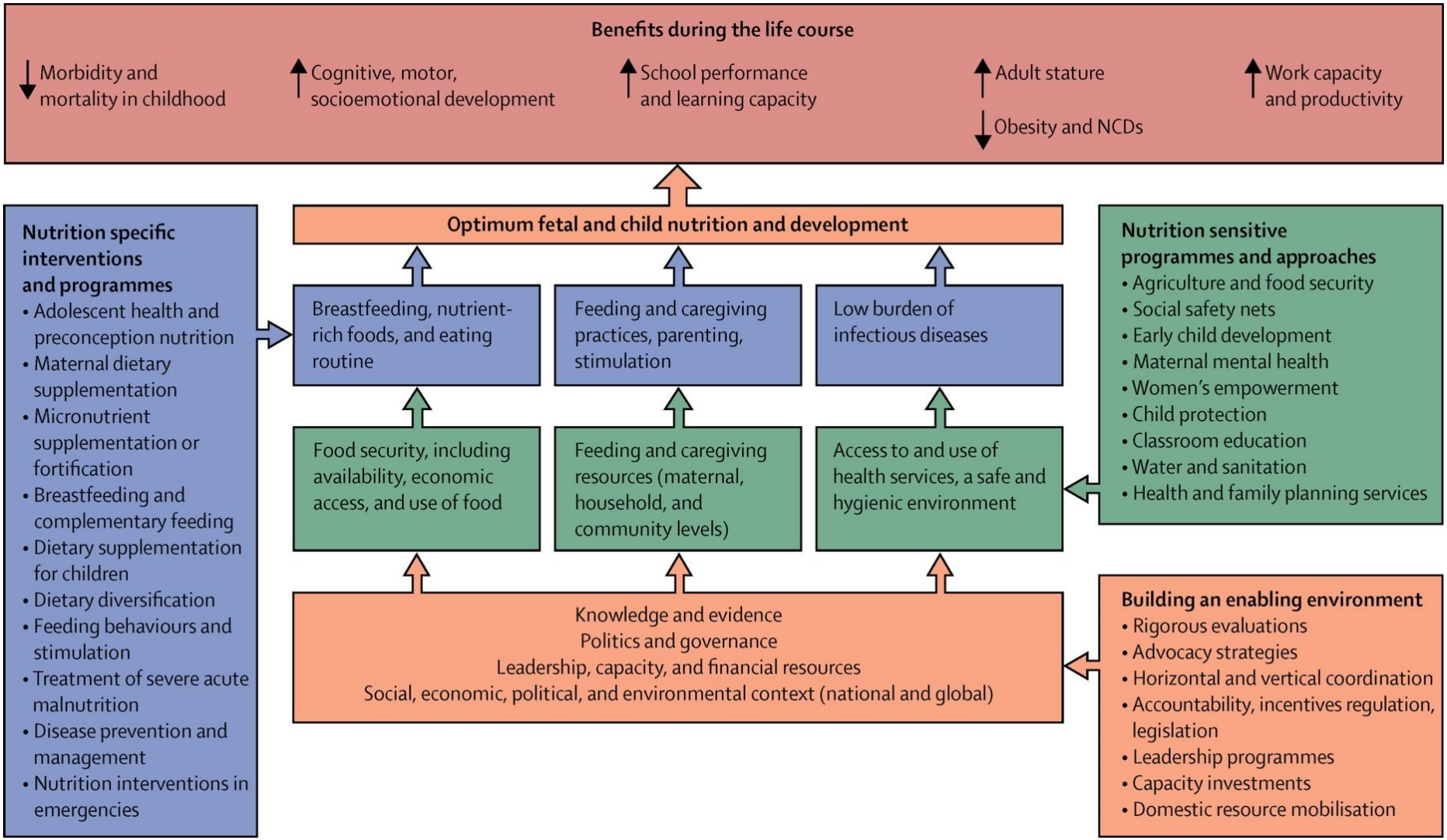

Figure 2: The Lancet conceptual framework for actions to achieve optimum fetal and child nutrition and development (1)

The framework provides a more comprehensive and goal-oriented approach to addressing maternal and child health, with the focus on achieving optimal nutrition and development through multi-level platforms (from individually focused nutrition and lifestyle interventions to building enabling environments) rather than simply avoiding adverse health outcomes. For this reason it has become the key framework for research – and potential intervention – in the area of maternal and child nutrition, and will therefore, form the foundation upon which this thesis is built. The key aspects of the framework to which this research relates are highlighted in Figure 3. Firstly, it contextualises the research in relation to the Developmental Origins of Health and Disease (DOHaD) paradigm by highlighting the benefits of optimal fetal and childhood growth and development in reducing long-term risk of obesity and NCDs. Secondly, it depicts the potential relationship between the main exposure of interest (maternal nutrition during pregnancy) and the key outcomes to be explored, i.e. optimal fetal nutrition and growth, assessed using birth size (weight and length) and neonatal body composition (adiposity). Lastly, it introduces the influence that other maternal demographic, health and socioeconomic variables may have on the complex associations between MNS, dietary patterns and infant outcomes. Specifically the extent to whether maternal HIV/ART exposure acts as a potential modifier or confounder must be explored in order to gain a comprehensive understanding of these complex associations, as well as the possible means of intervention to optimise both short and long term health outcomes.

Due to the focus on maternal and neonatal adiposity, this framework is therefore complemented by the conceptual model modified and presented by the Institute of Medicine (IoM) in their 2009 updated GWG guidelines. This framework provides a more focused picture, placing MNS and diet (and other influential maternal factors) as determinants of GWG (including fat and fat-free mass in both the mother and fetus) and, subsequently, overall neonatal size and body composition, as well as long-term obesity and disease risk.

The specific factors included in this thesis – as they fit within the IoM framework - are presented in figure 4; therefore providing a final schematic for the thesis itself.

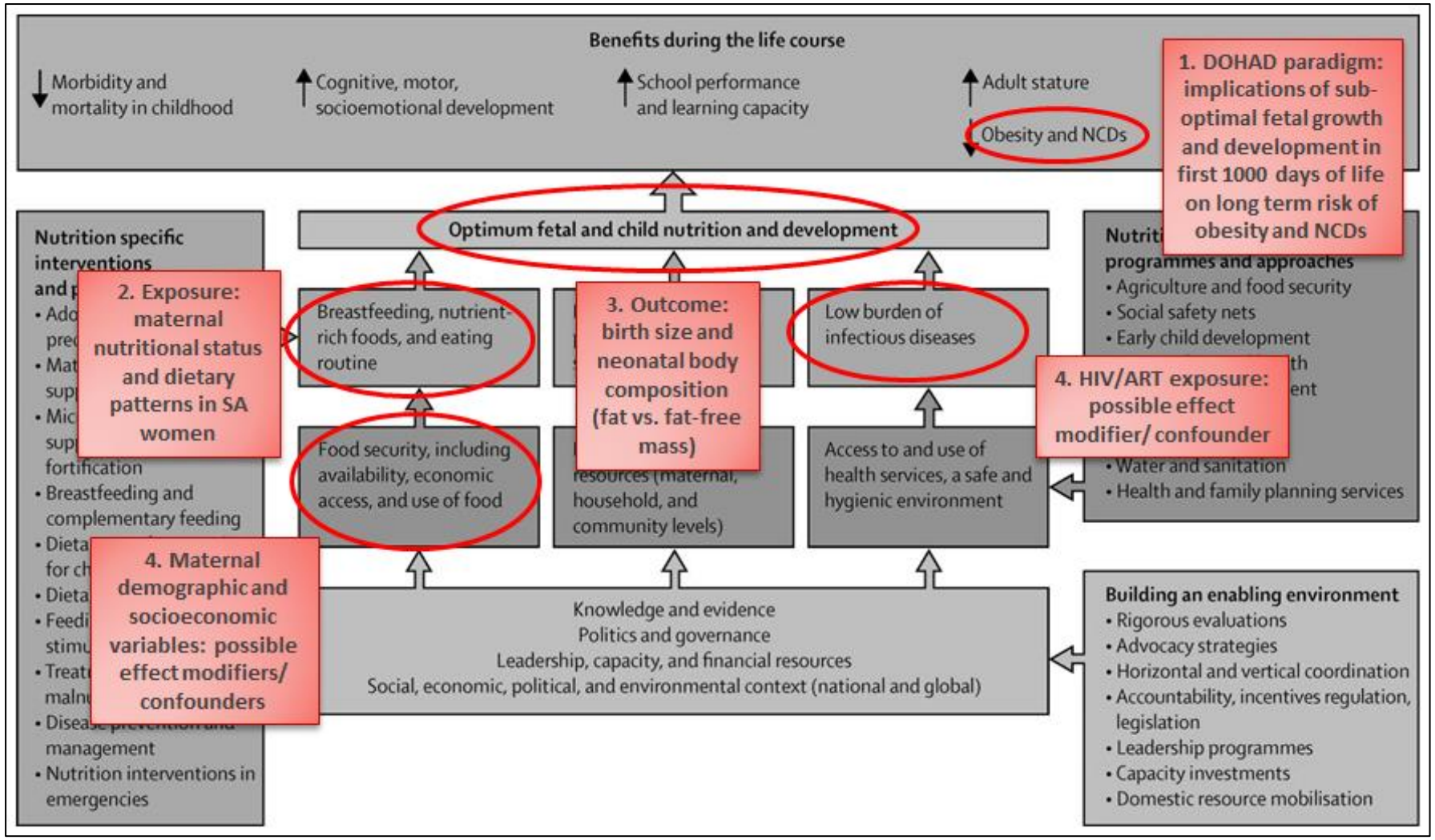

Figure 3: Link between The Lancet conceptual framework and PhD thesis (1)

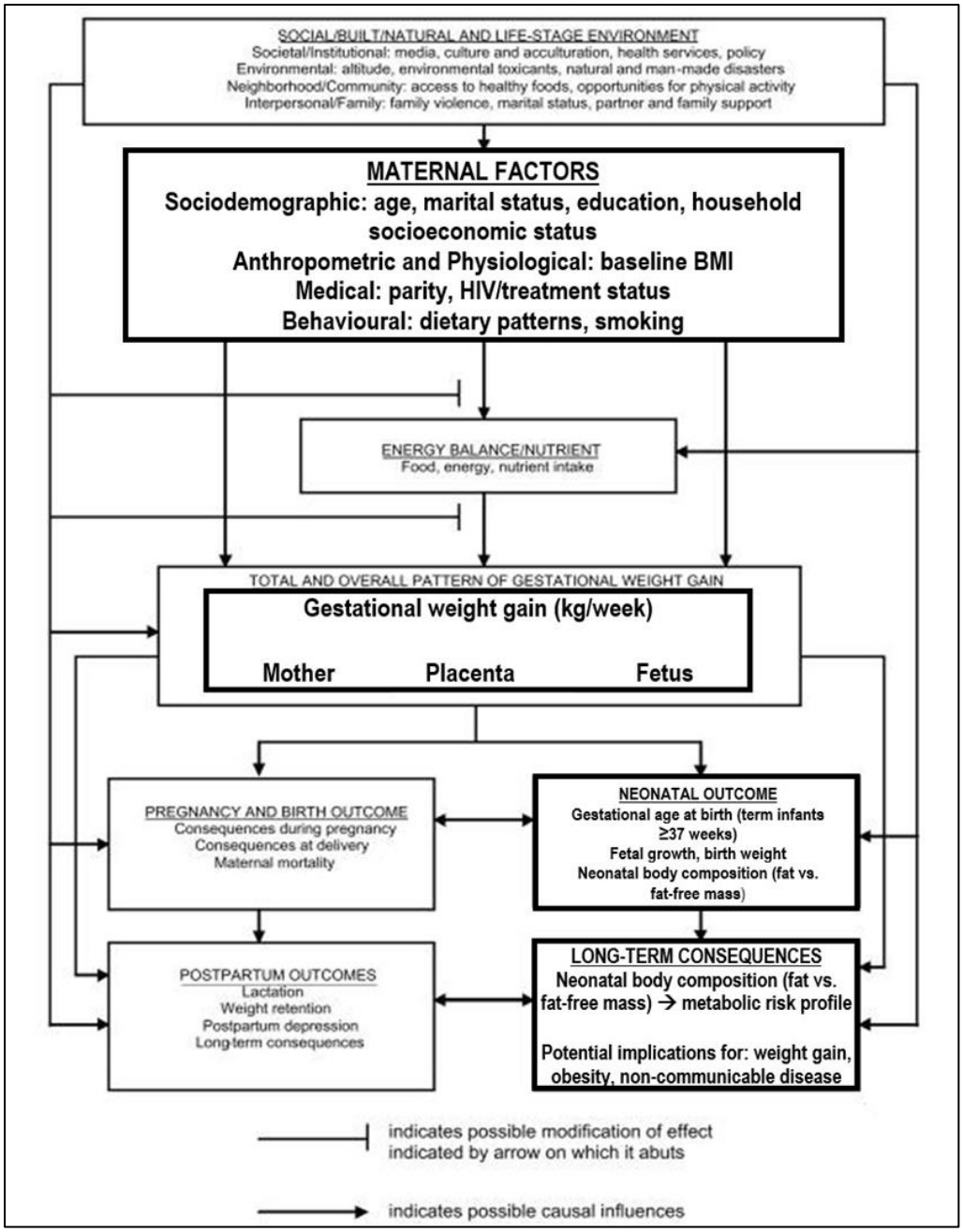

Figure 4: Conceptual model for PhD thesis within the Institute of Medicine (IoM) framework for gestational weight gain (GWG) (25)

1.3 Literature review

1.3.1 The nutrition transition

As LMICs become increasingly urbanised, significant demographic and lifestyle changes – characterised by a transition to typically westernised diets and decreased levels of physical activity – occur (26). Such shifts in consumption to energy-dense diets high in saturated fat, added sugar and salt and processed/convenience foods low in fibre and essential micronutrients have been implicated in the rapidly rising overweight and obesity prevalence in these populations, as well as the emerging burdens of T2DM and CVD (26–28).

Although urbanisation has progressed across Sub-Saharan Africa, the extent to which this has occurred – as well as the magnitude of the resulting metabolic and health consequences – differs between settings. In countries such as South Africa, where the transition has been most profound, the implications for maternal and child nutrition are extensive. Although undernutrition persists, and one in four children are stunted by the age of two, approximately 39% of adult women are obese and 25% of young children (2-4 years) are either overweight or obese (2). These co-existing burdens of childhood stunting and maternal obesity are not only present at a population and community level, but are evident in mother-child pairs within single households (1,29–31).

1.3.2 The DOHaD paradigm

The DOHaD paradigm describes a transgenerational effect between maternal environment and fetal development, leading to altered susceptibility of the infant to NCDs in later life (32). Early environmental factors, such as those experienced in utero and in early infancy, have been shown to programme the growth and development of body tissues via changes in gene expression during these critical periods of developmental plasticity. These changes may lead to permanently altered body function and metabolism, thereby influencing the ability of

individuals to cope with the adult environment later in life and increasing the risk of obesity, T2DM, metabolic syndrome and CVD (32–34).

1.3.2.1 The first 1 000 days

The first 1000 days – constituting the period of pregnancy plus the first two years of infancy – has been proposed as a critical window of developmental plasticity during which growth and development can be targeted for long-term health benefits (35,36). Evidence shows that improving environmental influences during this period to facilitate optimal nutrition and growth has lasting effects throughout life. These include reductions in childhood morbidity and mortality, increased cognitive and motor development, increased school performance and later work capacity, higher adult stature and reduced obesity and NCD risk (Figure 5) (1,37).

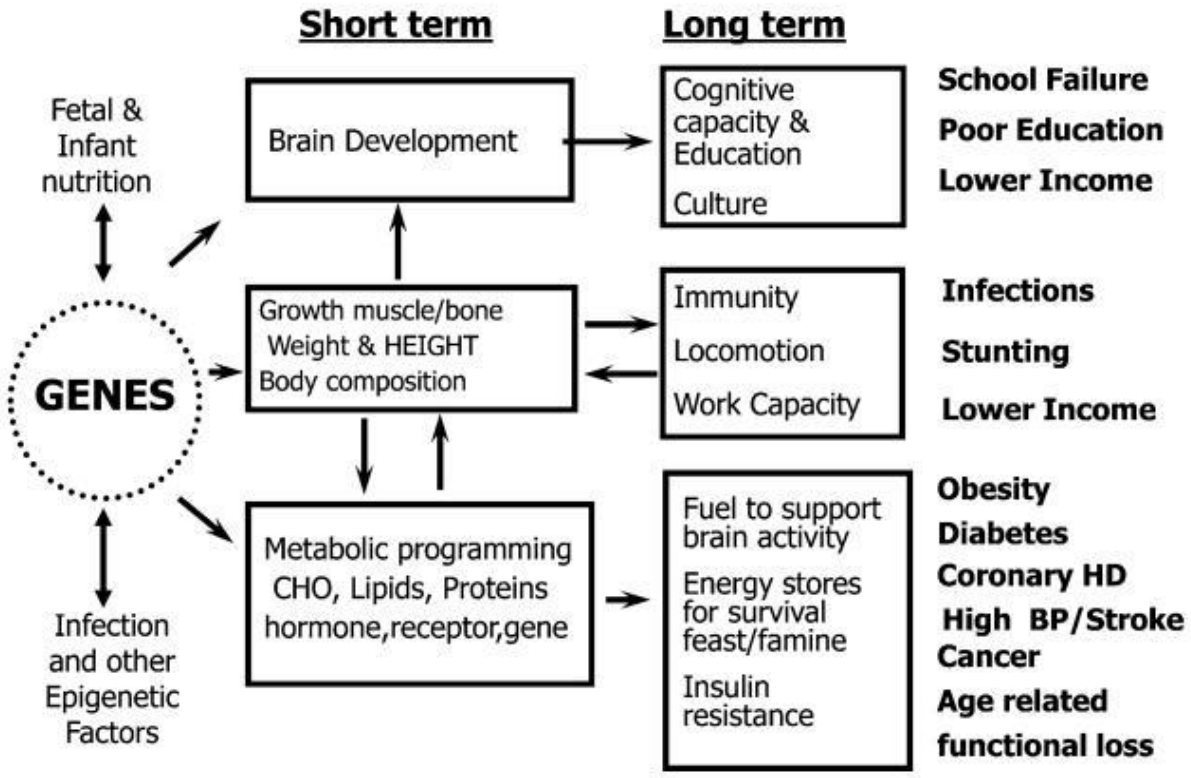

Figure 5: The Developmental Origins of Health and Disease (DOHaD) paradigm: short- and long-term effects on health outcomes (37)

1.3.3 MNS, fetal growth and birth outcomes

1.3.3.1 Anthropometry

Maternal underweight

Traditionally, the risks associated with pregnancy in LMICs have been focused on maternal underweight and short adult stature, which may result in a high risk of adverse birth outcomes, such as maternal death, intrauterine growth restriction (IUGR), LBW and small-for-gestational-age (SGA) infants (20). Although maternal underweight and stunting rates have declined in the developing world, they continue to persist in Africa, with over 10% of adult women underweight in 2008 (1). However, due to the low burden of underweight in South African adult females, approximately 3% of women 15 years and older in the 2016 South African Demographic and Health Survey (SADHS) (3), this thesis focuses on over- rather than underweight.

Maternal overweight and obesity

With the rise in obesity prevalence worldwide – predicted to reach approximately 21% in adult women by 2025 – maternal obesity has been recognised as a pertinent global issue that affects not only the health of the mother and her immediate offspring, but may have longstanding consequences across generations (5). In 2016, the need to address obesity early in the lifespan – i.e. in the mother prior to, and during, pregnancy – was highlighted in the Lancet Diabetes and Endocrinology series on Maternal Obesity (5,38–41). By prioritising early intervention, this series proposed that we may be able to reduce the immediate and longer-term risks to maternal and infant health while curbing the propagation of obesity through the life course and potentially in future generations.

Although overweight and obesity have traditionally been viewed as an epidemic of high-income countries (HICs), thanks to a prevalence ranging between 61.9%, 56.8% and 47.6%

for adult women in the USA, Eastern and Western Europe respectively in 2013, the growing burden of overweight and obesity in LMICs has resulted in prevalence estimates of 28.3% in Southeast Asia and 34.5% and 63.7% respectively in Western and Southern Sub-Saharan Africa in recent years (42). Such rapid changes in the nutritional profile in LMICs – attributed to the transitioning lifestyles in these settings and exhibited predominantly in women – pose a serious threat to maternal and child health, as well as to the long-term risk of NCDs in the most vulnerable settings where health systems are least equipped to respond (5,43). In South Africa in particular, substantial effects have already been seen in recent years, with the International Diabetes Federation estimating the number of diagnosed diabetes cases in adults (20-79 years) at 2.3 million in 2015 (4) and 44% of black women aged 15 years and older being identified as hypertensive in the 2016 SADHS (3).

Maternal obesity prior to pregnancy is associated with a wide range of pregnancy and delivery complications, as well as with morbidity and mortality in the offspring. Specifically, obesity is associated with an increased risk of GDM and pre-eclampsia during pregnancy, maternal death, haemorrhage and infection during delivery and weight retention post-partum, as well as poor infant outcomes, such as neonatal death, birth trauma and macrosomia (1,10,11,44). Focusing specifically on birth size – used commonly as a proxy for growth and development in utero, as well as for long-term NCD risk – a Canadian study found that a 10% higher pre-pregnancy body mass index (BMI) was associated with an approximately 10% increased risk of macrosomia (45), while researchers in Italy showed maternal obesity to be an independent predictor of macrosomia (46). Even within a group of obese women in the US, a dose-dependent effect of increased adiposity has been shown, with a 30% higher risk of large-for-gestational age (LGA) shown for class III vs. class I obesity sub-groups compared to normal-weight women (47). Similar results have been shown in Asian settings, where higher pre-pregnancy BMI was associated with higher macrosomia risk in China (48). Due

to the differences in under- and overweight/obesity prevalence across the African continent, higher pre-pregnancy BMI has been beneficially associated with increased birth weight in countries such as Sudan (49). However, in regions such as Zambia and Ghana, where the nutrition transition has progressed further, results have shown similar effects of maternal overweight and obesity increasing macrosomia risk, as seen in more developed settings (50,51). In South Africa, maternal overweight and obesity have been associated with dual burdens of risk for the new born, with elevated risk of both macrosomia and IUGR evident from previous studies (52,53).

Gestational weight gain

In addition to maternal BMI at conception, weight gain during pregnancy has been shown to have significant effects on pregnancy outcomes, with both insufficient and excessive weight gain increasing risk for both mother and infant (25). The most commonly utilised guidelines for assessing pregnancy-associated weight gain in both clinical and research contexts were published by the IoM in 2009 (Table 1) (25). These prescribe recommended ranges of maternal weight gain according to a woman's BMI status (underweight, normal weight, overweight or obese) prior to conception, with the capacity for gain decreasing with increased BMI.

Table 1: Institute of Medicine (IoM) guidelines for total and rate of gestational weight gain (GWG) according to pre-pregnancy body mass index (BMI) (25)

Pre-pregnancy BMI	**Total weight gain**	**Rates of weight gain (2^{nd} and 3^{rd} trimester)**
	Range (kg)	**Total range (kg/week)**
Underweight (<18.5 kg/m^2)	12.5-18	0.51 (0.44-0.58)
Normal weight (18.5-24.9 kg/m^2)	11.5-16	0.42 (0.35-0.50)
Overweight (25.0-29.9 kg/m^2)	7-11	0.28 (0.23-0.33)
Obese (≥30.0 kg/m^2)	5-9	0.22 (0.17-0.27)

In a recent systematic review and meta-analysis across the US, Europe and Asia, pooled estimates showed that, compared to weight gain within IoM-recommended ranges, inadequate gain was associated with a 5% higher risk of SGA and a 2% lower risk of both LGA and macrosomia (54). The increase in SGA risk associated with inadequate weight gain was greatest for underweight women. Conversely, GWG that exceeded recommendations was associated with a 2% reduction in SGA risk and 4% and 6% increases in the risk of LGA and macrosomia respectively. These findings demonstrate the complexity of the association between baseline MNS and GWG and fetal growth and birth outcomes. While reductions in weight gain may improve birth outcomes at one end of the risk spectrum, they may simultaneously increase risk at the other, with the resulting effects on birth size being strongly influenced by pre-pregnancy BMI.

Although this systematic review and meta-analysis included data on 1,309,136 pregnancies from 23 studies across 10 countries, no data was available from the African context. While not using the IoM guideline ranges, Mochhoury et al demonstrated an increased risk of macrosomia in women who gained >8kg weight compared to those who gained <8kg during pregnancy in Morocco (55). However, limited evidence of an association between GWG and high birth weight, as well as a lack of comparability in the weight-gain cut-offs used in this and other studies, makes it impossible to draw conclusions around the applicability of these associations to African settings.

Although excessive weight gain during pregnancy is associated with increased risk regardless of pre-pregnancy weight, and women in high BMI categories have reduced capacity for healthy weight gain, studies show that women who are overweight or obese are more likely to experience high GWG than their normal weight counterparts. In a study across Australia, New Zealand and Ireland, overweight and obese women were 3 and 2.5 times more likely to exceed the IoM recommended weight-gain ranges respectively than those within normal

weight categories at the start of pregnancy (56). In addition to the risks associated with high GWG during pregnancy and delivery, weight gain above IoM-recommended ranges has been linked to higher risk of postpartum weight retention over 2kg (57). This may have implications for the development of overweight or obesity after pregnancy, as well as increase the risk profile of women during subsequent pregnancies. This was demonstrated in the USA, where one third of women who had a normal pre-pregnancy BMI were found to be overweight or obese at one year postpartum (58).

1.3.3.2 Diet

Dietary intake and quality

Energy and nutrient requirements increase during pregnancy in order to meet the needs of both the mother and the growing fetus. It is recommended that the requirements are met through increased consumption of a healthy, balanced diet rich in essential micronutrients. Specific recommendations relate only to certain nutrients that are likely to be insufficient in supply from the diet, such as iron and folate. However, these recommendations are based on the assumption that the pre-pregnancy diet adequately meets the mother's baseline nutritional needs while providing an appropriate supply of energy, and that an increase in quantity will result in an increase in overall diet quality during pregnancy.

Although pregnancy-associated risk has been largely linked to overnutrition in recent years, high energy intakes have not been coupled with improvements in diet quality. In Australia, pregnant women have been shown to consume diets containing less than the recommended levels of fruit, vegetables, breads and cereals and above recommended levels of energy-dense "non-core" convenience foods, as well as low intakes of fibre and key micronutrients such as iron, folate and calcium (59–61). Studies in the USA have found similarly low-quality diets in pregnant populations, with lower than recommended servings of vegetables, fruit and

grains consumed and higher than recommended total fat and saturated fat servings, as well as moderate folate and low iron levels (62,63). In Israel, pregnant women were shown to have adequate total energy intakes, with 42% being overweight and obese, but at least 50% of women had insufficient protein and fibre intakes and 95% consumed less than the recommended levels of calcium, iron and folate (64).

Studies from LMICs, while demonstrating lower energy intakes in some cases than those seen in developed countries due to limited food availability, also show this dual burden of high overweight and obesity prevalence coupled with poor dietary diversity and low micronutrient intakes (5). In Swaziland, only 6%, 12%, 43% and 41% of participants reported eating grains, dairy products, vegetables and fruits respectively at least four times during the previous week and only 30% reported consuming at least four different food groups the previous day, suggesting low-quality diets in this African population (65). Although literature depicting dietary intake in South African pregnant women is limited, May et al (2014) showed that urban pregnant women, while consuming diets sufficient in carbohydrate and protein, have low fibre and essential fatty acid intakes, as well as inadequate intakes of vitamins A, C, D and E and minerals such as iron, folate, calcium and zinc (66).

Evidence for the association between maternal dietary intake and fetal growth and birth outcomes is more limited and inconsistent than that for anthropometric measures of nutritional status. In the USA, while energy and macronutrient intakes were significantly associated with GWG by the second trimester, and this weight gain was positively associated with birth size, no relationship was found between dietary intake variables and infant size at delivery (67). However, when using a healthy-eating index to depict maternal diet quality in Spain, higher scores were associated with beneficial increases in birth weight and length, as well as reduced risk of fetal growth restriction (68). While limiting growth restriction is

beneficial, excessive increases in birth weight are associated with risk of both maternal and infant morbidity and mortality and should also be prevented. In France, high carbohydrate intake (and low fat intake) was negatively associated with birth weight in women with gestational diabetes and reduced the incidence of macrosomia (69). However, potential benefits of this macronutrient profile to non-diabetic pregnancies have not been adequately shown. Data from India showed that, although energy and protein intakes during pregnancy were not associated with birth size, higher fat intake in the second trimester was associated with neonatal length, birth weight and triceps skinfold thickness, while in Jordan, energy and carbohydrate intakes were associated negatively with gestational age (70,71).

Although evidence characterising maternal food and nutrient intakes during pregnancy exists, few studies – particularly in the African context – have looked at the associations between these factors and any fetal, birth or neonatal outcomes. In a review of dietary interventions – largely using nutrition education and counselling – in overweight/obese pregnant women, Flynn et al showed that, while improvements in dietary behaviour and quality, as well as reductions in GWG were observed, only one study showed a significant reduction in the likelihood of macrosomia in the intervention group (72). However, the methodological differences between studies highlighted in this review makes comparability of intervention strategies and assessment of outcomes difficult to ascertain, ultimately limiting the conclusions that can be drawn from any beneficial effects of dietary change on offspring health.

Dietary patterns

The aforementioned studies represent traditional approaches to the assessment of dietary intake; namely, through analysis of individual macro- and/or micronutrient consumption. Although these methods have provided some useful exploration into dietary component effects, they have come under scrutiny in recent years, as diets consist of a range foods and

nutrients eaten in combination and while there is a high level of correlation between some nutrients, individual nutrients may have only small effects, so the risk of confounding in the analysis of single nutrients by the overall pattern of intake is high (67,68). This not only makes associations with outcomes of interest difficult to ascertain, but may limit the applicability of findings to the development of dietary recommendations and public health messaging (73,74). As a result, recent studies have focused on the analysis of overall patterns of intake by applying various statistical methods to dietary assessment data, e.g. from food frequency questionnaires (FFQs). Although a number of dietary pattern analysis methods such as principal component analysis (PCA) and cluster analysis have been used in epidemiology – each with its own strengths and weaknesses – PCA has been identified as the beneficial method in a number of studies, as it allows for better interpretation of dietary patterns and provides factor scores per pattern that are continuous in nature and may provide for more robust and informative analyses (75,76).

Although studies exploring the associations between dietary patterns in pregnant women and fetal growth exist, they have been largely limited to HICs and show inconsistent results. In the UK, New Zealand and the Netherlands, higher adherence to "traditional" or "Mediterranean" dietary patterns – characterised by high vegetable, fruit and whole grain intakes – was associated with higher birth weight and reduced odds of SGA births (77–79). Conversely a "western" dietary pattern – high in red and processed meat, refined grains and full-fat dairy products – was associated with an increase in the odds of SGA in Denmark (80). However, in Brazil and Australia, energy-dense and highly processed "snack" and "junk food" patterns were associated with higher birth weight and increased odds of macrosomia respectively (81,82).

These differences in dietary-outcome relationships reflect the complex nature of the associations between patterns of intake and fetal growth, which may be strongly influenced

by other factors, such as demographics, BMI, GWG and any pre-existing conditions such as HIV status, as well as socioeconomic and lifestyle factors (73). It is therefore critical to explore such relationships in Africa, due to the continent's unique nutrition and health context.

1.3.3.3 Neonatal body composition

Methods for measuring body composition

In addition to birth weight – which has traditionally been the focus for assessing neonatal outcomes – recent studies have explored, not only total size, but body composition, i.e. fat mass and fat-free mass. The ability to differentiate between the components of body weight using objective measures has been shown to provide more sensitive markers of in-utero nutrition and growth and may better reflect long term metabolic risk (83,84). The most common methods of assessing neonatal body composition are dual-energy x-ray absorptiometry (DXA) and air displacement plethysmography (ADP) using the Peapod, due to their non-invasive nature, ease and efficiency of use and low cost relative to other objective techniques (85–87). DXA has been shown to provide reliable measurements of fat mass and fat-free mass in neonates (88), but concerns around the risks of radiation exposure to the infant for repeated measures and the need to prevent movement of the subject during testing restrict its use in paediatric populations. Although the Peapod can only be used to measure infants up to approximately six months of age who weigh less than 8 kgs, it may be a more practical method of body composition assessment in early life (89). However, due to low access to these objective methods, use of the techniques has been limited in LMICs and the accuracy and reliability of ADP in comparison to DXA has not been examined in African infants. In the USA, a strong correlation was demonstrated between DXA and ADP at six months of age; however, percentage fat and fat mass were significantly lower and fat-free mass significantly higher, when using the Peapod (89). Due to the lack of evidence to

support the use of one method above the other, as well as the lack of neonatal body composition data in Africa, comparison of the two methods in African neonates is needed.

MNS and neonatal body composition

The associations between MNS during pregnancy, as measured by BMI and GWG, and neonatal body composition within the first three days of life have been studied in the US. Pre-pregnancy BMI was associated with neonate size and body composition, with a 1kg/m^2 increase in BMI associated with 5.2g and 7.7g increases in fat and fat-free mass respectively, as well as a 0.12% increase in body fat percentage. In addition, each 0.1kg/week increase in GWG was associated with a 24g increase in fat mass, a 34g increase in fat-free mass and a 0.6% increase in percentage fat (90). Data from Australia showed similar results, with pre-pregnancy BMI and GWG both being associated with neonate body fat percentage within 48 hours of birth (91). One study from Ethiopia demonstrated a positive association between birth weight and Peapod-estimated fat mass in neonates within 48 hours of birth; however the association between body composition variables and MNS was not explored in this setting (92).

Only one study in the US has explored the associations between maternal diet and neonatal adiposity assessed objectively via ADP (93). This showed that a diet high in starchy vegetables, solid fats and refined grains and low in dairy and green vegetables was associated with higher birth weight, as well as greater fat mass and body fat percentage (%fat).

Literature therefore suggests a relationship between maternal pre-pregnancy BMI and GWG and neonatal adiposity, with higher maternal BMI and weight-gain increasing both fat mass and body fat percentages in early life. In addition, there is data to suggest that refined and high-fat maternal dietary patterns have a role in greater fat mass accrual in utero. However,

data is scarce, with only one study exploring the role of dietary patterns in these relationships, and none examining these associations in the African context.

1.3.4 The role of HIV and ART

In addition to the relationship between MNS and fetal, birth and neonatal outcomes, these outcomes may be influenced by maternal HIV, as well as ART, in countries such as South Africa, where the HIV prevalence was estimated at 22.3% in women aged 15-49 in 2016 (94). HIV infection has been associated with both MNS and birth outcomes, but the influence of infection, particularly with treatment, on the relationship between these factors is yet to be thoroughly explored.

The current literature on HIV infection and MNS has largely concentrated on maternal anthropometry prior to, and during, pregnancy, with a particular focus on low pre-pregnancy weight and/or poor GWG in predominantly ART naïve, HIV-positive women (95). However, data from HIV-positive pregnant women in three African countries (Malawi, Nigeria and Zambia) suggests more of a spread across anthropometric risk categories than is traditionally associated with HIV during pregnancy, with approximately equal numbers of pregnant women being underweight, normal weight and overweight at enrolment and GWG ranging between weight loss/insufficient weight gain and excessive weight gain in these settings (96). Although the proportions of underweight women at baseline, as well as the high number experiencing weight loss/low weight gain during pregnancy, is still of concern, it is coupled with an increasing number of overweight women who may be experiencing a different spectrum of pregnancy-associated risk.

The limited number of studies that have assessed the relationship between maternal anthropometry and birth outcomes in HIV-positive and HIV-negative women indicate an association between infection and increased risk of preterm birth and LBW (15,16). In

Rwanda, HIV-positive women were at increased risk of giving birth to premature, LBW or growth-restricted infants than HIV-negative women (97).

The aforementioned studies focused on the associations between HIV infection and MNS during pregnancy prior to the increase in ART treatment availability in Africa. In high-risk populations such as South Africa, health policy now prescribes mandatory ART initiation with fixed-dose combination antiretrovirals for all pregnant women, regardless of their CD4 count. This makes exploration into the effects of infection and treatment on pregnancy critical for the South African setting. In Botswana, HIV was associated with increased risk of stillbirth, preterm birth, SGA infants and neonatal death, with women who had been receiving highly active ART prior to conception being at greatest risk of adverse outcomes. However, low CD4 counts during pregnancy were also independently associated with risk of stillbirth and SGA infants (98). Although the effectiveness of ART use in reducing HIV transmission rates is undeniable, the effects of maternal HIV and ART use on MNS or neonatal outcomes is limited, with the available literature focusing on these in isolation, rather than on the interplay between maternal pre-pregnancy nutritional status, diet and HIV/treatment and the subsequent effect on fetal growth and development, as well as on metabolic risk, in the longer term. Both HIV and antiretroviral use have been associated with changes in weight and fat distribution, as well as with altered glucose and lipid metabolism, in adults during recent years (17–19). ART exposure in particular has been associated with central fat deposition and dyslipidaemia, as well as with disordered glucose metabolism and insulin resistance, with obese individuals being at greatest risk of adverse metabolic outcomes (17–19). This makes the inclusion of HIV and treatment in the exploration of the diet-outcome associations in increasingly overweight/obese populations such as South Africa all the more critical, as the resulting impact on fetal growth and metabolic risk is yet to be explored.

1.4 Research gaps

It is clear that in LMICs, maternal and child health are influenced by a number of complex and often conflicting factors, such as the dual burdens of under- and overnutrition, as well as communicable and non-communicable diseases. Although evidence exists for the relationships between isolated maternal factors, such as obesity, specific micronutrient deficiencies and HIV infection, and the associated fetal, neonatal and infant outcomes, very little is known about the interplay between these factors, particularly maternal nutritional intake and dietary patterns, and their associations with birth outcomes and neonatal body composition within an African context.

1.5 Aim and objectives

1.5.1 Aim

The overall aim of this study is to examine maternal nutrition (nutritional status; dietary patterns) of urban, black South African women and to explore the relationship between maternal dietary patterns during pregnancy and birth outcomes and neonatal body composition. Furthermore, the extent to which other maternal factors – i.e. HIV/ART status, BMI at recruitment, GWG, demographics, SES etc. - act as confounders or effect modifiers to these associations will be explored.

1.5.2 Objectives

1. To review and report on MNS in African women and its associations with fetal, birth, neonatal and infant outcomes in the first 1000 days.

2. To compare body composition measurements using two methods, namely (i) DXA and (ii) ADP (Peapod), in black South African neonates.

3. To characterise, depict and report on maternal dietary intakes and patterns during pregnancy using multivariate dimension reduction techniques in urban black South African women

4. To examine the associations between maternal dietary intakes and patterns with neonatal body composition and to explore how specific maternal factors i.e. HIV, maternal BMI and GWG may influence these associations

CHAPTER 2: Review of the importance of nutrition during the first 1000 days: Maternal nutritional status and its associations with fetal growth and birth, neonatal and infant outcomes among African woman[1]

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[1]Wrottesley SV, Lamper C, Pisa PT. Review of the importance of nutrition during the first 1000 days: maternal nutritional status and its associations with fetal growth and birth, neonatal and infant outcomes among African women. J Dev Orig Health Dis. 2016 Apr;7(2):144–62. (99)

2.1 Introduction

Nutrition, among other factors, seems to be one of the pivotal drivers and determinants of maternal and child health. Maternal nutritional status (MNS) has been shown to be an important predictor of maternal health (7–9,11,100), fetal growth (11,101,102), birth outcomes (10,101,103,104) and infant growth (10) in both high-income countries (HICs) and low-or middle-income countries (LMICs). However, the association between maternal nutrition and these multifaceted outcomes is complex and is influenced by many other factors, including genetic, socioeconomic, and demographic variables which differ greatly between populations (100,105). Increased prevalence of non-communicable diseases (NCDs) in LMICs, including Africa, is attributed mainly to the epidemiological health transition. Poor maternal and child health has been associated with increased risk of NCDs, including obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome and cardiovascular disease (CVD) in various studies (106–109).

Additionally, restricted fetal growth, adverse birth outcomes and poor growth in infancy have been associated with increased risk of developing NCDs in adulthood (109). Malnutrition and/or other adverse exposures during critical periods of plasticity (fetal and infant development) may alter gene expression and permanently restructure the body's tissues, thereby resetting metabolism and function, with long-term consequences (110). Maternal undernutrition has long been thought to play a role in phenotypic programming of the growing fetus, which results in intrauterine growth restriction (IUGR) and low birth weight (LBW) babies with increased risk of developing adult NCDs. Maternal obesity, adiposity and weight gain are associated with negative outcomes for: (i) women, including increased risk of gestational diabetes mellitus (GDM), pre-eclampsia, pre-term births, stillbirths and low breast-feeding rates, (ii) fetal growth and (iii) birth and infant outcomes. Although the importance of maternal nutrition in fetal development and birth outcomes has been clearly

demonstrated in experimental animal studies, the findings of studies in humans are less consistent.

The first 1000 days of life – defined as the period from conception to 2 years of age – seems to be an optimistic window for intervention to prevent/reverse programming and improve both maternal, fetal, birth and infant outcomes; ultimately reducing the risk of infants developing NCDs in later life. Evidence seems to suggest that, where mother and child are concerned, chronic conditions have a transgenerational effect (110). However, the extent to which maternal biological factors independently and interactively relate to patterns and proportionality of fetal growth, birth outcomes, and infant growth, remains unclear.

To date, most of the studies and literature reviews dealing with maternal nutrition and its various outcomes have investigated single nutrients in isolation. Though important, nutrient deficiencies are generally found in low socioeconomic status (SES) populations, where they present as multiple, rather than single deficiencies. Studies addressing and pulling together the broader picture of multiple nutrient intakes or deficiencies are lacking. In addition, studies reporting on associations between MNS and maternal, fetal, birth and infant outcomes in Africa are few.

In this review, our aim was to provide and report on the available data from Africa, using a systematic approach, to illustrate whether maternal nutrition during the first 1000 days of life is important to this unique continent, undergoing rapid urbanization and characterized by a triple burden of disease, including infection-related undernutrition illnesses, human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) and the emergence of NCDs (111). The specific objectives were to:

i) Report on MNS of pregnant African women

ii) Examine the associations between MNS (using anthropometric indicators) and fetal growth and birth, neonatal and infant outcomes

iii) Examine the associations between MNS (using nutritional biomarkers) and fetal growth and birth, neonatal and infant outcomes

iv) Examine the associations between MNS (using reported dietary intakes) and fetal growth and birth, neonatal and infant outcomes

v) Explore the evidence from randomised/quasi-randomised clinical trials on the associations between maternal nutritional interventions and fetal growth and birth, neonatal and infant outcomes

vi) Explore the evidence from randomised/quasi-randomised clinical trials on the associations between nutritional interventions in the first two years of life and any later adolescent or adult health outcomes

2.2 Methods

2.2.1 Search strategy

Comprehensive literature searches were independently performed in May 2015 by a team of researchers. Although this is not a generic systematic review, this paper followed a systematic approach to select all available studies describing MNS and how it associates with fetal, birth, neonatal and/or infant outcomes in Africa. Databases used to conduct the searches included: Medline, EMBASE, Web of Science, Google Scholar, Science Direct, SciSearch and Cochrane Library. Search terms and phrases included the following, as well as variations of the following where applicable: prenatal/anthropometry/[specific anthropometric measure of interest, e.g. body mass index]/maternal nutrition/[specific nutrient of interest, e.g. protein or iron]/[specific micronutrient deficiency of interest, e.g. anaemia]/[specific nutritional biomarker of interest, e.g. ferritin]/[specific dietary intake assessment method of interest, e.g. food frequency questionnaire] and birth

outcome/pregnancy outcome/[specific adverse outcome of interest, e.g. low birth weight]/[specific growth or body composition variable of interest, e.g. head circumference or fat mass] and Africa. These terms and phrases were used in different combinations to be identified in titles and abstracts. Full text articles were obtained and reviewed to identify those which met selection criteria below and data was extracted from relevant publications into tables appropriately.

2.2.2 Selection criteria

Studies which met the following criteria were considered relevant for inclusion:

- Studies conducted in African countries
- Any study design
- For observational studies:
    - Studies that described MNS (defined by reported dietary intakes, anthropometric data and biochemical indicators) in pregnant women of any age
    - Studies that associated MNS in pregnant women of any age with any fetal, birth, neonatal or infant outcome
- For intervention studies:
    - Nutritional interventions done in pregnancy with dietary values and/or where biochemical indicators and fetal, birth, neonatal and/or infant outcome data from both the intervention and control group could be extracted
    - Nutritional interventions done in infancy with later adolescent or adult outcomes reported
- Studies reporting data in a format that enabled daily mean or median nutrient intake for the population to be extracted

Studies were excluded from the review according to the following criteria:

- Studies conducted in animals
- Studies in subjects with health conditions that may have influenced dietary intake (i.e. GDM, celiac disease)
- Interventions (including any supplements) in which MNS and fetal, birth, neonatal and/or infancy outcomes were reported for the intervention group only

2.3 Results

The results of the scientific papers included in this review are presented and structured according to the specific aims:

2.3.1 MNS of pregnant African women (Table 2)

Nineteen studies met the inclusion criteria (49,50,52,112–127). The publication year ranged from 2002 to 2014. The number of pregnant women examined in the studies ranged from 30 to 191 834 and the gestational age at MNS assessment varied between 18 and 39 weeks. Six studies used anthropometric measurements to describe MNS in pregnant women (49,50,52,113,117,118), one used a biomarker of anaemia (haemoglobin (Hb)) (127), two used reported dietary intakes (114,119) and the remaining ten used a combination of anthropometry, biomarkers and reported dietary intakes (112,115,116,120–126) (Table 2).

Of the studies including anthropometric measures of MNS, five provided data for the mean/median body mass index (BMI). BMI varied from being within the normal range (18.5-24.9) in Tanzania (115), Ethiopia (112) and Zambia (50), to being within the overweight category (25.0-29.9) in South Africa (124), Sudan (49,117) and Zambia (50). No studies reported mean/median BMI in either the underweight or obese categories. BMI was described according to World Health Organisation (WHO) classification in two studies, with one describing a prevalence of 79.1% overweight and obesity in South African women and the other showing prevalence's of 34.1% and 60.2% overweight and obesity in women who

gave birth to normal weight and macrosomic babies respectively in Zambia (50,122). Weight gain was 228g/week from approximately 23 week's gestational age in Malawi (118) and 1.06kg/week during the third trimester in Liberia (120). In Sudan, the mean mid-upper arm circumference (MUAC) of pregnant women at delivery was 26.9 cm (49,117), while in Ethiopia 52.7% of women had a MUAC of <23 cm (113) (Table 2).

Hb was used as a biomarker of iron status in pregnant women in five studies.(115) All studies described mean Hb values above the threshold for diagnosis of anaemia in pregnant women (<11g/dL), with the exception of one study in Tanzania (115). Based on this cut-off point 66.7% of rural and 26.7% of urban women were classified as anaemic in one study in Ghana and 32% were anaemic in another study in the same setting (125,127). Kenyan pregnant women had a 32% anaemia prevalence in one study (121), while 42.2% and 21.8% of women from pastoral and farming communities respectively were diagnosed with anaemia in a another study (123). In addition to anaemia diagnosed via Hb concentrations, Keverenge-Ettyang (2006) also assessed iron stores in pregnant women using serum ferritin concentrations. Pregnant women from pastoral communities had significantly higher serum ferritin concentrations than those from farming communities, although the difference was relatively small (25.8µg/L v. 24.4µg/L, P<0.05). The prevalence of low maternal iron stores (serum ferritin <32µg/L) was high in both groups (77% in pastoral and 85.9% in farming communities) (123). 27.9% and 24.2% of women from pastoral and farming communities respectively had low vitamin A status (serum retinol). Iron deficiency and iron deficiency anaemia (IDA) prevalence's were 41.6% and 50% respectively in pregnant South African women based on a combination of biochemical markers (serum iron, ferritin, transferrin, Hb, haematocrit, mean corpuscular volume and red blood cell count) (122).

Red cell folate concentrations were between 166 and 183nmol/L in rural and between 158 and 177nmol/L in urban Nigerian women (126). Mean calcium concentrations were 8.9mg/dL in Egyptian pregnant women (116).

Of the ten studies reporting dietary intake in pregnant women, most used 24 hour recall and/or food frequency questionnaires (FFQs) as the assessment method (114,115,119,121,122,124,125), while two used weighed food records (112,126) and one used a food survey questionnaire for calcium intake specifically (116). Mean energy intake ranged between 952 and 3981 kcal/day across study sites. Mean macronutrient intakes ranged as follows: carbohydrate 231-350 g/d, protein 15-104 g/d and fat 7-62 g/d; with the lowest intakes of all macronutrients found in the same Ethiopia population (112). Mean intake of the key pregnancy micronutrients analysed ranged between 7-41 mg/d of iron, 194-424 µg/d of folate, 355-974 mg/d of calcium and 5-13 mg/d of zinc.

Table 2: Results from observational studies describing maternal nutritional status (MNS) of pregnant African women

					MNS Measures [mean(SD)/ median(range)]						
					Anthropometry		Biomarkers		Dietary Intake		
First Author, Year	Country	Main Objective(s)	Sample Size	Gestational Age	Measurement(s)	Value(s)	Type(s)	Value(s)	Energy, kcal/day	Macronutrient(s)/ Fibre, g/day	Micronutrient(s), mg/day
Changamire, 2014	Tanzania	To examine the effect of maternal macronutrient intake on GWG	8428	12-27 weeks	Weight, kg	59.5 (10.7)	Hb, g/dL	10.2 (1.6)	2108 (804)	CHO: 344 (132)	
					BMI, kg/m^2	24.6 (3.9)				Protein: 53 (35)	
										Fat: 59 (37)	
Kesa, 2005	South Africa	Explore anthropometry and nutritional intake of pregnant and lactating women (table includes only pregnant women)	315	1st, 2nd and 3rd trimesters	Overweight/ obese (BMI >25 kg/m^2) (%)	79.12%	Fe deficient (%)[b]	41.6%	8425.71 (2279)[a]	CHO: 292.45 (72.2)	Fe: 9.74 (3.8)
							Fe deficiency anaemia (%)[b]	50%		Protein: 73.18 (23)	
										Fat: 62.29 (23.7)	
Darwish, 2009	Egypt	To assess the prevalence and predictors of low calcium intake in pregnant women	503	34 (2) weeks	Weight, kg	67.5 (42-107)	Ca, mg/dL	8.9 (1.6)			Ca: 879.1 (504.9)
					Height, m	160.6 (143-173)					
Abebe, 2007	Ethiopia	To assess the prevalence of zinc inadequacy based on dietary intakes and plasma zinc concentrations	99	>28 weeks	Weight, kg	52.1 (6.1)			3981 (3156, 5211)[a]	CHO: 231.2 (178.2, 299.7)	Fe: 27.1 (20.7, 33.2)
					Height, m	154.8 (6.5)				Protein: 15.5 (10.4, 20.1)	Ca: 479 (220, 680)
					BMI, kg/m^2	21.7 (2.0)				Fat: 7.7 (4.7, 11.7)	Zn: 5.0 (3.3, 7.2)
										Fibre: 24.4 (15.3, 32.8)	Vit C: 2.2 (0.8, 4.7)
Keverenge-Ettyang, 2006	Kenya	To assess differences in maternal body composition, iron and vitamin A status during pregnancy and postpartum in pastoral	122 P, 128 F	3rd trimester (28-36 weeks)	Weight, kg	P: 51.9 (5.5) F: 51.6 (7.1)	Hb, g/L	P: 119 (11.3) F: 124 (15.0)			
					Height, m	P: 160 (5.6)	Hematocrit	P: 33 (3.95) F: 32 (5.42)			

First Author, Year	Country	Main Objective(s)	Sample Size	Gestational Age	MNS Measures [mean(SD)/ median(range)]						
					Anthropometry		Biomarkers		Dietary Intake		
					Measurement(s)	Value(s)	Type(s)	Value(s)	Energy, kcal/day	Macronutrient(s)/ Fibre, g/day	Micronutrient(s), mg/day
		(P) and farming (F) communities				F: 160 (6.8)	Serum ferritin (SF), µg/L Serum retinol (SR), µmol/L Anaemia (Hb <110g/L), n (%) Low Fe stores (SF <32 µg/L), n (%) Low vit A status (SR <0.70 µmol/L), n (%)	P: 25.8 (4.82) F: 24.4 (4.87) P: 0.92 (0.43) F: 0.92 (0.35) P: 49 (42.2%) F: 27 (21.8%) P: 95 (77%) F: 110 (85.9%) P: 34 (27.9%) F: 31 (24.2%)			
Kamau-Mbuthia, 2007	Kenya	To determine diet quality and common food sources of nutrients in pregnant women	716	1st antenatal visit			Hb, g/dLd Anaemia (Hb <11g/dL), n (%)	11.7 (1.98) 18 (32%)	2055 (537)	CHO: 350 (96.8) Protein: 59.3 (22.4) Fat: 51 (24.5) PUFA: 13.8 (8.3) Fibre: 38.8 (15.1)	Fe: 16.1 (5.4) Folate (µg/d): 317 (161) Ca: 441 (386) Zn: 9.4 (3.4) Vit A (µg/d): 1187 (878) Vit C: 110 (71.9)
Belgnaoui, 2006	Morocco	To assess dietary intake and nutrient adequacy in an agricultural population of pregnant women	172	First (19.4%), second (49%), 3rd (31.6%) trimesters					2947.0 (827.9)	Protein: 104.1 (38.7)	Fe: 17.2 (5.1) Folate (µg/d): 423.8 (140.4) Ca: 832.3 (397.4) Zn: 10.4 (4.3) Vit C: 127.6 (112.5) Vit B1: 1.55 (0.50)

First Author, Year	Country	Main Objective(s)	Sample Size	Gestational Age	MNS Measures [mean(SD)/ median(range)]						
					Anthropometry		Biomarkers		Dietary Intake		
					Measurement(s)	Value(s)	Type(s)	Value(s)	Energy, kcal/day	Macronutrient(s)/ Fibre, g/day	Micronutrient(s), mg/day
											Ph: 1867.0 (519.8) Mg: 528.2 (192.9) Vit E: 22.7 (24.1)
Oguntona, 2002	Nigeria	To assess the food intake and nutrition status of rural and urban pregnant adolescents in the south-western region of Nigeria	54 rural (R), 47 urban (U)	>28 weeks			Red Cell Folate (nmol/L)	R <17y: 166 (18.4) R >17y: 183 (41.4) U <17y: 158 (28.5) U >17y: 177 (24.7)	R <17y: 5571 (307)[a] R >17y: 5693 (481)[a] U <17y: 5209 (214)[a] U >17y: 5864 (416)[a]	Protein: R <17y: 33.6 (2.4) R >17y: 37.0 (3.0) U <17y: 33.1 (2.2) U >17y: 42.2 (3.9) Fat: R <17y: 11.2 (1.1) R >17y: 24.4 (2.1) U <17y: 9.0 (1.0) U >17y: 26.5 (5.8)	Fe: R <17y: 9.7 (2.1) R >17y: 10.9 (1.5) U <17y: 9.9 (1.0) U >17y: 11.8 (3.3) Zn: R <17y: 10.6 (2.6) R >17y: 12.0 (2.1) U <17y: 10.8 (2.0) U >17y: 13.6 (2.1) Ca: R <17y: 662.6 (200.3) R >17y: 609.4 (210.5) U <17y: 623.8 (176.8) U >17y: 739.5 (119.0)
Nti, 2002	Ghana	To determine the food consumption, diet quality and awareness of	15 rural (R), 15 suburba	6 months			Anaemia (Hb <11g/dL), n (%)	R: 10 (66.7%) S: 4 (26.7%)	R: 2640 (2189–3205)	Protein: R: 41.6 (35.0–57.3)	Fe: R: 7.3 4.9–9.3 S: 10.6 7.6–13.7

First Author, Year	Country	Main Objective(s)	Sample Size n (S)	Gestational Age	MNS Measures [mean(SD)/ median(range)]						
					Anthropometry		Biomarkers		Dietary Intake		
					Measurement(s)	Value(s)	Type(s)	Value(s)	Energy, kcal/day	Macronutrient(s)/ Fibre, g/day	Micronutrient(s), mg/day
		nutritional requirements in rural and suburban pregnant women							S: 3018 (2314–3783)	S: 59.6 (46.2–83.7)	Ca: R: 860.0 419.1–1744.5 S: 974.4 453.2–1700.0 Vit A (IU/d): R: 13 825 1 396–37 310 S: 21 893 3 118–71643 Thiamin: R: 1.6 0.7–2.4 S: 2.2 1.3–4.6 Riboflavin: R: 1.5 0.5–2.1 S: 1.4 0.8–1.9 Niacin: R: 16.6 5.7–25.6 S: 20.2 14.9–31.7 Vit C: R: 42 18.0–85.0 S: 36 22.3–51.2
Huybregts, 2009	Burkina Faso	To assess potential changes in dietary habits in during pregnancy in a sample of rural women	218	126 in 1/2nd trimester (T1/2), 92 in 3rd trimester (T3)					T1/2: 8600 (6800; 11100)[a] T3: 9000 (7000; 11300)[a]	Protein: T1/2: 60.5 (44.8; 76.8) T3: 61.2 (46.5; 77.8) Fat: T1/2: 31.2 (17.1; 47.5) T3: 27.0 (15.4;	Fe: T1/2: 40.6 (28.9; 57.7) T3: 40.1 (25.4; 54.6) Folate (µg/d): T1/2: 234.7 (152.1; 367.5) T3: 217.2 (150.3; 316.9)

First Author, Year	Country	Main Objective(s)	Sample Size	Gestational Age	MNS Measures [mean(SD)/ median(range)]						
					Anthropometry		Biomarkers		Dietary Intake		
					Measurement(s)	Value(s)	Type(s)	Value(s)	Energy, kcal/day	Macronutrient(s)/ Fibre, g/day	Micronutrient(s), mg/day
										46.5)	Ca T1/2: 574.3 (335.3; 1081.5) T3: 468.6 (231.0; 708.5) Zn: T1/2: 13.1 (10.1; 16.2) T3: 13.3 (10.4; 16.6) Thiamine: T1/2: 0.85 (0.53; 1.1) T3: 0.81 (0.57; 1.1) Riboflavin: T1/2: 0.20 (0.13; 0.34) T3: 0.25 (0.14; 0.5) Niacin: T1/2: 7.3 (5.2; 10.0) T3: 7.8 (5.5; 10.3) Ph: T1/2: 839.7 (616.9; 1135.7) T3: 852.1 (670.7; 1180.7) Vit A (µg/d): T1/2: 118.9 (60.5; 249.6) T3: 117.6 (53.8; 242.3)

First Author, Year	Country	Main Objective(s)	Sample Size	Gestational Age	MNS Measures [mean(SD)/ median(range)]						
					Anthropometry		Biomarkers		Dietary Intake		
					Measurement(s)	Value(s)	Type(s)	Value(s)	Energy, kcal/day	Macronutrient(s)/ Fibre, g/day	Micronutrient(s), mg/day
Mostert, 2005	South Africa	i) To determine the dietary intake of poor rural women during pregnancy and lactation ii) To determine the nutritional status and dietary intake of their infants at 6 months	46	Anthropometry: 1st visit (4-6 months) Dietary intakes during 2nd and 3rd trimesters	BMI, kg/m^2	27.2 (5.5)			7760 (2059)[a]	CHO: 306.2 (88.0) Protein: 66.8 (30.3) Fat: 55.5 (47.3) Fibre: 21.7 (5.5)	Vit B6: T1/2: 0.84 (0.63; 1.14) T3: 0.88 (0.68; 1.26) Vit C: T1/2: 11.0 (5.7; 21.2) T3: 10.4 (6.1; 28.4) Fe: 9.6 (4.3) Folate (µg/d): 194.5 (75.3) Ca: 354.8 (245.5) Zn: 8.1 (4.3) Vit A (µg/d): 574.2 (428.4) Vit C: 34.7(26.6) Vit E: 5.6 (3.2) Vit B6: 0.87 (0.39) Niacin: 10.94 (4.97)
Assefa, 2012	Ethiopia	To measure the incidence and determinants of LBW in a rural population	956		MUAC <23 cm, n(%) MUAC ≥23 cm, n(%)	504 (52.7%) 452 (47.3%)					
Elshibly, 2008 (i) and Elshibly 2009 (ii)	Sudan	i) To quantify the effect of maternal anthropometry, education and socioeconomic status on gestational age and birth weight	1000	39.1 (1.8)	Weight, kg Height, cm BMI, kg/m^2 MUAC, cm Lean body mass	65.2 (13.0) 159.6 (6.2) 25.5 (4.8) 26.9 (3.9) 44.2 (4.9)					

First Author, Year	Country	Main Objective(s)	Sample Size	Gestational Age	MNS Measures [mean(SD)/ median(range)]						
					Anthropometry		Biomarkers		Dietary Intake		
					Measurement(s)	Value(s)	Type(s)	Value(s)	Energy, kcal/day	Macronutrient(s)/ Fibre, g/day	Micronutrient(s), mg/day
		ii) To investigate the relationship between maternal and newborn anthropometry									
Liu, 2013	Zambia	To identify predictors and outcomes associated with a birth weight of 4000 g or more in Lusaka. Women were analysed according to those who gave birth to normal weight (N) compared to macrosomic (M) babies	191834	First antenatal visit	BMI, kg/m^2	N: 23.5 (21.5–26.1)[c] M: 26.2 (23.4-29.8)[c]					
					Underweight (BMI <18.5 kg/m^2), n (%)	N: 3289 (2.8%) M: 41 (1.4%)					
					Normal weight (BMI 18.5-24.9 kg/m^2), n (%)	N: 75 043 (63.2%) M: 1 135 (38.4%)					
					Overweight (BMI 25-29.9 kg/m^2), n (%)	N: 30 464 (25.7%) M: 1 057 (35.7%)					
					Obese (BMI ≥30 kg/m^2), n (%)	N: 9 934 (8.4%) M: 724 (24.5%)					
Hartikainen, 2005	Malawi		1032	23 (25th, 75th percentiles: 20, 26)	Weight, kg	52 (48; 55)[d]					
					Weight gain, g/week	228 (111; 355)[d]					

First Author, Year	Country	Main Objective(s)	Sample Size	Gestational Age	MNS Measures [mean(SD)/ median(range)] Anthropometry Measurement(s)	Anthropometry Value(s)	Biomarkers Type(s)	Biomarkers Value(s)	Dietary Intake Energy, kcal/day	Dietary Intake Macronutrient(s)/ Fibre, g/day	Dietary Intake Micronutrient(s), mg/day
Nieuwoudt, 2014	South Africa	To investigate whether differences exist in adverse pregnancy outcomes between morbidly obese (MO, BMI 40-49.9) and super-obese (SO, BMI ≥50) women	66 MO, 46 SO	MO: 29 (range: 9 - 40) SO: 29 (range: 10 - 40)	Height, cm	MO: 160 (142-175) SO: 159 (136-172)					
					Weight, kg	MO: 114 (89-144) SO: 135 (111-193)					
Stephens, 2014	Ghana	To establish the prevalence of pregnancy-associated malaria and its associated consequences	320	18.5 (95% CI: 17.12-19.05)			Hb, g/dL	11.44 (95% CI 11.29 - 11.80)			
							Anaemia (Hb <11g/dL), n (%)	102 (32%)			
Jackson, 2010	Liberia	i) Estimate the proportion of LBW infants ii) Study the relationship between mothers' health complaints and pregnancy outcomes iii) Examine the relationship between GWG and pregnancy outcome iv) Determine the optimal weight gain associated with a favourable birth weight range	80	6 and 9 months	3rd trimester GWG, kg	3.18	Hb, g/dL	6 months: 9.9 (1.1)			
					3rd trimester GWG, kg/ week	1.06		9 months: 11.5 (1.2)[e]			

Abbreviations: LBW, low birth weight; GWG, gestational weight gain; BMI, body mass index; MUAC, mid-upper arm circumference; Fe, iron; Ca, calcium; Zn, zinc; Vit, vitamin; Hb, haemoglobin; Ph, phosphorus; Mg, magnesium

[a]Reported as kJ/day

[b]Based on serum Fe, ferritin, transferrin, Hb, hematocrit, mean corpuscular volume and red blood cell count
[c]Median (interquartile range)
[d]Median (25^{th}, 75^{th} percentiles)
[e]Routine Fe supplementation received between 6 and 9 months

2.3.2 Associations between MNS (anthropometry) and fetal growth and birth, neonatal and infant outcomes (Table 3)

Eight studies met the inclusion criteria (49,50,52,113,117,118,120,123). The publication year ranged from 2005 to 2014. Four studies followed a prospective cohort design (52,113,120,123), two studies used retrospective data (50,118) and two were cross-sectional studies (49,117). The number of pregnant women included in the studies ranged from 80 to 191 834. Anthropometric measurements used to describe MNS in study participants included weight and height (52,123), BMI (50), gestational weight gain (GWG) (118,120), MUAC (113) and lean body mass; with two studies describing all of these variables in pregnant women (49,117). All offspring outcomes were assessed at birth or within the first 30 days of life; with the most commonly examined outcomes being birth weight and gestational age (Table 3).

In Sudan, postpartum maternal weight and BMI were positively associated with birth weight ($P<0.001$), but neither variable predicted risk of LBW (<2 500g) (117). Although risk of LBW in Kenya was 2.4 times greater in infants born in farming than in pastoral communities, there was no difference in mean weight of the pregnant women during the third trimester of pregnancy (123). GWG in the second and third trimesters showed a strong seasonality effect in rural Malawian women, with those delivering in the rainy season gaining significantly less weight than those delivering in temperate/dry months (100-200g/week compared to 250-300 g/week; $P<0.001$); however this was not reflected as strongly in birth weight ($P<0.05$) and GWG was therefore only weakly correlated with birth weight (Pearson's correlation coefficient 0.13; significance not reported) (118). In contrast, although birth weight was correlated with maternal weight at 6 ($r=0.54$, $P=0.01$) and 9 months ($r=0.53$, $P=0.01$) in Liberia, there was a stronger, positive correlation with net weight gain between the two time points ($\beta=0.059$, $P<0.001$) (120).

Table 3: Results from observational studies of the associations between maternal nutritional status (anthropometry) and fetal growth and birth, neonatal and infant outcomes

					Anthropometry			Outcome			
First Author, Year	Country	Study Design	Sample Size	Participant Characteristics	Timing	Measurement(s)	Mean (SD)/ Median (range)	Timing	Variable	Mean (SD)/ Median (range)	Conclusions
Assefa, 2012	Ethiopia	Prospective cohort	956	Urban and rural Ethiopian pregnant women		MUAC <23 cm, n (%)	504 (52.7%)	Birth	LBW, n (%)	271 (28.3%)	MUAC < 23cm significantly associated with LBW and increased odds of LBW by 1.6 times (95% CI: 1.19, 2.19)
						MUAC ≥23 cm, n (%)	452 (47.3%)				
Elshibly, 2008 and Elshibly 2009	Sudan	Cross-sectional study	1000	Urban non-diabetic Sudan women with singleton births	Birth, 39.1 (1.8) weeks gestational age	Weight, kg	65.2 (13.0)	Neonatal (within 24 hours of birth)	Gestational age, weeks	39.1 (1.8)	Maternal height identified as strongest anthropometric predictor of neonatal outcomes; associated positively with gestational age (P<0.002), limb length (P<0.001) and birth weight (P<0.001)
						Height, cm	159.6 (6.2)		Birth weight, g	3131.7 (538.9)	
						BMI, kg/m^2	25.5 (4.8)		LBW, n (%)	83 (8.3%)	
						MUAC, cm	26.9 (3.9)		Preterm, n (%)	57 (5.7%)	
						Lean body mass	44.2 (4.9)		Supine Length, cm	49.3 (2.9)	
									Crown-rump length, cm	33.6 (2.2)	Height <156 cm increased RR of LBW by 52%
									Limb length, cm	15.0 (1.0)	Maternal weight, BMI and MUAC positively correlated with birth weight (p<0.001), but didn't predict LBW
									Head circumference, cm	34.4 (1.7)	
									Chest circumference, cm	31.7 (2.4)	Maternal BMI significantly associated with skinfold thicknesses (P<0.001)
									Abdominal circumference, cm	28.2(2.7)	Postpartum maternal lean body mass positively associated with birth weight, body length and body circumference (P<0.001)
									MUAC, cm	10.0 (1.1)	
									Mid-thigh circumference,	15.0 (1.7)	

First Author, Year	Country	Study Design	Sample Size	Participant Characteristics	Anthropometry			Outcome			Conclusions
					Timing	Measurement(s)	Mean (SD)/ Median (range)	Timing	Variable	Mean (SD)/ Median (range)	
									cm		
									Triceps skinfold thickness, mm	0.81 (0.21)	
									Subscapular skinfold thickness, mm	0.83 (0.24)	
									Ponderal index, g/cm^3	2.61 (0.45)	
Liu, 2013	Zambia	Retrospective cohort	191 834	Women with singleton births >2500g and at least one documented prenatal visit. Analysed as two groups: normal birth weight (N, 2 500-3 999g) and macrosomia (M, ≥4 000g)	First antenatal visit	BMI, kg/m^2	N: 23.5 (21.5-26.1)[a] M: 26.2 (23.4-29.8)[a]	Birth	Macrosomia, n (%)	4 717 (2.5%)	Mean BMI higher in women who gave birth to macrosomic infants (P<0.01) Overweight and obesity at baseline associated with 1.72 and 2.88 times greater odds of giving birth to a macrosomic infant respectively
						Underweight (BMI <18.5 kg/m^2), n (%)	N: 3289 (2.8%) M: 41 (1.4%)				
						Normal weight (BMI 18.5-24.9 kg/m^2), n (%)	N: 75 043 (63.2%) M: 1 135 (38.4%)				
						Overweight BMI 25-29.9 kg/m^2), n (%)	N: 30 464 (25.7%) M: 1 057 (35.7%)				
						Obese (BMI ≥30), n (%)	N: 9 934 (8.4%) M: 724 (24.5%)				

First Author, Year	Country	Study Design	Sample Size	Participant Characteristics	Anthropometry			Outcome			Conclusions
					Timing	Measurement(s)	Mean (SD)/ Median (range)	Timing	Variable	Mean (SD)/ Median (range)	
Hartikainen, 2005	Malawi	Retrospective cohort	1032	Rural Malawian women with singleton births	23 weeks (25th, 75th percentiles : 20, 26)	Weight, kg Weight gain, g/week	52 (48; 55)[b] 228 (111; 355)[b]	Neonatal (within 30 days of birth)	Birth weight, g Gestational age, weeks	3400 40 (38; 41)[b]	Seasonal variation in maternal weight gain: highest in those who delivered in the third quarter of the year (250-300 g/week) and lowest in the first quarter (100-200 g/week) (p<0.001) Weaker correlation for seasonality in newborn weight (p<0.05) GWG showed a modest correlation with newborn weight (Pearson's correlation coefficient: 0.13)
Keverenge-Ettyang, 2006	Kenya	Prospective cohort	122 P, 128 F	Rural pregnant women in their third trimester from pastoral (P) and farming (F) communities	3rd trimester (28-36 weeks gestational age)	Weight, kg Height, cm	P: 51.9 (5.5) F: 51.6 (7.1) P: 160 (5.6) F: 160 (6.8)	Neonatal (within 7 days of birth)	Birth weight, kg LBW, n (%)	2.856 (0.314) P: 19 (16.8%) F: 35 (31.3%)	Mean infant weight significantly lower in the farming communities (P<0.01) and LBW prevalence significantly higher in farming villages RR of death was 2.4 times greater for neonates born in farming compared to pastoral communities
Nieuwoudt, 2014	South Africa	Prospective cohort	66 MO, 46 SO	Pregnant women attending a high risk antenatal clinic with BMIs ≥40 kg/m^2	Gestational age, weeks: MO: 29 (range: 9-40) SO: 29 (range: 10-4(	Height, cm Weight, kg	MO: 160 (142-175) SO: 159 (136-172) MO: 114 (89-144) SO: 135 (111-193)	Neonatal	Symphysis-fundal height ≥90th centile, n (%) IUGR, n (%)	MO: 44 (66.7) SO: 28 (60.9) MO: 1 (1.5%) SO: 6 (13%)	Incidence of IUGR greater in the SO than the MO group (13% vs. 2%; p=0.02) No differences in macrosomia incidence between groups

First Author, Year	Country	Study Design	Sample Size	Participant Characteristics	Anthropometry			Outcome			Conclusions
					Timing	Measurement(s)	Mean (SD)/ Median (range)	Timing	Variable	Mean (SD)/ Median (range)	
									Macrosomia, n (%)	MO: 5 (7.6%) SO: 3 (6.5%)	
									Weight, g	MO: 3200 (525-4330) SO: 3430 (640-4690)	
Jackson, 2010	Liberia	Prospective cohort	80	Generally healthy women with no previous antenatal care for current pregnancy	6 and 9 months	3rd trimester GWG, kg	3.18	Birth	Birth weight, g (mean)	3311	Infant birth weight weakly correlated with maternal weight at 6 (r=0.54, P=0.01) and 9 months (r=0.53, P=0.01) Net weight gain between 6 and 9 months was strongly correlated with birth weight (b=0.059, P<0.001)
						3rd trimester GWG, kg/ week	1.06		LBW, n (%)	1 (1.3%)	

Abbreviations: LBW, low birth weight; IUGR, intrauterine growth restriction; GWG, gestational weight gain; BMI, body mass index; MUAC, mid-upper arm circumference ; MO, morbidly obese (BMI 40-49.9 kg/m^2); SO, super obese (BMI ≥50 kg/m^2); RR, risk ratio

[a]Median (interquartile range)

[b]Median (25^{th}, 75^{th} percentiles)

In Zambia, where maternal overweight was more prevalent than the aforementioned studies, overweight and obesity were associated with 1.72 and 2.88 times greater odds of giving birth to a macrosomic infant respectively (50). In South African women with BMIs ≥40kg/m^2, incidence of IUGR was significantly higher in those who had BMIs ≥ 50kg/m^2 than those with BMIs between 40 and 49.9kg/m^2 (52).

MUAC was associated with birth weight in two studies, with a MUAC <23cm (suggestive of maternal underweight) (128) increasing odds of LBW by 1.6 times (49,113). In addition to the aforementioned findings, maternal height was identified as the strongest anthropometric predictor of neonatal outcomes in Liberia. Maternal height had positive associations with gestational age (P<0.002), limb length (P<0.001) and birth weight (P<0.001), while height <156 cm increased the relative risk of LBW by 52%. In the same study sample, positive associations were found between maternal lean body mass and birth weight, body length and body circumference within 24 hours of birth (P<0.001) (49).

2.3.3 The associations between MNS (nutritional biomarkers) and fetal growth and birth, neonatal and infant outcomes (Table 4)

Three studies met the inclusion criteria (120,123,127). The publication years were 2006, 2010 and 2014. All studies included were prospective cohort studies and the sample size ranged between 80 and 320. All studies used biomarkers to assess anaemia and/or iron status of pregnant women, with two studies using Hb concentrations only and one study including haematocrit and serum ferritin concentrations (123). The latter also assessed maternal vitamin A status using serum retinol concentrations. Birth weight was the outcome of interest in all studies (Table 4).

Data from Kenya reports pregnant women from pastoral communities having lower Hb concentrations and higher anaemia prevalences than those from farming communities

Table 4: Results from observational studies of the associations between maternal nutritional status (biomarkers) and fetal growth and birth, neonatal and infant outcomes

					Biomarkers			Outcome			
First Author, Year	Country	Study Design	Sample Size	Participant Characteristics	Timing	Type(s)	Mean (SD)/ Median (range)	Timin	Variable	Mean (SD)/ Median (range)	Conclusions
Stephens, 2014	Ghana	Prospective cohort	320	Pregnant women from a low malaria transmission area of suburban, coastal Ghana who had not received Intermittent Preventive Treatment for malaria prevention at enrolment	First antenatal visit, 18.5 weeks gestation (95% CI: 17.12-19.05)	Hb, g/dL Anaemia (Hb <11g/dL), n (%)	11.44 (95% CI 11.29 - 11.80) 102 (32%)	Birth	LBW, n (%)	11 (3.3%)	None of the LBW babies were born to women who had anaemia in the first trimester
Keverenge-Ettyang, 2006	Kenya	Prospective cohort	122 P, 128 F	Rural pregnant women in their third trimester from pastoral (P) and farming (F) communities	3rd trimester (28-36 weeks gestational age)	Hb, g/L Hematocrit Serum ferritin (SF), µg/L Serum retinol (SR), µmol/L Anaemia (Hb <110g/L), n (%)	P: 119 (11.3) F: 124 (15.0) P: 33 (3.95) F: 32 (5.42) P: 25.8 (4.82) F: 24.4 (4.87) P: 0.92 (0.43) F: 0.92 (0.35) P: 49 (42.2%) F: 27 (21.8%)	Within 7 days of birth	Birth weight, kg LBW, n (%)	P: 2.9 (0.4) F: 2.8 (0.4) P: 19 (16.8%) F: 35 (31.3%)	Women from the pastoral community had lower Hb concentrations (P<0.05) and higher anaemia prevalence (P<0.01), but higher serum ferritin concentrations (p<0.05) There were no differences in serum retinol levels Mean birth weight was significantly lower (p<0.01) in the farming than in the

First Author, Year	Country	Study Design	Sample Size	Participant Characteristics	Biomarkers			Outcome			Conclusions
					Timing	Type(s)	Mean (SD)/ Median (range)	Timin	Variable	Mean (SD)/ Median (range)	
						Low Fe stores (SF <32 μg/L), n (%) Low Vit A status (SR <0.70 μmol/L), n (%)	P: 95 (77%) F: 110 (85.9%) P: 34 (27.9%) F: 31 (24.2%)				pastoral community and a significantly higher proportion of newborns in the farming community were LBW (P<0.05)
Jackson, 2010	Liberia	Prospective cohort	80	Generally healthy women with no previous antenatal care for the current pregnancy	6 and 9 months	Hb, g/dL	6 months: 9.9 (1.1) 9 months: 11.5 (1.2)[a]	Birth	Birth weight, g (mean) LBW, n (%)	3311 1 (1.3%)	There was a significant (but weak), positive correlation between Hb at 6 months and infant birth weight (P=0.042), but the correlation was not significant at 9 months

Abbreviations: LBW, low birth weight; Fe, iron; Vit, vitamin; Hb, haemoglobin
[a]Routine Fe supplementation received between 6 and 9 months

[(119g/L v. 124g/L, P<0.05); (42.2% vs. 21.8%, P<0.01)] respectively, but serum ferritin concentrations were higher in the pastoral community (25.8µg/L vs. 24.4µg/L, p<0.05). Mean infant birth weight was significantly lower (2.9 kg vs. 2.8 kg, P<0.01) and prevalence of LBW significantly higher (31.3% vs. 16.8%, P<0.05) for babies born to mothers from farming than from pastoral communities (123). A positive correlation between maternal Hb concentrations at 6 months gestation and infant birth weight (P=0.042) was shown in Liberia; however this was not significant at 9 months (all women had been routinely supplemented with 180mg Fe/day between the 6 and 9 month assessment) (120). In Ghana, none of the women who were anaemic in the first trimester of pregnancy gave birth to LBW babies (127).

2.3.4 The associations between MNS (dietary intake) and fetal growth and birth, neonatal and infant outcomes

No studies were identified which met the inclusion criteria.

2.3.5 Randomised/quasi-randomised clinical trials on the associations between maternal nutritional interventions and fetal growth, birth, neonatal and infant outcomes (Table 5)

Six studies met the inclusion criteria (129–134). The publication years ranged between 1997 and 2011. Four studies were double-blind randomised controlled trials (RCTs) (131–134), one was a cluster RCT (129) and one study used data from both a double-blind RCT and a cluster RCT (130). The sample sizes ranged between 125 and 2 100 and gestational age of the subjects at baseline ranged between 20 and 28 weeks. Interventions included iron (134), multiple micronutrient (133), calcium (130–132) and protein-energy supplementation (129,130) (Table 5).

Protein-energy supplementation in chronically undernourished Gambian women from 20 weeks gestational age was associated with 136g higher pregnancy weight gain than in the

Table 5: Results from randomized/quasi-randomized clinical trials on the associations between maternal nutritional interventions and fetal growth and birth, neonatal and infant outcomes

First Author, Year	Country	Experimental Design	Sample Size	Baseline MNS	Intervention Targeted	Gestational Age at Initiation, weeks	Intervention Prescribed, per day	Intervention Duration	Outcome Assessment	Conclusions
Preziosi, 1997	Niger	Double-blind, randomized, placebo-controlled trial	197 pregnant women (99 intervention, 98 control)	Apparently healthy low- or middle-class civil servants and rural women with no obstetric complications. Anaemia (Hb <110g/L): Intervention: 65.7% Control: 69.4%	Fe supplementation	28 (±21 days)	100 mg elemental Fe	Until delivery	Birth, 3, 6 months postpartum	No difference in birth weight Birth length significantly higher in intervention group (P<0.05) No differences in infant length at 3 or 6 months postpartum Serum ferritin concentrations higher in infants born to supplemented mothers at 3 [99(63) vs. 80(53)µg/L] and 6 months [26(27) vs. 15(20)µg/L] (P<0.05)
Kaestel, 2005	Guinea-Bissau	Double-blind randomized, controlled trial	2100 pregnant women	Baseline BMI, mean (SD): Micronutrient intervention (MN)-1: 23.3 (3.4) MN-2: 23.3 (3.3) Control: 23.2 (3.3) Anaemia (Hb <100g/L): MN-1: 30% MN-2: 31% Control: 31%	Multiple micronutrient supplementation	Mean (SD): MN-1: 22.3 (6.6) MN-2: 22.1 (6.8) Control: 21.9 (6.9)	MN-1: one RDA of 15 micronutrients MN-2: two RDA of 14 micronutrients (Fe at one RDA) Control: standard Fe-folic acid supplement	Until delivery, mean (SD): 16.6 (7.1) weeks	Birth, neonatal	Mean birth weight was 53g higher in MN-1 and 95g higher in MN-2 than the control group Non-significant effect on LBW: 10.1% MN-2, 12% MN-1 and 13.6% control (P=0.33) Birth weight increased by 218g and risk of LBW decreased by 69% in anaemic women receiving MN-2 compared to the control group

First Author, Year	Country	Experimental Design	Sample Size	Baseline MNS	Intervention Targeted	Gestational Age at Initiation, weeks	Intervention Prescribed, per day	Intervention Duration	Outcome Assessment	Conclusions
										No effect on perinatal mortality
Ceesay, 1997	The Gambia	Cluster randomized controlled trial	1460 pregnant women (yielded 2047 live singleton births over 5 years)	Chronically undernourished, rural pregnant women	Protein-energy supplement	Intervention: 20 Control: after delivery	Energy: 1015 kcal Protein: 22 g Fat: 56g Calcium: 47mg Fe: 1.8mg	Intervention: until delivery Control: 20 weeks	Birth, neonatal	GWG increased by 136g in the supplementation group (P<0.001): higher increases in the hungry (201g, P<0.001) than the harvest season (94g, P<0.01) Odds of LBW was 0.61 (95% CI: 0.47-0.79, P<0.001) and head circumference was 3.1mm higher (P<0.01) in the supplemented group Supplementation reduced perinatal mortality: OR 0.54 (95% CI: 0.35-0.85, P 0.01) for all deaths in first week of life
Hawkesworth, 2011			1267 term births followed up						Childhood (11-17 years)	No differences between groups childhood body composition (BMI, fat mass or lean mass), blood pressure, insulin or cholesterol concentrations Children of pregnancy-supplemented mothers: -0.05 mmol/L (95% CI: -0.10, -0.001 mmol/L) lower fasting plasma

First Author, Year	Country	Experimental Design	Sample Size	Baseline MNS	Intervention Targeted	Gestational Age at Initiation, weeks	Intervention Prescribed, per day	Intervention Duration	Outcome Assessment	Conclusions
										glucose
Jarjou, 2006	The Gambia	Double-blind, randomized, placebo-controlled trial	125 pregnant women (subsample of main study, n=536)	Rural Gambian women with previously documented low calcium intakes (approximately 350 mg/d)	Calcium	20	1500 mg	Until delivery	Birth, neonatal, infant (birth, <5 days, 2, 13, 52 weeks of age)	No differences in birth weight between groups No differences in infant weight, body length, head circumference or bone mineral status in the first year of life
Hawkesworth, 2010 and Hawkesworth, 2011			350 term births followed up of main study sample						Chilldhood (5-10 years)	No differences in blood pressure between infants born to supplemented compared to un-supplemented mothers. No interaction between childhood BMI and supplementation for blood pressure variables

Abbreviations: MNS, maternal nutritional status; LBW, low birth weight; Fe, iron; BMI, body mass index; OR, odds ratio

control group (P<0.001) (129). Increases were higher in the hungry (201g, P<0.001) than in the harvest season (94g, p<0.01). Odds of perinatal mortality (death within the first 7 days of life) (OR: 0.54, 95% CI: 0.35;0.85, P<0.01) and LBW (0.61 95%CI 0.47;0.79, P<0.001) were respectively lower in the supplementation group. There was also a 3.1 mm increase in head circumference (P<0.01) in those who received the intervention (129). During the follow-up study in 11-17 year olds, no differences in BMI, fat mass, lean mass, blood pressure, insulin or cholesterol concentrations were found between those whose mothers had received protein-energy supplementation during pregnancy and controls; however those born to supplemented mothers had -0.05 mmol/L (95% CI: -0.10, -0.001 mmol/L) lower fasting glucose concentrations (130).

In Gambian women, no differences in weight, body length, head circumference or bone mineral content between infants born to women who received calcium supplementation and those who received a placebo from 20 weeks gestational age were observed. Follow-up of infants at 5-10 years of age showed no differences in blood pressure and no interaction between BMI and calcium supplementation for blood pressure variables (130,131).

A trial of iron supplementation to a cohort of pregnant women with a high anaemia prevalence in Niger found no differences in birth weight between babies born to the intervention and control groups (134). Birth length and Apgar scores were significantly higher in babies born to supplemented mothers; however the difference in length did not persist at 3 and 6 months. Serum ferritin concentrations at 3 and 6 months of age were higher in infants whose mothers received iron supplementation compared to those whose mothers received the placebo (P<0.05) (134).

Pregnant women in Guinea-Bissau received either one of two possible interventions: a tablet with one recommended daily allowance (RDA) of 15 micronutrients (MN-1) or a tablet with

two RDAs of the same micronutrients (but one RDA of iron) (MN-2), or a standard iron-folic acid supplement (control group) from approximately 22 weeks gestation (133). Mean birth weight was 53g and 95g higher in the MN-1 and MN-2 groups respectively than in the control group, suggesting a dose-response effect of supplementation. Supplementation had a positive effect on LBW, with 10.1%, 12% and 13.6% LBW prevalence found in the MN-2, MN-1 and control groups respectively; however this was not significant (P=0.33). Birth weight was 218g higher and risk of LBW 69% lower for babies born to anaemic women in the MN-2 group compared to the control group (133).

2.3.6 Randomised/quasi-randomised clinical trials on the associations between nutritional interventions in the first 2 years of life and any adolescent and/or adult health outcomes

No studies were identified which met the inclusion criteria.

2.4 Discussion

Using a systematic approach, this review aimed to provide and report on available data on MNS among Africans and illustrate whether the first 1000 days of life are nutritionally important for Africa. We focused on the role of MNS during this period and how it associates with fetal growth and birth, neonatal and infant outcomes. The results are conveniently discussed by sub headings addressing each specific objective set for this review.

2.4.1 MNS in Africa

Using BMI most African women in the reported studies were within the normal weight to overweight category during pregnancy, with maternal overweight or obesity being more prevalent than underweight. This was supported by high energy intakes in some countries; however mean energy intakes varied greatly between populations. Carbohydrate, protein and fat contributed between 58-87%, 5.9-14.5% and 6.3-27.9% respectively to total energy intake

across study sites. The most prevalent micronutrient deficiency in African pregnant women was iron.

Studies included in this review indicate a low burden of maternal underweight, and comparatively high overweight and obesity prevalence, typical of the epidemiological health transition across African countries. Although GWG was approximately half the Institute of Medicine (IoM) recommended level for normal weight women in Malawi (118), much higher weekly gain than recommended for any BMI category was found in Liberian pregnant women (25,120). Though the above findings provide good proxies for maternal obesity status, interpretation of the findings should be done with care, as a limitation exits in that obesity status (being underweight, normal, overweight or obese) was categorized by using BMI cut-offs of non-pregnant women.

Nutritional biomarkers showed a persisting high prevalence of micronutrient deficiencies in pregnant African women. Data suggest anaemia and/or iron deficiency prevalence to be high. Although comparison between rural and urban sites in Ghana suggests significantly higher anaemia prevalence in rural women, close to 30% of pregnant women were anaemic in urban settings (125). This is a much higher than the prevalence seen in HICs (Europe: 16.2%, America and the Caribbean: 15.2%) and other LMIC (Asia: 19.8%) settings (1). This may be due to a chronic intake of low absorbable iron and insufficient iron stores to support both maternal and fetal requirements or to high levels of infection in African communities; or a combination of both (101).

Reported dietary intakes of pregnant women varied significantly across African countries, with studies showing energy consumption below and above the American Dietetic Association recommended range of 2 200-2 900 kcal/d (135). However, the shift towards higher energy intakes in populations with traditionally low food access was not reflected in

adequate protein intake, which was lower than the IoM's recommended daily allowance (RDA, 71g) in all but one study (136). Mean dietary iron intakes were much lower than the IoM RDA of 27 mg/d (137) in all but two studies (112,119). Folate intake was much lower, on average, than the 600 µg/d recommended for pregnant women (138), with most studies reporting intakes less than half this. Majority of the women studied either did not receive or did not comply with micronutrient supplementation during pregnancy, even in countries where iron and/or folic acid supplementation should have been a routine part of antenatal care. Higher energy consumption, coupled with inadequate protein and micronutrient intakes, may be a result of poor diet quality and/or food availability in communities in transition. This was demonstrated in South Africa where one study showed pregnant women to consume predominantly cereal-based diets high in energy and refined sugar, with low intakes of more expensive protein/micronutrient rich foods such as meat, poultry, and seafood, as well as legumes and non-starchy vegetables (122).

2.4.2 Associations of MNS (using anthropometric parameters) and fetal growth and birth, neonatal and infant outcomes

Maternal weight, BMI and weight gain during pregnancy were positively associated with birth weight in African studies. However, maternal overweight and obesity increased the risk of macrosomia in Zambia (50) and higher BMIs were associated with increased risk of IUGR in a sample of very obese women from South Africa (BMI >40kg/m^2) (52).

These findings are consistent with studies from other parts of the world. A systematic review including data from both HICs and LMICs showed a significant risk of LBW in women who were underweight during pregnancy compared to those who were within normal weight categories (104) and GWG has been positively associated with birth weight in a number of studies (139–141). Substantial evidence supports the association between maternal obesity and macrosomia, with a 2-3-fold increase in risk of macrosomia being observed in obese

women (8,10,100). There is also evidence to support the association between maternal obesity and IUGR; however fewer studies have documented this (142,143).

Although the findings of this review have been supported by literature from other settings, the strength of and comparability between the included studies is limited due to differences in study design, exposure variables, and sample sizes which were relatively low in prospective cohort designs. In addition, the timing in assessment of anthropometric parameters in pregnancy and outcome measurements varied greatly between studies, with maternal assessments being done between the first antenatal visit and delivery across studies and birth outcome measurements being taken any time between birth and the first 30 days of life.

Although the underweight prevalence was low overall in African settings, risk of adverse fetal and birth outcomes remained high in populations where low pre-pregnancy weight is a key issue, for example in Ethiopia where 52.7% of women had MUAC measurements <23 cm, LBW prevalence was high (28.3%). However, as maternal overweight and obesity continue to rise, high pre-pregnancy weight and excessive GWG and their associated risks should become the pivotal focus for maternal and child nutrition.

2.4.3 Associations of MNS (nutritional biomarkers) and fetal growth and birth, neonatal and infant outcomes

Data on the associations between nutritional biomarkers and outcomes of interest were very limited. The limited data available seem to suggest associations between low Hb and serum ferritin concentrations with lower birth weights in African settings (120,123).

Global evidence associating nutritional biomarkers with outcomes of interest show mixed results. Maternal anaemia in Indians was associated with increased risk of LBW and IDA predicted a three times higher risk of preterm birth (144). Low maternal Hb, but not serum ferritin concentrations were associated with lower birth weight in Iran (145). However, in a

multicentre study across four HICs (New Zealand, Australia, England and Ireland), as well as in Sri Lanka (LMIC), there were no associations observed between anaemia (Hb <11 g/dL) and risk of preterm birth, LBW or small-for-gestational-age (SGA) infants (146,147).

Although all included studies focused on the association between anaemia and birth weight using prospective cohort designs, the late assessment of biomarker status in two of the three studies provided poor proxies of pre-pregnancy status and the variation in timing of measurements between studies limited comparability. Sample sizes were low in all studies, which may have limited the power to detect associations between the biomarker(s) and outcome(s) of interest.

More evidence is needed to understand the associations between maternal micronutrient status and deficiencies on outcomes of interest in the first 1000 days; however use of individual biomarkers of nutritional status in isolation may be impractical in Africa where diet quality is poor and pregnant women are likely to experience multiple nutrient deficiencies. Thus identifying nutritional biomarker patterns using dimension reduction techniques could be essential to employ in such studies.

2.4.4 Associations of MNS (reported dietary intakes) and fetal growth and birth, neonatal and infant outcomes

Although no articles were retrieved for Africa on associations between reported dietary intakes and outcomes of interest, the use of reported dietary assessment has a number of challenges. Repeated 24 hour recalls and FFQs are the most commonly used methods for assessing habitual dietary intakes in Africa (148). The inherent errors associated with reported dietary intakes and the strengths and limitations of different dietary assessment (DA) methods cannot be ignored (148). Limitations include recall bias, assuming temporarily regular eating habits, seasonality and providing inaccurate estimations of portion size etc.

(149–151) However, very few DA tools used in Africa have been validated or tested for reliability which presents a huge challenge for effective assessment and monitoring of dietary intake, as well as for comparison of intakes within and between African settings (148).

2.4.5 Randomised clinical trials of maternal nutritional interventions and fetal growth and birth, neonatal and infant outcomes

Evidence from an African study suggests positive associations between protein-energy supplementation during pregnancy and higher GWG, birth weight and lower risk of perinatal mortality (129). However, no longer term effects were seen on CVD risk during the 11-17 year follow-up (130). Multiple micronutrient supplementations had a dose-response effect on birth weight and significantly reduced LBW risk in anaemic women (133). Although iron supplementation was associated with an increase in birth length, no improvement in birth weight was found (134). Prenatal calcium supplementation had no effect on any birth, neonatal, infant or childhood health outcomes (130–132).

A review on protein-energy supplementation trials including both HICs and LMICs showed positive effects on birth weight in the supplemented compared to control groups, with the greatest effects seen in undernourished populations (152). This supports the findings of the Gambian study where pregnant women were chronically undernourished at baseline (129). Similarly, a meta-analysis on multi-micronutrient supplementation trials (153) supported the data from Guinea-Bissau (133) by showing significant reductions in LBW, SGA incidence and increased mean birth weight for women in the intervention compared to the control group (mostly receiving iron-folate supplements) (153). Data from the iron supplementation trial in Niger (134) contradicted the general findings from a meta-analysis which showed that daily iron supplementation during pregnancy (alone or in combination with folate) reduced incidence of LBW by 20% compared to controls (154). The effects of supplementation were most pronounced in populations with higher baseline anaemia prevalence (154). Although

positive effects on birth length were seen in the supplementation group in Niger, the lack of improvement in birth weight in this population with high anaemia prevalence is not a common finding compared to the literature. However, this finding could be, in part, attributed to the late start of the intervention during pregnancy (38 weeks, ±21 days) and/or the small sample size (134). No studies in Africa were found which suggested any long term benefits of nutritional supplementation during pregnancy.

RCTs are considered the most robust designs for assessing the relationship between exposure and outcome, because they ensure comparability between those exposed and those unexposed to the intervention and allow for causal links to be made since the intervention always precedes the outcome of interest. However, variability between RCT designs can alter the strength of individual studies. The following are important factors of concern associated with maternal nutritional intervention studies that make comparability of findings difficult: (i) sample size (ii) dose of intervention (iii) timing of intervention during pregnancy (iv) baseline nutritional status of pregnant woman and (v) an appropriate control group. Cumulatively the data available seems to suggest significant benefits of macronutrient and/or micronutrient supplementation during pregnancy on fetal/birth outcome (specifically birth weight), particularly in undernourished women.

The results presented in this review illustrate that data available for Africa ranges from 18 weeks gestational age onwards. This highlights that an important critical phase (less than 18 weeks) has not been investigated in this setting.

2.4.6 The most important nutrients of concern for the first 1000 days for African women

Energy and nutrient requirements increase during pregnancy in order to meet the needs of both the mother and the growing fetus. Inadequate intakes of macro- and/or micronutrients

prior to, and during, pregnancy result in limited growth and development and therefore poor pregnancy outcomes. Nutrient sufficiency is similarly required during early infancy to prevent growth faltering. Certain nutrients are of particular importance, due to the critical functions that they perform and the plasticity during the first 1000 days of life. Energy requirements increase during pregnancy to support increases in basal metabolic rate as a result of growth and expansion of new and existing tissue (fetus, placenta and maternal tissues), as well as the higher work rate of the maternal cardiovascular, respiratory and renal systems. Adequate energy is also needed to support periods of rapid growth and development in the first two years of life. Protein requirements are high during pregnancy and infancy for deposition and maintenance of maternal and fetal tissue (101,155). Omega-3 and omega-6 fatty acids are essential to new tissue formation, due to their structural role in cell membranes, with omega-3 fatty acids being particularly important for brain and central nervous system development (156). Micronutrients of key concern in pregnancy are iron and folate, as they are unlikely to be in sufficient supply from the diet. Additional iron is required to support the increase in red cell mass and ensure sufficient oxygen supply during tissue synthesis and growth. Folate is an important co-factor in cellular function, including DNA and nucleic acid synthesis and cell division (101,157,158).

2.4.7 Known consequences associated with poor MNS

Inadequate maternal nutrition – underweight and overweight, as well as micronutrient insufficiency has been strongly linked with adverse maternal and infant outcomes, with both short and long term consequences. Maternal obesity and adiposity and high GWG are associated with increased risk of GDM, preeclampsia, maternal weight retention post-partum and poor infant outcomes such as prolonged labour, birth trauma, neonatal death and contrasting burdens of both macrosomia and SGA. Higher neonatal fat mass has been associated with adiposity in childhood and adulthood and therefore increased metabolic risk

in later life (10,109). Premature delivery has been shown to be associated with both maternal underweight and overweight and is strongly associated with increased risk of perinatal morbidity and mortality, as well as impaired cognitive and emotional development later in childhood and adolescence (103,104,159). Low maternal weight-for-height, poor GWG and micronutrient deficiencies such as IDA increase risk of IUGR which is associated with neonatal mortality in the short term and sub-optimal growth and development in the long term; e.g. in cognition, learning disabilities, academic achievement and psychosocial maturation (160). For those infants defined as SGA, neonatal mortality risk is higher than those born appropriate for gestational age, even if born at term (101). LBW, a result of preterm birth and/or growth restriction in utero, is associated with increased risk of perinatal morbidity and mortality, as well as of long term health risk. Sufficient evidence exits to suggest that impaired growth in utero increases long term risk of NCDs such as T2DM, hypertension and CVD, with the highest level of risk being seen in those who subsequently experience rapid and/or excessive of weight gain (101).

2.4.8 Challenges of appropriate interventions in the first 1000 days to reduce childhood obesity and adult NCDs in African women

MNS, childhood obesity and adult NCD risk is complex and influenced by multiple factors at various life stages, making it difficult to reverse once highly prevalent in populations. Exposure to a poor nutrition environment in the first 1000 days (critical periods of plasticity) seems to have significant effects on body function, metabolism and a programming phenotypic effect thereby influencing susceptibility to obesity, as well as to NCDs, in the longer term. This is of critical importance in the African setting where maternal obesity, coupled with poor micronutrient status and diet quality, continues to grow (1,161,162). Although the plastic nature of this period makes it vulnerable to poor environmental exposures, it also provides a unique window for intervention. Ensuring optimal growth and

development during this window, when women are highly motivated and tend to experience greater contact with health services, should therefore be prioritised in Africa to improve long term health trajectories (163). The main challenges for appropriate nutritional interventions in first 1000 days include, (i) when to intervene to get the best returns (pre-pregnancy vs. early pregnancy vs. after birth vs. infancy) and (ii) which nutrients and what doses to include. The Lancet series on maternal and child nutrition has provided a new conceptual framework that shows and elucidates on the means to optimum fetal and child growth and development (1). This framework outlines the dietary, behavioural, and health determinants of optimum nutrition, growth, and development, and how they are affected by various underlying conditions, which are in turn shaped by economic and social conditions, national and global contexts, capacity, resources, and governance. Additionally the series outlines and discusses how determinants can be changed to enhance maternal and childhood outcomes, including nutrition specific interventions that address the immediate and underlying causes of malnutrition (164,165).

There is a lack of data associating MNS with outcomes beyond birth in Africa. A need exists for longitudinal data from pregnancy through infancy to two years of age, and beyond. Without this evidence we cannot adequately influence policy or strengthen health systems.

2.5 Conclusion

Although improvements in MNS are evident in African countries, such as low maternal underweight prevalences, rapid transition has widened the spectrum of risk associated with maternal and child health to include high levels of overweight and obesity alongside sustained macro- and micronutrient insufficiency (hidden hunger). While robust evidence to support the associations between MNS and fetal, birth, neonatal and infant outcomes is limited in Africa, data does support the relationships seen globally between maternal anthropometry and outcomes in this setting. In addition, the high prevalence of deficiencies

in critical pregnancy-related nutrients, as well as the benefits seen in supplementation trials of women does suggest that improvements in MNS could have significant effects on outcomes of interest. This review therefore confirms the importance of the first 1 000 days within the African setting, but highlights that this area still remains under-researched as well as the need to focus on this window to optimise not only maternal and child health in the short term, but potentially reduce the burden of both undernutrition and NCD risk in current and future generations.

CHAPTER 3: Methodology

This chapter provides background context for the study, which took place in Soweto, Johannesburg, South Africa. Although specific methods for the subsequent study objectives (objectives 2-4) are discussed within their respective chapters (chapters 4-6), this section provides an overall view of the study setting and design for more comprehensive interpretation of the study as a whole.

3.1 Study setting

South Africa is a middle income country in Sub-Saharan Africa with a total population of 55.9 million people (166). While the gross domestic product (GDP) was estimated at $294.8 billion in 2016, growth in economic development has somewhat stagnated in recent years due to high dependence on international investment and markets, poor educational and training outputs, corruption, policy uncertainty and persistent inequality (166). This has serious implications for the continued levels of unemployment and poverty in South Africa, and therefore for access to adequate and sustainable public health care in the context of an overburdened health system with limited resources (167,168).

Soweto is a large urban area approximately 15 km from the inner city of Johannesburg in the Gauteng province (Figure 6). Although the name originated as an abbreviation for South Western Townships, it has also been believed to be derived from the phrase “so where to?” due to its origins as an informal settlement for migrant mine workers and black people displaced from other areas of Johannesburg during the Apartheid era. This cluster of townships grew rapidly and is now the most densely populated area in the country, with a predominantly black African population (98.5%) of approximately 1.2 million within an area of 200km^2. Although democracy was introduced in South Africa in 1994, the lasting inequality means that the income distribution between households is still one of the widest in the world (169). The levels of inequality are clearly evident within Soweto itself; with homes ranging from affluent mansions in some areas to makeshift shacks (Figure 7).

However, regardless of some economic growth in the area, approximately a third of residents are unemployed and the majority still live in low income households – 40% of which are female-led - with poor access to basic services (170).

Figure 6: Location of Soweto within the Gauteng province of South Africa (171)

Soweto is a prime example of the nutrition transition taking place across Africa; where rapid urbanisation has resulted in a shift towards more energy dense westernised diets high in saturated fat, sugar, processed/convenience foods and edible oils and low in fibre and essential micronutrients, alongside low levels of physical activity (26,28). A previous study of Sowetan adult women showed that they exhibit particularly high intakes of refined carbohydrates, added sugar and fat (particularly brick/hard margarine and sunflower oil), sugar sweetened beverages, processed meats and fast food products (172). Such shifts in the nutrition environment and the growing overweight/obesity prevalence, alongside persisting

infectious diseases (HIV/AIDS and tuberculosis; TB) and emerging NCD burdens are particularly prevalent in adult women; making maternal and child health particularly vulnerable in this population (3,94). This is further complicated by limited access to health education and services.

Figure 7: Basic housing in Soweto (173)

3.2 Study design and participants

This PhD was nested within the wider Soweto First 1000-Day Study (S1000) conducted by the Medical Research Council (MRC)/University of the Witwatersrand (Wits) Developmental Pathways for Health Research Unit (DPHRU). S1000 was a longitudinal maternal pregnancy cohort study with the overall aim of understanding the complexities between multiple

maternal factors and fetal and infant outcomes, and identifying the levers that could optimize maternal and child health within the first 1000 days, in an urban-poor African context. This study recruited approximately 1000 pregnant women from Chris Hani Baragwanath Academic Hospital (CHBH) between 2013 and 2016.

CHBH – based in Soweto – is the third largest hospital in the world, with approximately 3 200 beds. Approximately 60 000 patients attend the Maternity Hospital and 17 000 babies are delivered each year. CHBH is a tertiary public health facility which is government funded and acts as a teaching hospital for University of the Witwatersrand medical students. This is the only hospital serving the residents of Soweto and therefore all primary healthcare facilities refer into CHBH; providing a sample of women that represent the range of co-morbidities experienced by urban, black pregnant women living in Soweto. In addition, it is the only healthcare facility offering fetal ultrasounds. For this reason, CHBH provided the only option for accurate assessment of gestational age via ultrasound at recruitment.

Women were recruited into the wider S1000 study according to the following inclusion criteria: pregnant with a singleton, naturally conceived pregnancy, 18 years or older, resident of Soweto, or the Greater Soweto area, <18 weeks gestational age at recruitment, non-epileptic, non-diabetic, and able to give consent. Additionally, women were excluded if they had any detected fetal abnormalities.

3.3 Data collection

S1000 was separated into two main components: the Soweto Fetal Growth Study (SFGS) and the Soweto Baby Growth Study (SBGS). Data collection for SFGS took place at six time points during pregnancy (<14 weeks; 14–18 weeks; 19–23 weeks; 24–28 weeks; 29–33 weeks and 34–38 weeks) and at delivery. SBGS followed up both mother and infant at eight time points after delivery (<14 days; 6 weeks; 2 months; 3 months; 6 months; 12 months; 18

months and 24 months). S1000 collected a vast range of data on both the mother and infant across the study visits; including demographics, behavioural factors (diet and physical activity), anthropometry, body composition, health and psychosocial factors and neurocognitive development, as well as collection of biological samples (e.g. blood, urine and faeces) (Appendix A and B). The flow of participants through this PhD sub-study, as well as attrition rates across the longitudinal follow up (from recruitment through to delivery and neonatal data collection) are depicted in Figure 8. Data collection sheets and questionnaires for PhD specific data collection are included in Appendices C-E. However, detailed descriptions of the data collection methods for the variables used in this PhD are provided in the individual objective chapters to follow (Chapters 4-6).

3.4 Ethical approval

All women were provided with information and consent sheets prior to their inclusion in SFGS (Appendix F and G), as well as prior to the inclusion of themselves and their infants SBGS (Appendix H and I). Where necessary a trained member of research staff explained these documents to them in their native language. Written informed consent was then provided by all women prior to enrolment in each study component. All questionnaires were coded to ensure that participant names are not used and confidentiality was maintained. A document containing the coding "links" was kept separately by the Principal Investigator of SFGS and SBGS. Ethical approval was obtained from the University of the Witwatersrand's Research Ethics Committee (Medical) for both components of the wider S1000 study (SFGS: M120524 and SBGS: M130905) (Appendix J and K) as well as for this PhD (M150720) (Appendix L).

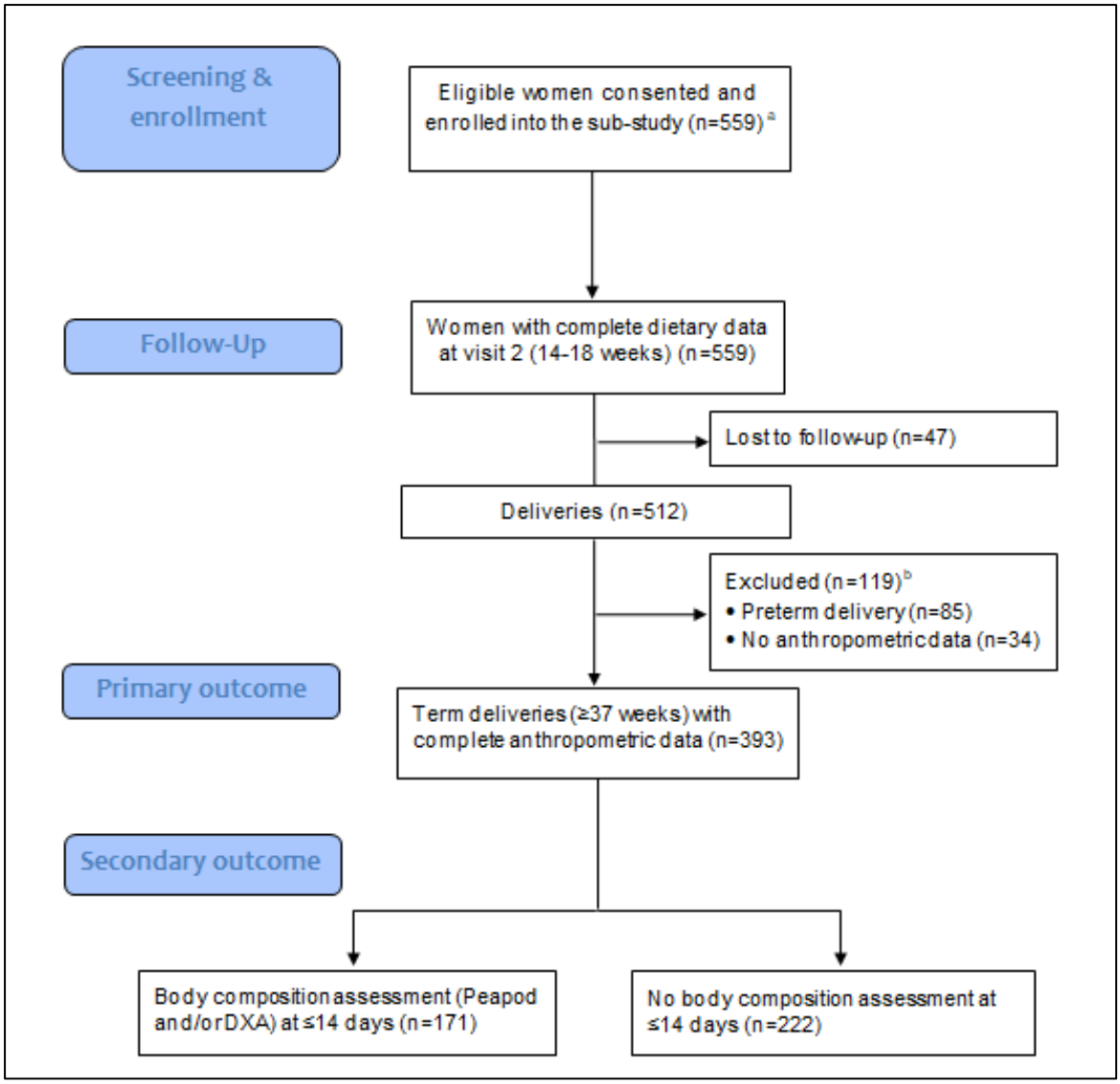

Figure 8: Flow chart of participants within the Soweto First 1000-Day Study (S1000) at Chris Hani Baragwanath Academic Hospital (CHBH)

[a]Of eligible women approached at the Fetal Medicine Unit at Chris Hani Baragwanath Academic Hospital (CHBH), 85% consented to participate. Women who refused to participate were not different in age, BMI, or education, but participants were more likely to be married

[b]Mother-neonate pairs excluded from the final analyses were not different in any baseline maternal characteristics: age, parity, HIV/treatment status, smoking, marital status, education, household SES, weight, height, BMI

SECTION 2: EMPIRICAL CHAPTERS

CHAPTER 4: A comparison of body composition estimates using dual-energy X-ray absorptiometry and air-displacement plethysmography in South African neonates[2]

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[2]Wrottesley SV, Pisa PT, Micklesfield LK, Pettifor JM, Norris SA. A comparison of body composition estimates using dual-energy x-ray absorptiometry and air-displacement plethysmography in South African neonates. Eur J Clin Nutr. 2016 Nov;70(11):1254-1258. (174)

4.1 Introduction

Fetal growth and subsequently birth weight, have been shown to be key determinants of neonatal, infant and childhood growth and development, as well as, long-term risk of non-communicable diseases (NCDs) in adulthood; with both insufficient and excessive growth in utero increasing the risk of adverse outcomes across the life course (10,175–178). Although birth weight is the most commonly utilised measure of prenatal growth, particularly in low-or middle-income countries (LMICs), it provides a crude estimate which does not differentiate between the various components of body composition i.e. fat mass and fat-free mass. As a result, objective body composition measures at birth, and particularly neonatal adiposity, are becoming increasingly important outcomes to assess, as they are more sensitive markers of fetal nutrition and growth and therefore possibly stronger predictors of future metabolic health risk (84,179). Furthermore, neonatal and infant body composition assessments can better inform early and targeted interventions during pregnancy and early infancy to reduce metabolic disease risk during the life course.

The most common methods used to assess neonatal body composition are dual-energy x-ray absorptiometry (DXA) and, more recently, air displacement plethysmography (ADP) using the Peapod, due to ease and efficiency of use, low operator skill requirements and the non-invasive nature of the techniques (85,88,180). Although DXA has been shown to reliably estimate fat mass and fat-free mass during repeated neonatal measurements, validation studies in piglets found that fat mass was overestimated in smaller subjects (88,181–183). In addition, the risk of radiation exposure and the need to prevent movement of the subject during assessment have limited the use of DXA in paediatric populations. In contrast, ADP has been shown to provide accurate and reliable estimates of %fat in infants when compared to both deuterium dilution and a 4-compartment model, independent of body size and infant behavioural state (9,10). Although the Peapod is only suitable for assessment of infants up to

6 months of age and for body weights under 8 kg (86,185), which restricts its use in longitudinal studies, it may be a more practical and reliable method of body composition assessment in early life.

In a USA sample of 84 infants, strong correlations were observed between DXA and ADP measurements at 6 months of age, however, ADP estimates were significantly lower than those by DXA for fat mass and body-fat percentage (%fat), and significantly higher for fat-free mass (89). This suggests that, although the two methods are able to reliably detect increases or decreases in fat and fat-free mass, there are large inter-method differences in absolute and relative fat and fat-free mass measurements. To our knowledge, this is the only study comparing these techniques in infants, with no published data available for neonates. Comparison of DXA and ADP is important for neonates due to the increasing use of neonatal adiposity to assess fetal growth and future metabolic risk, the documented differences in ADP and DXA body composition estimates in older infants and the inaccuracies in DXA estimated fat mass in small subjects, as well as the concerns around the safety and practicality of using the techniques in this population.

Assessment of body composition during infancy by ADP and DXA has been limited in LMICs and the accuracy and reliability of the techniques has never been explored in African paediatric populations. These comparisons are important for this unique setting where rapid urbanization is characterised by a triple burden of disease, including infection-related under-nutrition illnesses, human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDs) and tuberculosis, and the emergence of NCDs (111). Pilot data from our on-going Soweto First 1000-Day Study (S1000) shows that urban black South African women exhibit a high clustering of disease during pregnancy, including 67% overweight and obesity, 14% gestational diabetes mellitus (GDM), 31% anaemia and 30% HIV prevalence (Norris et al, unpublished). In addition, black women demonstrate different patterns of body

fat distribution and associated metabolic risk than their white counterparts in both South Africa and the USA (186–188). The presence of these co-morbidities, together with the adiposity and risk profiles, in black South African women may influence in utero phenotypic programming of fetal growth and tissue deposition and have distinct effects on neonatal body composition.

The aim of this study was therefore to compare estimates of fat mass and fat-free mass by ADP and DXA in black South African neonates within the first two weeks of life and to determine whether the techniques provide comparable measurements of neonatal body composition.

4.2 Methods

4.2.1 Study setting and participants

Body composition data was collected for 92 neonates born to women from the S1000 study which is currently in progress in Soweto, Johannesburg, South Africa. S1000 is a longitudinal maternal pregnancy cohort study with the overall aim of understanding the complexities between multiple maternal factors and fetal and infant outcomes, and identifying the levers that could optimize maternal and child health within the first 1000 days, in an urban-poor African context. Women are considered eligible for inclusion in the study if they live in Soweto, or the Greater Soweto area, and are <20 weeks gestational age at recruitment, non-epileptic, non-diabetic, 18 years or older and pregnant with singleton, naturally conceived pregnancies. Neonates were included in this sub-study if they had body composition measurements using ADP and DXA within the first two weeks of life before the end of June 2015. All testing took place on the same day and infants who were not asleep on arrival were typically fed prior to body composition assessments. All mothers gave written

informed consent for their infants to participate and ethical approval was obtained from the University of the Witwatersrand's Research Ethics Committee (Medical) (M130905).

4.2.2 Neonatal measurements

Neonatal crown-to-heel length was measured in duplicate using a calibrated Harpenden Infantometer. Weight of nude neonates was measured using the scale on the ADP Peapod machine (Cosmed, USA) and fat mass, fat-free mass and %fat were estimated using both the Peapod and DXA (Hologic DiscoveryA S/N 86254, APEX software version 4.0.2, Hologic Inc., USA).

4.2.2.1 ADP

Body composition assessments using the Peapod were performed according to standard procedures as previously described (86,189,190). Participants were placed inside the Peapod chamber wearing only a wig cap if necessary. ADP measurements lasted approximately 2 minutes, during which body volume was estimated using pressure and volume changes (air displacement) within the chamber. Neonatal body density was calculated using body mass and volume measurements, and fat mass, fat-free mass and %fat were subsequently derived using sex-specific equations. All included ADP assessments passed the quality check on the Peapod machine.

4.2.2.2 DXA

Standard procedures for performing infant DXA scans are described elsewhere (191). Neonates were placed supine on the scanning bed wearing only a disposable diaper and swaddled in a cotton blanket. Scans were performed according to standard procedures and were satisfactory for use if the participant's body lay within the scanning region and there was minimal movement during the procedure. Whole body measurements of fat mass, fat-free mass and %fat were extracted for use in analyses. All outliers for the comparison

between ADP and DXA fat mass measurements were identified and checked against their raw DXA assessments for scan quality. Where the quality of a DXA scan was compromised, i.e. excessive movement or inappropriate positioning of the neonate on the scanning bed or folding of the arms or legs, these results were excluded from body-composition analyses.

4.2.3 Statistical analysis

Continuous and categorical variables were summarised as mean (SD) and n (%) respectively. Sex and gestational age independent standard deviation scores were calculated for neonatal weight and height using the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) Project standards (192). Paired t-tests were used to compare differences between DXA and ADP on the following estimated body composition variables: (i) fat mass (ii) fat-free mass and (iii) %fat. Scatter plots with 95% confidence intervals were generated for each body composition variable from both techniques, and Pearson correlation coefficient analyses were used to determine the association between these variables measured using ADP and DXA. The level of agreement between DXA and ADP measurements of fat mass, fat-free mass and %fat were assessed using Bland-Altman analyses. Data were analysed using Stata 13.0 (StataCorp, USA) and p-values less than 0.05 were considered statistically significant.

4.3 Results

Four outliers were excluded from ADP and DXA comparisons due to the compromised quality of their DXA scans. A total of 88 neonates were therefore included in all further analyses. Growth and body composition variables measured by ADP and DXA are presented in Table 6. ADP estimates of fat mass and %fat were significantly higher ($p<0.001$), and fat-free mass estimates significantly lower ($p<0.001$) than those by DXA. Specifically, ADP estimates were 21.1% higher for fat mass and 9.7% lower for fat-free mass than those by DXA.

Table 6: Growth and body composition variables in South African neonates (n=88)

Variable	Mean (SD)
Gestational age at birth (weeks)	38.7 (1.8)
Age at scanning (days)	7.6 (3.8)
Sex, %	
Male	55.7
Female	44.3
Anthropometry	
Weight (g)	3089 (456)
Weight SDS[a]	-0.11 (0.88)
Length (cm)	49 (2.4)
Length SDS[a]	0.09 (1.09)
Body composition	
ADP fat mass (g)	408 (172)*
ADP fat-free mass (g)	2681 (348)*
ADP %fat	12.9 (4.4)*
DXA fat mass (g)	337 (165)
DXA fat-free mass (g)	2969 (375)
DXA %fat	9.9 (4)

Abbreviations: ADP, air displacement plethysmography; DXA, dual-energy x-ray absorptiometry; SDS, standard deviation score

[a]Independent of sex and gestational age

*p<0.001 ADP vs. DXA

Scatter plots of ADP and DXA body composition estimates for fat mass, fat-free mass and %fat showed a positive linear relationship between the two methods for all of the variables (Figure 9). The corresponding Pearson correlation coefficients indicated significant correlations between ADP and DXA measurements of fat mass, fat-free mass and %fat ($p<0.001$) However, fat-free mass estimates showed much stronger positive correlations between the two methods than those for fat mass and %fat. 88.7% of the variance in DXA fat-free mass was therefore explained by ADP fat-free mass; whereas ADP estimates predicted only 58.7% and 39.7% of the variability in DXA fat mass and %fat respectively.

Bland-Altman analyses of fat mass, fat-free mass and %fat estimates by ADP and DXA are presented in Figure 10. DXA showed a tendency to under-predict fat-mass (mean difference, 95% limits of agreement; 72 g, -159 g to 302 g) and %fat (mean difference, 95% limits of

agreement; 3%, -4.2% to 10.2%) in comparison to ADP. There were no correlations between the differences in ADP and DXA estimates and their averages for fat mass ($r=0.062$, $p=0.565$) or %fat ($r=0.101$, $p=0.348$) and therefore no bias shown across the range of fatness between the two methods. Fat-free mass was over-predicted by DXA in comparison to ADP for all subjects (mean difference, 95% limits of agreement; -288 g, -541 g to -35 g). There was a significant correlation between the difference in fat-free mass measured by ADP and DXA and the average of these estimates ($r= -0.220$, $p<0.05$), suggesting a bias (i.e. a systematic difference in measurements) between the techniques for fat-free mass estimates.

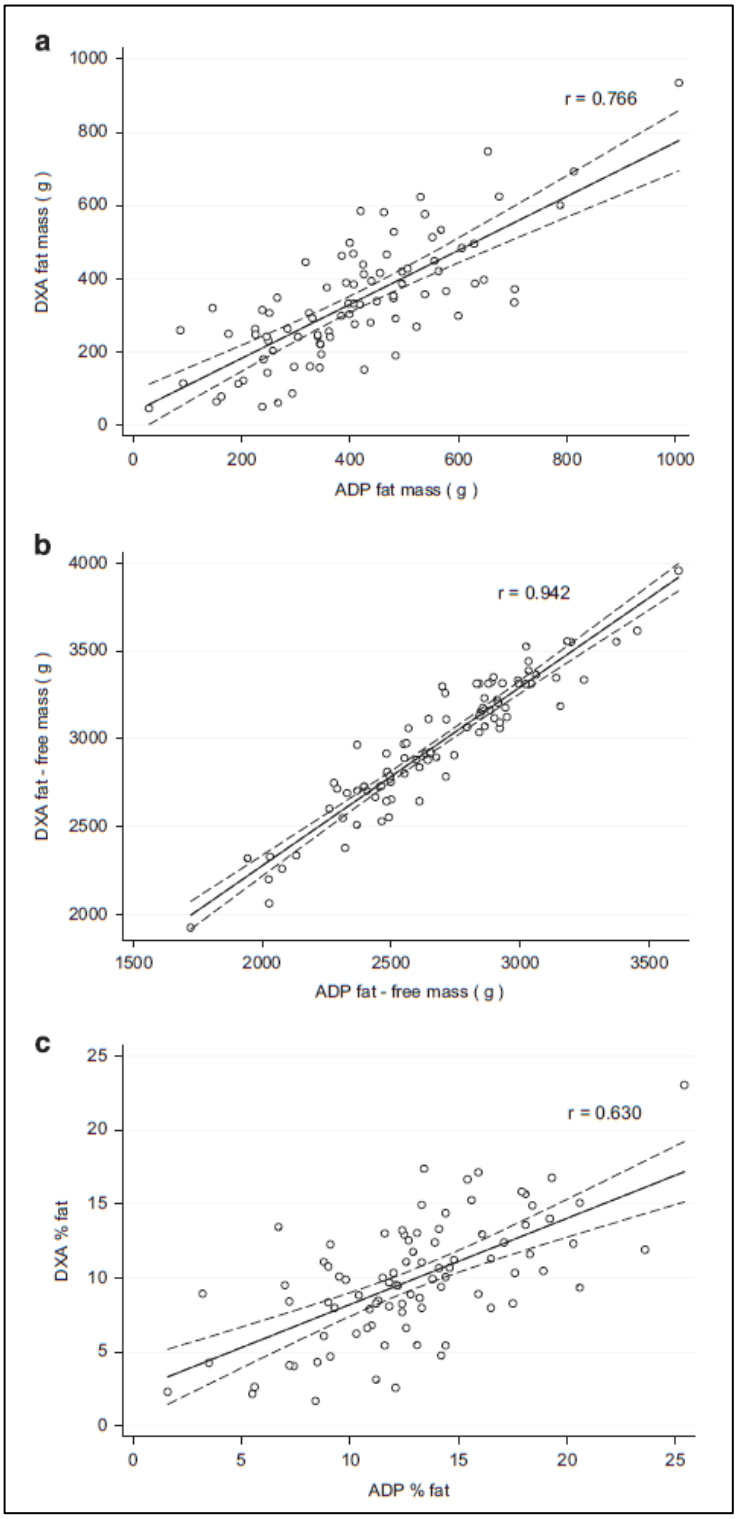

Figure 9: Scatter plots of air displacement plethysmography (ADP) versus dual-energy x-ray absorptiometry (DXA) with 95% confidence intervals and Pearson correlation coefficients for body composition variables of interest; (a) fat mass, (b) fat-free mass, and (c) %fat

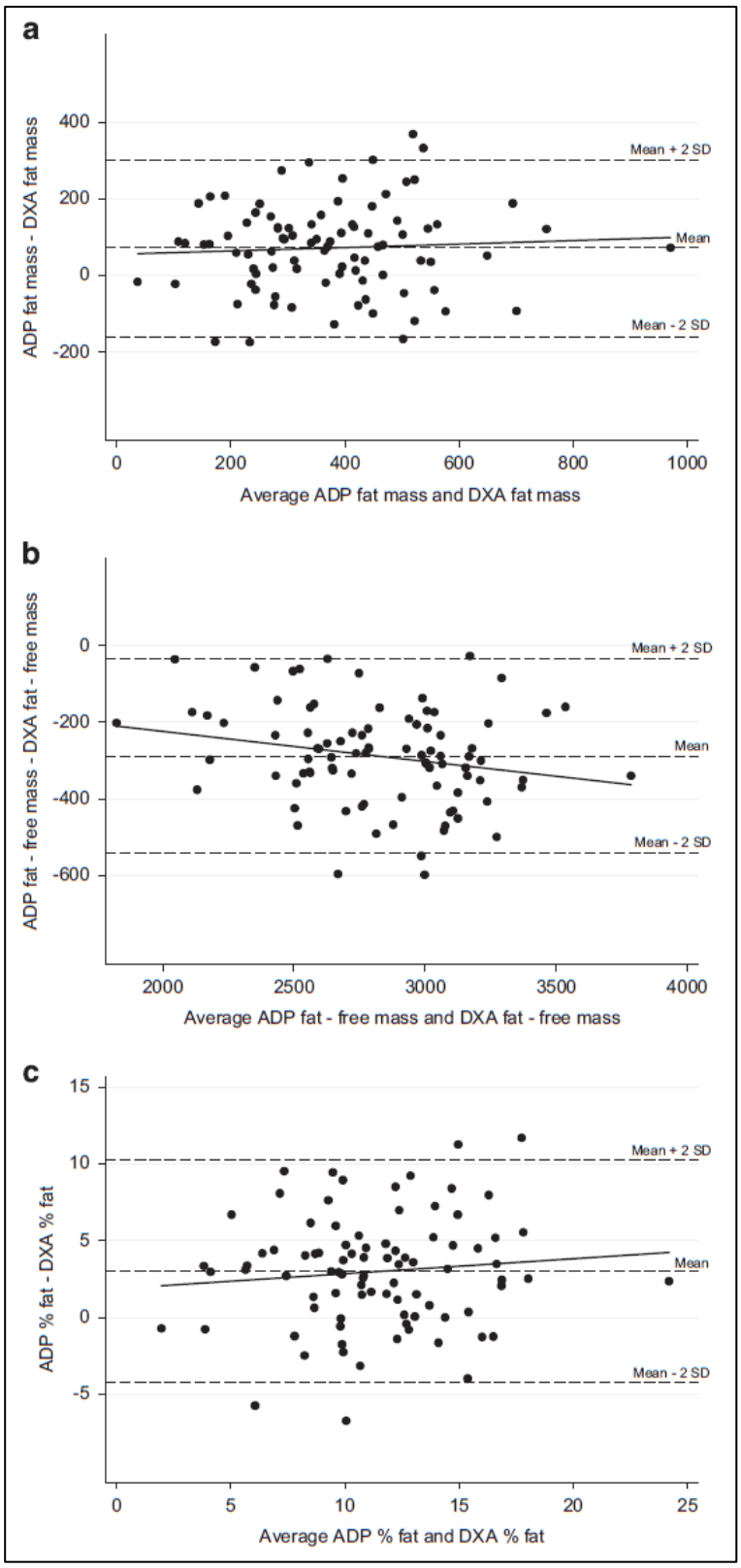

Figure 10: Bland–Altman analyses between air displacement plethysmography (ADP) and dual-energy x-ray absorptiometry (DXA) estimates of body composition variables of interest; (a) fat mass, (g); (b) fat-free mass, (g); and (c) %fat

4.4 Discussion

This study aimed to determine whether ADP and DXA provide comparable measurements of fat mass and fat-free mass in black South African neonates. We found that, while body composition variables were highly correlated, fat mass and %fat estimates were significantly higher and fat-free mass was significantly lower when measured by ADP compared to DXA. Fat-free mass estimates showed stronger agreement between the two methods, with DXA estimated fat-free mass being consistently higher than that by ADP compared to fat and %fat estimates where the differences between methods were less predictable. To our knowledge, this is the first study comparing these increasingly popular methods of infant body composition assessment in neonates, as well as in an African population.

Although both ADP and DXA have been suggested to provide reliable estimates of fat mass and fat-free mass in infants (88,180,184), accessibility of these methods in LMICs is limited. Use of DXA has also been restricted below two years of age, due to the difficulties in preventing movement of infants during scanning. While use of the Peapod does not have these limitations, it can only be used in infants up to approximately 6 months of age and/or 8kg body weight, which restricts its use in longitudinal follow-up and comparisons. In addition, ADP does not distinguish between lean mass and bone constituents of fat-free mass which has been suggested as a source of inaccuracy in fat-free mass estimates when compared to DXA (193). Due to the strengths and limitations of DXA and ADP, as well as the lack of data to support the use of one method over the other, the best practice for body composition assessment in neonates is currently not known.

Our findings are in contrast to those in US 6 month old infants, where fat mass and %fat were significantly lower and fat-free mass was significantly higher when estimated by ADP compared to DXA (89). In addition, although similarly strong correlations were shown between fat-free mass estimates by ADP and DXA in these studies, fat mass and %fat

estimates by the two techniques were more highly correlated in the older US population (r = 0.925-0.969). A possible explanation for the differences may be the age of the participants studied, with Fields et al (2012) demonstrating larger inter-method differences in those infants who were younger and smaller (89).

Early life growth and development of body tissues, including fat and lean mass deposition and bone mineralisation, is rapid, with the greatest increases in %fat seen between birth and 6 weeks of age (194). The substantial changes in tissue development and formation may affect the accuracy with which measurement techniques are able to distinguish between the different components of body mass at different stages of infancy. This is particularly relevant for DXA, which has documented improvements in the accuracy of body composition estimates in larger subjects, particularly with higher bone mineral content (BMC) and fat mass (181).

This is supported by DXA validation studies in piglets which show that, while DXA provides reasonable estimates of lean tissue irrespective of body size, accuracy in bone and fat mass estimates is less consistent (181–183). Similarly, and unlike our sample, studies conducted in both obese and non-obese children and adolescents show an overestimation of %fat measured by DXA in comparison to ADP (195–198).

The aforementioned studies focused on the accuracy and comparability of ADP and DXA estimates of whole body fat mass, rather than in detection of fat and fat-free mass across different body compartments. In adult women, DXA has been shown to underestimate trunk fat and overestimate thigh lean mass, particularly in those with greater adiposity (199). This may be due to reduced accuracy in distinguishing between fat and fat-free tissue and potentially the inclusion of adipose tissue in the fat-free mass measurement in certain body regions. At birth, the trunk constitutes the largest proportion of neonatal body weight and

therefore fat mass, with the limbs contributing comparatively little (200). If fat mass is underestimated in this region, and fat-free mass overestimated in the limbs, it may explain the differences seen in neonates compared to older infants who have experienced rapid changes in body proportions and fat deposition. This suggests that ADP-estimated body composition, based on total body mass and volume, may provide more reliable estimates of fat and fat-free mass in early life when the ability of DXA scans to accurately depict fat mass is not consistent. ADP validation studies support this, showing Peapod to provide accurate and reliable estimates of %fat in infants, independent of body size and fatness, compared to both deuterium dilution and a 4-compartment model (180,184).

Another potential contributor to the differences in body composition estimates by ADP and DXA could be the effect of movement and/or material (i.e. cotton blanket swaddling) artefacts on DXA accuracy during scanning. Animal studies show tightly bound swaddling to be included in DXA scans as fat and tissue mass, with disproportionately higher inclusion (63-95%) as lean mass (201), thereby reducing the accuracy of tissue estimates. However the systematically lower estimates of fat mass by DXA compared to ADP suggest a minimal effect of any inconsistencies in movement or placing of neonatal swaddling on DXA accuracy.

Both ADP and DXA fat mass estimates are based on the assumption that hydration of fat-free mass remains constant. This has been proposed as a possible source of error in paediatric body composition assessment by the two methods, as the level of tissue hydration is high and rapidly changing during infancy (202). However, studies examining the effects of changes in tissue hydration on ADP and DXA accuracy show only marginal fluctuations in fat mass estimates (184,202). As neonatal ADP and DXA measurements in our study were taken on the same day, tissue hydration changes would be minimal and unlikely to make a substantial contribution to the differences in body composition estimates by the two methods.

In addition to comparison of ADP and DXA techniques, our study provided novel data on neonatal body composition in South Africa suggestive of higher %fat at birth than has been seen elsewhere in Africa. Although data in African populations is scarce, Ethiopian neonates have comparatively lower ADP fat mass and %fat (boys, girls: 7%, 7.6%) at similar total body mass (boys, girls: 3106 ± 407 g, 2973 ± 414 g) than shown in our South African sample, regardless of whether ADP or DXA estimates are used (92). Body composition estimates in our sample were more comparable to those in HICs such as USA, where neonates have an estimated 14% body fat (203). This provides unique insight into the effects that the rapid nutritional transition and resulting co-morbidity profile experienced by South African pregnant women may have had on neonatal body composition and potentially on longer-term metabolic risk. Further research into the effects of this clustering of risk factors during pregnancy on both maternal and infant health and disease risk, as well as on accurate, reliable and practical methods of assessment and intervention in African settings, is critical and on-going in the S1000 cohort.

4.5 Conclusion

In conclusion, our study showed that, although estimates of fat and fat-free mass by ADP and DXA were highly correlated, fat mass and %fat were significantly lower and fat-free mass was significantly higher when measured by DXA in South African neonates. These findings are the opposite to those by Fields et al (2012) in infants at 6 months of age (89). This may be due to a limited ability to distinguish between different body tissues (fat mass, lean mass and bone) in early life and suggests a preferential basis for use of the Peapod in neonatal body composition assessment. However, further investigation is needed to elucidate on the mechanical basis for these differences and to determine how inter-method differences may be minimised so that body composition assessment is comparable across longitudinal study follow-up.

CHAPTER 5: The influence of maternal dietary patterns on gestational weight gain in urban black South African women[3]

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[3]Wrottesley SV, Pisa PT, Norris SA. The influence of maternal dietary patterns on body mass index and gestational weight gain in urban black South African women. Nutrients. 2017 Jul 11;9(7). (204)

5.1 Introduction

Maternal nutritional status (MNS), principally defined by body mass index (BMI) prior to pregnancy, is a strong predictor of maternal and infant health; with both underweight and overweight/obesity being associated with increased risk of maternal complications during pregnancy and delivery, sub-optimal fetal growth and adverse birth outcomes (1,5,10,11,99,100). In addition to the immediate risks posed to mother and infant, adverse exposures such as malnutrition during critical periods of developmental plasticity—including fetal growth and development—have been shown to alter gene expression, thereby modifying growth and development of body tissues (110). Such changes may lead to permanently altered metabolism and body function and increased susceptibility to non-communicable diseases (NCDs) including obesity, type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) in later life (107,108,110,205).

In addition to pre-pregnancy BMI, weight gain during pregnancy has been shown to independently influence both maternal and infant outcomes; with inadequate weight gain, according to the Institute of Medicine (IoM) BMI-specific recommended ranges, increasing the risk of preterm birth, low birth weight (LBW) and small-for-gestational-age (SGA) infants (48,206). In contrast, excessive gestational weight gain (GWG) is associated with an increased risk of pregnancy-induced hypertension, pre-eclampsia, fetal macrosomia and requiring an emergency caesarean delivery (48,55,207). Additionally, women who gain excessive weight during pregnancy are at higher risk of postpartum weight retention, which may influence their susceptibility to developing overweight and obesity in the longer term and increase their risk profile in subsequent pregnancies (5,57,58).

Studies indicate that overweight and obese pregnant women are more likely to gain excessive weight than their normal-weight counterparts (56,208,209). This is particularly relevant for low- or middle-income countries (LMICs) such as South Africa, where the prevalence of

overweight and obesity has increased substantially as a result of rapid urbanisation and a nutrition transition characterised by a shift towards typically westernised diets high in saturated fat, sugar, salt, processed foods and edible oils, as well as decreased levels of physical activity (27,28). Pilot data from our Soweto First 1000-Day Study (S1000) shows that urban black South African women exhibit a high clustering of disease during pregnancy, including 67% overweight and obesity, 14% gestational diabetes mellitus (GDM), 31% anaemia and 30% human immunodeficiency virus (HIV) prevalence (Norris et al., unpublished). This high-risk profile is coupled with the poor quality, predominantly cereal-based diets that are high in energy and refined sugar and low in protein and micronutrient-rich foods such as meat, poultry, seafood, legumes and non-starchy vegetables that have been demonstrated in South African pregnant women (122).

In high income countries (HICs) some studies have shown that a high protein: carbohydrate ratio, partly driven by a reduced added-sugar intake, is associated with lower GWG and that high adherence to a margarine, sugar and snacks dietary pattern is associated with excessive GWG when compared to low adherence to this pattern (210,211). Additionally, in Puerto Rican women, frequent consumption of high-sugar fruit drinks was associated with excessive GWG (212). To our knowledge, however, dietary patterns and their relationships with MNS prior to and during pregnancy are yet to be explored in urban African populations where the associations may be further complicated by existing co-morbidities, such as the high burden of HIV, which may independently predict low pre-pregnancy BMI, poor GWG and micronutrient deficiencies (95,96,213,214).

While the relationship between maternal anthropometry prior to and during pregnancy and maternal and infant outcomes is well documented, the influence of dietary intake and patterns on BMI-specific GWG, and therefore maternal and infant outcomes, is unclear. Hence in this study, we aimed to identify patterns of habitual dietary intake in urban, black South African

pregnant women and further explored their associations with first trimester BMI and GWG in the context of HIV and other sociodemographic factors.

5.2 Methods

5.2.1 Study setting and participants

This study was nested within a larger longitudinal pregnancy cohort study (Soweto First 1000-Day Study; S1000), based at the CHBH in Soweto, Johannesburg, South Africa. The overall aim of S1000 was to understand the complexities between multiple maternal factors and fetal and infant outcomes, and to identify the levers that could optimise maternal and child health within the first 1000 days in an urban-poor African context. Women were considered eligible for inclusion in the study if they lived in Soweto, or the Greater Soweto area, and were <20 weeks gestational age at recruitment, non-epileptic, non-diabetic, 18 years or older and pregnant with singleton, naturally conceived pregnancies. Data collection for S1000 took place at six time points during pregnancy: <14 weeks; 14–18 weeks; 19–23 weeks; 24–28 weeks; 29–33 weeks and 34–38 weeks. All the women provided written informed consent prior to their inclusion in the study and ethical approval was obtained from the University of the Witwatersrand's Research Ethics Committee (Medical) (M120524).

5.2.2 Demographic, health and socioeconomic variables

Maternal demographic and socioeconomic variables were collected using an interviewer-administered questionnaire at the first visit (<14 weeks gestational age). All questionnaires were administered by trained members of research staff. Socioeconomic status (SES) was assessed using a household asset index which scored each participant according to the number of assets that they possessed out of a possible 9 (electricity, radio, television, refrigerator, mobile phone, personal computer, bicycle, motorcycle/scooter, car). Maternal education was assessed according to the highest level of education (primary, secondary or

tertiary) completed. Parity was defined as the number of times a subject had given birth to a fetus with a gestational age of 24 weeks or more, regardless of whether the child was born alive or was stillborn, and smoking status was assessed according to whether the participant reported smoking and/or chewing tobacco at baseline. HIV status was determined via self-report at each pregnancy visit and, if HIV-positive, the date of antiretroviral treatment (ART) initiation was recorded. According to South Africa's national Prevention of Mother-to-Child Transmission (PMTCT) guidelines during this time, all HIV-positive pregnant women, if not already on treatment, were initiated on lifelong ART regardless of their CD4 count and therefore all HIV-positive participants were receiving ART. HIV-positive women were therefore stratified according to whether they were initiated prior to pregnancy (pre-pregnancy ART) or during the current pregnancy (antenatal ART).

5.2.3 Anthropometry

Maternal weight was measured to the nearest 0.1 kg using a digital scale at each pregnancy visit and maternal height was measured to the nearest 1 mm using a wall-mounted Stadiometer (Holtain, UK) at the first visit. Weight at the first visit (<14 weeks) was used as a proxy for pre-pregnancy weight and, together with height, used to calculate maternal BMI (weight (kg)/height (m^2)). As there were no underweight women in this sample, BMI was classified as either normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2) or obese (≥30.0 kg/m^2). GWG (kg/week) was calculated as: (weight at last visit − weight at recruitment)/duration of follow-up. Women were classified as having inadequate, adequate or excessive GWG using the BMI-specific IoM recommended ranges (25).

5.2.4 Dietary intake

Habitual dietary intake was assessed using an interviewer-administered quantitative food-frequency questionnaire (QFFQ) during the second pregnancy visit (14–18 weeks). The QFFQ has been extensively utilised in this setting and results are published elsewhere

(172,215). Data was collected retrospectively on the previous week's intake of local South African foods, including convenience food products. Frequency and quantity—defined using a combination of household measures, two-dimensional life-size drawings of foods and utensils and three-dimensional food models, as described by Steyn et al. (216), and then converted to an average intake in grams per day—of each food item consumed was recorded during the interview and captured electronically using REDCap electronic data capture tools hosted at The University of the Witwatersrand (217).

5.2.5 Statistical analysis

Data were analysed for 538 pregnant women with complete data using Stata 13.0 (StataCorp, College Station, TX, USA). Principal component analysis (PCA) was the dimension reduction technique used to depict dietary patterns (218). This is a statistical method that provides a set of coefficients for each of the food items/groups which reflect intakebased on the level of correlation with each other; thereby forming new linear components that represent the combinations (patterns) of intake in a population (73,219). PCA was conducted on the weekly frequency of consumption of the food items listed in the QFFQ, classified into 48 food groups according to their nutrient composition and usage. These groupings were based on those used and described extensively by Crozier et al. (76,220). PCA was applied using orthogonal (varimax) rotation and the Kaiser-Meyer-Olkin measure of sampling adequacy (0.65) and Bartlett's test of sphericity ($p<0.001$) confirmed PCA to be an appropriate dimension reduction technique to apply in this sample. Eigen values, as well as their visual inflections on a scree plot (i.e. those values situated above the point where the downward curve levels off; Supplementary Figure F1; Appendix M), and the total variance explained (percentages) were used to identify retained patterns. As described elsewhere, foods or food groups that had factor loading scores ≥0.2 on the PCA matrix reflected strong associations with principal components and these were used to name dietary patterns (211). A

dietary pattern score for each pattern was generated for all participants by multiplying factor loadings by the intake of each food/food group and standardising the summed scores. Mean factor scores for the patterns were zero; with positive and negative scores therefore representing high and low intakes of each dietary pattern respectively (219).

Maternal characteristics of the sample are presented as median (interquartile range) and percentages (%) for continuous and categorical variables respectively. The Kruskal-Wallis and chi-squared tests were used to compare all continuous and categorical variables respectively across the three IoM defined BMI-specific GWG categories; namely inadequate, adequate and excessive weight gain. Potential covariates for the associations between dietary patterns and GWG were identified by comparing factor scores for each pattern using either an independent t-test (two categories) or analysis of variance (ANOVA) (more than two categories) according to the following maternal factors: maternal age, parity, HIV status, education, marital status, SES, BMI at recruitment and GWG. Furthermore, in cases where there were more than two categories for a given characteristic, a Tukey post-hoc test was carried out to identify where the differences occurred between categories (data presented as a supplementary Table S1; Appendix M).

The associations between depicted dietary pattern scores and GWG were tested using multivariable linear regressions and multinomial logistic regressions for the total sample and then across three BMI categories; namely normal weight, overweight and obese. Linear regression analyses were based on continuous dietary pattern scores (exposure) and GWG (g/week) (outcome) and multinomial logistic regression analyses were based on dietary pattern scores (exposure) and adequacy of GWG according to the IoM recommended ranges (inadequate and excessive GWG vs. adequate GWG; reference) (outcome). Due to the fact that the diet patterns are not mutually exclusive and it is possible for individuals to have high

scores for multiple patterns, all regression analyses (crude and adjusted models) included all diet patterns.

Regression coefficients for linear regressions are therefore presented for a 1 SD increase in dietary pattern scores across three models namely; Model 1 (M1): crude analysis adjusted for the other diet patterns; Model 2 (M2): M1 adjusted for covariates shown to be significantly associated with dietary patterns and Model 3 (M3): M2 adjusted for total energy intake. Due to the similarities observed between M1 and M2 during multinomial logistic regression analyses, odds ratios are presented for only two models; i.e., replacing M2 with M3.

5.3 Results

5.3.1 Maternal characteristics

Maternal demographic, health, SES and anthropometric variables stratified according to IoM BMI-specific GWG categories are presented in Table 7. 24%, 21% and 55% of women were categorised as experiencing inadequate, adequate and excessive GWG respectively. Median maternal age decreased across GWG categories (p=0.038); however there were no significant differences in the distribution of women across age range categories between the GWG groups. HIV status was significantly associated with GWG, with HIV-negative women being more likely to exhibit excessive weight gain and HIV-positive women more likely to exhibit inadequate weight gain; regardless of whether they had been initiated on ART prior to the current pregnancy. Household SES increased across categories of GWG. Median BMI was lowest in women who gained adequate weight during pregnancy, with the prevalence of excessive weight gain being significantly higher in overweight and obese women than in their normal weight counterparts who were more likely to gain either inadequate or adequate weight. There were no significant differences in parity, smoking status, education or marital status across GWG adequacy groups.

Table 7: Maternal characteristics of South African women according to Institute of Medicine (IoM) BMI-specific gestational weight gain categories

Variable	BMI-Specific Gestational Weight Gain (kg/week)				p-value [a]
	Total (*n* = 538)	**Inadequate (*n* = 128)**	**Adequate (*n* = 113)**	**Excessive (*n* = 297)**	
Maternal characteristics					
Age, year	30 (25–34)	31 (27–36)	30 (26–35)	29 (25–34)	**0.038**
<25	115 (21)	19 (15)	25 (22)	71 (24)	0.216
25–29	153 (29)	39 (30)	28 (25)	86 (29)	
30–34	137 (26)	31 (24)	31 (28)	75 (25)	
35–39	99 (18)	25 (20)	23 (20)	51 (17)	
≥40	34 (6)	14 (11)	6 (5)	14 (5)	
Parity					
Para 0	134 (25)	22 (17)	33 (29)	79 (26)	0.189
Para 1	236 (44)	62 (49)	44 (39)	130 (44)	
Para ≥2	168 (31)	44 (34)	36 (32)	88 (30)	
HIV status					
HIV-negative	357 (66)	66 (52)	72 (64)	219 (74)	**<0.001**
HIV-positive (pre-pregnancy ART)	65 (12)	22 (17)	15 (13)	28 (9)	
HIV-positive (antenatal ART)	116 (22)	40 (31)	26 (23)	50 (17)	
Smokes/chews tobacco					
No	467 (87)	108 (84)	100 (88)	259 (87)	0.612
Yes	71 (13)	20 (16)	13 (12)	38 (13)	
Socioeconomic characteristics					
Maternal education					
Primary	10 (2)	4 (3)	2 (2)	4 (1)	0.214
Secondary	377 (70)	96 (75)	82 (72)	199 (67)	
Tertiary	151 (28)	28 (22)	29 (26)	94 (32)	
Marital status (*n* = 529)					
Single	326 (62)	73 (59)	70 (62.5)	183 (62)	0.771
Married/cohabiting	203 (38)	51 (41)	42 (37.5)	110 (38)	
Household SES					
Low	77 (14)	25 (20)	16 (14)	36 (12)	**0.048**
Medium	430 (80)	100 (78)	86 (76)	244 (82)	
High	31 (6)	3 (2)	11 (10)	17 (6)	
Anthropometry					
Height, cm	158.3 (154.5–162.4)	156.7 (153.5–161.3)	158.8 (154.8–162.8)	159 (154.8–163)	**0.042**
Weight at recruitment, kg (<14 weeks)	68.8 (59.7–78.4)	66.9 (57.7–76.4)	64 (56.5–76.4)	70.8 (62–79.3)	**<0.001**
BMI at recruitment, kg/m^2 (<14 weeks)	27.5 (23.9–31.0)	27.3 (23.28–30.9)	25.4 (22.2–29.5)	28.2 (25.1–31.2)	**0.001**
Normal weight (BMI 18.5–24.9 kg/m^2)	182 (34)	54 (42)	54 (48)	74 (25)	**<0.001**
Overweight (BMI 25–29.9 kg/m$^{2)}$	190 (35)	38 (30)	32 (28)	120 (40)	
Obese (BMI ≥30 kg/m$^{2)}$	166 (31)	36 (28)	27 (24)	103 (35)	
GWG, kg/week	0.40 (0.27–0.55)	0.14 (0.06–0.22)	0.32 (0.27–0.42)	0.54 (0.42–0.64)	**<0.001**

Data are summarised as median (IQR) or n (%); IoM gestational weight gain (GWG) ranges (kg/m^2), Inadequate: normal weight < 0.35, overweight < 0.23, obese < 0.17; Adequate: normal weight 0.35–0.50, overweight 0.23–0.33, obese 0.17–0.27; Excessive: normal weight > 0.50, overweight > 0.33, obese > 0.27;
[a]Kruskal-Wallis test (continuous variables), Chi2 test (categorical variables).

5.3.2 Identification and description of depicted dietary patterns

Three distinct dietary patterns were identified which explained 20.5% of the variation in food intakes (Table 8 and Supplementary Figure F1; Appendix M). The first principal component (PC) was characterised by high factor loadings for energy dense, processed, high sugar/fat foods (white bread, processed and red meat, roast potatoes and chips, sweets and chocolate, soft drinks and cheese) and was labelled the "western" pattern (8.7% variance). The second PC, labelled the "traditional" pattern, had high factor loadings for beans and legumes, vegetables, traditional meats and porridge/pap (6.4% variance). The final PC was heterogeneous in composition, with both classically healthy foods such as whole grains, nuts and dairy as well as high sugar items (added sugar and sweet spreads) loading high (5.4% variance). This was labelled the "mixed" pattern.

5.3.3 Associations between maternal demographic, health, SES and anthropometric characteristics and dietary pattern scores

Of the maternal demographic, HIV status, SES and anthropometric variables tested as potential confounders or effect modifiers of the association between dietary patterns and GWG, only parity and marital status were shown to conclusively influence diet; with younger women with no previous births consuming a more "western" pattern and married/cohabiting women consuming a more "traditional" diet vs. single women who scored higher for the "western" diet pattern (data not shown; Supplementary Table S1; Appendix M).

Table 8: Factor loadings of various foods or food groups characteristic to the principal dietary components identified in pregnant South African women (n = 538)

Food or food group	PC1	PC2	PC3
	Western pattern	*Traditional pattern*	*Mixed pattern*
White bread	0.318		
Cheese and cottage cheese	0.244		
Red meat	0.212		
Processed meat	0.335		
Roast potatoes and chips	0.353		
Sweets and chocolate	0.245		
Soft drinks	0.249		
Miscellaneous (soup powder, condiments, sauces, etc.)	0.325		
Maize, sorghum and oat porridge		0.229	
Offal and traditional meats		0.251	
Salad vegetables		0.262	
Green vegetables		0.336	
Root vegetables		0.248	
Other vegetables		0.340	
Vegetable dishes		0.251	
Beans and pulses		0.273	
Boiled and baked potatoes		0.207	
Other fruit		0.211	
Brown and wholemeal bread			0.325
Breakfast cereals			0.208
Full-fat milk			0.368
Reduced-fat spread			0.307
Nuts and nut spreads			0.259
Added sugar (teaspoons)			0.361
Sweet spreads			0.327
Decaffeinated tea and coffee			0.211
Explained variance (%)	8.7	6.4	5.4
Cumulative explained variance (%)	8.7	15.1	20.5

Abbreviations: PC, principal component
Foods or food groups presented had factor loadings ≥0.2 and were therefore used to describe each dietary pattern

5.3.4 The effect of identified dietary patterns on GWG

In the total sample of pregnant women, only the "mixed" diet pattern showed a significant, positive association with rate of GWG in both crude (M1: 22 (7.3) g/week; p=0.003) and adjusted models (M2: 22 (7.3) g/week; p=0.003; M3: 22 (7.6) g/week; p=0.004) (Table 9). This positive association was maintained in obese women for all models (M1: 25 (11.4) g/week; p=0.029; M2: 23 (11.4) g/week; p=0.042; M3: 24 (11.6) g/week; p=0.041), but was not shown in normal weight or overweight women. While the "western" diet pattern was not

associated with rate of GWG in the total sample of pregnant women or in the overweight/obese subsamples, it was significantly associated with higher weight gain in normal weight women in all models. The “traditional” pattern showed significant, inverse associations with GWG in M1 (−27 (11.1) g/week; p=0.015) and in M2 (−27 (11.5) g/week; p=0.02); however, this association was no longer significant after adjustment for total energy intake.

During crude multinomial logistic regression analyses, a higher “western” diet pattern score was associated with an increased odds of excessive weight gain in normal weight women (M1: 1.30; p=0.014) (Table 10). However, this association was not maintained after adjustment for covariates. A 1SD increase in “traditional” diet pattern score was associated with a 19% reduction in the odds of excessive weight gain in the total sample after adjustment for covariates (M2: 0.81; p=0.006). This association was similarly shown in the normal weight women in both crude (M1: 0.77; p=0.021) and adjusted models (M2: 0.68; p=0.003). “Mixed” diet pattern scores were not associated with GWG adequacy in the total sample, or across any BMI categories.

Table 9: Associations between dietary pattern scores and rate of gestational weight gain in South African women

Dietary pattern	Gestational weight gain (g/week)											
	Total (n = 538)			***Normal weight (n = 182)***			***Overweight (n = 190)***			***Obese (n = 166)***		
	B	**SE**	**p-value[a]**	**B**	**SE**	**p-value[a]**	**B**	**SE**	**p-value[a]**	**B**	**SE**	**p-value[a]**
Western												
Model 1	8	5.8	0.181	27	10.6	**0.013**	5	9.9	0.588	-7	9.3	0.469
Model 2	4	5.9	0.527	25	10.9	**0.025**	-1	10.2	0.887	-7	9.4	0.443
Model 3	4	8.6	0.640	35	14.9	**0.021**	-8	14.5	0.593	-4	15.9	0.795
Traditional												
Model 1	-12	6.8	0.077	-27	11.1	**0.015**	-3	12.0	0.787	-5	11.8	0.683
Model 2	-7	7.0	0.311	-27	11.5	**0.020**	4	12.5	0.751	-1	12.0	0.935
Model 3	-7	7.5	0.353	-23	12.2	0.063	2	13.1	0.900	1	13.7	0.964
Mixed												
Model 1	22	7.3	**0.003**	17	13.3	0.215	20	12.8	0.111	25	11.4	**0.029**
Model 2	22	7.3	**0.003**	15	13.7	0.281	21	12.9	0.107	23	11.4	**0.042**
Model 3	22	7.6	**0.004**	19	14.4	0.182	18	13.7	0.187	24	11.6	**0.041**

Values are regression coefficients (β) with standard errors (SE) that represent the difference in rate of gestational weight gain (g/week) for a 1SD increase in dietary pattern scores
[a]Multivariable linear regression analyses; significant results presented in bold ($p<0.05$)
Model 1: crude analysis, adjusted for other diet patterns; Model 2: M1 adjusted for parity and marital status; Model 3: M2 adjusted for total energy intake.

Table 10: Associations between dietary pattern scores and adequacy of gestational weight gain in South African women

Dietary pattern	Gestational weight gain category															
	Total (n = 538)				*Normal weight (n = 182)*				*Overweight (n = 190)*				*Obese (n = 166)*			
	Inadequate		Excessive		Inadequate		Excessive		Inadequate		Excessive		Inadequate		Excessive	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Western																
Model 1	0.98	0.85; 1.12	1.06	0.94; 1.19	0.98	0.78; 1.24	1.30	1.06; 1.61	0.94	0.72; 1.23	1.05	0.84; 1.31	0.98	0.77; 1.25	0.91	0.74; 1.13
p-value[a]		0.717		0.334		0.877		**0.014**		0.658		0.666		0.892		0.403
Model 2	0.82	0.66; 1.02	0.84	0.70; 1.01	0.81	0.58; 1.12	1.07	0.78; 1.45	0.86	0.56; 1.31	0.80	0.56; 1.15	0.83	0.52; 1.30	0.70	0.47; 1.05
p-value[a]		0.077		0.068		0.202		0.682		0.471		0.224		0.410		0.084
Traditional																
Model 1	1.03	0.89; 1.20	0.90	0.79; 1.03	0.97	0.79; 1.21	0.77	0.62; 0.96	1.09	0.81; 1.48	0.90	0.69; 1.17	1.16	0.83; 1.61	1.18	0.89; 1.57
p-value[a]		0.700		0.132		0.803		**0.021**		0.560		0.426		0.383		0.256
Model 2	0.90	0.75; 1.07	0.81	0.69; 0.94	0.85	0.67; 1.09	0.68	0.53; 0.87	0.92	0.64; 1.33	0.77	0.56; 1.04	1.04	0.70; 1.54	1.07	0.77; 1.50
p-value[a]		0.216		**0.006**		0.205		**0.003**		0.675		0.090		0.853		0.681
Mixed																
Model 1	0.95	0.80; 1.13	1.03	0.90; 1.19	0.94	0.72; 1.21	0.87	0.68; 1.12	1.00	0.70; 1.44	1.31	0.97; 1.78	0.95	0.69; 1.30	0.99	0.69; 1.30
p-value[a]		0.557		0.651		0.619		0.273		0.994		0.077		0.744		0.952
Model 2	0.89	0.74; 1.07	0.96	0.82; 1.12	0.83	0.61; 1.12	0.77	0.58; 1.03	0.96	0.64; 1.44	1.25	0.89; 1.75	0.93	0.67; 1.28	0.94	0.71; 1.25
p-value[a]		0.231		0.597		0.213		0.078		0.836		0.201		0.648		0.669

Values are odds ratios with 95% confidence intervals that represent the association between a 1 SD increase in dietary pattern scores and inadequate or excessive gestational weight gain relative to adequate weight gain (reference);

[a]Multinomial logistic regression analyses; significant results are presented in bold ($p<0.05$)

Model 1: crude analysis, adjusted for other diet patterns; Model 2: M1 adjusted for parity, marital status and total energy intake.

5.4 Discussion

To our knowledge this is the first study to identify dietary patterns in urban, black South African pregnant women and to explore their relationships with first trimester BMI and GWG. We found that, higher intake of a "mixed" diet pattern was associated with increased rate of GWG in both the total sample and in the subgroup of obese women, while a "western" diet pattern was associated with higher weight gain in normal weight women. In addition, high intake of a "traditional" diet pattern was associated with a reduction in the odds of excessive GWG, predominantly in normal weight women.

Observed positive associations between both the "western" and "mixed" diet patterns and rate of GWG are supported by Uusatilo et al. who found that greater adherence to a "fast food" diet pattern—characterised by high intakes of fast food items such as hamburgers and pizza, as well as sweets, soft drinks and added sugar—was positively associated with maternal weight gain (221). Although our "mixed" diet pattern was heterogeneous in nature, the high added sugar content may be driving this association and potentially negate some of the benefits of the healthier items such as whole grains and nuts. This is supported by previous studies in which higher intakes of a "margarine, sugar and snacks" diet pattern and more frequent consumption of high-sugar fruit drinks have been positively associated with excessive GWG (211,212).

The association identified between the "traditional" diet pattern and adequacy of GWG is unique to this study and, to our knowledge, has not been shown in other settings. Previous studies have reported contrasting findings for associations between the typically healthy dietary patterns identified; with Uusitalo et al. showing no association between a "healthy" diet pattern (high in vegetables, fish, poultry, legumes, fruit and eggs) and rate of GWG and Tielemans et al. showing a positive association between a "vegetable, oil and fish" pattern and GWG (211,221). However, when assessing diet using a quality index (i.e., level of

adherence to a pre-defined "healthy" diet; in this case a diet high in fruit, vegetables, whole grains, potatoes, fish, game and milk) Hillesund et al. found that diets of higher nutrient density and healthier macronutrient distribution were associated with reduced odds of excessive GWG in normal weight women (222). As with our study, the associations between maternal diet and GWG adequacy were not seen in overweight/obese women.

While high "mixed" diet pattern scores were significantly associated with higher rate of weight gain in both the total sample and the obese subgroup, we found that high "western" pattern intake was only associated with lower GWG in the normal weight women. In addition, the reduction in odds of excessive GWG associated with a "traditional" diet pattern in the total sample was evident in normal weight, but not in overweight or obese women after stratification. Differential associations between diet patterns and GWG across BMI categories have been shown previously, with both Tielemans et al. and Hillesund et al. finding that the associations between diet patterns and either rate of weight gain or GWG adequacy were specific to normal weight women (211,222). Due to the complexity of the relationship between diet patterns, BMI and GWG, the reason for this is difficult to ascertain; however it is possible that the effects of diet quality on GWG may be somewhat negated if quantity remains high; particularly in overweight/obese women who are already at an increased risk of higher weight gain. In addition, overweight and obese women have a reduced capacity for weight gain according to IoM recommended ranges than their normal weight counterparts and this may limit the effect of improved diet quality on adequacy of weight gain in these women (222). Differences may also be a result of the higher degree of under-reporting of dietary intake associated with increasing BMI (223). Although under- and over-reporting has largely focused on overall energy and macronutrient intakes, women have been shown to preferentially under-report foods that they believe to be unhealthy or fattening—such as high

sugar and snack foods—which may result in greater inaccuracies in the dietary data reported by overweight and obese women when compared to those of normal weight (224,225).

As previously mentioned, the relationship between diet and weight gain during pregnancy is complex; as reflected by the effect of adjusting for identified covariates in the regression models. Both dietary patterns and GWG are associated with a number of other sociodemographic and health factors which may confound or modify the associations between identified patterns and weight gain. In our sample of pregnant women we show that, although HIV status is not associated with dietary patterns, HIV-positive women are more likely to gain inadequate weight during pregnancy than their HIV-negative counterparts; regardless of whether they were initiated on ART prior to, or during, the current pregnancy. Although inadequate weight gain is not the key focus in this highly overweight/obese population, the high prevalence of HIV (34%) and increased risk of adverse birth outcomes associated with inadequate weight gain during pregnancy (1) highlight a need to tailor dietary advice to promote higher weight gain where necessary, while potentially monitoring weight gain throughout pregnancy, in this vulnerable population.

Alongside HIV status, our results highlight additional factors which should be considered when tailoring advice or interventions to promote improved diet quality and optimal weight gain in urban black South African pregnant women. We found that younger, nulliparous, single women had higher adherence to a "western" (i.e. "less healthy") pattern, while women who had a previous birth and were married or cohabiting had higher adherence to a "traditional" (i.e. "healthier") diet pattern. Similar results have been shown previously for age and marital status (219,226,227).

Although less convincing than the contrasting associations we have shown between "western" and "traditional" diet patterns and both parity and marital status, we found that

"western" diet pattern scores were higher in more educated women. This is opposite to relationships shown between patterns of dietary intake and education in HICs; with more educated women being more likely to consume the studies respective "healthy" diet pattern in these cases (219,226). This may be due to the influence of urbanisation in our setting, where improved education and higher household SES have been positively associated with obesity risk; potentially as a result of increased intakes of more refined, high fat foods and reduced levels of physical activity (228,229). This is reflected by the positive association identified between household SES and the "mixed" diet pattern in our study which was high in full fat dairy, margarine and added sugar.

While dietary intake has been explored in pregnant South African women, studies have been limited to the use of traditional analyses that assess macro- and micronutrient or food item intakes in isolation rather than overall patterns of dietary intake. This is a widely recognised flaw in the classic approaches to dietary assessment, as diets consist of multiple combinations of foods/nutrients consumed together, nutrient intakes are highly correlated and individual nutrients may have very small effects (73,219). This makes independent nutrient-outcome associations difficult to ascertain, while limiting their practicality and relevance for use in public-health messaging. Use of PCA to depict dietary patterns therefore provides strength to our study in that we are able to capture both the diversity and quality of population diets and therefore contribute to a more comprehensive understanding of the relationships between habitual eating behaviours and outcomes of interest. In addition, it provides a more accessible basis for comparison with food based dietary guidelines, as well as for recommendations to the public who may have limited understanding of the nutrient composition of foods (73,227). However, this approach is not free from limitations; with the majority being linked to the subjective nature of decisions made on the grouping of food items and formatting of the input variables, as well as the number and naming of the derived patterns (230,231).

Further limitations of our study included the use of BMI at recruitment to classify maternal pre-pregnancy BMI status. Although first trimester weight has been identified as an adequate proxy for pre-pregnancy weight, BMI specific differences in the amount of weight gained—although low overall—have been shown in black women and may result in a degree of misclassification (232,233). In addition, differences in the timing of anthropometric measurements resulted in variations in follow-up duration for GWG assessment between participants which were dependent on gestational age at both recruitment and the final visit prior to delivery. However, this was addressed through using the rate of gain per week rather than total gain through pregnancy; thereby increasing accuracy and comparability of GWG in the sample. While we included a number of covariates in our study which were shown to be associated with diet pattern(s) and/or GWG, we did not have data on all behavioural factors which may influence these relationships; for example physical activity which, alongside change in dietary intake and smoking, has been proposed as a determinant of GWG (234). Lastly, although our sample size was sufficiently robust for analysis of associations between diet and GWG in the total sample, the numbers of women within specific BMI categories were substantially lower and may be a contributing factor to the differences in the associations observed after stratification.

5.5 Conclusion

Our findings suggest that increased intakes of a "traditional" diet pattern – high in whole grains, legumes, vegetables and traditional meats and low in processed foods - and reduced intakes of highly refined diets - high in fat, added sugar and convenience food products - may significantly reduce gestational weight gain (including risk of excessive weight gain) in urban black South African women. However, due to the high prevalence of overweight and obesity in this population, these results emphasize the need for early intervention to improve diet quality and promote a healthy weight prior to conception. This would not only reduce the risk

of excessive GWG inherently associated maternal obesity, but enhance the effects of good quality diets on weight gain adequacy during pregnancy to potentially improve both maternal and infant outcomes.

CHAPTER 6: Maternal traditional dietary pattern and antiretroviral treatment exposure are associated with neonatal size and adiposity in urban, black South Africans[4]

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[4]Wrottesley SV, Ong KK, Pisa PT, Norris SA. Maternal traditional dietary pattern and antiretroviral treatment exposure are associated with neonatal adiposity in urban, black South Africans. Br J Nutr (accepted: 30/05/2018)

6.1 Introduction

Maternal pre-pregnancy body mass index (BMI) and gestational weight gain (GWG) are established predictors of fetal growth and birth outcomes; with both obesity and excessive weight gain being associated with high birth weight and large-for-gestational age (LGA) deliveries, as well as with obesity and cardiometabolic disease risk in later life (1,5,10,99,235). Although the effects of anthropometrically defined maternal nutritional status on birth weight have been well documented, the influence of dietary patterns on birth size – particularly in increasingly urbanised low-and-middle income countries (LMICs) such as South Africa – is not known. In addition, the predominant use of birth weight as a proxy for fetal growth fails to elucidate the effects of diet on adiposity (i.e. fat vs. fat-free mass) and therefore provides only a weak indication of newborn metabolic risk (84,236). Studies have shown that adiposity in early infancy tends to track through childhood and is associated with long term risk of central adiposity, as well as of elevated triglyceride levels and insulin resistance (237,238). Finally, the extent to which high human immunodeficiency virus (HIV) and antiretroviral treatment (ART) exposure in this setting prior to, and during, pregnancy may impact these associations is yet to be explored.

During a previous study in urban, black South Africans, we used principal component analysis (PCA) to identify three distinct dietary patterns in pregnant women; namely “western”, “traditional” and “mixed” (204). These patterns were consistent with those expected for a transitioning African population experiencing a shift towards increasingly westernised diets high in saturated fat, sugar, salt, processed/convenience foods and edible oils and low in essential micronutrients (26,28). We further showed that adherence to the “traditional" dietary pattern – characterised by high intakes of vegetables, beans and legumes, traditional meats and whole grains - was associated with lower rate of GWG and reduced odds of excessive weight gain. These associations remained evident in women of normal

weight after stratification, but not in overweight or obese subgroups. This suggests that the relationship between diet and maternal adiposity may be modified by baseline BMI. However, whether "traditional" pattern intake is similarly associated with beneficial reductions in neonatal adiposity – either independently or via interactions with maternal nutritional status - is not known.

The maternal high risk profile in this setting – where 66% of women are overweight or obese and 55% experience excessive gestational weight gain - is further complicated by a 33% prevalence of HIV (204). While ART initiation is mandatory in South Africa for all HIV positive pregnant women not yet receiving treatment, and this has had undeniable benefits in the prevention of vertical transmission, the metabolic consequences for both mother and infant are not clear. Both HIV infection and ART exposure have been positively associated with weight and fat distribution changes and altered glucose and lipid metabolism in both adults and children, as well as with adverse birth outcomes (17–19,239,240). However, little is known about the possible effects of HIV/treatment on fetal growth and adiposity, as well the risk of non-communicable disease in the long term.

The aim of this study was therefore to examine the associations between maternal "traditional" dietary pattern adherence and HIV/treatment with neonatal size and adiposity in urban, black South Africans, as well as how specific maternal factors - i.e. BMI and GWG - may influence these associations.

6.2 Methods

6.2.1 Study setting and participants

This study was nested within a large pregnancy cohort study (Soweto First 1000-Day Study; S 1000), based at the Chris Hani Baragwanath Hospital in Soweto, Johannesburg, South Africa between 2013 and 2016. Overall, S 1000 aimed to understand the complex

associations between multiple maternal factors and fetal and infant outcomes in an urban-poor African context, and to identify the levers that could optimise maternal and child health within the first 1000 days. Inclusion criteria for S 1000 were as follows: resident of Soweto, or the Greater Soweto area, <20 weeks gestational age at recruitment, non-epileptic, non-diabetic, 18 years or older and pregnant with a singleton, naturally conceived pregnancy. Data collection for S 1000 took place at six time points during pregnancy (<14 weeks; 14–18 weeks; 19–23 weeks; 24–28 weeks; 29–33 weeks and 34–38 weeks) and eight time points after delivery (<14 days; 6 weeks; 2 months; 3 months; 6 months; 12 months; 18 months and 24 months) . All women provided written informed consent prior to their inclusion in the pregnancy component of the study (Soweto Fetal Growth Study), as well as prior to the inclusion of themselves and their infants in the post-delivery follow-up (Soweto Baby Growth Study). Ethical approval was obtained from the University of the Witwatersrand's Research Ethics Committee (Medical) for both components of S 1000 (M120524 and M130905). 559 women were recruited into this sub-study and had dietary intake assessed at 14-18 weeks.

6.2.2 Maternal variables

6.2.2.1 Demographic, health and socioeconomic variables

Maternal demographic and socioeconomic variables were collected by trained members of research staff using interviewer-administered questionnaires at the first pregnancy visit (<14 weeks gestational age). Parity was defined as the number of previous births at a gestational age of 24 weeks or more - regardless of whether the infant was born alive or was stillborn. Smoking and/or chewing tobacco was reported at baseline. HIV-status was self-reported at each pregnancy visit and confirmed using the results recorded in the participant's antenatal clinic card. According to South Africa's national Prevention of Mother-to-Child Transmission (PMTCT) guidelines, routine HIV counselling and testing is required during

pregnancy; for any HIV-positive woman who is not already receiving treatment, ART is initiated. All HIV-positive participants in this study were therefore receiving ART and were stratified according to whether they had been initiated on ART prior to pregnancy (pre-pregnancy ART) or during the current pregnancy (antenatal ART). Household socioeconomic status (SES) was assessed using an asset index which scored each participant according to the number of assets that they possessed out of a possible 9 (electricity, radio, television, refrigerator, mobile phone, personal computer, bicycle, motorcycle/scooter, car). This was based on standard measures used in the Demographic and Health Surveys household questionnaire (available at: www.measuredhs.com) and has been extensively utilised in this setting (241,242). Asset index scores were subsequently grouped into low (<5), medium (5-7) and high (>7) SES categories. Maternal education was defined according to the highest level of completed (primary, secondary or tertiary).

6.2.2.2 Anthropometry

A wall-mounted Stadiometer (Holtain, UK) was used to measure maternal height to the nearest 1 mm at baseline. Maternal weight was measured to the nearest 0.1 kg at each visit during pregnancy using a digital scale. Weight at recruitment (<14 weeks) was used as a proxy for pre-pregnancy weight and, together with height, was used to calculate maternal BMI (weight (kg)/height (m^2)). There were no underweight women in this sample and therefore BMI was classified according to the following categories: normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2) or obese (≥30.0 kg/m^2). GWG (kg/week) was calculated as: [(weight at final pregnancy visit − weight at recruitment)/duration of follow-up]. GWG was classified as inadequate, adequate or excessive according to the BMI-specific Institute of Medicine recommended weight gain ranges (25).

6.2.2.3 Dietary intake

Habitual dietary intake was assessed at the second pregnancy visit (14-18 weeks) using an interviewer-administered quantitative food-frequency questionnaire (QFFQ). This nationally utilised QFFQ was developed by the South African Medical Research Council (SAMRC) based on analyses of 11 dietary surveys conducted in rural and urban South Africa and includes all foods consumed by at least 3% of the population (243). Retrospective data was collected on the frequency and quantity of food and beverage intake during the previous week using food flash cards (high quality photographs of food items) and a combination of household measures, two-dimensional life-size drawings of foods and utensils, and three-dimensional food models as described and validated by Steyn et al (216). According to the criteria developed by Dennis et al, this QFFQ is a very high quality tool – scoring a total of 13 points (high quality classified as a score of seven or higher) (244). This QFFQ has been extensively piloted and utilised in this setting and results are published elsewhere (172,204,215). QFFQ data was captured electronically using REDCap electronic data capture tools hosted at The University of the Witwatersrand (217).

6.2.3 Neonatal variables

Neonates were included in this study if they were born at term (≥37 weeks) and had complete delivery outcome data. Additionally, body composition was analysed for a sub-sample who had assessments via either air displacement plethysmography (ADP) using the Peapod (Cosmed, USA) or dual-energy x-ray absorptiometry (DXA; (Hologic DiscoveryA S/N 86254, APEX software version 4.0.2, Hologic Inc., USA) within the first two weeks of life.

6.2.3.1 Birth outcomes

Gestational age at delivery (weeks) was calculated as: [duration of pregnancy follow-up (date of delivery – date of baseline ultrasound dating scan) + gestational age at baseline (crown-to-rump length measured by ultrasound; days)]. Birth weight and length were measured by trained research nurses within 24 hours of delivery for 82% of neonates. Where assessment

within this window was not possible – for example due to the infant being admitted to the hospital for observation – measurements were taken within 48 hours. Weight to length ratio (WLR; kg/m) was calculated to represent the best anthropometric predictor of neonatal body composition (fat-free mass and fat mass) as described by Villar et al (245).

6.2.3.2 Neonatal body composition

Weight and length were measured and fat mass and fat-free mass estimated for nude neonates within 14 days of birth. According to a previous study in which we demonstrated the level of agreement between ADP and DXA estimated body composition in this population, ADP was utilised where available (174). In cases where a neonate had only DXA measurements, fat mass and fat-free mass were converted to their ADP equivalent estimates using the following linear equations:

Fat mass (ADP equivalent) = 139.8311 + 0.7974718 * DXA fat mass

Fat-free mass (ADP equivalent) = 89.40371 + 0.8728309 * DXA fat-free mass

These equations were generated for the population from the regression of ADP on DXA measurements in the aforementioned ADP/DXA comparison study and provided reliable ADP equivalent estimates (174). Fat mass index (FMI; kg/m^3) was calculated from these estimates to describe adiposity in neonates. As described by Villar et al, the applicability of exponents in body composition indices to address the relationship between body composition and size may vary across populations (245). We therefore regressed fat mass on length (data in natural logarithms) to confirm that this index (kg/m^3) provided the best description of the relationship between weight and length in the study population. This was indeed confirmed - regression power exponent: 2.8±0.6 (SE) (246).

ADP

Peapod assessments were performed according to standard procedures as previously described (86,189). Participants were placed inside the Peapod chamber wearing only a wig cap if necessary. Body volume was estimated using pressure and volume changes (air displacement) within the chamber and body density calculated using body mass and volume measurements. Fat mass, and fat-free mass were subsequently derived using gender-specific equations developed by Fomon et al (247).

DXA

DXA scanning was performed according to standard procedures as described elsewhere (191). Typically, neonates were fed prior to DXA scanning and were sleeping during the procedure. Neonates were placed supine on the scanning bed wearing only a disposable diaper and swaddled in a cotton blanket. Scans were satisfactory for use if the subject's body lay within the scanning region and there was minimal movement during assessment. Whole body measurements of fat mass and fat-free mass were extracted for use in analyses.

6.2.4 Statistical analysis

Data were analysed for 393 mother-neonate pairs with complete data using Stata 13.0 (StataCorp, College Station, TX, USA). As previously described, the flow of participants through the sub-study to reach the final sample sizes for the primary and secondary outcomes (WLR and FMI respectively) is depicted in Figure 8. Mother-neonate pairs included in the final analyses did not differ in any baseline maternal characteristics (demographics, SES and anthropometry) from those excluded.

The dietary patterns previously identified in this population – namely "western", "traditional" and "mixed" – were confirmed in this sub-sample using PCA as described elsewhere (204). PCA was conducted using orthogonal (varimax) rotation on the weekly frequency of consumption of the QFFQ food items, classified as 48 food items/groups based on those

described by Crozier et al (76,220). The Kaiser-Meyer-Olkin measure of sampling adequacy (0.68) and Bartlett's test of sphericity ($p<0.001$) confirmed PCA as an appropriate dimension reduction technique for use in this sample. Eigen values, as well as their visual inflections on a scree plot, and the percentage of total variance explained were used to retain patterns. Specifically, those Eigen values situated above the point where the downward curve levels off on the scree plot were considered for retention (Supplementary Figure F1; Appendix M). As described elsewhere, foods or food groups with factor loadings ≥0.2 reflected strong associations with principal components and were used to name the dietary patterns (211). Dietary pattern scores for each pattern were generated by multiplying factor loadings by the intakes of each food/food group and then generating standardised summed scores. Mean factor scores for the patterns were zero; with positive and negative scores representing high and low adherence respectively of each dietary pattern (219). Due to the associations previously demonstrated between the "traditional" diet pattern and odds of excessive GWG in this population, as well as the independent associations demonstrated between excessive weight gain and both birth size and neonatal adiposity in other studies (248,249), maternal diet was classified according to adherence to the "traditional" pattern ("traditional" pattern score) in all subsequent study analyses.

Maternal and neonatal characteristics of the sample are presented as median (interquartile range) and percentages (%) for continuous and categorical variables respectively. The Kruskal-Wallis test was used to compare neonatal WLR and FMI according to the following maternal and infant factors: maternal age, parity, HIV/treatment status, smoking status, education, marital status, SES, BMI at recruitment, GWG, "traditional" diet pattern adherence, neonate sex and gestational age at birth.

Based on known associations between maternal factors and birth size, as well as previously described associations in this population (204), we proposed a conceptual framework for the

associations between maternal "traditional" pattern adherence (continuous: diet pattern score), BMI (categorical: normal weight (ref) vs. overweight and obese), GWG (continuous: kg/week), HIV/treatment status (categorical: HIV negative (ref) vs. HIV positive (antenatal ART) and HIV positive (pre-pregnancy ART) and neonatal WLR (continuous: kg/m) and FMI (continuous: kg/m^3). The bivariate associations between these maternal factors and each neonatal outcome were tested using linear regression analyses.

In order to identify the independent associations between diet, BMI, GWG, HIV/treatment status and WLR and FMI we performed hierarchical regression analyses per outcome. Covariates included in analyses were maternal or infant variables conclusively associated with infant outcome(s) (namely parity, newborn sex and gestational age at birth). Additionally, for FMI, age at scan (days) was included to adjust for variation across the two week assessment period. Regression coefficients and R^2 values are therefore presented across three models for WLR and FMI. Variables included in the models were as follows: Model 1 (M1): neonate sex and "traditional" diet pattern score; Model 2 (M2): M1 with HIV/treatment status, BMI and GWG; Model 3 (M3): M2 with parity and gestational age at delivery (and age at scan for FMI). In order to test for an interaction between BMI and dietary pattern adherence, a fourth model per outcome was run as follows: M3 with the interaction term BMI category*traditional pattern score (data not shown). P-values less than 0.05 were considered statistically significant.

6.3 Results

Maternal and infant characteristics are presented in Table 11. The median age of pregnant women was 30 years. 35% and 30% of women were overweight and obese at recruitment, respectively, while 58% gained excessive weight according to the IoM BMI-specific guidelines. 34% of women were HIV positive; with 23% in total being initiated on ART

during the current pregnancy. 52% of newborns were male. Neonates had a median birth weight of 3100 g and WLR and FMI were 6.4 kg/m and 3.6 kg/m^3 respectively.

As previously described, for the purpose of this study maternal diet was classified according to "traditional" diet pattern adherence (i.e. "traditional" diet pattern score). This dietary pattern was characterised by high factor loadings for vegetables, beans and legumes, traditional meats and porridge/pap (Supplementary Table S2; Appendix M).

Of the maternal variables described as potential covariates in this population, only parity was consistently associated with neonatal outcomes; with higher WLR and FMI seen in infants born to mothers who had experienced at least one previous birth (Supplementary Table S3; Appendix M). Female neonates had significantly higher FMI than males and WLR increased with gestational age.

Conceptual models for the bivariate associations between maternal "traditional" diet pattern, BMI, GWG and HIV/treatment status and outcomes of interest are presented in Figures 1 (WLR) and 2 (FMI). Compared to those of normal weight at recruitment, overweight and obese women exhibited significantly lower GWG. GWG was also lower in HIV positive (antenatal ART initiation) vs. HIV negative women. Additionally, in HIV positive women, pre-pregnancy ART initiation was associated with higher adherence to the "traditional" diet pattern. Maternal obese vs. normal weight BMI was positively associated with WLR during bivariate analyses, while HIV positive (pre-pregnancy ART) status was positively associated with FMI when compared to HIV negative status.

Table 11: Maternal and neonatal characteristics of urban, black South Africans (n=393)

	Median (IQR) or %
Maternal variables	
Demographic and health characteristics	
Maternal age, y	30 (25-35)
Parity	
Para 0	25
Para 1	45
Para ≥2	30
HIV status	
HIV-negative	66
HIV-positive (antenatal ART)	23
HIV-positive (pre-pregnancy ART)	11
Smokes/chews tobacco	
No	87
Yes	13
Socioeconomic characteristics	
Maternal education	
Primary	2
Secondary	70
Tertiary	28
Marital status [n=387]	
Single	61
Married/cohabiting	39
Household SES	
Low	13
Medium	80
High	7
Anthropometry	
BMI at recruitment, kg/m^2 (<14 weeks)	27.2 (23.8-30.9)
Normal weight (18.5-24.9)	35
Overweight (25-29.9)	35
Obese (≥30)	30
GWG, kg/week	0.41 (0.28-0.56)
Inadequate	22
Adequate	20
Excessive	58
Neonatal variables	
Sex	
Male	52
Female	48
Gestational age at delivery, weeks	39 (38-40)
Anthropometry	
Birth weight, g	3100 (2850-3365)
Birth length, cm	48.9 (47.3-50.3)
Weight to length ratio (kg/m)	6.4 (5.9-6.8)
Body composition [n=171][a]	
Age at scanning, d	8 (4)
Fat mass, g	435 (331-548)
Fat-free mass, g	2774 (2530-2929)
Fat mass index, kg/m^3	3.6 (2.9-4.6)

Abbreviations: BMI, body mass index; GWG, gestational weight gain; ART, antiretroviral treatment

IoM GWG ranges (kg/week): inadequate, normal weight <0.35, overweight <0.23, obese <0.17; adequate, normal weight 0.35-0.50, overweight 0.23-0.33, obese 0.17-0.27; excessive, normal weight >0.50, overweight >0.33, obese >0.27

[a]Measured by air displacement plethysmography (ADP; Peapod) or dual-energy x-ray absorptiometry (DXA) corrected for the measurement differences between techniques

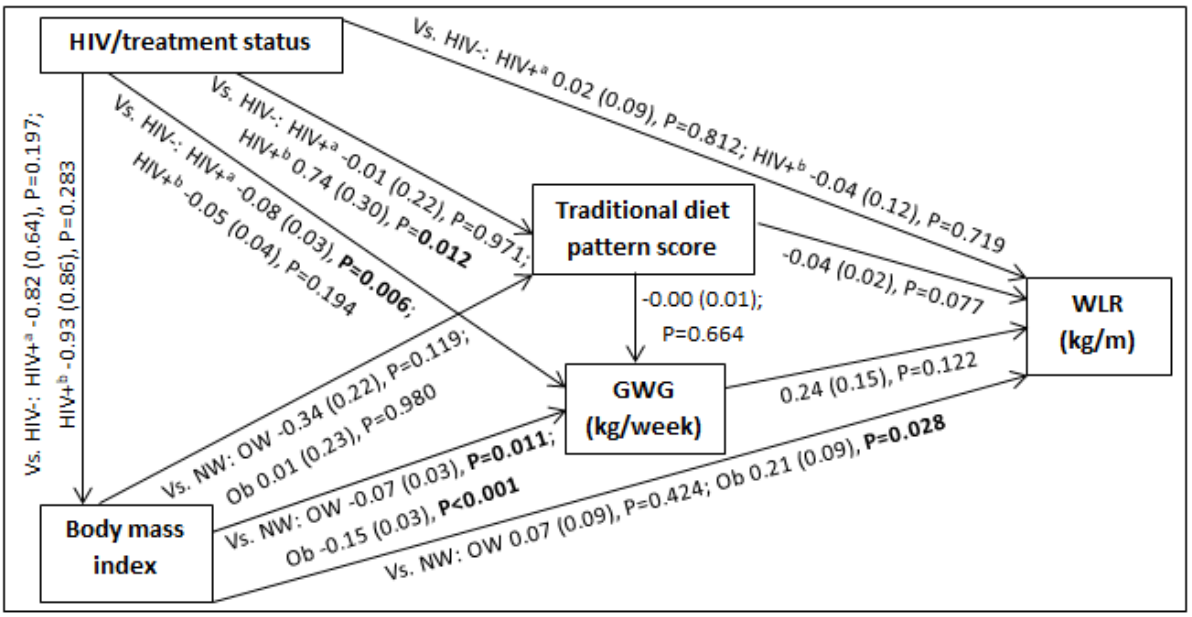

Figure 11: Conceptual model with bivariate associations between maternal factors and neonatal weight-to-length ratio (kg/m) in urban, black South Africans

Abbreviations: GWG, gestational weight gain; HIV-, HIV negative; HIV+a, HIV positive (antenatal ART); HIV+b, HIV positive (pre-pregnancy ART); NW, normal weight; OW, overweight; Ob, obese; Vs, versus (i.e. compared to the following reference category); WLR, newborn weight-to-length ratio

Values are regression coefficients with standard errors [β(SE)] from linear regression analyses; significant results presented in bold (P<0.05)

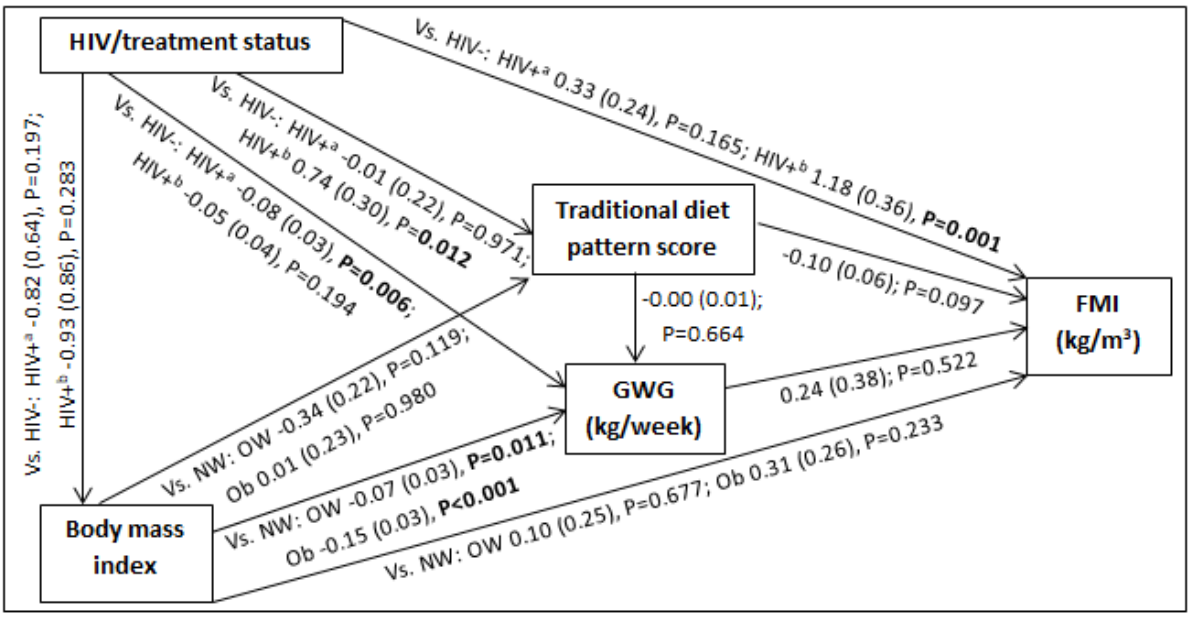

Figure 12: Conceptual model with bivariate associations between maternal factors and neonatal fat mass index (kg/m^3) in urban, black South Africans

Abbreviations: FMI, neonatal fat mass index; GWG, gestational weight gain; HIV-, HIV negative; HIV+a, HIV positive (antenatal ART); HIV+b, HIV positive (pre-pregnancy ART); NW, normal weight; OW, overweight; Ob, obese; Vs, versus (i.e. compared to the following reference category)

Values are regression coefficients with standard errors [β(SE)] from linear regression analyses; significant results presented in bold (P<0.05)

Table 12 presents the results of hierarchical regression analyses of maternal variables on newborn WLR. In fully adjusted models (M3: adjusted for neonate sex, "traditional" diet pattern score, HIV/treatment status, BMI, GWG, parity and gestational age at delivery) a 1 SD increase in "traditional" diet pattern score was inversely associated with newborn WLR (-0.04 kg/m; P=0.033). In addition, compared to a normal weight BMI at recruitment, maternal obesity was positively associated with WLR (M3: 0.25 kg/m; P=0.008) and a 1 kg/week increase in GWG was associated with a 0.48 kg/m increase in newborn WLR (M3: P=0.002). M3 explained approximately 14% of the variation in newborn WLR.

The results of hierarchical regression analyses of maternal variables on newborn FMI are presented in Table 13. "Traditional" diet pattern adherence was associated with lower FMI (-0.13 kg/m^3 per +1 SD; P=0.027) after full adjustment for covariates (M3: adjusted for neonate sex, "traditional" diet pattern score, HIV/treatment status, BMI, GWG, parity, gestational age at delivery and age at scan). HIV positive (pre-pregnancy ART) vs. HIV negative status was associated with 1.18 kg/m^3 (P=0.001) higher neonatal FMI in M2 (adjusted for neonate sex, "traditional" diet pattern score, HIV/treatment status, BMI, GWG). This association remained significant in the fully adjusted model (M3: 1.11 kg/m^3; P=0.002). Approximately 19% of the variation in newborn FMI was explained in M3.

Although BMI and GWG were not associated with newborn FMI in any of the presented regression models; we confirmed an interaction between "traditional" diet pattern adherence and maternal BMI on FMI (vs. normal weight: overweight*traditional diet pattern, P=0.203; obese*traditional diet pattern, P=0.024). Therefore, while dietary pattern adherence was associated with a significant reduction in FMI for infants born to normal weight women (-0.32 kg/m^3; P=0.005), these effects were not seen among newborns of overweight or obese women (data not shown).

Table 12: Hierarchical regression for the associations between maternal factors and neonatal weight to length ratio (n=393)

WEIGHT TO LENGTH RATIO (kg/m)									
Independent variables	**Model 1**			**Model 2**			**Model 3**		
	B	**95% CI**	**p-value[b]**	**B**	**95% CI**	**p-value[b]**	**B**	**95% CI**	**p-value[b]**
Neonate sex									
Male		Ref			Ref			Ref	
Female	-0.02	-0.17; 0.13	0.769	-0.05	-0.19; 0.10	0.533	-0.06	-0.20; 0.08	0.396
Maternal dietary pattern									
Traditional pattern score	-0.04	-0.08; 0.00	0.078	-0.04	-0.08; 0.00	0.081	-0.04	-0.08; -0.00	**0.033**
HIV/treatment status									
HIV negative					Ref			Ref	
HIV-positive (antenatal ART)				0.07	-0.11; 0.25	0.422	0.07	-0.10; 0.24	0.436
HIV-positive (pre-pregnancy ART)				0.01	-0.23; 0.26	0.906	-0.02	-0.26; 0.21	0.833
BMI category									
Normal weight					Ref			Ref	
Overweight				0.09	-0.09; 0.26	0.343	0.09	-0.08; 0.26	0.301
Obese				0.27	0.08; 0.46	**0.005**	0.25	0.07; 0.43	**0.008**
Gestational weight gain							.		
Rate, kg/week				0.36	0.05; 0.67	**0.024**	0.48	0.18; 0.78	**0.002**
Parity									
Para 0								Ref	
Para 1							0.33	0.15; 0.50	**<0.001**
Para ≥2							0.32	0.13; 0.52	**0.001**
Gestational age									
Gestational age at birth, weeks							0.18	0.12; 0.24	**<0.001**
R2 per model		*0.008*			*0.035*			*0.137*	

Values are regression coefficients with 95% confidence intervals

[a]Multiple linear regression analyses; significant results are presented in bold ($p<0.05$)

Table 13: Hierarchical regression for the associations between maternal factors and neonatal fat mass index (n=171)

Independent variables	FAT MASS INDEX (kg/m^3)								
	Model 1			Model 2			Model 3		
	B	95% CI	p-value[b]	β	95% CI	p-value[b]	B	95% CI	p-value[b]
Neonate sex									
Male		Ref			Ref			Ref	
Female	0.44	0.04; 0.84	**0.033**	0.40	0.00; 0.79	**0.049**	0.39	0.01; 0.78	**0.044**
Maternal dietary pattern									
Traditional pattern score	-0.09	-0.22; 0.03	0.130	-0.10	-0.22; 0.02	0.086	-0.13	-0.25; -0.02	**0.027**
HIV/treatment status									
HIV negative					Ref			Ref	
HIV-positive (antenatal ART)				0.37	-0.10; 0.84	0.123	0.29	-0.17; 0.75	0.218
HIV-positive (pre-pregnancy ART)				1.18	0.48; 1.87	**0.001**	1.11	0.42; 1.81	**0.002**
Gestational weight gain									
Rate, kg/week				0.44	-0.29; 1.18	0.238	0.46	-0.26; 1.18	0.213
BMI category									
Normal weight					Ref			Ref	
Overweight				0.17	-0.30; 0.65	0.469	0.20	-0.26; 0.66	0.390
Obese				0.37	-0.13; 0.87	0.147	0.24	-0.25; 0.74	0.335
Parity									
Para 0								Ref	
Para 1							0.76	0.27; 1.25	**0.002**
Para ≥2							0.64	0.11; 1.17	**0.018**
Neonate age									
Gestational age at birth, weeks							0.09	-0.08; 0.26	0.286
Age at examination, days							0.05	0.00; 0.10	**0.035**
R2 per model		*0.043*			*0.121*			*0.194*	

Values are regression coefficients with 95% confidence intervals

[a]Multiple linear regression analyses; significant results are presented in bold ($p<0.05$)

6.4 Discussion

To our knowledge this is the first study to explore the relationships between maternal nutritional status, dietary patterns and HIV/treatment exposure in African women and to examine their effects on neonatal adiposity within the first two weeks of life. We found that, although maternal obesity and GWG were associated with neonatal body composition, they predicted overall birth size rather than increased fat mass in particular. In contrast, adherence to a "traditional" dietary pattern during pregnancy was associated with lower WLR and FMI; suggestive of a predominant effect on fat mass in the neonate. Finally, we showed that duration of ART exposure (pre-pregnancy vs. antenatal initiation) in HIV positive women was positively associated with newborn adiposity in this setting.

Our findings build on data previously reported on the association between "traditional" dietary pattern adherence and reduced GWG (including odds of excessive weight gain) in this population (204). Here we show that, not only is a dietary pattern high in vegetables, beans and legumes, traditional meats and whole grains associated with beneficial reductions in maternal adiposity during pregnancy, but also in fetal fat deposition. Such effects have important implications for the long term health trajectory of the infant; with previous studies showing a tendency towards tracking of adiposity through infancy, as well as of an increased risk of obesity and elevated metabolic risk profiles (including higher fasting triglyceride concentrations and insulin resistance) in later life (237,238). In addition, while Catalano et al demonstrated a significant correlation between body fat percentage at birth and at childhood follow up (mean age: 8.8 ± 1.8 years), there was no correlation in total weights at these two time points (238). This highlights the importance of neonatal adiposity as a potential predictor for longer-term obesity and metabolic disease risk.

Although the associations between a "traditional" diet pattern and neonatal WLR and FMI are unique to our study, they are supported by Starling et al who found that intake of a diet

pattern with lower consumption of green vegetables and dairy and higher in refined grains was associated with higher birth weight, fat mass and body fat percentage in the US (93). However, conflicting results have also been shown; with typically healthier diet patterns ("Mediterranean" and "traditional") being associated with higher birth weight and reduced SGA risk, while more processed or "western" dietary patterns have been associated with increased risk of SGA and lower weight-for-age z-score in some high income settings (77–80,250).

The relationship between maternal diet and birth size and adiposity is complex and the variation in findings across populations may reflect an influence of baseline nutritional status on these associations. For example, while energy dense, processed diets have been shown to restrict fetal growth and increase SGA risk in certain populations, they may increase the risk of high birth weight and adiposity in others (80,81,250). As both ends of the spectrum - i.e. being born too small or too large for gestational age – are associated with long term disease risk (205,251–253), identifying patterns of intake which facilitate optimal fetal growth and limit excess fat deposition in increasingly urbanised African populations, is critical. Given the high level of urbanisation in Africa - and particularly South Africa - to date, as well as the representativeness of the study population to that of an urban-poor community, our findings contribute substantially to understanding these contexts. Here we confirm that, not only do the effects of maternal dietary patterns on GWG differ across BMI categories (204), but the association between "traditional" diet pattern adherence and neonatal adiposity is similarly modified by maternal BMI at baseline; with the effects seen predominantly in the normal weight subgroup. This suggests that, in increasingly obesogenic populations, the beneficial effects of improved diet quality may be limited in women who exhibit excess adiposity and associated metabolic risk profiles prior to conception. Obesity induces a chronic inflammatory state that has been suggested as a key driver of insulin resistance and may

intensify the naturally occurring insulin resistant profile during pregnancy (84,254,255). Reduced insulin sensitivity in the obese pregnant woman – and the subsequent increase in availability of glucose and lipids – may potentially facilitate excess substrate transfer to the fetus and, thus, fat deposition irrespective of the current dietary pattern.

While we found no differences in WLR or FMI between infants born to HIV positive women with antenatal ART initiation compared to their HIV negative counterparts, we showed a significant increase in FMI for infants whose mothers were HIV positive and initiated on ART prior to the current pregnancy. These associations were independent of baseline BMI, GWG and diet and therefore suggest a strong treatment effect in this population. ART associated metabolic complications have been widely documented in HIV positive patients, with fat redistribution (reduced subcutaneous and increased central adiposity), impaired glucose tolerance and insulin resistance, as well as dyslipidaemia being common side effects (256,257). Such changes may substantially impact the metabolic risk profile of women prior to conception; further exacerbating the pregnancy associated insulin resistant state and increasing the risk of gestational diabetes mellitus (GDM) and associated complications. These findings have important implications for HIV positive pregnancies in South Africa, during which duration of ART exposure (increasing with each subsequent pregnancy) may elevate the risk profile for both mother and infant in an already high risk population. Targeted monitoring and care strategies are therefore needed in order to minimise the adverse effects of treatment exposure on adiposity and associated metabolic risk in the newborn; with interventions designed to optimise nutritional status and diet quality pre-conception being potentially more vital in these women.

Although we showed significant effects of maternal nutritional status, diet and HIV/treatment exposure on birth size and/or neonatal adiposity, our final models explained only 14% and 19% respectively of the variability in infant outcomes. While maternal adult size, adiposity

and metabolic profile are predictive of infant outcomes, these characteristics are highly influenced by a mothers own growth and development; with studies showing strong intergenerational associations between maternal and offspring birth weight (258–261). This suggests that the gestational environment of the mother – and the resulting consequences on maternal birth size and longer term metabolic risk profile – may be an important factor in further explaining differences in neonatal size and adiposity in our study. In addition, there may be other behavioural factors which require exploration in these models, such as physical activity. Although there was no association found between physical activity and birth outcomes (including birth weight and ponderal index) in a previous study of this population (262), any potential indirect effects on birth size – for example through reductions in GWG and/or risk of GDM – as well as possible associations with fetal fat deposition and neonatal adiposity should be explored (51–53).

Other limitations of our study include the use of baseline BMI as a proxy for pre-pregnancy BMI and the variation in timing of maternal anthropometric measurements for assessing maternal GWG as previously described (204). Although first trimester weight has been identified as an adequate proxy for pre-pregnancy weight – correctly classifying 91-95% of women according to pre-pregnancy BMI - BMI specific differences in weight gain during trimester one have been shown (232,233). While use of baseline weight may have resulted in a degree of misclassification in our study, any effects on study findings were likely to be negligible due to the low overall amount of weight gained prior to 14 weeks; particularly in black women at higher BMIs (233). In addition, although the categorisation of pregnant women into three HIV/treatment status groups (i.e. HIV negative, HIV positive (antenatal ART) and HIV positive (pre-pregnancy ART)) allowed for effective comparison of overall treatment exposure in our sample, inclusion of additional measures of duration and/or adherence to ART would allow for more robust comparison of ART exposure on a

continuous scale. While we present strong evidence for an effect of ART exposure on neonatal adiposity, use of an objective measure such as viral load – an established proxy for ART adherence/effectiveness (266) - would be beneficial in further explaining the influence of treatment on fetal fat deposition and metabolic risk in future research. Given the focus of our study and the differential patterns of growth (both in fat mass and fat free mass) between pre-term and term infants, we included only term neonates in our analyses (245). While this reduced the final sample size, it allowed for better interpretation of the effects of maternal factors (principally diet and HIV/treatment status) on newborn adiposity. However, future studies should explore these associations in pre-term infants in order to further elucidate the relationships with newborn size and adiposity according to gestational age. Lastly, neonatal body composition was measured using two techniques (Peapod and DXA) within the first two weeks of life which may reduce comparability across the sample; particularly due to the reduced sample size for these objective assessments. However, the comparability between techniques previously shown in this population allowed for correction of DXA to Peapod measurements and therefore equivalent fat mass estimates in the sub-sample (174). Although the variation in day of neonatal body composition assessment must be considered - with physiological weight loss occurring during this period – any changes in body composition are predominantly due to reductions in body water and would therefore have little effect on the comparability of fat mass estimates between subjects (267).

6.5 Conclusion

Our findings suggest that increased adherence to a "traditional" diet pattern - high in whole grains, beans and legumes, vegetables and traditional meats and low in processed/convenience foods— may reduce neonatal adiposity in urban, black South Africans. However, early intervention to ensure a healthy BMI prior to pregnancy is needed in order to optimise the beneficial effects of diet quality on adiposity and associated

metabolic risk for both mother and infant. Although ART initiation and adherence is critical for both maternal and infant health, the effects of treatment exposure on maternal metabolic risk and neonatal adiposity highlights the vulnerability of HIV positive pregnant women and the importance of tailored care in this population. Targeted monitoring and management strategies are therefore necessary to limit treatment-associated effects on in utero fat deposition and to potentially reduce metabolic risk profiles and poor health trajectories of infants in both the short and longer term.

SECTION 3: INTEGRATED DISCUSSION

CHAPTER 7: Discussion and conclusion

7.1 Introduction

Overall, this thesis described MNS and dietary patterns in urban, black South African women and examined their associations with birth size and neonatal adiposity using the critical framework of the first 1000 days and its implications for optimal growth and development and reduced risk of metabolic disease in the long term. The South African setting was of particular relevance to the study findings, as it provided an opportunity to explore these associations within the context of rapid urbanisation and transitioning nutrition environments in Africa. These epidemiological shifts have serious consequences for the clustering of disease burdens experienced by pregnant women in these unique and vulnerable populations.

Chapter 1 provided the theoretical framework for the research and established the important role that nutrition plays in maternal and child health. However, it highlighted the need for a holistic approach to examining the associations between maternal nutrition and outcomes that are highly influenced by multi-level health determinants, such as demographics, SES and baseline burdens of disease. In addition, it presented the findings of objective one of this thesis: a review of the literature base for Africa in particular. This review highlighted the extensive gaps in our understanding of the importance of maternal nutrition in the first 1000 days in rapidly urbanising African settings (Figure 13). In terms of MNS, these gaps included a focus on nutrient intakes and biomarkers in isolation and a lack of understanding of the effects on GWG and maternal metabolic outcomes. For the infant, the predominant use of birth weight as a study outcome provided limited interrogation of both the short- and long-term metabolic consequences of sub-optimal growth and development. While it was outside the scope of this thesis to address all of these gaps, we were able to add significantly to the understanding of these relationships within an urban-poor African context by providing novel data on dietary patterns in pregnant South African women and the association between these patterns and both maternal and neonatal adiposity (Chapters 4-6).

This final chapter presents a consolidated overview of the thesis, specifically summarising the key findings according to each individual study objective and highlighting the overarching themes that emerge. In addition, it provides interpretation of the thematic areas, with particular focus on recommendations for intervention. Lastly, it discusses the strengths and weaknesses of the study design and methods, as well as potential routes for future research.

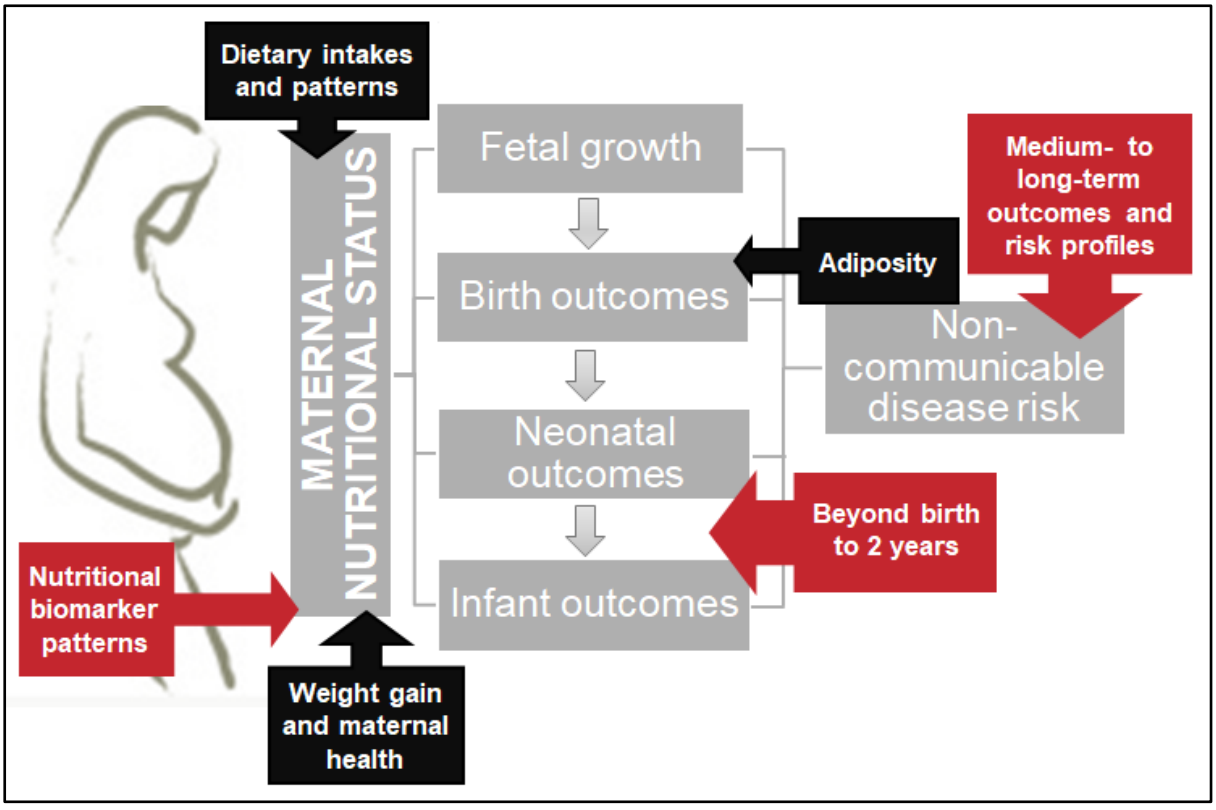

Figure 13: Research gaps identified in existing African literature and those addressed in the current study (illustrated in black)

Table 14: Summary of research findings per study objective

Thesis chapter	Study objective	Key findings
Chapter 2	To review and report on maternal nutritional status (MNS) in African women and its associations with fetal, birth, neonatal and infant outcomes in the first 1000 days.	MNS in Africa showed features typical of the epidemiological health transition; i.e. low burdens of maternal underweight and comparatively high overweight/obesity prevalences, as well as persistently high prevalences of micronutrient deficiencies. Additionally, reported maternal dietary intakes were high in energy-dense foods and low in protein/micronutrient-rich foods. Robust evidence to support associations between MNS and fetal, birth, neonatal and infant outcomes was limited in Africa; however available data supported the globally observed relationships between maternal BMI and birth outcomes (particularly birth weight) in this setting. A high prevalence of deficiencies in critical pregnancy-related nutrients (particularly iron), as well as benefits observed in micronutrient supplementation trials of undernourished pregnant women suggest that improvements in MNS could have significant effects on outcomes of interest. However, this review highlighted a need for data examining the associations between maternal dietary intakes and patterns and offspring outcomes, as well as longitudinal infant-outcome data through to two years of age.
Chapter 4	To compare body composition measurements using two methods, namely (i) dual-energy x-ray absorptiometry (DXA) and (ii) air displacement plethysmography (ADP; Peapod), in black South African neonates.	ADP and DXA estimates of fat and fat-free mass were highly correlated; however fat mass and %fat were significantly lower and fat-free mass was significantly higher when measured by DXA in South African neonates. This may potentially be explained by a limited ability to distinguish between different body tissues (i.e. fat mass, lean mass and bone) in early life and therefore suggests a preferential basis for use of the Pea Pod in neonatal body composition assessment.
Chapter 5	To characterise, depict and report on maternal dietary intakes and patterns during pregnancy using multivariate dimension reduction techniques in urban black South African women.	At recruitment (<14 weeks gestational age) 66% of pregnant women were overweight/obese. In addition, 24%, 21% and 55% of women were categorised as experiencing inadequate, adequate and excessive GWG respectively, according to Institute of Medicine (IoM) guidelines. Three distinct dietary patterns were identified which explained 20.5% of the variation in food intakes: "western" (energy dense, processed, high sugar/fat foods), "traditional" (beans and legumes, vegetables, traditional meats and porridge/pap) and "mixed" (whole grains, nuts and dairy as well as high sugar items). "Western" and "mixed" diet patterns were associated with 35 g/week (per 1 SD; p=0.021) and 24 g/week (per 1 SD; p=0.041) higher GWG in normal-weight and obese women respectively. A 1 SD increase in "traditional" diet pattern score was associated with reduced odds of excessive weight gain in the total sample (OR: 0.81; p=0.006) and in normal-weight women (OR: 0.68; p=0.003).
Chapter 6	To examine the associations between maternal dietary intakes and patterns with neonatal body composition and how specific modifying factors i.e. HIV, maternal BMI and GWG modify or confound these associations.	Maternal obesity and GWG were associated with 0.25 kg/m (P=0.008) and 0.48 kg/m (P=0.002) higher newborn WLR, while "traditional" diet pattern score was associated with lower newborn WLR (-0.04 kg/m per 1 SD; P=0.033). Additionally, "traditional" pattern score was associated with 0.13 kg/m^3 (P=0.027) and 0.32 kg/m^3 (P=0.005) lower FMI in the total sample and in newborns of normal-weight women, respectively. HIV-positive (pre-pregnancy ART) vs. HIV-negative status (ref) was associated with 1.11 kg/m^3 (P=0.002) higher newborn FMI.

7.2 Summary of study findings

An overview of the study findings per objective is provided in Table 14. Overall, two main themes emerged from these finding, as follows: (1) typical transition-associated dietary patterns are firmly established in pregnant South African women and influenced by multiple maternal factors, and (2) the associations between MNS, dietary patterns (and overall metabolic health profiles) and adiposity are highly complex and play a fundamental role in maternal and child health. Together, these themes highlight the need for early interventions that take a holistic approach to improving nutritional status and diet quality while ensuring the optimal health and wellbeing of women prior to, and during, pregnancy, as well as in the post-partum period.

7.2.1 Expanded empirical study findings: maternal dietary patterns

From data presented in empirical chapters (specifically Chapter 5 and 6) it is important to expand on the possible determinants of maternal dietary patterns. Although outside the scope of the individual objectives of these chapters and therefore only briefly touched on in the chapters themselves, understanding the factors that may drive dietary behaviour in urban African women is an important component which should not be overlooked. While urbanisation and subsequent nutrition transitions are well-established in African settings, with increases in obesity and NCD burdens documented across the continent, the maternal factors associated with depicted dietary patterns and the potential consequences for MNS and fetal growth have not been thoroughly explored.

The three dietary patterns identified in pregnant South African women in this study – namely "western", "traditional" and "mixed" – typify those of a transitioning population and confirm urbanisation-associated changes in patterns of intake in this setting (Chapter 5). Specifically, the findings demonstrate the continued presence of a "traditional" dietary pattern high in

staple grains (predominantly maize porridge/pap), vegetables, beans and legumes and traditional meats, alongside substantial emergence of a "western" pattern characterised by energy-dense, refined, high-sugar/fat and convenience foods. While such dietary shifts typically result in considerable increases in energy intake – and therefore a positive energy balance and weight gain – they are also associated with intakes of poor nutritional value. For this reason, populations such as this demonstrate a form of malnutrition known as "hidden hunger", in which two thirds of women are overweight or obese in early pregnancy, yet micronutrient deficiencies are common. This has serious implications for pregnant women, who may demonstrate energy-dense/nutrient-poor dietary patterns, excess adiposity and increased risk of GDM and fetal adiposity, while simultaneously being deficient in the essential micronutrients required to support optimal fetal growth and development.

Of the maternal factors included as potential confounders in this study, parity and marital status were conclusively associated with dietary pattern adherence, with married/cohabiting women who had had two or more previous pregnancies demonstrating higher adherence to the "traditional" diet pattern. In contrast, "western"-pattern adherence was higher for single women with no previous births. As one would expect, these women also tended to be younger. Similar relationships have been shown in previous studies, with diets high in refined grains, confectionery, processed/convenience food products and sugar-sweetened beverages associated with younger maternal age and the absence of a partner; a "health conscious" diet negatively associated with being single; and a "traditional" diet associated with increasing age and parity (226,227,268).

These findings suggest that younger women who are single and have not yet had their first baby may be more susceptible to the shift towards western diets. This may be the result of more limited experience of traditional dietary practices and greater exposure to the urbanisation-associated effects of food promotion and availability in their experience than in

the experience of their older counterparts (269,270). In addition, these women may be less financially stable and more likely to work (and to work longer hours), resulting in less stable lifestyles (271). This may reduce the likelihood of household-based food preparation and increase consumption of convenience and fast-food products that are quickly and easily accessible, as well as more affordable. Lastly, the pregnancy period is a key healthcare access point for women in South Africa, who may otherwise have little or no contact with the health system. Nutrition education and support is limited within the South African public health system and it is possible that increased access to healthcare services and health information would play a role in encouraging healthy lifestyles – including healthy dietary patterns – in the population as a whole. Such access might be particularly effective during pregnancy, when women are more motivated to make health improvements.

Although higher educational attainment and SES are associated with improvements in diet quality and decreased obesity risk in HICs, rural-to-urban transition facilitates the opposite effects in LMICs. In the landscape of poverty and deprivation in these settings, economic improvement, western acculturation and weight gain are viewed as symbols of status and wealth – as well as of an absence of disease (predominantly HIV and/or TB) – which fuels the obesity epidemic (272,273). This has been shown in a previous study of rural South African adolescents, with higher education driving improvements in household SES and, subsequently, obesity risk (111). Although no significant associations were shown between household SES and either the "western" or "traditional" diet patterns in our study, there was a positive association between SES and the "mixed" pattern score. While this pattern was labelled according to its heterogeneous nature – i.e. mixing both classically healthy and unhealthy foods/food groups – high levels of sugar and sugar-sweetened products represented a dominant transition-associated influence.

The move towards high intakes of empty calories is of concern, with our study showing a significant association between the "mixed" pattern and maternal adiposity. While improvements in education and SES are critical to alleviating poverty in South Africa, they need to be coupled with nutrition education to shift the persisting cultural perceptions driving obesogenic dietary habits.

Overall, these findings highlight the importance of promoting improvements in diet quality early (i.e. prior to a woman's first pregnancy) in order to reach those most susceptible to poor dietary practices, as well as to optimise health benefits for the woman and for any (or all) future pregnancies. In addition, they emphasise the need to take a holistic approach to tackling obesity and improving diet quality and MNS, as substantial and sustained improvements cannot be made without targeted, multi-level approaches that incorporate individual and societal determinants.

7.3 MNS, dietary patterns and adiposity: a complex paradigm

As described by the DOHaD theory, neonatal size is an important predictor of long-term susceptibility to disease. However, the significance of adiposity (i.e. "fatness") in early life – although recognised as a more robust indicator of the in utero nutrition environment and newborn metabolic risk profile – has been widely overlooked, particularly in African settings. While data describing the associations between MNS (and, to a lesser extent, dietary intake) and birth weight exists, this study provided novel data on the influence of maternal pre-pregnancy anthropometry and dietary patterns on both maternal adiposity and particular parameters of fetal growth and, therefore, newborn size and body composition. In addition, these relationships were established within the context of high HIV/ART exposure in South African women, adding an additional, unique level of complexity.

While the conceptual models proposed in Chapter 6 – built according to the conceptual frameworks for this thesis as a whole (with the literature to support these), as well as the findings in Chapter 5 – suggested comparable relationships between maternal factors and newborn size (WLR) and adiposity (FMI), our final analyses highlighted considerable differences per outcome. This allows for outcome-specific tailoring of the models and presentation of a refined framework for the associations between the maternal factors studied and WLR and FMI (Figure 14). Insight into the individual predictors of body size and adiposity is critical in order to ensure that excess fat deposition is minimised without compromising linear fetal growth.

Overall, these models confirm the importance of maternal pre-pregnancy BMI and weight gain in predicting overall birth size in urban African women, with maternal obesity and increased GWG potentially increasing the risk of fetal overgrowth. In addition, they show that adherence to the "traditional" dietary pattern is associated with beneficial reductions in maternal adiposity and birth size; with its predominant effect on newborn fat mass.

A possible explanation for these associations is related to improvements in the metabolic profile of women prior to, and during, pregnancy, which affect glycaemic control and therefore risk of GDM. While obesity is a well-established risk factor for GDM, maternal dietary intake and dietary patterns are increasingly being linked to impaired glucose tolerance and insulin resistance during pregnancy; with dietary patterns high in refined grains, fat and sugar and low in fruit and vegetables being associated with increased odds of GDM (274). The resulting hyperglycaemic state during pregnancy – further amplified by obesity/GDM co-morbidity – is associated with fetal overgrowth and, more particularly, with a tendency towards central fat deposition (275).

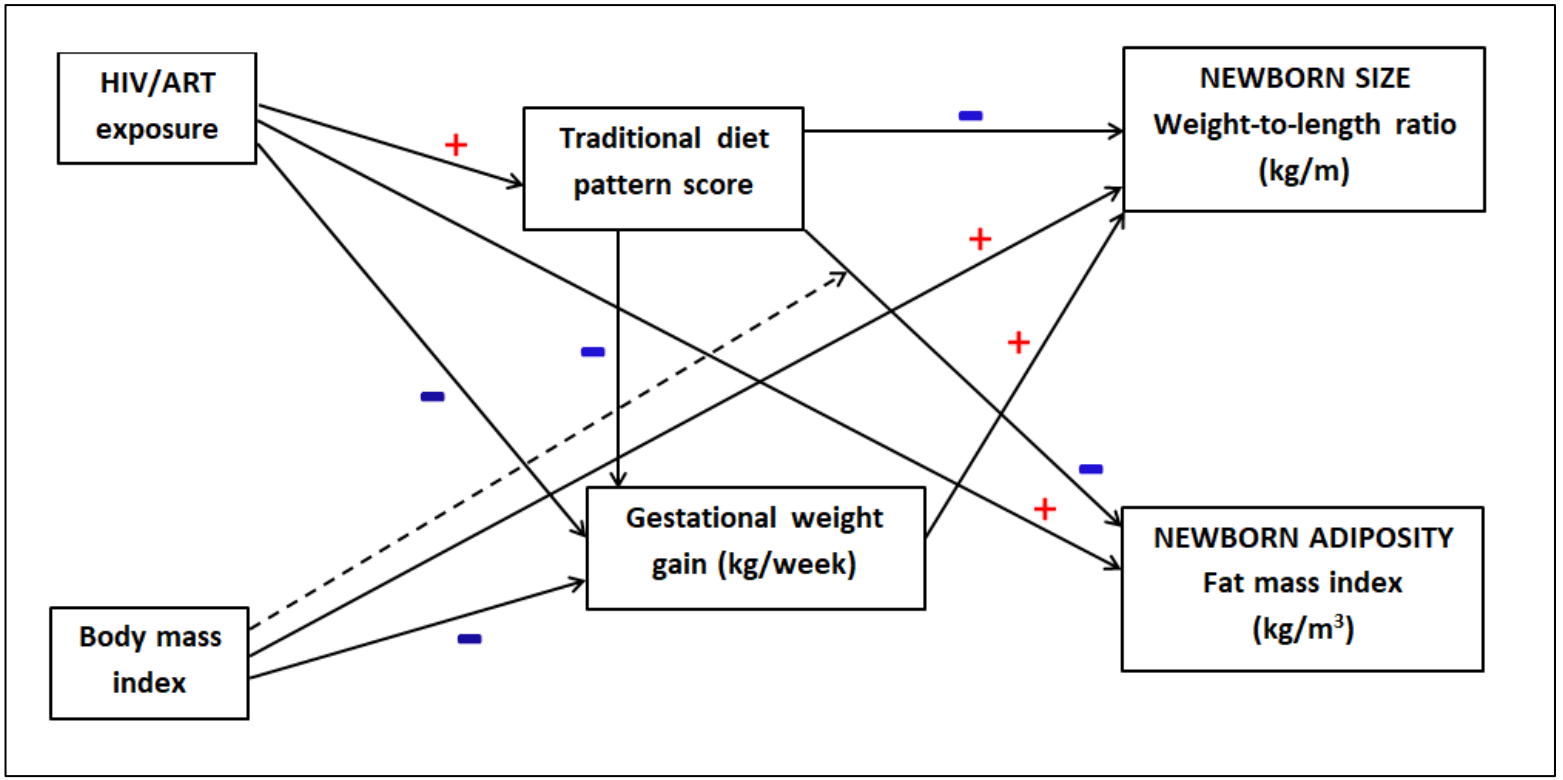

Figure 14: Revised conceptual model for the associations between maternal factors and neonatal size (weight-to-length ratio, kg/m) and adiposity (fat mass index, kg/m^3)

Solid arrows indicate associations between model variables, with + (red) and – (blue) symbols depicting positive and negative associations respectively. Dashed arrows represent effect modification of the identified association.

This link between the maternal glycaemic profile and newborn adiposity is further supported by Veena et al, who showed that even women who are not diagnosed with diabetes during pregnancy, but deliver higher birthweight babies with greater adiposity, are at increased risk of developing diabetes approximately nine years later (276). The effects on long-term disease risk are not limited to the mother, with studies showing significant associations between maternal hyperglycaemia and newborn adiposity and increased risk of overweight/obesity, abnormal glucose tolerance and higher blood pressure in children up to five years of age (277,278).

The association observed between maternal HIV/treatment exposure and neonatal adiposity adds an additional level of complexity to our model. The independent effect of ART on neonatal adiposity suggests that documented effects on maternal adiposity and glucose and lipid metabolism may exacerbate the maternal risk profile, potentially amplifying the pregnancy-associated effects (256,257). As HIV-positive women are increasingly exposed to ART – often initiated in their first pregnancy, when HIV is most likely to be diagnosed – this may have serious implications for maternal and infant health, particularly when coupled with obesity and poor-quality diets.

Our findings provide important and unique insights into potential intervention strategies, with promotion of a shift away from the urbanisation-associated dietary practices and towards more traditional patterns (dominated by coarse grains, vegetables, beans and legumes and lean, unprocessed meat) potentially improving the body composition and metabolic profile of mothers and newborns without adverse consequences for fetal growth. However, such intervention will be effective only if promotion of healthy pre-pregnancy weight is prioritised, thereby reducing obesity-related risk while optimising the beneficial effects of improved diet quality on both maternal and neonatal adiposity. In addition, special care must be taken when it comes to HIV positive women, whose exposure to ART makes them even

more susceptible to the advanced metabolic changes that increase the risk of excess adiposity in the newborn.

7.4 Recommendations for interventions

Poor diet quality and subsequent malnutrition – including under- and overweight – is the leading driver of the global disease burden, with approximately 88% of countries experiencing dual – or even triple – burdens of malnutrition (childhood stunting, anaemia in women of reproductive age, and/or overweight in adult women) (279). While tackling childhood undernutrition is an established global priority and was at the forefront of the Millennium Development Goals (MDGs) – with some countries successfully meeting targets aimed at eradicating extreme hunger – universal progress has been slow and has somewhat stagnated (279,280). In addition, the growing obesity epidemic – and subsequent NCD burdens – pose a serious threat to growth and development, with NCDs being the leading cause of morbidity and mortality worldwide (281). Although this has more recently been incorporated into global development plans – with the Sustainable Development Goals (SDGs) including targets to end malnutrition in all its forms and to reduce premature mortality from NCDs by one third by 2030 – little progress has been made towards integrating these targets into effective intervention planning and implementation strategies at country levels (280).

Evidence shows that implementation of prevention strategies to minimise behavioural risk factors (the greatest being poor diet and nutrition status), alongside effective care and management programmes, can successfully diminish NCD burdens. In addition, the benefits for health, productivity, economic growth and poverty reduction greatly outweigh the cost of preventative intervention (281). This is the basis upon which the United Nations (UN) has declared 2016-2025 the "Decade of Action on Nutrition", the overarching aim being to achieve scalable nutrition impact and ultimately eliminate all forms of malnutrition and, in

turn, nutrition-related NCDs. However, as emphasised by the findings of this thesis, the success of the UN declaration will depend on multi-level approaches to improving nutrition determinants and outcomes across sectors, including education, agriculture and food systems, health systems, water and sanitation and social protection (282). In particular, strengthening the health system to support the implementation of effective interventions, early risk assessment and monitoring and surveillance strategies will play a critical role in improving nutrition and disease outcomes.

In South Africa, malnutrition and NCD burdens – as well as the critical link between the two – are acknowledged at government levels and strategic plans have been developed in response to these. In the Strategic Plan of the National Department of Health 2015/16-2019/20, it is acknowledged that "strengthening the effectiveness of the health system is the foundation on which successful interventions to improve health outcomes must be built" (283). This is aligned with the National Development Plan for 2030, which was proposed by the National Planning Commission to "eliminate poverty and reduce inequality" through multi-sector action (284) as well as implementation of the World Health Organisation's (WHO's) Health Systems Strengthening Framework (285). However, regardless of the prioritisation of health-system strengthening in multiple government – particularly Department of Health – strategic plans over the years, little progress has been made in translating these plans into health-system reform. Commitment to, and directed and enforced action towards, strengthening health systems – particularly public healthcare services – is therefore a critical platform for maternal and child nutrition interventions.

7.4.1 Health system strengthening for maternal and child nutrition

7.4.1.1 Strategies, targets and indicators

Improvements in dietary intake (and other lifestyle factors) are important at all stages of the lifecycle; however, substantial progress in nutrition and health outcomes can be achieved only via adoption of defined targets and interventions in high-risk – and therefore high-impact – groups. In South Africa, multi-faceted health burdens and thinly stretched resources present a challenge for effective prioritisation and implementation of health-system strategies. As a result, strategic plans aimed at addressing various health issues (e.g. maternal and child health, obesity and NCDs) are developed continually in response to international research and health agendas, as well as local disease burdens and healthcare needs. However, these strategies tend to be compartmentalised, focusing on specific health outcomes across different timelines, and are therefore not sufficiently aligned with targets that facilitate the greatest impact on overall population health. Additionally, plans do not necessarily build on the strategies and progress (or lack thereof) that came before, and tend to provide very limited guidance on implementation at provincial, district and community levels. As a result, public healthcare systems have become overburdened with goals, objectives, targets and indicators that they do not have the knowledge or capacity to implement and drive within facilities. To achieve the maximum impact on malnutrition and associated disease burdens across the life course, it is essential to ensure that national strategic plans are aligned and prescribe globally relevant targets. In addition, targets must be supported by evidence-based and culturally relevant interventions that can be implemented effectively via the public healthcare system. Lastly, measurement indicators per target are critical to ensuring effective monitoring and evaluation of progress, as well as to facilitate responsive updates to future planning.

The WHO's Comprehensive Implementation Plan for Improving Maternal, Infant and Young Child Nutrition (MIYCN) (286) provides a universally recognised platform for effectively

addressing the double burden of malnutrition by prioritising adequate early-life nutrition to optimise growth and development and reduce the associated risk of disease throughout the life course. This plan specifies six targets, which broadly aim to reduce maternal anaemia and LBW, reduce stunting, wasting and overweight in children under the age of five, and increase exclusive breastfeeding rates (Table 15). All six of the targets – and their respective indicators – are highly influenced by MNS, making interventions that improve nutritional status and dietary intake prior to, and during, pregnancy critical drivers of reduced malnutrition at individual country levels.

Although some equivalent targets and indicators exist within South Africa's strategic plans for maternal and child health and the prevention and control of both obesity and NCDs, streamlining health-system targets to better complement these, and ensuring that effective interventions are put in place, could substantially improve maternal and infant nutritional status and optimise health trajectories. Additionally, this would ensure that effective existing intervention strategies are prioritised, while the process is underway to establish specific, measurable, achievable, relevant and time-bound (SMART) targets and indicators that allow for globally comparable monitoring of progress. SMART commitments to, and targets for, improving nutrition are critical to ensuring that the focus on these commitments is maintained while accountability at stakeholder level is enforced (282).

Table 15: Global targets and indicators to improve nutritional status and behaviours within the structure of the health system

Targets for maternal, infant and young child nutrition (MIYCN) (2025)	
Target 1 *Under-5 stunting*	Achieve a 40% reduction in the number of children under 5 who are stunted Stunting[a] among children under 5 years of age
Target 2 *Anaemia*	Achieve a 50% reduction of anaemia in women of reproductive age Women aged 15–49 years with haemoglobin <12 g/dL (non-pregnant) or <11 g/dL (pregnant)
Target 3 *Low birth weight*	Achieve a 30% reduction in low birth weight Infants born with a birth weight <2,500 g
Target 4 *Under-5 overweight*	Ensure that there is no increase in childhood overweight Overweight[b] among children under 5 years of age
Target 5 *Exclusive breastfeeding*	Increase the rate of exclusive breastfeeding in the first 6 months up to at least 50% Infants 0–5 months of age who are fed exclusively with breast milk
Target 6 *Under-5 wasting*	Reduce and maintain childhood wasting to less than 5% Wasting[c] among children under 5 years of age

[a]Stunting, length/height-for-age z-score >2 SD below the median of the WHO Child Growth Standards
[b]Childhood overweight, weight/height-for-length z-score >2 SD above the median of the WHO Child Growth Standards
[c]Wasting, weight-for-length/height z-score >2SD below the median of the WHO Child Growth Standards

7.4.1.2 Nutrition education and health promotion

The WHO has shown that, alongside promotion of good breastfeeding and complementary feeding practices in the first two years of life, improving nutrition in pregnant and lactating women is critical to achieving the targets set out in the Comprehensive Implementation Plan for Improving MIYCN (286,287). In addition, the Global Nutrition Report 2017 recognised that "a well-functioning health system is vital not just to treat, but to deliver preventative interventions at scale" and that at universal levels "much more effort and focus is needed for health systems to include nutrition and diet-related NCD programmes and interventions in universal health coverage" (279). In order to facilitate improvements in nutrition targets and indicators – and therefore reductions in morbidity and mortality – the South African health system must play an active role in preventative (rather than strictly curative) healthcare

services. The only nutrition-based intervention for pregnant women currently being implemented in the South African health system is routine iron/folic acid supplementation. While this is important, there is a lack of focus on improving dietary intakes and patterns in order to optimise overall nutritional status during pregnancy and lactation. Healthcare practitioners should therefore be trained to educate women about the importance of nutrition – including weight management and diet quality – at the earliest opportunity; for example, via family planning services when possible. Although addressing overweight/obesity and diet prior to pregnancy will ensure optimum benefits, pregnancy itself should be treated as a key target period for intervention, due to the relatively low access that women have to pre-conception care in low-to-middle income settings such as this (288). Improving patterns of dietary intake at this time would not only incur benefits for maternal and neonatal adiposity in the current pregnancy, but could have knock-on effects for maternal weight management and metabolic health in the future, as well as for fetal and infant health during subsequent pregnancies. In addition, women tend to adopt healthier lifestyle behaviours during pregnancy (e.g. reducing smoking and alcohol intake), making it an optimal period for promoting and establishing better dietary practices (289).

Many women – and healthcare providers themselves – may not be aware of the strong association between diet and fetal growth and development. Additionally, even when women are aware that certain foods are unhealthy, they may not know the best options for replacing these foods in their diet. The findings of this study therefore provide a good foundation for tailoring dietary advice and, specifically, encouraging a reduction in typically westernised diets high in processed, high-sugar and fatty foods, as well as convenience food products, and a shift towards dietary patterns dominated by whole grains, beans and legumes, vegetables and lean/unprocessed meat.

7.4.1.3 Identification and monitoring of high risk women

While universal education aimed at improving nutrition and health behaviours is important for pregnant women, identification of those at high risk (e.g. obese, GDM-positive and HIV-positive women) is important for the effective monitoring of maternal and fetal health during pregnancy, as well as for the tailoring of dietary advice. The independent association identified between ART exposure and neonatal adiposity, as well as the established risks associated with obesity and GDM for both mother and infant, mean that improvements in diet quality and management of weight gain is even more critical in these groups.

However – while the risks associated with maternal obesity for both maternal and infant health are widely established – research into optimal management of these women is limited; with available guidelines and recommendations during pregnancy being largely based on women of normal weight. While the IoM guidelines do provide overweight- and obese-specific GWG ranges, appropriate nutrition (and physical activity) based strategies for managing and monitoring MNS in these cases is needed. As the increasing majority of pregnant women attending health facilities in South Africa are overweight or obese, the development of guidelines for optimising maternal nutrition – both in terms of weight and micronutrient and metabolic profiles - during pregnancy is critical.

7.4.1.4 Facility-level accountability and service delivery

While streamlining targets and health indicators and prioritising nutrition-based interventions at national, provincial and district levels is important, successful implementation will not be possible without effective dissemination and buy-in across the health system. High disease burdens, coupled with insufficient and poorly allocated resources and ineffective management strategies in the South African public health system, place extremely high burdens on the physicians and healthcare practitioners working at ground level (290).

In such environments – in which the provision of basic day-to-day reactive care is a challenge – maintaining preventative education and health-promotion efforts, consistent and accurate recording of data, and monitoring and evaluation of nutrition and health indicators is close to impossible. In addition, the nurses and midwives who provide frontline care during pregnancy are often not sufficiently educated about the social, economic and environmental determinants of malnutrition and associated diseases, as well as the role that lifestyle intervention must play on reducing NCD burdens. The importance of preventative nutrition education and counselling, as well as of accurate data entry, indicator monitoring and evaluation of health promotion programmes and outcomes is therefore widely overlooked by those responsible for these activities (290,291). Therefore, ensuring that healthcare providers have sufficient knowledge and understanding to facilitate ownership of, and accountability for, nutrition-based interventions and monitoring strategies, and that they have the capacity to effectively and consistently implement these efforts, must be critical components of any plan to strengthen health systems going forward.

7.5 Strengths, limitations and research gaps

This study is the first to examine nutritional status and **dietary patterns in pregnant African women** and to explore the associations with **birth size** and **neonatal body composition** in the **context of HIV**. It presents not just novel data on the current status of pregnant South African women and the benefits that **improved diet quality** – alongside **healthy pre-pregnancy BMIs** – could have on both **maternal and neonatal adiposity** in this setting, but evidence of the **complexity that HIV/treatment brings** to the relationships between MNS, metabolic risk and newborn adiposity.

MNS and dietary patterns were assessed in pregnant women from the larger S1000 study. As CHBH is the only hospital serving the Soweto area, recruitment from the Fetal Medicine Unit allowed us access to a sample of pregnant women who were experiencing the full range of

co-morbidities currently experienced by the population. This recruitment process also provided the opportunity for regular pregnancy monitoring, accurate pregnancy dating via ultrasound, and timely and accurate measurement of birth outcomes after delivery. However, due to a lack of recruitment at clinic level and the specifically urban-poor setting, this was a convenient sample which was not representative of the Sowetan pregnant population as a whole, or of South African women living in higher-income or rural settings. In addition, the homogeneous nature of the sample – particularly with respect to race, education and SES–- may have restricted diversity of food access and dietary behaviours and the associated risk profiles, thereby limiting the scope for generalising the findings and subsequent development of nationally inclusive interventions. However, given the high risk profiles of urban, black, low-to-middle income women of reproductive age in South Africa, as well as the increasing exposure to urban contexts and their consequences, the importance of interventions within such population groups is undeniable.

Maternal dietary assessment via reported dietary intakes was useful in our study, as these questionnaire-based methods can be applied to large samples, including illiterate populations via interview, and they place a relatively low burden on the respondent. In addition, our QFFQ provided data on the reported consumption of local South African foods – including convenience-food products – which could then be used to derive habitual dietary patterns via PCA. While the use of dietary-pattern analysis techniques such as PCA have limitations – including subjectivity in the grouping of food items and in selecting the number and names of the derived patterns (230,231) – this method added substantial strength to our study when compared to classic dietary assessment methods. This was due to the ability of dietary patterns to capture the diversity and quality of overall diets within the sample and to provide an accessible basis for interventions and public recommendations based on foods, rather than on individual macro- and micronutrients (73,227).

It is widely acknowledged that using a QFFQ such as ours has a number of limitations; including being prone to recall bias, assuming regularity of eating habits over time, and providing inaccurate estimations of portion size (145–147). Additionally – as with the majority of reported dietary assessment tools currently used in Africa – our QFFQ has not been validated or tested for reliability (144). However, by using a seven-day recall period (including assessment of week- and weekend-day intakes) and incorporating frequency (rather than quantity) of consumption into our analyses, we were able to reduce the probability of inaccurate and biased recall, as well as the risks associated with portion-size estimation. Further, while not validated, the criteria developed by Dennis et al for assessing the quality of FFQs used in observational studies, classifies our questionnaire as a very high-quality tool, with a total of 13 points (high quality is classified as a score of seven or higher) (Table 16) (244).

Dietary patterns – although useful – provide only a subjective measure of dietary intake, which does not directly reflect nutritional status in the population studied. It is also widely acknowledged that under- and over-reporting is common in FFQs; with women tending to under-report intakes of unhealthy high-fat and high-sugar foods (224,225). This is particularly relevant where overweight and obese women are concerned, making reported dietary data potentially less accurate in highly obese populations such as ours (223). While assessment of nutritional biomarkers is scarce in Africa, due to the expensive nature of sample collection and analysis, use of these objective measures is critical for more accurate assessment of nutritional status. Not only would it provide a useful validation tool for reported intakes, but it would facilitate more accurate assessment of nutritional status – particularly in pregnancy, when micronutrient levels are critical for fetal growth and development.

Table 16: Evaluation of the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU) Food Frequency Questionnaire (FFQ; SA MRC) according to criteria adapted from Dennis et al (244)

Assessment items	Scoring categories		Total category score	FFQ summary score
	Points	Criteria		
Is the FFQ a quantitative instrument?	0	Frequency only	2	2
	1	Frequency (including average amount consumed)		
	2	Quantitative		
How many food items are measured by the FFQ?	0	Not stated or unknown	4	4
	1	≤ 70 items		
	2	71-110 items		
	3	111-150 items		
	4	> 150 items		
Is the FFQ completed by an interviewer or is it self-administered?	0	Not stated or unclear	2	2
	1	Self-administered		
	2	Interviewer administered		
Has the FFQ been pretested in the study population?	0	Not stated or unknown	1	1
	1	Yes		
Is the FFQ a validated instrument (either by the authors or has a standard FFQ been used)?	0	Not stated or unknown	2	0
	2	Yes or referenced use of Willet, Block or Jain FFQ		
Does FFQ coding make use of a nutrient database that is specified and can be referenced?	0	Not stated or unknown	1	1
	1	Yes		
Is there any measure of quality control incorporated into the FFQ tool?	0	Not stated	1	1
	1	Any measure incorporated		
Is the FFQ focused on a recalled dietary period that predates any diagnosis?	0	Not stated or unclear	1	1
	1	Yes or not applicable to study design		
Can the survey completion time be stated for either the FFQ or the whole questionnaire?	0	No	1	1
	1	Yes		
Total			15	13[a]

[a]Total quality score: low < 7; high ≥7

While this study provides information on maternal dietary patterns and their determinants, it was outside the scope of our research to explore the knowledge and perceptions of healthy/unhealthy eating that may be driving certain food choices. In addition, dietary patterns in this population may be highly influenced by both the availability and expense of

healthy food choices in urban-poor environments such as Soweto. An understanding of the reasons why women make particular dietary choices – including the influence of social, cultural, economic and environmental factors – is a critical component of developing acceptable, realistic and sustainable interventions.

As previously mentioned, current nutrition guidelines and recommendations for pregnant women are largely focused on normal weight women; with a lack of understanding of appropriate management of obese women. In addition our findings show that, while improving diet quality during pregnancy may have beneficial effects on both maternal and neonatal adiposity, these benefits are substantially reduced in overweight and obese women. While strategies aimed at ensuring appropriate maternal weight pre-pregnancy in South African women are critical, further research needs to focus on the development of guidelines for nutritional management of the substantial number of overweight and obese pregnant women for which this is not possible. In addition, while our findings identified the potential interaction between dietary intake and pre-pregnancy BMI on neonatal adiposity (FMI); this could not be further explored using stratification due to the sample size available for body composition analyses. Future research should therefore expand on these analyses by investigating how the effect of diet on FMI may change across BMI categories. In order to accurately elucidate the effects of diet on FMI, study design should focus on adiposity as a primary outcome and ensure that sample size is calculated to achieve sufficient power to detect differences in FMI within stratified groups (i.e. a minimum of n=171 per group; with a confidence level of 5% and a power of 80%; calculated using Stata 13.0, StataCorp, USA).

Our study highlights the importance of HIV/ART exposure in predicting fetal fat deposition, potentially via changes in maternal metabolic profiles and therefore fetal programming in utero. As ART exposure was not the main focus of our study, objective measures of treatment duration and/or adherence to treatment – for example, measurements of viral load –

were not collected. Such measures are important if we are to further understand the associations between HIV/ART exposure and neonatal adiposity in the future. Additionally, they should be complemented by exploration of the potential effects on maternal metabolism – for example, glucose and lipid profiles – in order to elucidate possible mechanisms to explain these relationships.

As previously described, studies in the African setting have been largely focused on the outcome of birth weight, with data lacking on neonatal adiposity, as well as on the influence of MNS and diet on infant outcomes to two years (and beyond). While our study begins to fill these gaps, long-term follow-up to two years (and beyond) was not possible within the scope of the study. Longitudinal data is therefore needed in this population, in order to explore whether the effects on adiposity are propagated through infancy and into childhood, thereby influencing metabolic trajectories and risk of obesity and NCDs in the longer term.

The hierarchical regression models used in our study provide a good basis for the differential associations between maternal factors and neonatal size and adiposity and, therefore, support the development of the refined conceptual framework proposed in Figure 14. However, future work using techniques such as structural equation modelling (SEM) - able to pull together the multiple exposures and outcomes (as well as confounding factors) into a single model - should be explored in order to more adequately test the framework as a whole.

7.6 Future research

Given the limitations of this thesis, future work should aim to fill the identified research gaps, particularly building on our findings in the following ways:

i. Explore the associations between MNS, dietary patterns and neonatal size and adiposity across **other South African population subgroups**, focusing

particularly on other ethnic groups, geographical areas (e.g. rural settings) and levels of SES.

ii. Assess MNS using **objectively measured nutritional biomarkers**, particularly examining biomarker patterns in pregnant women.

iii. Expand understanding of **determinants of dietary patterns in South Africa**, including a) research into the economic and environmental determinants – including household and community-level food availability and access, and b) qualitative research into the social and cultural determinants – particularly norms and beliefs about body size, food choices and perceived barriers to healthy eating.

iv. Investigate the **potential interaction between diet and maternal BMI** on both maternal and neonatal adiposity through **robust stratified analyses** across BMI categories

v. Examine the effects of **objectively measured maternal HIV/ART-exposure** on **maternal metabolism** (glucose and lipid profiles) and **infant growth and adiposity** from birth to two years (and beyond).

vi. Explore the associations between MNS and dietary patterns and **infant growth and adiposity from birth to two years** (and beyond).

vii. Explore the associations between MNS and dietary patterns, and adiposity and **NCD risk factors** (e.g. blood pressure, glucose and lipid profiles) in childhood/adolescence

7.7 Conclusion

In conclusion, this thesis shows that promotion of a "traditional" diet pattern—high in whole grains, legumes, vegetables and traditional meats and low in processed foods – alongside a healthy preconception weight in urban, black African women would significantly improve

both maternal and infant adiposity profiles. This may have substantial benefits in reducing long-term risk of NCDs in both current and future generations.

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APPENDICES

Appendix A: S1000 data collection process

Recruited from Fetal Med at CHBH	• Discuss study with her & give her a **Fetal Growth Study Information Sheet** • Complete **BAS** • Get her contact details and nurse to give her contact details. Plan an appointment.
Visit 1 **<14 weeks**	• Pregnancy test - if positive continue • Discuss study with her • Give her a **Fetal Growth Study Information Sheet** • Get her to sign the **Fetal Growth Study Consent Sheet** • Give her a **Fetal Growth Study DNA & RNA Information Sheet** • Get her to sign a **Fetal Growth Study DNA & RNA Consent Sheet** • Complete a **Contact Sheet** • Send her for a **Dating Scan- USD** • Complete **BAS** and **MSE** – (for 3G there is no restriction on BMI) • Complete **BPMS** • Complete **page 2** of the **Pregnancy and Delivery** form **(DEV)**- SES questions • Complete **HHP** • Complete **SSS** • Complete **Visit 1 Lab Sheet** • Nurse to give participant her contact details • Make a follow-up appointment for 5 weeks' time/ for when she is 14-18 weeks pregnant • Give her a refreshment & R50 transport money
Visit 2 **14-18weeks**	• Complete **PFU** • Complete **BPMS** • Complete **UFU** and **UMS** • Complete **GPAQ** and **SBQ** • Complete **FFQ** • Complete **Visit 2 Lab Sheet** • Complete **BAS** and **MSE (if not completed previously)** • Send her for a Scan- **USD (if not completed previously)** • Complete **Visit 1 Lab Sheet (if not completed previously)** • Nurse to give participant her contact details (if not given previously) • Make a follow-up appointment for 5 weeks' time/ for when she is 19-23 weeks pregnant • Give her a refreshment & R50 transport money
Visit 3 **19-23 weeks**	• Complete **PFU** • Complete **BPMS** • Complete **UFU** and **UMS** • Complete **Visit 3 Lab Sheet** • Complete **BAS** and **MSE (if not completed previously)** • Send her for a Scan- **USD (if not completed previously)** • Complete **Visit 1 Lab Sheet (if not completed before)** • Nurse to give participant her contact details (if not given previously) • Make a follow-up appointment for 5 weeks' time/ for when she is 24-28 weeks pregnant • Remind her to fast overnight (from 10pm; can have water) for her next appointment • Give her a refreshment & R50 transport money
Visit 4 **24- 28 weeks**	• Begin with OGTT • Complete **GLUQ** • Complete **PFU**

	• Complete **BPMS** • Complete **SSS** • Complete **UFU** and **UMS** • Complete **Visit 4 Lab Sheet** • Complete **BAS** and **MSE (if not completed previously)** • Send her for a Scan- **USD (if not completed previously)** • Complete **Visit 1 Lab Sheet (if not completed before)** • Nurse to give participant her contact details (if not given previously) • Make a follow-up appointment for 5 weeks' time/ for when she is 29-33 weeks pregnant • Give her a refreshment & R50 transport money
Visit 5 **29-33 weeks**	• Complete **PFU** • Complete **BPMS** • Complete **UFU** and **UMS** • Complete **GPAQ** and **SBQ** • Complete **Visit 5 Lab Sheet** • Complete **BAS** and **MSE (if not completed previously)** • Send her for a Scan- **USD (if not completed previously)** • Complete **Visit 1 Lab Sheet (if not completed previously)** • Nurse to give participant her contact details (if not given previously) • Give instructions for delivery • Make a follow-up appointment for 5 weeks' time/ for when she is 34-38 weeks pregnant • Instructions to be given around delivery • Give her a refreshment & R50 transport money
Visit 6 **34-38 weeks**	• Complete **PFU** • Complete **BPMS** • Complete **UFU** and **UMS** • Complete **PAI** • Complete **Visit 6 Lab Sheet** • Complete **BAS** and **MSE (if not completed previously)** • Send her for a Scan- **USD (if not completed previously)** • Complete **Visit 1 Lab Sheet (if not completed previously)** • Nurse to give participant her contact details (if not given previously) • Make a follow-up appointment for 5 weeks' time/ for when she is 39-42 weeks pregnant (tell her she should come to that appointment if she is still pregnant) • Instructions to be given around delivery • Give her a refreshment & R50 transport money
Visit 7 **39-42 weeks**	• Complete **PFU** • Complete **BPMS** • Complete **UFU** and **UMS** • Complete **Visit 7 Lab Sheet** • Complete **BAS** and **MSE (if not completed previously)** • Send her for a Scan- **USD (if not completed previously)** • Complete **Visit 1 Lab Sheet (if not completed previously)** • Nurse to give participant her contact details (if not given previously) • Instructions to be given around delivery • Give her a refreshment & R50 transport money
Delivery	• Complete **DEV**

Appendix B: S1000 sample collection table

Measurement	Time Point																Key:
	Pregnancy							Delivery	Neonate	Infant Follow-up							Wks = Weeks
	<14 Wks	14-18 Wks	19-23 Wks	24-28 Wks	29-33 Wks	34-38 Wks	38-42 Wks	Del	NN	6 Wks	2 Mnth	3 Mnth	6 Mnth	12 Mnth	18 Mnth	24 Mnth	Mnth = Months
Length/Height	M								I	M I	I	I	I	I	I	I	M = Mother
Weight	M	M	M	M	M	M	M		I	M I	M I	M I	M I	M I	M I	M I	
Head Circumference	F	F	F	F	F	F			I	I	I	I	I	I	I	I	F = Foetus
Baseline Info	M																
Urine	M	M	M	M	M	M	M										I = Infant
Haemoglobin	M	M	M	M	M	M	M										
Whole Blood	M			M				M									FOB = Father of the Baby
Serum	M			M		M		M									
Plasma	M			M				M									
Buffy Coat	M			M				M									BPD = Biparietal Diameter
HBA1C				M		M											
Ultrasound (BPD, OFD, HC, TAD, APAD, AC, FL, AFI)	F	F	F	F	F	F	F										OFD = Occipito-Frontal Diameter
Blood Pressure	M	M	M	M	M	M	M			M I	M I	M I	M I	M I	M I	M I	
Capillary (Heel Prick) Dried Blood									I	I		I	I	I		I	
Oral Glucose Tolerance Test				M													HC = Head Circumference
Glucose Metabolism				M													
Fasting Glucose										M			M				
Lipids				M			M										SAT = Subcutaneous Adipose Tissue
Cord Blood								M									
Placental Tissue								M									
Faeces								M									
PEAPOD									I	I	I	I	I				VAT = Visceral
DXA (Full Body Scan)									I	M			M I	I		M I	

Breast Milk									M	M		M					Adipose Tissue
PCR HIV Test										I							
Prenatal Attachment Inventory						M											TAD = Transverse Abdominal Diameter
Food Frequency		M															
Sedentary Behaviour		M			M												
Global Physical Activity		M			M												
Breastfeeding Intentions									M								
Diet History & 24 Hour Recall & Weaning										M		M	M	M	M		APAD = Anterior-Posterior Abdominal Diameter
Household Composition and Pregnancy	M							M									
SES & Household Composition								FOB						M		M	
Morbidity & Illness Child Assessment										M	M	M	M	M	M	M	AC = Abdominal Circumference
Social Support Stress & Depression	M			M				FOB		M			M			M	
Ages and Stages: Social Emotional													M				FL = Femur Length
Ages and Stages													M	M		M	
Attachment														M			
Home Screening																M	AFI = Amniotic Fluid Index
Reactivity/Temperament												M					
Clinic Link-Up	M									I	I	I	I	I	I	I	
Executive Functioning																I	
Parenting Stress Index - Short Form															M		
Role of the Father Questionnaire													M				
Infant VAT and SAT by ultrasound										I	I	I	I	I	I	I	
Maternal beliefs Questionnaire													M	M			
Maternal reported infant physical activity												I	I	I	I		
Home environment Questionnaire												M	M	M	M		

Objective physical activity (Accelerometry)												M I	M I	M I	M I	M I	
Age at the attainment of developmental milestones												I	I	I	I	I	
InterBio Infant Follow-up Questionnaire														M		M	
Buccal Swabs														I		I	
Arm Circumference														I		I	
Tricep Skinfold														I		I	
Subscapular Skinfold														I		I	
Postnatal Abnormality Questionnaire														M		M	
24-Hour Food Recall														M			
InterBio FFQ														M			
Motor Development Assessment														I		I	
Food Intake Questionnaire																I	
Cognitive Functioning																I	

Appendix C: PhD relevant data collection sheet

Study Number		Date	

Please answer all yes/no questions by placing a "x" in the corresponding box

Section 1: Demographic, socio-economic and nutritional characteristics

1. Age: (years) ☐☐ yrs
2. Maternal height: (cm) ☐☐☐.☐ cm
3. 1st trimester or pre-pregnancy weight: (kg) ☐☐☐.☐ kg

During this pregnancy:

4. Has she smoked? yes ☐ no ☐ If yes, how many cigarettes/cigars per day? ☐☐
5. Has she sniffed/chewed tobacco? yes ☐ no ☐ If yes, how many times per day? ☐☐
6. Has she chewed betelnut? yes ☐ no ☐ If yes, how many nuts per day? ☐☐
7. On average, how many units of alcohol per week has she had? ☐☐
   (1 unit = small glass (125ml) of wine or one bottle/can (330ml) of beer; see table)
8. Has she used any of the following recreational drugs? (cross all that apply; see table)

Heroin	☐	Amphetamines	☐	Benzodiazepines	☐
Methadone	☐	Hallucinogens	☐	Inhalants/Solvents	☐
Crack/Cocaine	☐	Cannabis	☐	Other recreational drugs	☐

9. Has she been involved in any of the following high-risk occupations or activities? (cross all that apply; see table)
   - Frequent exposure to chemical/toxic substances ☐
   - Frequent physically demanding work ☐
   - Frequent high-risk sports/vigorous exercise ☐
10. Has she followed any of the following special diets? (cross all that apply; see table)

Vegetarian with no animal products	☐	Gluten-free	☐
Weight loss programme	☐	Malabsorption treatment	☐

11. Marital status: (cross one box only)

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

12. Total number of years of formal education: ☐☐
13. Highest level of education attended: (cross one box only)

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

14. Which of the following best describes her occupational status? (cross one box only)

Housework	☐	Skilled manual work	☐	Managerial/professional/technical	☐
Student	☐	Unskilled manual work	☐	Clerical support, service or sales	☐
Other	☐				

15. *Please answer all parts (A to I) of Question 15 to help provide an indication of the woman's economic situation.*

A. Does the woman's household have or own any of the following:

Electricity	yes / no	Cell phone	yes / no	Bicycle	yes / no
Radio	yes / no	Personal computer	yes / no	Motorcycle/Scooter	yes / no
Television	yes / no	Farm animals	yes / no	Car/Truck/Tractor	yes / no
Refrigerator	yes / no	Agricultural land	yes / no		

B. Number of people living in the woman's household: ☐☐

C. Number of rooms used for sleeping in the woman's household: ☐☐

D. Main fuel used for cooking in the woman's household: (cross one box only)

Electricity	☐	Kerosene	☐	Animal dung	☐
Liquid propane gas (LPG)	☐	Charcoal	☐	Other	☐
Natural gas	☐	Wood	☐	No cooking	☐
Biogas	☐	Straw/shrubs/grass	☐		

E. Main source of drinking water in the woman's household: (cross one box only, unless selecting 'Bottled water' - see below)

Bottled water *(if so, please also cross the box corresponding to the main source for cooking/washing)*	☐		
Piped water into dwelling	☐	Tanker truck/Cart with small tank	☐
Piped water into yard/plot	☐	Unprotected dug well	☐
Protected dug well	☐	Unprotected spring	☐
Protected spring	☐	Surface water	☐
Rainwater	☐	Other	☐
Public tap/standpipe	☐		

F. Type of toilet facility in the woman's household: (cross one box only)

Flush to piped sewer system	☐	Ventilated improved pit (VIP) latrine	☐
Flush to septic tank	☐	No facility or bush or field	☐
Traditional pit toilet	☐	Other	☐

G. Is the toilet facility shared with other households? yes / no

H. Main flooring material in the woman's household: (cross one box only)

Earth/sand/mud	☐	Vinyl/linoleum	☐	Carpet	☐
Wood planks	☐	Ceramic tiles	☐	Other	☐
Parquet or finished wood	☐	Cement	☐		

I. Main wall material in the woman's household: (cross one box only)

No walls	☐	Mud and cement	☐	Bare brick or cement block	☐
Plastic/cardboard	☐	Corrugated iron/zinc	☐	Plaster/finished	☐
Mud	☐	Prefab	☐	Other	☐

Section 2: Medical History

Before this pregnancy, was she diagnosed with, or treated for, any of the following conditions?

16. Diabetes yes no
17. Thyroid disease yes no
18. Other endocrinological condition yes no
19. Any type of malignancy/cancer (including leukaemia or lymphoma) yes no
20. Cardiac disease yes no
21. Epilepsy yes no
22. Mental illness e.g. Clinical depression yes no
23. Hypertension/chronic hypertension with treatment yes no
24. A chronic respiratory disease (including chronic asthma) yes no
25. Proteinuria, kidney disease or chronic renal disease yes no
26. Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition yes no
27. Lupus erythematosus yes no
28. HIV or AIDS yes no
29. Hepatitis B or C yes no
30. Malaria - *within past 5 years* yes no
31. Tuberculosis yes no
32. Thalassaemia yes no
33. Sickle-cell anaemia yes no
34. Thrombophilia yes no
35. Glucose-6-phosphate dehydrogenase deficiency yes no
36. Any congenital abnormality or genetic disease yes no
37. Any other clinically relevant condition yes no

Section 3: Gynaecological History

38. Did she have regular (24-32 day) menstrual cycles in the 3 months prior to this pregnancy? yes no
39. What is the average length of her menstrual cycle? ☐☐ days
40. Had she used hormonal contraceptives or been breastfeeding in the 2 months prior to this pregnancy? yes no
41. Is the first day of the last menstrual period (LMP) known? yes no
42. If yes, date: D D M M Y Y
43. Was she certain of the date of her LMP? yes no

Section 4: Obstetric History

44. Number of previous pregnancies, excluding this pregnancy *(if 0, skip to Question 57)* : ☐☐
45. Date of last delivery, miscarriage or termination: D D M M Y Y
46. Has she ever had a molar pregnancy or choriocarcinoma? yes no
47. Has she ever had an extrauterine or ectopic pregnancy? yes no
48. Number of previous miscarriages: ☐☐
49. Number of previous terminations: ☐☐
50. Number of previous births *(if 0, skip to Question 57)* : ☐☐
51. Birthweight of the immediately previous newborn: ☐☐☐☐ g
52. Gestational age at birth of the immediately previous newborn: ☐☐ weeks ☐ days
53. Have ANY of her other babies weighed less than 2500g? yes no
54. Have ANY of her other babies been born preterm (<37^{+0} weeks' gestation)? yes no
55. Has she had ANY previous stillbirths? yes no
56. Has she had ANY previous neonatal deaths? yes no

Section 5: Vaccination History

Has she been vaccinated against the following medical conditions?

Influenza: 57. Before this pregnancy: yes no 58. During this pregnancy: yes no

Tetanus: 59. Before this pregnancy: yes no 60. During this pregnancy: yes no

Section 6: Clinical Conditions

During this pregnancy was she diagnosed with, or treated for, any of the following conditions?

61. Diabetes, thyroid disease or any other endocrinological condition — yes / no
62. Any type of malignancy/cancer (including leukaemia or lymphoma) — yes / no
63. Cardiac disease — yes / no
64. Epilepsy — yes / no
65. Mental illness e.g. Clinical depression — yes / no
66. Symptomatic malaria — yes / no
67. Symptomatic malaria with parasite count — yes / no
68. Respiratory disease (including asthma) — yes / no
69. Pyelonephritis or kidney disease — yes / no
70. Lower urinary tract infection requiring antibiotic treatment — yes / no
71. Respiratory tract infection requiring antibiotic/antiviral treatment — yes / no
72. Any other infection requiring antibiotic/ antiviral treatment — yes / no
73. Group B streptococcus carrier — yes / no
74. Positive syphilis test — yes / no
75. HIV or AIDS — yes / no
76. Any genital tract or sexually transmitted infection — yes / no
77. Cholestasis — yes / no
78. Any other medical/surgical condition requiring treatment/referral — yes / no
79. Any accident or maternal trauma requiring hospital admission or referral to a higher level of care — yes / no

Section 7: Pregnancy-related complications

During this pregnancy was she diagnosed with, or treated for, any of the following conditions?

80. Severe vomiting requiring hospitalisation — yes / no
81. Gestational diabetes — yes / no
82. Vaginal bleeding before 15 weeks — yes / no
83. Vaginal bleeding at 15-27 weeks — yes / no
84. Vaginal bleeding after 27 weeks — yes / no
85. Pregnancy-induced hypertension (BP>140/90, no proteinuria) — yes / no
86. Preeclampsia (BP>140/90 and proteinuria) — yes / no
87. Severe preeclampsia/Eclampsia/ HELLP syndrome — yes / no
88. Rhesus disease or anti-Kell antibodies — yes / no
89. Preterm labour — yes / no
90. Fetal anaemia — yes / no
91. Fetal distress (abnormal fetal heart rate [FHR] or biophysical profile [BPP]) — yes / no
92. Suspected impaired fetal growth — yes / no
93. Oligohydramnios — yes / no
94. Polyhydramnios — yes / no
95. A condition requiring amniocentesis or fetal blood sampling (FBS) — yes / no
96. Abruptio placentae — yes / no
97. Clinical chorioamnionitis — yes / no
98. Other pregnancy-related infection — yes / no
99. Other pregnancy-related condition — yes / no

	<15 weeks	15-27 weeks	>27 weeks
100. Lowest haemoglobin level:	☐☐.☐ g/dl	☐☐.☐ g/dl	☐☐.☐ g/dl
OR Lowest haematocrit:	☐☐.☐ %	☐☐.☐ %	☐☐.☐ %

Section 9: Delivery

130. Onset of labour: (cross one box only) Spontaneous ☐ Induced ☐ No labour ☐

131. Prelabour premature rupture of membranes (PPROM)? yes ☐ no ☐

132. Place of delivery: (cross one box only) Home ☐ Health facility ☐

133. Mode of delivery: (cross one box only) Vaginal spontaneous ☐ Vaginal assisted (e.g. forceps, vacuum) ☐
Caesarean section ☐ Assisted breech or breech extraction ☐

If labour was induced or a Caesarean section performed, please cross all that apply:

134. Vaginal bleeding	yes ☐ no ☐	148. Worsening of a pre-existing clinical condition	yes ☐ no ☐
135. Placenta praevia	yes ☐ no ☐	149. Suspected intrauterine growth restriction (IUGR)	yes ☐ no ☐
136. Fetal death	yes ☐ no ☐	150. Post term (>42^{+0} weeks gestation)	yes ☐ no ☐
137. Pregnancy-induced hypertension (BP>140/90, no proteinuria)	yes ☐ no ☐	151. Rhesus disease or anti-Kell antibodies	yes ☐ no ☐
138. Preeclampsia (BP>140/90 and proteinuria)	yes ☐ no ☐	152. Intrahepatic cholestasis of pregnancy	yes ☐ no ☐
139. Severe preeclampsia/Eclampsia/ HELLP syndrome	yes ☐ no ☐	153. HIV or AIDS	yes ☐ no ☐
140. Breech presentation	yes ☐ no ☐	154. Any genital tract or sexually transmitted infection	yes ☐ no ☐
141. Fetal distress (abnormal fetal heart rate [FHR] or biophysical profile [BPP])	yes ☐ no ☐	155. Any infection requiring antibiotic/antiviral treatment	yes ☐ no ☐
142. Reduced fetal movement	yes ☐ no ☐	156. Any accident/maternal trauma	yes ☐ no ☐
143. Failure to progress	yes ☐ no ☐	157. Pregnancy termination	yes ☐ no ☐
144. Cephalo-pelvic disproportion	yes ☐ no ☐	158. Previous Caesarean section	yes ☐ no ☐
145. PPROM	yes ☐ no ☐	159. Maternal request	yes ☐ no ☐
146. Uterine rupture	yes ☐ no ☐	160. Any other maternal reason	yes ☐ no ☐
147. Abruptio placentae	yes ☐ no ☐	161. Any other fetal reason	yes ☐ no ☐

Section 10: Newborn outcomes and care

162. Date of delivery: D D M M Y Y

163. Time of delivery: H H : M M (24-hour clock)

164. Gestational age at birth based on the best obstetric estimate: ☐☐ weeks ☐ days

165. Fetal presentation at delivery: (cross one box only)
Cephalic ☐ Breech ☐ Other ☐

166. Newborn status at birth: (cross one box only)
Alive ☐ Intrapartum death ☐
Antepartum death ☐

167. Newborn sex: Male ☐
Female ☐

168. Apgar score at 5 minutes: ☐☐

169. Was the newborn admitted to intensive care or any special care unit? yes ☐ no ☐

170. If yes, total amount of days spent in intensive care or special care unit: (if less than 24 hours please enter 1 day) ☐☐☐ days

Was the newborn diagnosed with, or treated for, any of the following conditions before hospital discharge?

171. Respiratory distress syndrome	yes no	185. Fetal inflammatory response syndrome	yes no
172. Transient tachypnea of the newborn	yes no	186. Seizures	yes no
173. Apnea of prematurity	yes no	187. Necrotising enterocolitis, Bell's staging stage 2 or greater	yes no
174. Bronchopulmonary dysplasia	yes no	188. Meningitis	yes no
175. Pneumothorax	yes no	189. Hypoglycaemia	yes no
176. Meconium aspiration with respiratory distress	yes no	190. Anaemia (requiring transfusion)	yes no
177. No oral feeds for more than 24 hours	yes no	191. Hypotension (requiring inotropic treatment or stercids)	yes no
178. Retinopathy of prematurity	yes no	192. Intraventricular haemorrhage grade 2 or greater, periventricular haemorrhage or leukomalacia	yes no
179. Hypoxic-ischaemic encephalopathy	yes no		
180. Hyperbilirubinaemia	yes no	193. Polycythaemia	yes no
181. TORCH or any other intrauterine infection	yes no	194. Patent ductus arteriosus (requiring pharmacological treatment or surgery)	yes no
182. HIV	yes no	195. Any other serious condition	yes no
183. Neonatal sepsis	yes no	196. Congenital abnormality (complete a **Neonatal Abnormality Form**)	yes no
184. Fetal infection	yes no		

Section 11: Newborn Anthropometry (taken no longer than 48hrs after birth)

197. Date of measurement: D D M M Y Y

First set of anthropometric measurements		Repeat measurements (if required)	Repeat measurements (if required)
198. Weight:	g	g	g
199. Length:	. cm	. cm	. cm
200. Head circumference:	. cm	. cm	. cm

Second set of anthropometric measurements		Repeat measurements (if required)	Repeat measurements (if required)
201. Weight:	g	g	g
202. Length:	. cm	. cm	. cm
203. Head circumference:	. cm	. cm	. cm

Section 12: Newborn outcomes

204. Newborn status at hospital discharge: (cross one box only)

- Alive ☐
- Alive but referred to another centre for growth and development ☐
- Alive but referred to a higher level of care ☐
- Dead ☐

205. Date of neonatal hospital discharge or date of death:

Section 13: Maternal Outcomes

207. Was the mother admitted to intensive care or any special care unit after delivery? yes no

208. If yes, total number of days: (if less than 24 hours, please enter as 1 day)

209. Maternal status at hospital discharge: (cross one box only)

- Alive
- Alive but referred to a higher level of care
- Dead

Name of Researcher/Midwife			
Signature		Researcher Code	
	Anthropometrist-1 Code	Anthropometrist-2 Code	

Appendix D: Food frequency questionnaire

FV: FFQ

University of the Witwatersrand, Johannesburg

Developmental Pathways for Health Research Unit

Department of Paediatrics and Child Health

Soweto Fetal Growth Study
FOOD FREQUENCY QUESTIONNAIRE (FFQ) 2013

Participant's date of birth (DD/MM/YYYY): _____

BTT ID: _____

(Her or her ♂ partner's BTT ID)

Partner ID: _____

(♀partner of a ♂ BTT participant)

3G ID: _____

Or

INTERBIO ID: _____

Or

SFG ID: _____

Interviewer's name: _____

Interview date: _____

Food habits

1. Are you on a special diet that has been prescribed for you e.g. by a doctor or one that you have adopted from someone e.g. a TV star/magazine?

YES	1
NO	0

2. If NO, go to question 4.

If YES, describe what kind of diet you are on and where you got the diet from?

_____

3. How long have you been on that diet?_____months/years.

4. Do you currently take any vitamin and mineral supplements?

YES	1
NO	0

IF YES, what do you take?

	Name of product	Amount/day (or how many tablets)
Vitamins/vitamins and minerals		
Tonics		
Vitamin D		
Calcium		
Body building preparations		
Dietary fibre supplement		
Other: specify		

5. Which meals do you skip almost on a daily basis?

Breakfast	1
Lunch	2
Evening meal	3
None	4

6. Is salt added to your food while it is being cooked?

Always	1
Sometimes	2
Never	3
Don't know	4

7. Do you add salt to your food before you eat it?

YES	1
NO	0

8. If yes, how much salt do you add to your food each day?

¼ teaspoon	1
½ teaspoon	2
¾ teaspoon	3
1 teaspoon	4
Other specify:	5

9. Do you add Aromat to your food before you eat it?

YES	1
NO	0

10. If yes, how much Aromat do you add to your food each day?

¼ teaspoon	1
½ teaspoon	2
¾ teaspoon	3
1 teaspoon	4
Other specify:	5

11. There are some factors which influence the choice of foods we eat. Which of the following statements are true for you?

	Strongly agree	Agree	Disagree	Strongly Disagree
I choose to eat certain foods because they taste good	1	2	3	4
The food I eat depends on whether it is expensive	1	2	3	4
I choose to eat certain foods because it looks good	1	2	3	4
The food I choose to eat differs according to my mood (i.e. happy/sad)	1	2	3	4
My hunger level determines what type of food I eat.	1	2	3	4
I choose foods which are not time consuming to prepare	1	2	3	4
I consider whether a food is good for my health before eating the food.	1	2	3	4

12. Do you ever eat outside the home e.g. at fast food shops such as Nandos, KFC and Steers?

YES	1
NO	0

13. If YES, in an average month how often do you eat at the following places?

	Frequency of visits		
	Times/week	Times/month	Rarely/never
Nandos			
Spur			
Macdonalds			
Steers			
KFC			
Chicken Licken			
Debonaire's Pizza			
Romans			
Anat			
Wimpy			
Something fishy			
Fontana			
Chinese takeaway			
Other restaurants/takeaways			
(Quarters from tuck shop)			

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	DAIRY-BLUE									
	1.Tea	Ordinary		4038						
		Herbal		4053						
		Rooibos		4054						
	1. Sugar in tea			3989						
	2. Milk in tea	Full cream		2718						
		Low fat 2%		2772						
		Skim fat free		2775						
		Other								
	1. Coffee			4037						
	2. Milk in coffee	Full cream		2718						
		Low fat 2%		2772						
		Skim fat free		2775						
		Other								
	2. Sugar in coffee			3989						
	2. Milk as a drink	Full cream		2718						
		Low fat 2%		2772						
		Skim fat free		2775						
	3. Buttermilk/maas	Buttermilk		2713						
		Maas		2787						
	4. Milk drinks, flavoured									
X	**5. Yoghurt**	Fruit, LF, sweetened		2732						
		Plain LF		2734						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	3. Breakfast cereals									
	2. Milk added to cereal									
	1. Sugar added to cereal			3989						
	9. Ice cream	Full cream		3519						
		Low fat		4325						
	9. Ice lollies			3982						
	ASK ABOUT TYPE OF BREAD,THICKNESS OF SLICES AND THE SPREAD									
	1. Bread/rolls	White		3210						
		Brown		3211						
		Whole wheat		3212						
		Traditional		3210						
	Toppings:	Roti			___ x 54g					
Meat										
Veg?										
	Spread? Y / N	**Brand name**	*Do you use spread every day on your bread?*							
	1. Brick margarine	HM		3484						
	1. Tub margarine	PUM		3496						
	1. other tub e.g. floro			3521						
	1. Butter			3479						
	Cheese spread	WM		2730						
	Fish paste			3109						
	Honey/syrup			3988						
	Jam			3985						
circle	**Marmite/Bovril**									

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	Sandwich spread			3522						
	Peanut butter			3485						
	Chocolate spread									
	Cold meats	Ham		2967						
		Polony		2919						
		Salami		2948						
	6. Cottage cheese	Low fat		2729						
		Full fat		2759						
X	**7. Cheddar**			2722						
	7. Gouda			2723						
	7. Other cheese									
	8. Cheese wedges			2728						
	2. Fat cakes			3257						
	Any topping?									
(Red)	**QUARTER**	*Tick toppings:*								
	White bread			3210	¼ loaf = 225					
	Fried Chips			3740	1 portion = 90					
	Cheese			2722	___ slices x 20 =					
	Polony / French			2919	___ slices x 15=					
	Vienna (Russian)			2936	___ unit x 40 =					
	Fried egg			2869	___ medium x 45 =					
	Atchar			3117	___ Tsp x 40 =					
	Tomato sauce			3139	___Tsp x 25 =					
	Mashed potato			3876	¼ cup = 62.5					
	Mince meat, regular			2987						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	4. Maize porridge stiff			3400						
	4. Maize porridge soft									
	2. Milk on soft porridge									
	1. Sugar on soft porridge			3989						
	1. Fat on soft porridge									
	4. Mabele/maltabella stiff			3241						
	4. Mabele soft									
	2. Milk on Mabele									
	1. Sugar on Mabele			3989						
	1. Fat on Mabele									
	4. Oats			3239						
	2. Milk on Oats									
	1. Sugar on Oats			3989						
	4. Maize & pumpkin porridge									
	5. Pasta/Pasta dishes									
	Remember to ask about added cheese! Grated?									
	6. Spaghetti Bolognaise	Regular mince		3260						
	6. Macaroni and cheese	WH, HM		3301						
	6. Lasagne	Regular		3261						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	7. Rice	White		3247						
		Brown		3315						
circle *circle*	***Add anything?*** *Inkomazi /* *Plain yoghurt LF/WM*	*How much, t/T/S spoons?*								
Tick	**Biryani: Chicken**									
	Mutton									
	Vegetable									
	7. Samp/mealie rice	With fat?								
	7. Samp and beans	With fat?		3402						
	7. Wheat rice			3249						
	8. Pizza (toppings?)									
	8. Savoury tart									
X	**EGGS-YELLOW**									
	Boiled/poached			2867						
	Fried	HM		2877						
		PUM		2878						
		Sun oil		2869						
Tick	**Scrambled**	LFM, PUM		2888						
		LFM, Sun oil		2889						
		WM, HM		2890						
		WM, Sun oil		2873						
		WM		2872						
	Omelette (tick scrambled & add topping here)									

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	FRUITS-ORANGE									
X	**1. Apples**			4222						
X	**2. Bananas**	Fresh		3540						
		Fried		3611						
	3. Berries	Fresh								
		Candied								
X	**4. Figs/prickly pears &**	Fresh								
	Coconuts, dates	Candied								
	5. Fruit salad	Fresh								
		Sugar added?								
		Canned juice		3667						
		Canned syrup		3580						
X	**6. Grapes**			3550						
X	**7. Guavas**	Fresh		3551						
		Canned juice		3628						
		Canned syrup		3553						
X	**8. Mango**	Fresh		3556						
		Canned syrup		3633						
	8. Pawpaw	Fresh								
	8. Kiwi fruit			3660						
	8. Lychees			3632						
	9. Watermelons			3576						
X	**9. Sweet melon**									

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
X	**10. Naartjies**	Mineola fresh		4227						
		Naartjie fresh		3558						
		Naartjie canned in syrup		3635						
X	**11. Oranges**	Fresh		3560						
	11. Grapefruit	Fresh		3546						
X	**12. Peaches**	Fresh		3565						
		Canned juice		3640						
		Canned syrup		3567						
X	**12. Nectarines**	Fresh		4228						
X	**13. Pears**	Fresh		3582						
		Canned juice		3643						
		Canned syrup		3583						
	14. Pineapple	Fresh		3581						
		Canned juice		3647						
		Canned syrup		3648						
X	**15. Plums**	Fresh		3570						
	15. Apricots			3534						
	16. Dried fruit									
	16. Dry stewed fruit									
	16. Raisins			3552						
	17. Fruit juice	Fresh								
		Sweetened								
		Unsweetened								

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	
						Times/ day	Times/ week			
	SOUPS, LEGUMES, NUTS									
	1. Soups	Powder, average		3158						
		Reconstituted (power + water)		3165						
		Vegetable		3162						
		Meat, lentil veg		3153						
		Dried bean, meat, veg		3145						
Type?	**2. Beans** (baked or other)									
Type?	**2. Lentils**									
Type?	**3. Nuts**									
	3. Peanuts									
	Peanuts & raisins									
	FISH & SEAFOOD- BEIGE *ask which type of fish was eaten?* ***Or if they don't know what colour was the meat of the fish?***									
	1. Fried fish	?								
	1. Fish cakes									
	1. Fish fingers									
Circ le	**1. Calamari**	Batter/ No batter								
Circle one	**2. Grilled/smoked/dried fish**	*?*								
	2. Haddock									
Circle one	**3. Pilchards & sardines**	Canned water		3055						
	Canned	Tomato sauce		3102						
		Mayonnaise		3488						
	3. Tuna	Brine water		3054						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	3. Tuna	In oil		3056						
		Mayonnaise		3488						
	3. Pickled fish									
	MEAT- RED	*How do you eat meat? 1= with fat 2= fat trimmed*								
	1. Roast beef									
	1. Beef chops									
	1. Beef steak (bone)									
	1. Beef steak (no bone)									
	1. Beef stir-fry									
	1. Beef stew / vegetable			3020						
	1. Beef stew / cabbage			3006						
	2. Beef patties			2984						
	2. Mince	Regular		2987						
		With vegetables								
X	**2. Meatballs**	*Egg / no egg*								
	2. Cottage pie			3009						
	3. Burgers	Bun		3210						
	please tick, quantify, brand names (McDonalds bigmac)	Patty: Beef		2984						
		Cheese slice		2722						
		Tomato sauce		3139						
		Mayonnaise		3488						
Meal?	**Chips (fried)**	*Size?*		3740						
	Soft drink	*Size?*		3981						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	3. Hot dog	Roll								
		Vienna								
		Tomato sauce		3139						
	3. Pita with…									
	3. Chicken burger	Bun		3210						
	please tick, quantify, name of burger (e.g KFC twister)	Crumbed, fried		3011						
		Cheese slice		2722						
		Tomato sauce		3139						
		Mayonnaise		3488						
Meal?	**Chips (fried)**	*Size?*		3740						
	Soft drink	*Size?*		3981						
	3. Chicken nuggets			3018						
	4. Chicken stew									
	4. Fried chicken pieces	With skin								
		Without skin								
	4. Roast chicken	With skin								
		Without skin								
	4. Chicken stir-fry									
	6. Fat cakes & mince									
	7. Meat pies *which meat?*									
Circle!	**7. Samoosas**	Beef / lamb								
		Chicken								
Circle!		Veg / cheese & corn / potato								

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	7. Sausage rolls			2939						
	7. Spring rolls			2918						
	8. Mutton stew no veg									
	8. Mutton stew with veg									
	8. Mutton leg chop									
	8. Mutton loin chop			2927						
	8. Roast mutton			2947						
	9. Spare ribs			3010						
	9. Pork chops			2930						
	9. Bacon			2906						
	9. Roast pork									
	10. Pork sausages			2932						
	10. Viennas			2936						
Circle	**10. Frankfurters**	*Beef/pork/chicken*								
	10. Boerewors			2931						
	11. Traditional / organ meats									
	Chicken livers			2970						
	Chicken giblets			2998						
	Chicken head			2999						
	Chicken feet			2997						
	Liver & fat			2920						
	Sheep intestine / lungs			4342						
	Shank *pork/lamb/beef*									
Circle	**Mopani worms**	*Dried/ cooked*								

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	12. Vegetarian products									
	13. Dry sausages			2949						
	13. Biltong			2912						
	VEGETABLES – GREEN									
	1. Asparagus	Fresh boiled		3695						
		Canned boiled		4094						
	2. Avocado	Fresh		3656						
	3. Baby marrows	Fresh boiled		4171						
	Brinjal *(how cooked?)*									
	Okra *(how cooked?)*									
	4. Beetroot	Boiled		3698						
Circle	*Grated / sliced*	Salad		3699						
	Chutney, onion,	Salad								
	5. Butternut	Boiled		3759						
		Boiled Sugar added		3989						
		Sugar & fat added		4273						
	5.Pumpkin	Boiled		4164						
		Sugar & fat added		3893						
	6. Broccoli, *Fresh/frozen*	Boiled								
		Fat added		3808						
	6. Cauliflower	Boiled		3716						
		Boiled + cheddar		3715						
	Quantify white/cheese sauce Pg 23									

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	7. Cabbage	Boiled		3756						
	7. ..with potato, onion	HM		3813						
	7. ..with potato, onion	Sun oil		3815						
	7. coleslaw (mayo)			3705						
	7. Cabbage, *fried*	HM		3810						
	7. Cabbage, *fried*	Sun oil		3812						
	8. Carrots	Raw		3709						
	8. Carrots	Boiled		3757						
		Sugar added		3818						
	9. Gem squash	Boiled		3760						
		Boiled + sugar		3754						
	10. Green beans	Boiled		3696						
	10.. with potato, onion	No fat		3933						
	10.. with potato, onion	HM		3792						
	10.. with potato, onion	Sun oil		3794						
	11. Mealies	Whole, boiled		4133						
		Whole, boiled canned		4134						
	11. Sweet corn									
	12. Mixed vegetables	Canned, boiled		4264						
	carrot, corn, peas, green beans	Frozen, boiled		3727						
	caulif', carrot, green beans	Frozen, boiled		4265						
		Fresh boiled, PUM		3836						
		Fresh boiled, HM		3835						
	13. Mushrooms	Fried HM		3893						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	13. Mushrooms	Fried in Sun oil		3841						
	14. Peas	Frozen boiled		4146						
		Boiled, sugar, HM		3859						
	15. Potatoes, Baked	(ate flesh & skin)		3736						
		(ate skin only)		3970						
		(ate skin, sour cream & chives		3973						
	Boiled	(ate skin & flesh)		4155						
		(ate no skin)		3737						
		HM		3867						
		PUM		3868						
	Croquette/cake			3915						
	Fried/sautéed	HM		3871						
		Sun oil		3873						
	Frozen, boiled			4156						
	Mashed	(SM, PUM)		3875						
		(WM, HM)		3876						
	Roasted in..	.. beef fat		3878						
		.. chicken fat		3923						
		.. lamb fat		3735						
		.. pork fat		3956						
		..Sun oil		3979						
	15. Potato Salad	(mayonnaise)		3928						
	Potatoes, whole canned			4159						

Generic Sketch (look up)	A. Food items (with FPM numbers)	B. Description of food item	Tick for yes	C. Item code	D. Amount usually eaten(g) Generic/amount = g	E. Eaten every day	F. Eaten every week	G. Eaten Occasionally	H. Never eaten	Grams (g) /day
						Times/ day	Times/ week			
	16. Potato chips	Fried in Sun oil		3740						
		Oven baked		3945						
		Mayonnaise		3488						
		Tomato sauce		3139						
	If take away where from?									
	17. Salad vegetables	French		3921						
		Greek		4271						
	Dressing? See pg 19!	Mixed green		3927						
	Raita (yoghurt, LF/ WM cucumber, tomato, onion)									
	17. Raw tomato			3750						
	17. Cucumber			3718						
	17. Peppers raw, green			3733						
	17. Onion, raw			3755						
	17. Onion, fried	HM		3844						
	17. Onion, fried	Sun oil								
	18. Spinach/morogo	Boiled		3913						
		Boiled + HM								
		Boiled + PUM								
	18.. with potato, onion	HM		3901						
	18.. with potato, onion	Sun oil								
	19. Sweet potatoes	Baked, ate skin only		3748						
		Boiled, no skin		3903						
		Sugar + HM		3749						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	20. Tomatoes, cooked	Fried, onion		3925						
		Fried, onion, peppers								
		Fried, onion, peppers, carrots								
	Fried onions (only)									
	FATS-TAN 'prompt'									
Circle	**1. Tub margarine**	Where used								
Circle	**1. Butter / Ghee**	Number of spoons								
	1. Brick Margarine	Number in family								
	2. White margarine (type of fat)									
	3. Cream and substitutes (brand, real dairy/plant fats)									
	4. Oils	Where used								
		Number of spoons								
		Number in family								
	5. Salad dressings	Homemade								
		Shop bought								
	5. Mayonnaise	Where used								
		Number of spoons								
		Number in family								

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	BISCUITS, CAKE & PUDDING – PURPLE									
	1. Biscuits/cookies	Commercial plain		3216						
Brand		Commercial, filling		3217						
		Homemade, plain		3341						
	2. Biscuits/savoury	Provita		3235	___ x 6 g					
		Ry-vita		3236	___ x 5 g					
		Cream crackers		3230	___ x 8 g					
	High fat *(circle which one)*	Tuc/salticrax/kips		3331						
	Harvest wheat	Whole wheat		3391						
	Spread?									
	Topping?									
	3. Special buns *(hot cross, Danish, raisin)*			3409						
	3. Muffins	Bran		3407						
		Plain		3408						
	3. Scones	Plain		3237						
	4. Tart									
	4. Cake *(circle)*	Iced/Cream								
		Plain								
	5. Doughnuts	Plain		3232						
	5. Doughnuts	Jam		3423						
	5. Doughnuts	Icing		3422						
	5. éclairs			3268						
	5. Koeksisters			3231						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
X	**6. Pancakes/crumpets**	WM/Sun oil		3238						
	Toppings?									
	6. Waffles	WM/Sun oil		3238						
	Toppings?									
	7. Baked pudding									
	7. Trifle									
	7. Custard									
	7. Instant pudding									
	8. Rusks	Commercial		3329						
	9. Special breads	Banana loaf		3333						
		Date loaf		3256						
		Raisin bread		3214						
	Spread?									
	SNACKS, SWEETS & COLD DRINKS-PINK									
	1. Carbonated cold drinks e.g. coke			3981						
	1. Diet cold drinks e.g. diet coke			3990						
	2. Mageu			4056						
	2. Cold drinks (powder)									
	2. Energy drinks			4007						
	2. Squashes			3982						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
Brands	**3. Crisps, potato** *(lays)*			3417						
Size & Circle!	**3. Crisps Savoury,** *(nik naks, fritos, ghost pops)*			3418						
	3. Popcorn, plain			3332						
	3. Popcorn, sugar coated			3359						
	4. Sweets									
	Lollipops									
Brands	**4. Chocolates**									
Describe										
Sizes										
	SAUCES & CONDIMENTS-GREY									
	1. Cheese sauce	LFM, HM, cheese		3126						
		LFM, PUM, cheese		3127						
		SM, PUM, cheese		3128						
		WM, HM, cheese		3125						

Generic Sketch (look up)	**A.** Food items (with FPM numbers)	**B**. Description of food item	**Tick for yes**	**C**. Item code	**D**. Amount usually eaten(g) Generic/amount = g	**E**. Eaten every day	**F**. Eaten every week	**G**. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**
						Times/ day	Times/ week			
	1. White sauce	LFM, HM		3143						
		LFM, PUM		3164						
		SM, PUM		3141						
		WM, HM		3142						
	2. Chakalaka									
	2. Atjar			3117						
	2. Tomato sauce & other									
	2. Chutney									
	ALCOHOLIC DRINKS-GREY									
	1. Beer (regular/low alcohol)									
	1. Cider									
	Coolers									
	2. Wine									
	2. Champagne									
	3. Spirits (any carbonated drink e.g. coke added)									
	4. Liqueurs & Fortified wine									
	OTHER:									

Appendix E: Neonatal data collection sheet

BABY PEAPOD & DXA STUDY

DATE: | | | | | | | | | |

Day **Month** **Year**

BTT ID : _____

INTERBIO ID: _____

SFG ID: _____

NEONATAL ID: _____

DXA SCAN	**YES**	**NO**	**DONE?**

If Not done please describe :

_____
_____
_____
_____
_____

PEAPOD	**YES**	**NO**	**DONE?**

IF Not done please describe:

_____
_____
_____
_____
_____

Radiographer's **Name:** _____

Participants Name:

Gestational age:

DATE OF BIRTH: **Day** | | **Month** | | **Year** | | | | |

MEASUREMENTS

DATE OF | | | | | | | **MEASUREMENTS:**

Weight: | | | | | **G**

Length: | | | . | | **cm**

Head Circumference: | | | . | | **cm**

Research Assistant: | |

SECOND MEASUREMENTS:

Weight: | | | | | **G**

Length: | | | . | | **cm**

Head Circumference: | | | . | | **cm**

Research Assistant: | |

Appendix F: Soweto Fetal Growth Study (SFGS) information sheet

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

Corner of College and Clinic Road
Chris Hani Baragwanath Hospital

Telephone: +27-11-933-1122
Fax: +27-11-933-3242

FETAL GROWTH STUDY:

INFORMATION SHEET

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

What is involved in the study?

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

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

- 5 ultrasound scans at 4-6 weekly intervals. At each scan, we will measure your baby, and the blood flow to the placenta and the baby. Each ultrasound scan session will take approximately 30-60 minutes.
- Blood samples for micronutrients and glucose metabolism.
- Ask you a series of questions related to yourself and your pregnancy.
- **Weight and height for exploring weight gains and calculating BMI**
- **An ultrasound scan measuring your subcutaneous fat will be conducted and will take approximately 10 minutes**
- **Skinfold anthropometric measurements on your biceps and subscapular will be done and recorded and this will take approximately 10 minutes using callipers**

When you enroll in our study we will assign a Research Nurse to you so that you can contact her if you require any further advice or assistance during your pregnancy. You will also be required to

contact the nurse when you go into labour so that she can arrange to be present at the delivery of your baby. At the delivery, the research nurse will take a cord blood sample, a faecal sample and your placenta for investigations. When your baby is born, we would like to weigh your baby and measure his/her length and head circumference. This is to help us better understand how babies grow.

Procedures

Interviewer-completed questionnaires

At each visit at our offices, we will fill in some questionnaires with your help about your education, household circumstances and employment, your physical activity and dietary intake, events that have recently happened in your life and your health. We will also ask you questions around your pregnancy planning and menstrual and obstetric histories as well as some questions about your family. If you are uncomfortable about answering any of the questions you need not answer them.

Anthropometric measurements

Height (in mm) will be measured using a stadiometer (Holtain, UK) and converted to metres (m), and weight was measured to the nearest 0.1 kg using an electronic bathroom scale. All participants were measured wearing light clothing and without shoes. BMI will be calculated as weight in kilograms (kg) divided by height $(m)^2$.

Skinfold measurement will be taken on the bicep, triceps and subscapular sites using a skinfold caliper (Holtain, UK) to the nearest 0.2mm. Participants will be asked to remove excess clothing. All measurements will be carried out by a Research Assistant of the same sex as the participant.

Blood taking

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

1. Visit 1: (±15ml total; 3 teaspoons of blood) for biochemical analysis (e.g. Vitamin D, folate) and DNA analysis (see separate information and consent sheet).
2. **Visit 4: (±56ml total; 10 teaspoons) for glucose, insulin, and biochemical analysis (e.g. lipids and Vitamin D analysis etc).**
3. Visit 6: (±10ml total; 2 teaspoons) for DNA and RNA analysis (see separate consent sheet).

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

Haemoglobin

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

Ultrasound

We would like to perform 5 ultrasounds (sonar examinations) on your baby at various stages during your pregnancy. Ultrasound measurements are safe and carry no risk to you or your baby. If any problems are detected on ultrasound we will refer you to the appropriate doctors at Chris Hani Baragwanath Hospital. It is important to realise that ultrasound is a screening technique, which means that it is not 100% effective in detecting fetal abnormalities and so sometimes problems with the baby can be missed**. An ultrasound scan measuring your subcutaneous fat will be conducted and will take approximately 10 minutes and will be done at each visit for the pregnant woman.**

Urine dipstick test

As part of each follow-up visit, we will collect a urine sample from you to perform a urine dipstick test, which can indicate if there is protein, blood, and sugar in your urine. The nurse will explain the test to you and provide you with the results immediately and counsel you on what the results mean. These tests will be done at all of your 5 visits.

The Oral Glucose Tolerance Test

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

When you are around 24-28 weeks (6-7 months) pregnant an oral glucose tolerance test (OGTT) will be performed on you. You will have to have fasted from 10pm the night before you come for this visit. You may drink water but must not eat any food or drink anything else. The test will involve you drinking approximately 1 cup of a sweet sugary drink. Before you drink the liquid a nurse will prick your finger to check your fasting blood sugar level and she will take two blood samples from a vein in your arm (one for glucose and one for insulin testing). You will then be given the sugary liquid to drink. This has to be drunk within 5 minutes. Thereafter, two blood samples will be taken from you at 30 minutes, 1 hour and 2 hours after you have swallowed the drink.

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

Delivery Process

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

Possible risks

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

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

Possible benefits:

You will have 5 ultrasound scans which monitor the growth of your baby. If we find any problems during follow-up visits we will refer you to back to your antenatal clinic for further management and referral.

Costs to you:

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

Voluntary participation in research:

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

Records of your participation in this research:

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

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

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

Your questions:

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

YOU WILL HAVE A COPY OF THIS INFORMATION SHEET TO KEEP

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

Your signature on the consent form certifies the following:

· You have read the information provided in this consent form

· You have received answers to all of your questions.

· You have freely decided to participate in this research.

· You understand that you are not giving up any of your legal rights

Appendix G: Soweto Fetal Growth Study (SFGS) consent sheet

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

Corner of College and Clinic Road
Chris Hani Baragwanath Hospital

Telephone: +27-11-933-1122
Fax: +27-11-933-3242

FETAL GROWTH STUDY: CONSENT SHEET

I agree to myself being a participant in the study. The goals and methods of the study are clear to me.

I understand that the study will involve interviews, measurements, collection of biological samples, blood taking, an oral glucose tolerance test and ultrasound examinations. All the details and purposes of this study have been explained to me. I understand that I have the right to refuse to participate in the study.

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

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

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

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

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

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

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

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

PARTICIPANT

_____

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

RESEARCH ASSISTANT

_____

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

Appendix H: Soweto Baby Growth Study (SBGS) information sheet

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

Corner of College and Clinic Road
Chris Hani Baragwanath Hospital

Telephone: +27-11-933-1122
Fax: +27-11-933-3242

THE SOWETO BABY GROWTH STUDY:

INFORMATION SHEET

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

What is involved in the study?

The study will be conducted at our offices in Chris Hani Baragwanath Hospital (MRC/Wits Developmental Pathways for Health Research Unit). For this study there are certain investigations that we will be performing at our offices, but your baby will still need to go for his/her regular clinic and doctor checkups.

We would like to collect data from you and your baby at various time points over the next two years (at delivery and then when your baby is 6 weeks and 2, 3, 6, 12, 18 & 24 months of age)

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

- Your **baby's** weight and **length** at each of the visits
- Body composition measures on you and your baby **(DXA and PeaPod)**
- Fasting blood glucose samples on you
- Heel-pricks from your baby to test his/her glucose metabolism profile
- Ask you a series of questions related to yourself and your baby
- **Adiposity for the infants (non-invasive) - Triceps, sub scapular and arm circumference: Skinfold measurement will be taken on the bicep, triceps and subscapular sites using a skinfold caliper (Holtain, UK) to the nearest 0.2mm. Participants will be asked to**

remove excess clothing. All measurements will be carried out by a Research Assistant of the same sex as the participant.

- **A buccal swab from these infants at each 12 and 24 months will be collected. These samples would be used to compliment the samples already collected at delivery and allow us to investigate the effect of the intrauterine and early growth environments on the epigenome, which influences patterns of growth and development in infancy and childhood**

Procedures involving the mother

Measurements

- Your weight will be measured at every visit.
- **Adiposity: Biceps, Subscapular and subcutaneous fat (ultra sound). An ultrasound scan measuring your subcutaneous fat will be conducted and will take approximately 10 minutes and will be done at each visit for the mother.**
- **An ultrasound scan measuring your subcutaneous fat will be conducted and will take approximately 10 minutes**
- **Skinfold anthropometric measurements on your biceps and subscapular will be done and recorded and this will take approximately 10 minutes using callipers**

Interviewer-completed questionnaires

At each visit at our offices, we will fill in some questionnaires with your help about your education, household circumstances and employment, your dietary intake and events that have recently happened in your life. We will also ask you some questions around your feeding practices for you baby as well as some questions about your interaction with and feelings around your baby If you are uncomfortable about answering any of the questions you need not answer them. Each questionnaire will take approximately one hour to complete.

Blood taking for maternal fasting glucose

When we eat certain foods our bodies break the food down into sugar (glucose) which provides us with energy. When our blood sugar levels rise our bodies produce a hormone called insulin to control the sugar levels. However, sometimes there can be too much sugar in our blood stream which can cause problems. One of the most common problems resulting from too much sugar is a condition called diabetes. Diabetes can impact seriously on your health. Therefore, we want to determine your fasting glucose levels.

A nurse will collect blood from **you** (from a vein in your arm) at two visits (at the 6 week visit and at the six month visit). You will have to have fasted from 10pm the night before you come for these visits. Approximately 5ml of blood (1 teaspoon of blood) will be taken from you for glucose analysis.

Your samples will be analysed in the laboratory soon after they have been taken and results will be given to you at your next visit.

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

Duel-energy X-ray Absorptiometry (DXA)

DXA is a full body scan which determines your muscle, fat and bone mass. You will lie on a bed and then a machine will move over you (it does not touch you) and sends an image to the computer of your bone, fat and muscle composition. The levels of radiation in DXA are extremely low. A DXA scan will be performed on you at your 6 weeks, 6 month and 24 month visits.

Procedures involving the baby

Measurements

At every visit we would like to measure the baby's weight, length/height and head circumference.

Biological samples taken on your baby

- **Heel prick**

When your baby is 3, 12 and 24 months old we would like to take a heel prick from him/her. A heel prick involves pricking the heel of the baby with a small needles and putting the drop of blood on a special card. The blood dries on this card and we can then test the blood. The heel prick blood spots will be used to assess your baby's diabetes risk. Our laboratory will test the heel prick blood spots for insulin, glucose, C-Peptide and IGF1; all factors involved in glucose metabolism (how the body breaks down sugar).

Pricking the heel may cause the baby some discomfort and so some babies may cry when the heel prick is performed but, the actual procedure is not harmful to the baby. As with all blood taking procedures, a new clean sterile needle will be used.

Buccal swabs

A buccal swab from these infants at 12 and 24 months will be collected. These samples would be used to compliment the samples already collected at delivery and allow us to investigate the effect of the intrauterine and early growth environments on the epigenome, which influences patterns of growth and development in infancy and childhood

PeaPod

The PeaPod is a machine that determines infant body composition. The machine is safe and non-invasive. The process entails the baby being placed into a warmed test chamber where body mass and body volume is measured. The baby will lie inside the chamber for a few minutes and you will be able to see him/her through a glass lid. PeaPod assessments will be done at delivery then when the baby is 6 weeks old and again when he/she is 2, 3 and 6 months old.

Duel-energy X-ray Absorptiometry (DXA)

DXA is a full body scan which determines your muscle, fat and bone mass. Your baby will lie on a bed and then a machine will move over him/her (it does not touch the baby) and sends an image to the computer of the baby's bone, fat and muscle composition. The levels of radiation in DXA are extremely low. DXA scans will be performed on your baby at delivery and when he/she is 6, 12 and 24 months old.

Possible risks

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

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

There are chances that some of the questions we ask might trigger some emotional set-backs. If this does occur one of our midwives trained in counselling will be available to talk with you and then we can refer you to the Psychology Unit at Chris Hani Baragwanath Hospital where you will be given an appointment with a psychologist.

Possible benefits:

If we find any problems during the visits we will refer you to the appropriate medical professionals further management and referral.

Costs to you:

Participating in the research will involve no cost to you. You will be given a sandwich and fruit juice once your measurements and assessments have been completed. You will also be given **R75** for transport costs.

Voluntary participation in research:

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

Records of your participation in this research:

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

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

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

Your questions:

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

YOU WILL HAVE A COPY OF THIS INFORMATION SHEET TO KEEP

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

Your signature on the consent form certifies the following:

· You have read the information provided in this consent form

· You have received answers to all of your questions.

· You have freely decided to participate in this research.

· You understand that you are not giving up any of your legal rights.

Appendix I: Soweto Baby Growth Study (SBGS) consent sheet

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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

Corner of College and Clinic Road
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Telephone: +27-11-933-1122
Fax: +27-11-933-3242

THE SOWETO BABY GROWTH STUDY: CONSENT SHEET

I agree to myself and my child being participants in the study. The goals and methods of the study are clear to me.

I understand that the study will involve interviews, measurements, blood taking, and body composition assessments. All the details and purposes of this study have been explained to me. I understand that I have the right to refuse to participate in the study.

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

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

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

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

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

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

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

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

MOTHER OF CHILD

_____

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

RESEARCH ASSISTANT

_____

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

Appendix J: Soweto Fetal Growth Study (SFGS) ethical clearance certificate

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

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

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

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

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

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

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Appendix K: Soweto Baby Growth Study (SBGS) ethical clearance certificate

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130905

NAME: (Principal Investigator)	Prof Shane Norris & Shelley Macaulay
DEPARTMENT:	Developmental Pathways for Health Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	The Soweto Baby Growth Study
DATE CONSIDERED:	27/09/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	

APPROVED BY:

Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 25/03/2014

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

DECLARATION OF INVESTIGATORS

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

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix L: PhD study ethical clearance certificate

R14/49 Miss Stephanie Victoria Wrottesley

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150720

NAME: (Principal Investigator)	Miss Stephanie Victoria Wrottesley
DEPARTMENT:	Developmental Pathways for Health Research Unit Chris Hani baragwanath Academic Hospital
PROJECT TITLE:	Maternal Nutrition during Pregnancy and its Association with Birth Outcomes and Neonatal Body Composition in the Context of HIV in Urban Black South Africans
DATE CONSIDERED:	31/07/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shane Norris
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	16/09/2015

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

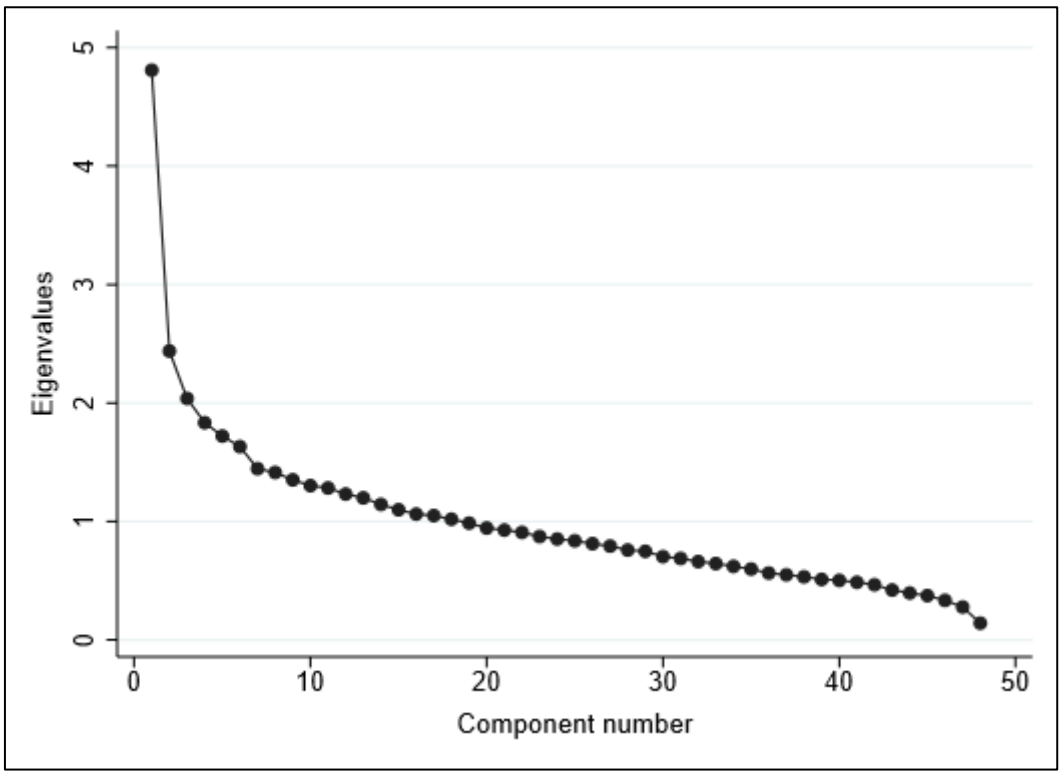
DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix M: Supplementary tables and figures



Supplementary figure 1: Scree plot of eigenvalues after principal component analysis (PCA)

Supplementary table 1: Associations between maternal characteristics and dietary pattern scores (n=538)

Variable	Dietary pattern		
	Western	**Traditional**	**Mixed**
Maternal characteristics			
Age, y			
<25	0.68 ± 2.27	-0.16 ± 1.78	0.10 ± 1.66
25-29	0.21 ± 2.12	-0.21 ± 1.81	-0.06 ± 1.52
30-34	-0.22 ± 1.96[b]	0.10 ± 1.68	0.06 ± 1.69
35-39	-0.47 ± 1.64[b]	0.37 ± 1.84	-0.00 ± 1.65
≥40	-0.97 ± 1.36[bc]	0.03 ± 1.36	-0.30 ± 1.41
p-value[a]	**<0.001**	0.094	0.745
Parity			
Para 0	0.15 ± 1.97	-0.39 ± 1.40	-0.11 ± 1.38
Para 1	0.23 ± 2.12	0.22 ± 1.91[e]	0.20 ± 1.74
Para ≥2	-0.44 ± 1.93[d]	-0.00 ± 1.74	-0.19 ± 1.57[f]
p-value[a]	**0.003**	**0.006**	**0.034**
HIV status			
HIV-negative	-0.06 ± 2.05	-0.03 ± 1.67	0.05 ± 1.56
HIV-positive (pre-pregnancy ART)	0.23 ± 2.19	0.40 ± 2.20	-0.02 ± 1.81
HIV-positive (antenatal ART)	0.05 ± 1.92	-0.13 ± 1.73	-0.14 ± 1.64
p-value[a]	0.560	0.126	0.538
Smokes/chews tobacco			
No	-0.02 ± 2.06	0.03 ± 1.76	0.06 ± 1.63
Yes	0.15 ± 1.91	-0.23 ± 1.71	-0.42 ± 1.40
p-value[a]	0.512	0.239	**0.019**
Socioeconomic characteristics			
Maternal education			
Primary	-1.54 ± 1.29	-0.41 ± 1.29	0.15 ± 1.92
Secondary	-0.07 ± 2.05	-0.01 ± 1.74	-0.04 ± 1.64
Tertiary	0.28 ± 2.01[g]	0.06 ± 1.84	0.08 ± 1.53
p-value[a]	**0.011**	0.704	0.700
Marital status (n=509)			
Single	0.15 ± 2.15	-0.14 ± 1.66	0.07 ± 1.61
Married/cohabiting	-0.24 ± 1.84	0.20 ± 1.88	-0.08 ± 1.61
p-value[a]	**0.035**	**0.032**	0.315
Household SES			
Low	-0.10 ± 2.15	0.00 ± 1.75	-0.67 ± 1.59
Medium	0.00 ± 2.01	0.03 ± 1.77	0.11 ± 1.61[h]
High	0.22 ± 1.82	-0.44 ± 1.57	0.15 ± 1.30[i]
p-value[a]	0.759	0.353	**<0.001**
Anthropometry			
BMI at recruitment, kg/m^2 (<14 weeks)			
Normal weight	0.06 ± 2.06	0.10 ± 1.91	0.09 ± 1.62
Overweight	-0.09 ± 1.98	-0.12 ± 1.63	-0.15 ± 1.47
Obese	0.03 ± 2.09	0.02 ± 1.72	0.06 ± 1.75
p-value[a]	0.736	0.460	0.289

Gestational weight gain, kg/w			
Normal weight			
Inadequate (<0.35)	-0.29 ± 2.02	0.22 ± 2.11	0.04 ± 1.73
Adequate (0.35-0.50)	-0.09 ± 2.01	0.43 ± 2.02	0.27 ± 1.51
Excessive (>0.50)	0.44 ± 2.09	-0.21 ± 1.63	0.01 ± 1.63
p-value[a]	0.117	0.152	0.313
Overweight			
Inadequate (<0.23)	-0.31 ± 1.55	0.10 ± 1.50	-0.47 ± 1.40
Adequate (0.23-0.33)	-0.19 ± 1.93	-0.08 ± 1.66	-0.48 ± 1.20
Excessive (>0.33)	0.01 ± 2.12	-0.20 ± 1.67	0.04 ± 1.52
p-value[a]	0.656	0.597	0.064
Obese			
Inadequate (<0.17)	0.21 ± 2.32	0.06 ± 2.05	-0.01 ± 1.96
Adequate (0.17-0.27)	0.18 ± 2.29	-0.28 ± 1.37	0.03 ± 1.50
Excessive (>0.27)	-0.06 ± 1.97	0.09 ± 1.69	0.10 ± 1.75
p-value[a]	0.742	0.614	0.951

Dietary pattern scores presented as mean ±SD
Normal weight, BMI 18.5-24.9kg/m^2; overweight, BMI 25-29.9kg/m^2; obese, BMI ≥30kg/m^2
[a]Independent t-test (two groups) or analysis of variance (ANOVA) (more than two groups)
[b]Tukey post-hoc test: p<0.01 vs. <25 years
[c]Tukey post-hoc test: p<0.05 vs. 25-29 years
[d]Tukey post-hoc test: p<0.05 vs. para 0; P<0.01 vs. para 1
[e]Tukey post-hoc test: p<0.01 vs. para 0
[f]Tukey post-hoc test: p<0.05 vs. para 1
[g]Tukey post-hoc test: p<0.05 vs. primary education
[h]Tukey post-hoc test: p<0.001 vs. low SES
[i]Tukey post-hoc test: p<0.05 vs. low SE

Supplementary table 2: Factor loadings of various foods or food groups in the principal dietary components identified in those pregnant black South African women included in analyses for objective 4 (n=393)

Traditional diet pattern	
Food or food group	***Factor loading***
Other vegetables	**0.366**
Beans and pulses	**0.319**
Green vegetables	**0.299**
Salad vegetables	**0.292**
Boiled and baked potatoes	**0.230**
Maize, sorghum and oat porridge	**0.220**
Vegetable dishes	**0.220**
Root vegetables	**0.218**
Offal and traditional meats	**0.204**
Chicken and turkey	0.196
Other fruit	0.175
Crackers	0.167
Citrus fruit	0.165
Crisps and popcorn	0.162
Diet soft drinks	0.162
Rice and pasta	0.141
Eggs and egg dishes	0.114
Tinned vegetables	0.112
Fish and seafood	0.095
Puddings	0.083
Red meat	0.075
Miscellaneous (soup powder, condiments, sauces, etc.)	0.073
Brown and wholemeal bread	0.072
Cakes and biscuits	0.063
Yoghurt, buttermilk and maas	0.063
Cream	0.061
Nuts and nut spreads	0.044
Soft drinks	0.044
Cooking fats and salad oils	0.043
Reduced-fat milk	0.041
Cooked and tinned fruit	0.029
Full-fat milk	0.012
Processed meat	0.009
Sweets and chocolate	0.009
Added sugar (teaspoons)	0.007
Decaffeinated tea and coffee	0.006
Dried fruit	-0.003
Fruit juice	-0.020
Tea and coffee	-0.024
Fat cakes and samosas	-0.045
Reduced-fat spread	-0.045
Roast potatoes and chips	-0.052
Full-fat spread	-0.056
Quiche and pizza	-0.056
Sweet spreads	-0.091
Breakfast cereals	-0.093
Cheese and cottage cheese	-0.103
White bread	-0.188
Explained variance (%)	6.8

Foods or food groups with factor loadings ≥0.2 were classified as characteristic to the dietary pattern and therefore used to describe it (illustrated in bold)

Supplementary table 3: Neonatal anthropometry and body composition according to maternal and neonatal characteristics in urban black South Africans

	Total sample (n=393) %	***Weight to length ratio (kg/m)*** **Median (IQR)**	**Sub-sample (n=171) %**	***Fat mass index (kg/m^3)^b*** **Median (IQR)**
Maternal variables				
Demographic and health characteristics				
Maternal age, y				
<25	91	6.4 (5.8-6.8)	34	3.6 (2.9-4.4)
25-29	105	6.3 (5.9-6.7)	42	3.8 (3.0-4.2)
30-34	97	6.5 (6.0-6.9)	46	3.9 (2.9-4.8)
35-39	79	6.2 (5.6-6.7)	39	3.6 (2.8-4.6)
≥40	21	6.4 (6.1-6.7)	10	3.2 (2.1-4.6)
p-value[a]		0.052		0.520
Parity				
Para 0	97	6.1 (5.6-6.6)	44	3.1 (2.6-3.7)
Para 1	177	6.4 (6.0-6.8)	71	3.9 (3.2-4.7)
Para ≥2	119	6.4 (6.0-6.8)	56	3.9 (2.9-4.8)
p-value[a]		**0.010**		**0.004**
HIV status				
HIV-negative	258	6.4 (6.0-6.8)	114	3.6 (2.9-4.2)
HIV-positive (antenatal ART)	92	6.4 (5.8-6.8)	42	3.9 (2.9-4.8)
HIV-positive (pre-pregnancy ART)	43	6.3 (5.8-6.7)	15	4.0 (3.5-5.5)
p-value[a]		0.826		0.066
Smokes/chews tobacco				
No	341	6.4 (5.9-6.8)	148	3.6 (3.0-4.6)
Yes	52	6.2 (5.9-6.6)	23	3.4 (2.4-4.7)
p-value[a]		0.450		0.489
Socioeconomic characteristics				
Maternal education				
Primary	7	6.4 (5.6-6.5)	4	4.1 (3.6-4.7)
Secondary	277	6.4 (5.9-6.8)	118	3.6 (2.9-4.6)
Tertiary	109	6.3 (6.0-6.8)	49	3.6 (2.9-4.6)
p-value[a]		0.496		0.655
Marital status [n=387]				
Single	236	6.4 (5.8-6.8)	103	3.6 (2.9-4.7)
Married/cohabiting	151	6.4 (5.9-6.8)	64	3.8 (3.0-4.6)
p-value[a]		0.613		0.594
Household socioeconomic status				
Low	51	6.2 (6.0-6.5)	23	3.7 (2.7-4.0)
Medium	315	6.4 (5.9-6.8)	135	3.6 (2.9-4.7)
High	27	6.4 (5.7-6.8)	13	3.1 (2.8-4.6)
p-value[a]		0.518		0.464
Anthropometry				
BMI at recruitment, kg/m^2 (<14 weeks)				
Normal weight (18.5-24.9)	136	6.3 (5.8-6.6)	55	3.6 (2.7-4.0)
Overweight (25-29.9)	137	6.3 (5.8-6.8)	62	3.9 (2.9-4.7)
Obese (≥30)	120	6.5 (6.0-6.9)	54	3.7 (3.2-4.6)
p-value[a]		**0.028**		0.488
GWG, kg/week				
Inadequate	79	6.3 (5.5-6.7)	35	3.6 (2.5-4.6)
Adequate	86	6.2 (5.7-6.6)	39	3.6 (2.5-4.6)
Excessive	228	6.4 (6.0-6.9)	97	3.7 (2.9-4.7)
p-value[a]		**0.007**		0.593
Traditional dietary pattern adherence				
Traditional pattern score				
T1 (high)	131	6.3 (5.9-6.8)	71	3.8 (3.1-4.6)

T2	131	6.4 (6.0-6.8)	55	3.7 (2.5-4.4)
T3 (low)	131	6.3 (5.8-6.7)	45	3.6 (2.8-4.6)
p-value[a]		0.368		0.441
Neonatal variables				
Sex				
Male	204	6.4 (5.9-6.8)	97	3.5 (2.5-4.4)
Female	189	6.4 (5.9-6.7)	74	3.7 (3.2-4.8)
p-value[a]		0.947		**0.034**
Gestational age at birth, w				
37-38	144	6.2 (5.7-6.7)	71	3.6 (2.9-4.6)
39-40	219	6.4 (6.0-6.8)	87	3.7 (2.8-4.6)
41-42	30	6.5 (6.1-7.2)	13	3.4 (3.0-3.7)
p-value[a]		**0.003**		0.744

Abbreviations: ART, antiretroviral treatment; BMI, body mass index; FMI, fat mass index; GWG, gestational weight gain; WLR, weight-to-length ratio

IoM GWG ranges (kg/week): inadequate, normal weight <0.35, overweight <0.23, obese <0.17; adequate, normal weight 0.35-0.50, overweight 0.23-0.33, obese 0.17-0.27; excessive, normal weight >0.50, overweight >0.33, obese >0.27

[a]Kruskal-Wallis test; significant results are presented in bold ($p<0.05$)

[b]Measured by air displacement plethysmography (ADP; Peapod) or dual-energy x-ray absorptiometry (DXA) corrected for the measurement differences between techniques

Appendix N: Plagiarism declaration

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I STEPHANIE VICTORIA WRATTESLEY (Student number: 702255) am a student registered for the degree of PhD in the academic year 2018.

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.
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