Growth of HIV-exposed and HIV-unexposed children in South Africa

Anthropometric nutritional status and growth rates

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Abbreviations

AIDS: Acquired immunodeficiency syndrome

ANC: Antenatal care

ARV: Antiretroviral

ART: Antiretroviral therapy

BMI: Body mass index

BMI-for-age: Body mass index-for-age

CDC: Centers for Disease Control and Prevention

EBF: Exclusive breastfeeding

EFF: Exclusive formula feeding

FAO: Food and Agriculture Organization

HAZ: Height-for-age Z-score

HIV: Human Immunodeficiency Virus

HEU: HIV exposed uninfected

HUU: HIV unexposed uninfected

Kg: Kilogram

LAZ: Length-for-age Z-score

LVZ: Length velocity Z-score

MAM: Moderate acute malnutrition

MBF: Mixed breastfed

MFF: Mixed formula fed

MGRS: Multicentre growth reference study

MTCT: Mother To Child Transmission

NCHS: National Center for Health Statistics

NVP: Nevirapine

PEM: Protein energy malnutrition

PMTCT: Prevention of Mother-To-Child HIV Transmission

SAPMTCTE: South African Prevention of Mother to Child HIV Transmission Evaluation

SDG: Sustainable Development Goals

SAM: Severe acute malnutrition

TDF: Tenofovir

UFA: Underweight-for-age

UNICEF: United Nations Children's Fund

WAZ: Weight-for-age Z-score

WHA: World Health Assembly

WHO: World Health Organization

WHZ: Weight-for-height Z-score

WLZ: Weight-for-length Z-score

WVZ: Weight velocity Z-score

ZDV: Zidovudine

Abstract

Background and objectives

South Africa is characterised by colliding epidemics of non-communicable and communicable diseases. Additionally, the prevalence of stunting, which affects almost a third of children under-five, has remained unchanged over the past decade. Regarding communicable diseases, infection with Human Immunodeficiency Virus (HIV) is endemic as South Africa is home to 1 in 5 people living with HIV globally, and approximately one in three pregnant women is HIV positive. Despite this, the success of the Prevention of the Mother to Child HIV Transmission (PMTCT) programme, particularly in the era of widespread use of triple antiretroviral treatment (ART) for PMTCT and maintenance of maternal health, has made it possible for most HIV-exposed children to remain uninfected. This has led to an emergence of a large population of HIV exposed uninfected (HEU) children for whom there are limited data on health outcomes. Notwithstanding the benefits of ART for PMTCT, in-utero exposure to ART may have risks that require further investigation. The significance of various postnatal growth velocities also needs further exploration as it is a research area that is not well understood and international growth velocity standards were not available prior to 2009. Thus very few studies have applied these standards.

Given this background, this thesis sought to describe child growth and its determinants in South Africa, a country with high malnutrition and HIV prevalences, under different PMTCT policies between 2002 and 2013, using the latest WHO growth standards. Specifically the thesis aimed to assess the effect of early infant feeding practices on growth velocity in the first 6 months, and the effect of both on body mass index-for-age-age Z-score (BMI-for-age Z-score) at 2 years of age (Paper I); to compare the longitudinal growth of HIV-exposed and -unexposed children in South Africa using the WHO growth velocity standards (Paper II) and to study the effect of infant in-utero HIV and ART exposure on preterm delivery (PTD), low birth weight (LBW) and small-for-gestational age (SGA) at birth, and underweight-for-age (UFA) at six weeks postpartum in children attending primary health care facilities in

South Africa during established implementation of WHO PMTCT Option A policy (Paper III).

Methods

Data from 3 studies conducted in South Africa were utilised. For Paper I HIV-negative women were recruited in pregnancy and followed-up at 3, 6, 12 and 24 weeks and 2 years postpartum with their children. For Paper II, the majority of the HIV-positive and -negative women were also recruited antenatally, with only a few recruited postnatally while still in hospital, and followed up with their children at 3, 24 and 36 weeks postpartum. For Paper III, data from a cross-sectional survey of HIV-exposed and -unexposed children attending their 6-week immunisation clinic visit were utilised.

Exposures

Only self-reported HIV-negative women and their children were considered in Paper I. The main exposures in this paper were infant feeding practices and growth velocity in the first few months of life. The child's HIV infection and exposure status, and the additional antiretroviral drug exposure status for children born to HIV-positive mothers, were the main exposures in Papers II and III. In these papers the child's HIV infection status was ascertained by the polymerase chain reaction test (PCR) using dried blood spot specimens obtained from the children by trained data collectors. In each primary study questionnaires were used to also collect information on key factors related to child health from mothers/caregivers, including child feeding practices established through 24-hour and seven-day recall questions.

Outcomes

Child weights and recumbent lengths/standing heights were measured by well-trained data collectors in Papers I and II and extracted from the patient-held road to health booklets in Paper III. Birthweights and gestational ages (based on fundal height and/or last menstrual period) were extracted from the road to health booklets in all three studies. Anthropometric

scoring was done using the 2006 WHO attained growth standards (used to estimate weight-for-age Z-scores (WAZ), length-for-age Z-scores (LAZ), weight-for-length Z-scores (WLZ) and body mass index(BMI)-for-age Z-score), 2009 WHO growth velocity standards (used to estimate weight and length velocity Z-scores (WVZ and LVZ respectively)) and recent Intergrowth-21st standards for assessing new born size for term and preterm infants (used to estimate birthweight-for-gestational-age Z-scores). Birth outcomes included low birth weight (LBW), preterm delivery (PTD) and small-for-gestational age (SGA).

Analysis

Simultaneous quantile regression was used to assess the effect of 1) early infant feeding practices on growth velocity in the first 6 months, and 2) both on BMI-for-age Z-score at 2 years of age in Paper I. Mixed effect regression was used to compare the mean growth velocity Z-scores of HIV-exposed and -unexposed children in Paper II. Logistic regression was used to study the effect of in-utero HIV and ART exposure on PTD, LBW and SGA at birth, and UFA at six weeks postpartum in Paper III.

Results

Paper I demonstrated that children who were not breastfed at 12 weeks had higher mean WVZ between 12 and 24 weeks, higher BMI-for-age Z-scores at 2 years and were more likely to be overweight or obese. Although most of the children were initiated on breastmilk early, the proportion of breastfed children decreased in the first 12 weeks of life while the frequency of formula feeding increased. The early introduction of solids such as cereals was also common. Paper II demonstrated that HIV-infected children were not only more underweight, wasted and stunted compared to HEU and HIV unexposed uninfected (HUU) children, but also had poorer growth velocity in the first few of months. The data also showed that maternal viral load, LBW and Nevirapine use were independent influencers of growth velocity in HIV-exposed children and that HIV-positive children had increased infectious morbidity compared to HUU children. Paper II also demonstrated that HEU children had similar attained growth and growth velocities compared to HUU children in the absence of maternal ART. Unlike in Paper II, data from Paper III demonstrated that HEU children had poorer birth and early

attained growth outcomes than HUU children. The results in Paper III also demonstrated that HEU children born to women with unmanaged HIV infection had an increased odds of being born preterm than children born to women on ART, and that children whose mothers initiated ART before conception had an increased odds of PTD than children whose mothers started ART after conception, but no increased odds for other outcomes.

Conclusion

This thesis highlights the importance of not only addressing the double burden of malnutrition in South Africa, but also the burden of colliding disease epidemic of communicable and non-communicable diseases. The strong association observed between infant HIV infection and poor growth highlights the importance of addressing the unfinished agenda of combating the HIV epidemic. While early initiation of HIV-positive women on ART is important for preventing MTCT and maintenance of maternal health, data does signal that pre-conception ART initiation may have an adverse effect on PTD. As access to ART increases routine surveillance system should be set up to monitor adverse outcomes. The emergence of a large population of HEU for whom there are limited data also warrants the urgent need for the close follow-up, through surveillance systems and in-depth cohort studies, of this sub-population of children. There is also an urgent need to tackle persistent undernutrition in both HIV-exposed and -unexposed South African children while curbing the concomitant rise of overnutrition, possibly thorough facility and community based support programmes.

Original papers

This dissertation is a synthesis of the following three papers, two of which have been published in peer reviewed journals. Paper III is in Press:

Paper I: RAMOKOLO, V., LOMBARD, C., CHHAGAN, M., ENGEBRETSEN, I. M., DOHERTY, T., GOGA, A. E., FADNES, L. T., ZEMBE, W., JACKSON, D. J. & VAN DEN BROECK, J. 2015. Effects of early feeding on growth velocity and overweight/obesity in a cohort of HIV unexposed South African infants and children. *International Breastfeeding Journal,* 10**,** 14. Weblink:https://internationalbreastfeedingjournal.biomedcentral.com/articles/10.1186/s13006-015-0041-x

Paper II: RAMOKOLO, V., LOMBARD, C., FADNES, L. T., DOHERTY, T., JACKSON, D. J., GOGA, A. E., CHHAGAN, M. & VAN DEN BROECK, J. 2014. HIV Infection, Viral Load, Low Birth Weight, and Nevirapine Are Independent Influences on Growth Velocity in HIV-Exposed South African Infants. *Journal of Nutrition,* 144**,** 42-48. Weblink: http://jn.nutrition.org/content/144/1/42.long

Paper III: RAMOKOLO, V., GOGA A.E., LOMBARD C., DOHERTY T., JACKSON D.J., ENGEBRETSEN I.M.S. In-utero ART exposure and birth and early growth outcomes amongst HIV exposed uninfected infants attending immunization services: Results from national PMTCT surveillance, South Africa .(In Press: *Open Forum Infectious Disease Journal.* Accepted for publication on 22 August 2017). Weblink:https://academic.oup.com/ofid/article/doi/10.1093/ofid/ofx187/4098288/In-utero-ART-exposure-and-birth-and-early-growth

The PhD candidate, being the lead author on all these papers, conducted data analysis and drafted each paper during the PhD registration period. All co-authors critically reviewed each manuscript and approved them before submission. The papers are all open access and are reproduced in this dissertation (please see Appendix).

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bless your name into eternity. I'll bless you every day, and keep it up from now to eternity. God is magnificent; He can never be praised enough" (Psalm 145:1-3).

Chapter 1: Background

The aim of this chapter is twofold. Firstly to define key terms and secondly to provide an overview of literature used to establish a theoretical framework for the research topic under study. To achieve this aim the chapter begins with a definition of malnutrition followed by a description of methods used to assess malnutrition and then an overview of the global and regional extent of child malnutrition. Given that HIV and inadequate feeding practices are risk factors for malnutrition, especially in low and middle-income countries (LMIC), the thesis then reviews literature on HIV and infant feeding practices.

1.1 Malnutrition

Malnutrition is defined as "an abnormal physiological condition caused by inadequate, unbalanced or excessive consumption of macronutrients and/or micronutrients. Malnutrition includes undernutrition and overnutrition as well as micronutrient deficiencies" (FAO. et al., 2015). Furthermore, malnutrition can also occur as a result of poor absorption or metabolism of nutrients or increased demand for nutrients as occurs during infection

1.1.1 The assessment of malnutrition

Malnutrition in an individual or population can be assessed using various approaches including biochemical, clinical, dietary history/ assessment (including duration and pattern of feeding) and anthropometrical methods (Norgan et al., 2012). For example a child with a WLZ<-3 would be regarded as severely malnourished in accordance with anthropometry; one clinical manifestation of this condition would be bilateral oedema (World Health Organization and UNICEF, 2009). Dietary assessments and anthropometry were considered in this thesis and are thus reviewed here.

Dietary assessment

One of the objectives of this thesis was to assess the effect of feeding on child growth. This necessitated a review of literature on infant feeding recommendations and definitions and dietary assessment methods. Human breast milk has sufficient energy and nutrients (including

protein, fats, vitamins and minerals) to support the growth and development of children in the first few months of life. It is estimated that 850ml/day of breast milk intake can meet the energy requirements of an infant growing along the 50th centile of the NCHS reference for 4 months. For children born in low-middle income country settings, who are generally born smaller than children in high income countries such as the United States, the intake would be adequate for 6 months if the infant was growing along the 25th centile (Whitehead and Paul, 2000). Prior to 2001 the World Health Organization (WHO) recommended exclusive breastfeeding (EBF) for 4-6 months followed by the introduction of complementary foods at 6 months and continued breastfeeding until 2 years (World Health Organization, 1995b). In 2001 the WHO recommended that the EBF period be extended to 6 months (World Health Organization, 2002a, World Health Organization, 2003), based on evidence from LMICs (Belarus, Iran and Nigeria) that demonstrated that EBF for 6 compared to 4 months is more protective against infectious disease morbidity and mortality in these settings (World Health Organization, 2002b, World Health Organization, 2002a, World Health Organization, 2003). Based on the known risk of vertical HIV transmission in the absence of ARV drug interventions (De Cock et al., 2000), the WHO also published separate infant feeding guidelines for HIV-positive women (World Health Organization, 2006a) and these are discussed later in this chapter. In 2008, following changes in the infant feeding recommendations, revised global guidelines for infant feeding indicators were also published and were the basis for the EBF, predominant breastfeeding (PBF) and ever breastfed indicators that were used in this thesis (World Health Organization, 2008a). A number of dietary assessment methods are used to collect dietary data for the construction of the feeding indicators. These include the use of dietary records (such as food diaries), food frequency questionnaires and dietary recall questionnaires that are used to assist the mother/caregiver recall foods/liquids the child had consumed in the preceding day or week (Thompson and Byers, 1994).

Anthropometric assessment

Physical "growth", is defined as a quantitative increase in cell number (due to cell division) or cell mass (due to an increase in cellular substance) (Himes, 2004). Poor physical growth in

children is one of the first visible signs of malnutrition (Ruel-Bergeron et al., 2015). Anthropometry compares the physical growth measurements of the child against those of a reference population to assess the growth rates or nutritional status of the child (World Health Organization, 1995a). However, anthropometric measurements have a limited diagnostic property, and therefore micronutrient deficiency can exist irrespectively of anthropometric status.

Growth measurements

The most commonly used measurements in children include weight, length or height, head and mid-upper arm circumference (MUAC). When performed at a given time point these measurements quantify attained size or mass at that particular time. This provides information on cumulative historical changes in the body size and mass (Himes, 2004). The individual human growth curves in Figure 1 illustrate the difference between the distance curve (which represents the height the child attained from birth until 18 years), the velocity growth curve (the rate of change in height expressed in units per time period) and the acceleration curve (the change in velocity through time) (Molinari and Gasser, 2004)

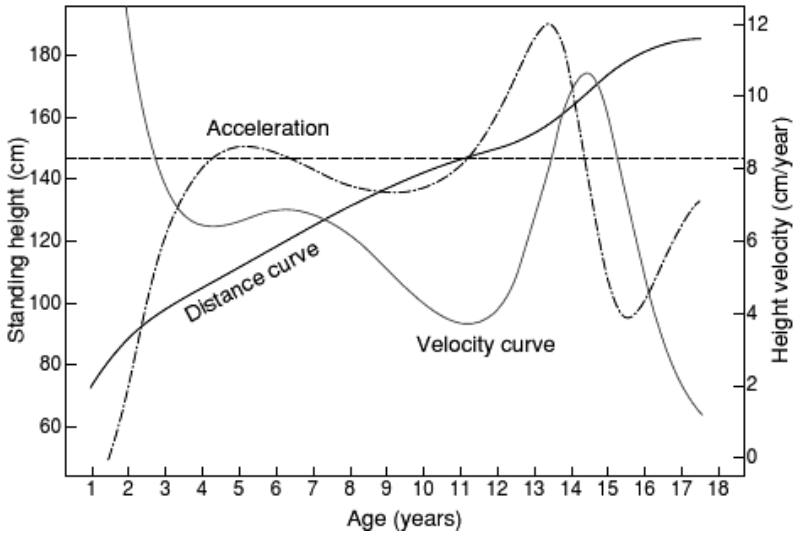

Figure 1: The relationship between the distance and velocity curves. Reproduced from (Molinari and Gasser, 2004) with permission from Cambridge University Press.

As child growth varies by sex and age anthropometric measurements need to be standardized for these characteristics. One approach is to convert these measurement values into anthropometric indices. The most commonly used anthropometric indicator in children is weight-for-age because of the relative ease of obtaining weight measurements. However, this indicator does not distinguish between children that are stunted (a measure of chronic undernutrition) and wasted (a measure of acute undernutrition). Height is therefore measured in addition to weight so height-for-age and weight-for-height can be calculated, and if the values of these indicators are low the child is regarded as stunted and wasted respectively. The use of the body mass index (BMI)-for-age, which is a ratio of weight (in kilograms) divided by recumbent length or standing height (in meters squared), indicator to screen for overweight/obesity in children is also becoming increasingly important given the rise in the circular trend of childhood overweight (World Health Organization, 2006b).

Anthropometric scoring

Given that malnutrition is a clinical syndrome with several phenotypes (for example stunting, wasting or obesity), it can be detected by scoring a child's anthropometric measurements within a distribution of values from a sample of children of the same age and sex. The scoring is generally used for three purposes: screening, surveillance or monitoring. Screening is commonly conducted at a community level to identify children with a disease risk. This activity is performed cross-sectionally using simple-to-use yet precise instruments with the purpose of referring identified children for further investigation or therapeutic assistance. For example, the MUAC tape is used by community health workers to identify children, aged 6-60 months, with severe acute malnutrition (SAM) in community settings. Children with MUAC measurements below 115mm are either referred for further assessment and treatment at a health facility or are managed at the community-level using Ready-to-Use-Therapeutic-Foods (RUTF). After treatment initiation repeat anthropometric measurements can be taken from the sample of malnourished children for surveillance purposes, i.e. to assess the group-level treatment effect on growth. Surveillance may identify an individual child requiring more intense follow-up and clinical examinations (Cameron, 2012, World Health Organization and UNICEF, 2009).

The position of a measurement within the distribution can be expressed using three classification systems: standard deviation scores (Z-scores), percentiles or percent-of-median. The Z-score system has the following characteristics that make it more agreeable to use than the other approaches: 1) for normally distributed measurements the Z-scores can be expressed as means and standard deviations to describe the group-level nutritional status or growth rates and 2) Z-scores can be used to assess longitudinal growth and 3) as the Z-score distribution is on a linear scale from -∞ to ∞, changes in Z-scores at the extremes of the distribution can be quantified. Although percentiles are easier to interpret in clinical practice they are less preferred in research because they are calculated on the rank scale that ranges from 0 to 100. This feature makes it difficult to quantify changes in percentiles at the extremes of distribution (Wang and Chen, 2012). The percent-of-median can also be used to describe non-normally distributed data and is easier to estimate than Z-scores and percentiles. One major disadvantage of using this system is that the estimation of the percent-of median does not take the distribution of the reference population into account therefore the interpretation of the estimate changes in accordance with the age of the child (Gorstein et al., 1994). The Z-score system was used in this thesis, and is defined as the number of standard deviations below or above the mean as seen in Equation 1, where z denotes the Z-score, x the measurement value, μ the mean of the measurement values and σ the standard deviation of the measurement values (World Health Organization, 1995a).

$$z = \frac{x-\mu}{\sigma} \qquad \textbf{[Equation 1]}$$

The lambda-mu-sigma (LMS) model values are added in the Z-score estimation to 1) allow μ and σ to change with age so that the Z-score is standardized for the child's age and 2) account for any skewness in the distribution of the measurement values, such as those for weight (Cole and Green, 1992), see Equation 2:

$$z = \frac{[\frac{(y+\delta)}{\mu(t)}]^{\lambda(t)}-1}{\sigma(t)\lambda(t)} \qquad \textbf{[Equation 2]}$$

In this equation lambda (λ) is the Box-Cox power that transforms skew measurement values to a normal distribution, mu (μ) is the mean, sigma (σ) is the coefficient of variation (ratio of standard deviation to the mean), t is the child's age and y the measurement value (absolute value for attained Z-score and increment for velocity Z-score). When λ=1 the standard deviation of x is equal to the coefficient of variation of y. For weight velocity Z-score calculations a constant, delta (δ), is added to the weight increments to ensure their values are above zero (Cole and Green, 1992, World Health Organization, 2009b). In this thesis, as in many research studies, external rather than internal Z-scores were computed using LMS values from growth standards.

Growth references and standards

The classification of a child as malnourished requires the clinician or researcher to compare the anthropometric values of the child against those of a child of the same age and sex in a growth reference or standard. The fundamental difference between growth references and standards is that the former is a descriptive distribution of anthropometric measurements taken from a group of children in a particular geographical region and time while the latter is a prescriptive set of standards that describes how children should grow under optimal conditions (de Onis et al., 2006). Examples of a growth reference include the 1977 National Centre for Health Statistics (NCHS) which has been used to analyse growth in several African studies (Bakaki P., 2001, Bobat et al., 2001, Isanaka et al., 2009, Lepage et al., 1996). However these references were inappropriate for assessing the growth of non-US based child populations, particularly those that are breastfed in the first few months of life (de Onis et al., 2007), highlighting the need for an international growth chart such as the 2006 WHO growth standard (de Onis et al., 2007, World Health Organization, 2009a).

WHO attained growth international standards

In 1997, WHO initiated the Multiple Growth Reference Study (MGRS) to collect growth data from children living in diverse settings using stringent inclusion criteria. Data collection took place between July 1997 and December 2003 in six countries (Brazil, Ghana, India, Norway, Oman and USA) so as to make the growth standards relevant for children living in different contexts worldwide. As a growth standard is a prescriptive distribution of how healthy children should grow when living in optimal conditions, only healthy children born to non-smoking mothers living in socio economic conditions that did not constrain growth and who were exclusively or predominantly breastfed for at least four months, were introduced to complementary foods by 6 months and were still breastfeeding at 12 months, were included in the study (de Onis et al., 2004). The WHO child growth standards were released in April 2006 and are available for weight, length/height and BMI (World Health Organization, 2006b).

WHO growth velocity international standards

In addition to the standards for attained growth, in 2009 the WHO also published prescriptive international standards for growth velocity , which is the rate of growth (Tanner, 1990), that made it possible to compare the growth rates of their study children against those of a sample of relatively healthy predominantly breastfed children (World Health Organization, 2009b).

Growth velocity standards are used to assess the rate of growth over a period of time so they measure growth rather than attained status. WHO growth velocity standards were developed from the longitudinal (birth till 24 months) data collected in the MGRS study. Weight and length measurements were taken at the following intervals: birth; 1, 2, 4, 6, and 8 weeks; and 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, 22, and 24 months. The WHO growth velocity curves are therefore appropriate for assessing the rapidly changing rate of growth in children in the first two years of life. These prescriptive growth velocity standards were published for velocity of weight, length and head circumference over various intervals (World Health Organization, 2009a) and have not been extensively applied yet.

Tanner (1978) suggests that using growth velocity, a measure of current rate of growth, to assess child growth would enable one to detect growth faltering earlier than if one were to use attained growth for age which assesses cumulative growth. The foetus develops in the uterus of the mother in a system, known as the feto-placental unit, which adapts to stimuli. These intrauterine growth adaptations often lead to catch-up or catch-down growth in the first six months of the postnatal period. Genetic, physiological and environmental factors also contribute towards the varying growth rates, characterised by periods of rapid and slow growth, observed in individual children in the postnatal period. Successive periods of consistently high or low growth velocity can be an indication that the infant is at risk of a disease or is diseased**.**

Intergrowth-21st standards for foetal and newborn growth

Similar to the WHO standards, the Intergrowth-21st international standards for newborn weight, length, and head circumference by gestational age and sex were generated using data from exclusively/predominantly breastfed infants from several countries (Brazil, Italy, Oman, UK, USA, China, India and Kenya) so they can be applied in different settings. These standards are prescriptive and can be used to assess foetal and infant growth for term and pre-term infants (Villar et al., 2014). Conceptually they are therefore an extension of the WHO standards which were constructed using data from term-born infants.

Cut-off points and definitions

Cut-off points for anthropometric indicators can be established using risk-based and statistical methods. The most commonly used statistically defined cut-off points of -2 and -3, which define moderate and severe undernutrition respectively, are arbitrary as in reality children that fall below these cut-off points are often a combination of physiologically undernourished and healthy children (Pelletier, 2006). A low weight-for-length Z-score (WLZ) or WHZ denotes that the child is wasted, a measure of acute malnutrition probably due to a sudden loss of

weight or a failure to gain weight as commonly seen in children with diarrhoeal episodes. Historically, wasting has been referred to as protein energy malnutrition (PEM), kwashiorkor (children with low WHZ or MUAC with oedema) or marasmus (children with wasting without oedema) (Lenters et al., 2016). These definitions have now been replaced with the term "severe acute malnutrition (SAM)", which defines children with WHZ<-3 or MUAC<115mm or bilateral oedema (World Health Organization, 2013b). Another term, "moderate acute malnutrition (MAM)", defines children with WHZ between -2 and -3 or MUAC 115mm to <125mm (World Health Organization, 2012a). A low length-for-age or height-for-age Z-score (LAZ or HAZ respectively) indicates that the child is stunted and is a measure of chronic undernutrition. A low weight-for-age Z-score (WAZ) indicates that the child is underweight for age (UFA) but does not distinguish between a wasted (tall thin child) and stunted (short child with an appropriate weight) child (Gorstein et al., 1994). As BMI changes with age in childhood and also differs between boys and girls, the BMI classification in children needs to take into account both the age and sex of the child (Dinsdale et al., 2011). Children under-five with BMI-for-age Z-score +1 are defined as "at risk for overweight", while those with BMI-for-age Z-score +2 and +3 are overweight and obese respectively (World Health Organization, 2008b).

For perinatal outcomes universally recognised cut-off points are used to define low birth weight (LBW) as birthweight below 2.5kg, preterm birth delivery (PTD) as birth before 37 completed weeks of gestation and small-for-gestational age (SGA) as birth-weight-for gestational-age Z-score below -1.28, which is equivalent to the 10th percentile (World Health Organization, 1977).

1.1.2 The global burden of under-and over-nutrition in children

Although the global number of undernourished people has decreased since the early 1990's, 795 million people still experience hunger (FAO. et al., 2015). It is for this reason that the second Sustainable Development Goal (SDG) is to 'end hunger, achieve food security and improved nutrition and promote sustainable agriculture' by 2030 (UNDESA, 2015).While global estimates of childhood undernutrition are showing a downward trend, progress towards achieving the World Health Assembly (WHA) goals of reducing stunting by 40% and wasting

to less than 5% by 2025 has been slow. Although the global prevalence of stunting declined from 32.7% in 2000 to 22.9% in 2016, 155 million children in the world are still stunted. Globally, MAM and SAM affected 7.7% (52 million) and 2.5% (17 million) of children under-five respectively in 2016. The global prevalence of childhood overweight increased from 5.0% in 2000 to 6.0% (41 million children) in 2016 and is still on the rise (UNICEF et al., 2017). Consequently more efforts are also required to reach the WHA goal of halting the increase in childhood overweight by 2025.

1.1.3 Double burden of malnutrition in Africa

Progress towards reducing malnutrition has been uneven, with regions such as Africa lagging behind. Africa, particularly sub-Saharan Africa, has some of the highest prevalences of stunting, underweight and wasting in children under-five in the world (Black et al., 2013). In 2016, the prevalences of stunting, wasting and overweight were 59%, 14% and 9.8% respectively in Africa. This region was not only home to more than a third (38%) of all stunted children globally but also the only region that experienced an increase in the absolute number of stunted children since 2000, largely due to the rise in stunting and population growth in Western Africa. In addition, almost a third (27%) of the world's wasted children lived in Africa in 2016. This region also has a high burden of overnutrition, and was home to almost a quarter (24%) of the world's overweight children in 2016 (UNICEF et al., 2017). In South Africa stunting in children persists with the latest estimates demonstrating that 27% children under-five are stunted while 3% are wasted. There is also a concomitant rise in overnutrition with the prevalence of childhood overweight estimated at 13% (National Department of Health et al., 2017).

These data suggest that both under- and over-nutrition often co-exist not just in high income countries, but also in LMICs, as they have common risk factors and etiological pathways that give rise to the double burden of malnutrition.

1.1.4 The developmental origins of malnutrition

As the growth and development of the foetus is influenced by, amongst others genetic and epigenetic factors, malnutrition can start in-utero and even pre-conception. It is estimated that

15-20% of children are born LBW each year globally. In Sub-Saharan Africa the incidence of LBW is approximately 13% (UNICEF, 2016, World Health Organization, 2012b). LBW can result from PTD and/or if an infant is born SGA (Kramer, 2013), both of which are important risk factors for childhood undernutrition in LMICs (Christian et al., 2013, Danaei et al., 2016). In addition, complications from PTD are a significant public health concern as they account for 28% of neonatal deaths globally (Lawn et al., 2005), and are the leading cause of neonatal deaths in South Africa (Msemburi et al., 2016).

Undernutrition in early life can also alter immune function and predispose children to the later-onset of non-communicable diseases (NCDs) and premature death. One of the first studies to explore this phenomenon reported a positive correlation between arteriosclerotic heart disease related adult mortality rates in Norway in 1964-1967, and the infant mortality rates in the same sample decades earlier (1896-1925) (Forsdahl, 1977). Similarly Barker and Osmond (1986) reported a correlation between infant mortality in 1921-1925 in England and Wales and coronary heart disease related adult mortality in the same regions 70 years later (1968-1978). These ecological observations were the precursors to numerous studies that have since showed that children born LBW, a proxy for poor foetal growth, are more likely to die from NCDs such as coronary heart disease , hypertension (Eriksson et al., 2007), stroke (Martyn et al., 1996) and type 2 diabetes (Barker, 1999) in adulthood. Undernutrition during critical intrauterine periods, characterised by rapid cell division and sensitivity to environmental stimuli, may lead to permanent changes in the structure and function of organs, alterations in hormone secretion and metabolism and changes in body size that lead to smaller or physiologically disproportioned children at birth (Barker, 2012). In accordance with the "thrifty phenotype" hypothesis, such changes in the foetus lead to a higher risk of developing NCDs in adulthood particularly if the infant experiences overnutrition in the postnatal environment (Barker and Lampl, 2013, Hales and Barker, 2001). This concept is illustrated in Figure 2, where we see that a LBW baby with limited access to good nutrition can develop into a stunted adolescent girl and if she consumes a calorie-dense diet later in life she can become an overweight or obese woman. Figure 2 is however incomplete as the overweight or obese woman can in turn give birth to a large baby.

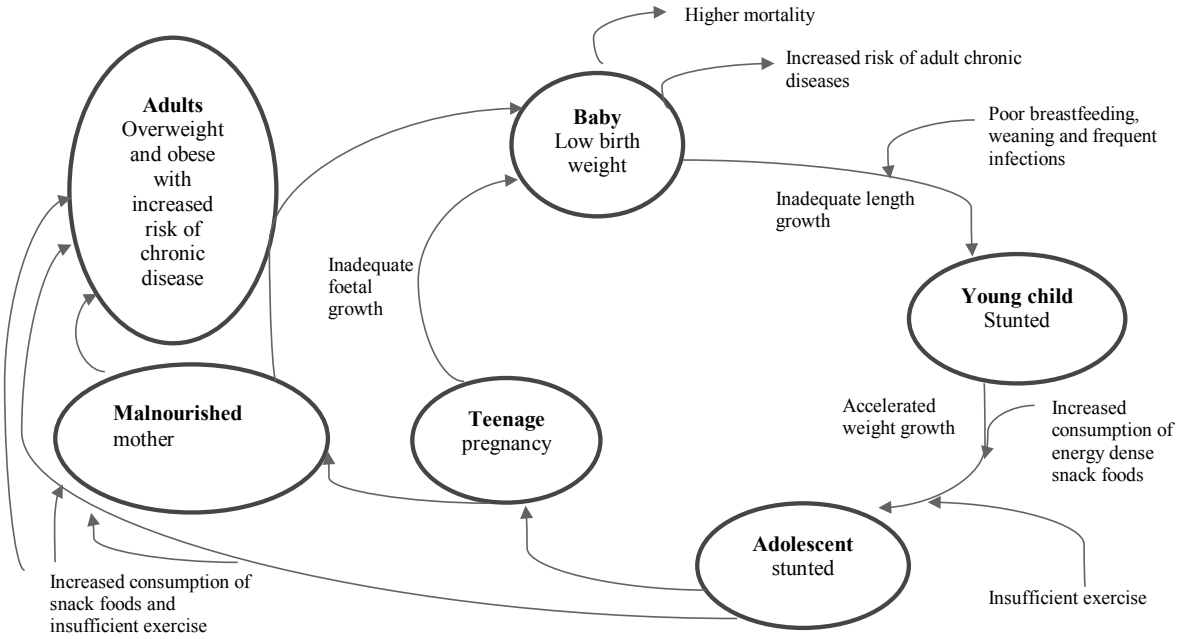

Figure 2: The double burden of malnutrition: causes and effects across the life course. Reproduced from (Shrimpton and Rokx, 2012) with permission from The World Bank.

Brenner and colleagues further hypothesized that poor foetal growth may lead to a reduction in cell numbers in the kidneys, which are prioritised less in-utero compared to organs such as the brain, thereby leading to the later development of hypertension and kidney disease (Brenner et al., 1988, Luyckx and Brenner, 2015). NCDs, including lung disease and cancer, are a major public health concern as they are a long-term burden on the health system, communities and affected households. In 2014 NCDs accounted for 16 million premature deaths in adults globally. The majority (82%) of these deaths occurred in LMIC. The situation in South Africa is also dire as it is estimated that adults aged between 30 and 70 years had a 27% probability of premature death from cardiovascular disease, cancer, diabetes or chronic respiratory disease in 2012 (World Health Organization, 2014b). The consequences of poor foetal growth can therefore be far-reaching across the life-span; it is for this reason that a global target has been set to reduce LBW by 30% by 2025.

1.1.5 The multifactorial nature of malnutrition

Malnutrition is a complex phenomenon with numerous risk factors operating at different levels (Figure 3). Although Figure 3 describes the main causes of undernutrition some of these risk factors also apply to overnutrition. At the immediate level are the individual causes of malnutrition and they include decreased intake of nutrients, poor absorption or metabolism of nutrients and increased demand for nutrients as occurs during infection.

1.1.6 Malnutrition and immune dysfunction

Malnutrition and infection are closely linked. When they exist in a synergistic relationship infection worsens malnutrition while, on the other hand, malnutrition increases the risk of infection and compromises the ability of the infected individual to fight infection. In such instances the combined effect of malnutrition and infection is more intense compared to the sum of the effects of malnutrition and infection alone (Scrimshaw et al., 1968). The development of the immune system is sensitive to nutritional cues, particularly in the first 1000 days of life (Bourke et al., 2016). In children severe undernutrition and various micronutrient deficiencies interfere with several immune defence mechanisms, such as antibody production and cell mediated immunity (Scrimshaw and SanGiovanni, 1997), thus increasing susceptibility to infections. As exemplified by diabetics who are prone to diabetic foot infections caused by pathogens such Staphylococcus aureus and Pseudomonas aeruginosa, overnutrition and related NCDs can also increase the risk of infection (Schaible and Kaufmann, 2007). Infections can lead to or worsen malnutrition by lowering the availability of nutrients, as seen in children that develop environmental enteropathy—a condition characterised by an impairment of the integrity and function of the small intestine through villous atrophy, increased permeability, inflammation and poor absorption of nutrients (Bourke et al., 2016, Humphrey, 2009). Infections may also lead to undernutrition by stimulating immune responses that require additional energy or by inducing a loss of appetite and food intake (Schaible and Kaufmann, 2007).

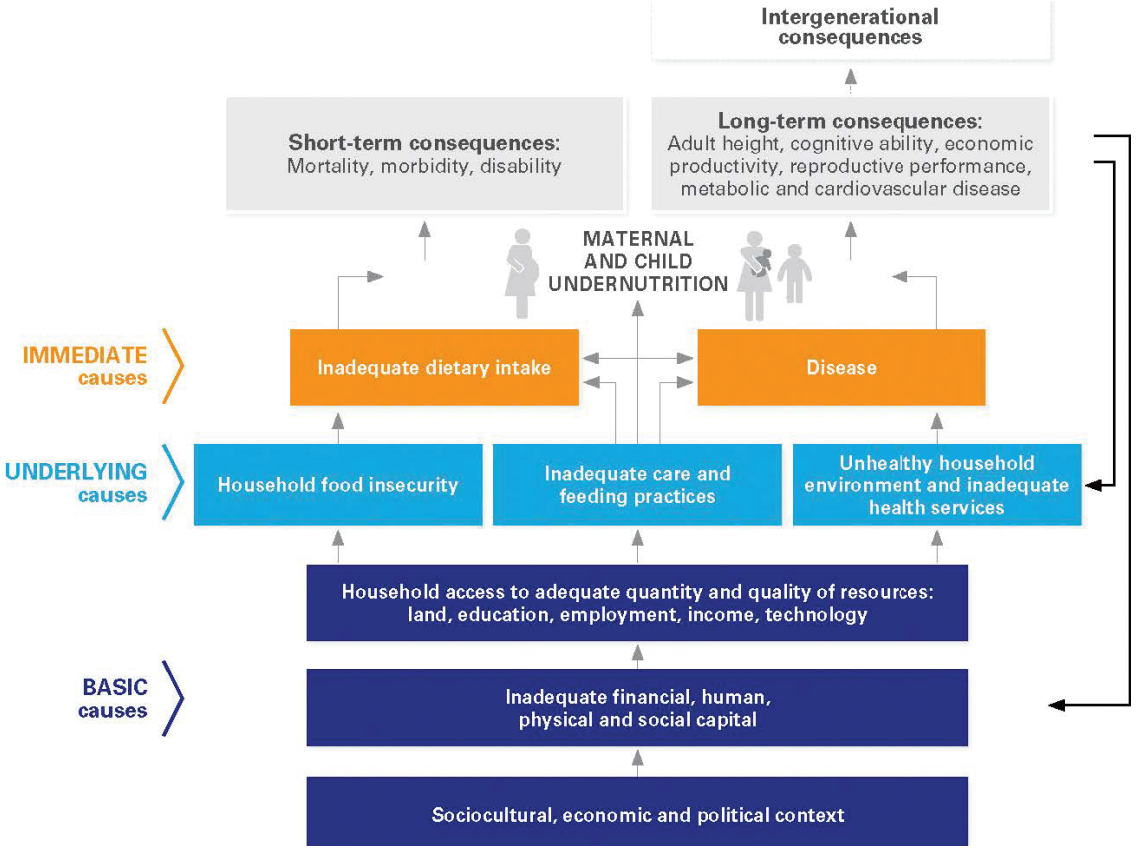

Figure 3: UNICEF conceptual framework for the causes of malnutrition. Reproduced from open source documents: (UNICEF, 1990, UNICEF, 2015)

1.2 HIV in South Africa

1.2.1 35 years of HIV and AIDS: policy development and outcomes

HIV/AIDS remains a global health problem, with 1.9 million new HIV infections reported in adults in 2015 and less than half (38%) of people living with HIV (PLHIV) virally suppressed (UNAIDS, 2016b). South Africa is home to 1 in 5 of PLHIV globally (South African National AIDS Council and National Department of Health, 2017) and has thus the highest number of people living with HIV/AIDS for a single country, estimated at 7.1 million in 2015. Further, it has the largest antiretroviral therapy (ART) programme in the world with approximately 3.7 million HIV-positive adults on ART (UNAIDS, 2016a).

The first cases of acquired immunodeficiency virus syndrome (AIDS) in South Africa were reported in two homosexual men in 1982 (Ras et al., 1983). By the early 1990's the HIV epidemic was rapidly increasing in the general population and mother to child HIV transmission (MTCT) from infected pregnant and breastfeeding women to their children was becoming a huge concern. Without any intervention, in breastfeeding populations, up to 45% of HIV-exposed children can be infected with HIV (De Cock et al., 2000). As transmission can take place in-utero, during labour and delivery or via breastfeeding, the prevention of mother to child transmission of HIV (PMTCT) programme involves interventions that target primary prevention in women of reproductive age, preventing unwanted and unintended pregnancy in HIV-positive women, reducing MTCT during pregnancy, delivery and breastfeeding and care and treatment of the mother and family (Luzuriaga and Mofenson, 2016). The early years (up to 2002) of the South African PMTCT and ART programme were marked with controversy around the government's reluctance to provide antiretroviral (ARV) drugs to infected people because of safety and affordability concerns. Once the Medicines Control Council (MCC) of South African had assessed the safety of Nevirapine (NVP) and registered the drug the government piloted the PMTCT programme in 18 purposively selected sites (two from each of the nine provinces) during 2000-2001. The PMTCT policy at the time recommended voluntary counselling and testing, NVP to HIV-positive mothers during labour

and to their infant during the first 72 hours of life, infant feeding counselling and free provision of commercial infant formula to avoid HIV transmission through breastfeeding (Burton et al., 2015). This programme was scaled-up nationally between 2002 and 2007 by order of the Constitutional Court (Abdool Karim et al., 2009). It has since evolved from providing i) Zidovudine (ZDV) prophylaxis or triple antiretroviral treatment (ART) using criteria-based strategies that determine eligibility for ART (2008 and 2010 (WHO PMTCT 'Option A') PMTCT guidelines); to ii) ART for all HIV-positive pregnant women throughout pregnancy and breastfeeding with ART cessation after the MTCT risk period ceases for those who do not need ART for their own health ('Option B' in the April 2013 guideline); to iii) 'Option B+' in January 2015 which recommends lifelong ART ('akin to 'universal test and treat') for all pregnant and lactating women irrespective of their CD4 cell count or clinical staging (Figure 6). Notwithstanding such advancements in reducing MTCT there is emerging evidence that ART use in pregnancy could carry risks for the foetus, including poor birth outcomes such as PTD (Jao and Abrams, 2014).

1.2.2. The consequences of malnutrition and HIV infection: mortality

In South Africa, the contribution of HIV/AIDS to deaths in children under-five and young adults (15-49 years) peaked in 2005 (47%), Figure 4. Following the scale-up in ART and PMTCT coverage and access (Johnson, 2012, Pillay et al., 2014), this has been sharply declining, contributing to 19% of under-five deaths in 2012 (Bradshaw et al., 2016). As the proportion of HIV/AIDS related under-five deaths has decreased in South Africa, other causes of child death have come to the fore (Figure 4). Vital registration mortality data for children under-five shows that the proportion of deaths directly caused by undernutrition, particularly severe undernutrition, has remained fairly unchanged at around 4% since 2000 (Figure 4). The impact of undernutrition on child mortality is however much greater as it is an underlying cause of approximately a third of child deaths (Bradshaw et al., 2003).

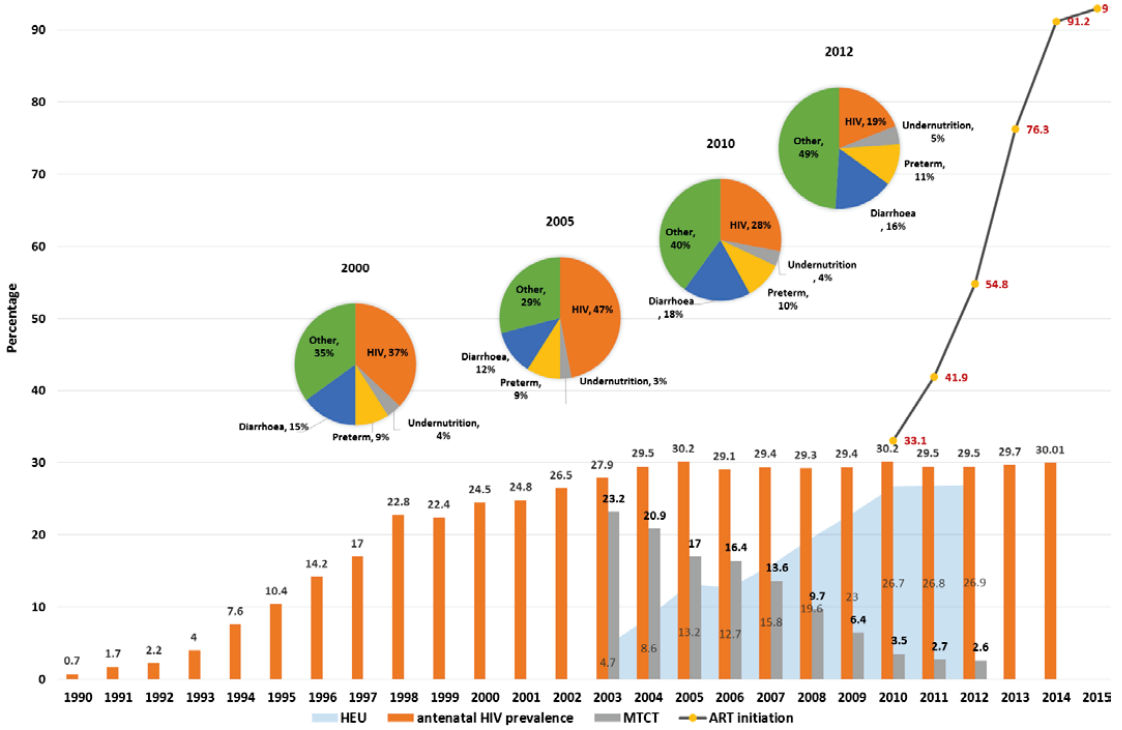

Figure 4: Antenatal HIV prevalence, ART initiation, MTCT, HEU children and causes of under-five mortality in South Africa. Note: Undernutrition includes children who had mild-to-moderate malnutrition, marasmus, kwashiorkor, marasmic kwashiorkor etc. Sources: (Bradshaw et al., 2016, Goga AE et al., 2014, Health Systems Trust, 2015, Health Systems Trust, 2016, Msemburi et al., 2014, Msemburi et al., 2016, National Department of Health, 2013, Sherman et al., 2014).

1.3 HIV and infant feeding

The underlying causes of malnutrition include household food insecurity, unhealthy household environment, inadequate health services and inadequate infant care and feeding practices. It is now widely recognised that breastfeeding is the ideal infant feeding mode in the first few months of life. Breastmilk not only has all the necessary nutrients to support optimal growth and development, but also has other advantages including antibodies to protect against common childhood infections such as those that cause diarrhoea. Additionally breastmilk is important for the maintenance of a normal microbiome that protects against dysbiosis which has been associated with allergies, auto-immune and chronic inflammatory conditions (Akre, 1989, Huang et al., 2017). This is particularly important in LMIC settings, where access to amenities such as a refrigerator and a stove for storing and preparing replacement foods may be limited, and where replacement feeding may not be affordable and safe.

Although there is limited national data on infant feeding practices in South Africa, up until recently the available historical data showed that more than 80% of children were ever breastfed and less than 10% were EBF until 6 months by the early 2000s (National Department of Health et al., 1998, National Department of Health et al., 2003). Although the proportion of EBF children still shows a decreasing pattern with infant age, data from the 2016 South African Demographic and Health Survey (SADHS) shows that the percentage of children that are EBF until 6 months increased from 8% in 2003 (National Department of Health, 2004) to 32% in 2016 (National Department of Health et al., 2017). The emergence of the HIV epidemic in South Africa further complicated infant feeding practices as ARVs were not widely available for all HIV-positive women in the 2000's. As 15-20% of HIV-exposed children could get infected through breastmilk in the absence of ARV drug interventions (De Cock et al., 2000), in October 2000 WHO recommended avoidance of breastfeeding for HIV-positive women if replacement feeding was acceptable, feasible, affordable, sustainable and safe (AFASS). If the AFASS criteria could not be met then EBF was recommended for the first 6 months of life and cessation of breastfeeding encouraged as soon as replacement feeding was AFASS to minimize MTCT risk. In 2006 the guidelines

were updated to include a recommendation that encouraged continued breastfeeding if replacement feeding was still not AFASS by 6 months postpartum (World Health Organization, 2006a). In line with these WHO recommendations the South African National Department of Health (NDoH), between 2001 and 2012, provided free commercial infant formula in public health facilities (Ijumba et al., 2013). Routine programme data from 2002 shows that 67% and 57% of rural and urban women enrolled in a PMTCT programme respectively expressed an intention to formula feed (Doherty et al., 2005). However, shortages of formula milk at the health facilities, cultural factors, lack of disclosure and the stigma associated with formula feeding made it difficult for mothers to exclusively formula feed for 6 months (Doherty et al., 2006). This strategy was associated with a "spillover" effect into the HIV-negative population, who generally initiated breastfeeding after delivery but did not exclusively breastfeed for six months because of early introduction of solid foods and/or infant formula (Goga et al., 2012). Furthermore early cessation of breastfeeding was prevalent among both HIV-positive and -negative women (Doherty et al., 2012).

The 2010 WHO HIV and infant feeding guidelines were consistent with previous iterations except that, where ARVs were available, HIV-positive women were encouraged to EBF for 6 months, introduce appropriate complementary foods at 6 months and continue to breastfeed until 12 months postpartum (World Health Organization, 2010). Following these guidelines the South African NDoH, in its August 2011 Tshwane Declaration of Support for Breastfeeding, adopted EBF for 6 months as the national infant feeding strategy for all women and by September 2012, discontinued the provision of free infant formula as part of the PMTCT programme in public health facilities.

In 2016 the WHO released an update of the 2013 consolidated ARV drug guidelines (World Health Organization, 2013a) that recommend lifelong ART for all HIV-positive people including pregnant and lactating women (World Health Organization, 2016). The risk of vertical transmission can be drastically reduced amongst HIV-positive breastfeeding women who are using ART. Furthermore it was noted that 1) replacement feeding is associated with a higher risk of child morbidity and mortality in resource constrained settings compared to

breastfeeding, 2) breastfeeding improves growth outcomes (Zunza et al., 2013), and 3) longer duration of breastfeeding is not only protective against infectious illnesses such as diarrhoea and pneumonia (Mallampati1 et al., 2015) but may also offer protection from later obesity (Singhal and Lanigan, 2007). Consequently the WHO 2016 guideline on HIV and infant feeding unequivocally recommends EBF for 6 months for all women, irrespective of their HIV status, provided HIV-positive women are on ART and are virally suppressed, and appropriate complementary feeding from 6 months onwards with continued breastfeeding until 2 years postpartum. Additionally, the update accepts any breastfeeding and mixed breastfeeding rather than avoiding breastfeeding (World Health Organization and United Nations Children's Fund, 2016). These new guidelines were adopted in South Africa in 2017 (Pillay, 2017). Recognising the current low EBF rates in many parts of the world, the WHA has now set a global target to increase the 0-6 months EBF rates to at least 50% by 2025 (World Health Organization, 2014a).

1.4 The rationale for the PhD

Given the contribution of malnutrition to childhood mortality and morbidity in the context of HIV, this thesis sought to answer specific questions (detailed in chapter 2) around infant growth in the context of HIV.

1.4.1 Analytical framework

As this thesis considered both maternal and infant factors in the assessment of infant growth, a life course approach was taken into consideration (Ben-Shlomo and Kuh, 2002) which covered the antenatal period up through to two years postpartum. This approach is based on the idea that prenatal or early life exposures can influence disease risk and later health outcomes. Two of the research studies in this thesis recruited participants antenatally and followed them up postnatally. The availability of repeat measures for both the exposures and outcomes in these studies made it possible to assess how early life exposures affect later growth rates and outcomes. The life course approach could also be applied in the third study which, although it had a cross-sectional design, had data on prenatal exposures, and birth and postnatal outcomes. With regard to the multifactorial nature of malnutrition, this thesis primary focussed on the immediate and underlying causes, within the context of PMTCT policy changes (Figures 5 and 6).

1.4.2 Paper I

Breastmilk is not only a good source of nutrition for the growing infant but also confer essential passive immunity to the infant. Furthermore, breastmilk has immunological properties that not only protect the infant from infections but also stimulates the development of the infant's immune system (Akre, 1989). Although the WHO recommends EBF for the first 6 months of life (World Health Organization and United Nations Children's Fund, 2016), South Africa, a country that endorsed this infant feeding strategy for both HIV-positive and –negative women in 2011 (National Department of Health, 2011), continues to have relatively

low EBF rates (National Department of Health et al., 2017) (Figure 6). Furthermore, early cessation of breastfeeding and introduction of complementary foods are common practices among both HIV-positive and -negative women. The first 1000 days of life from conception until 2 years postpartum, are recognised as a critical period whereby exposures can permanently alter the growth and developmental trajectory of a child. For example, evidence suggests that intrauterine growth retardation can "programme" the expression genes and ultimately lead to later non-communicable diseases such as cardiovascular disease and type 2 diabetes (Barker, 1991b, Barker, 1991a, Barker, 1993, Barker, 1999). Based on this background, this paper sought to understand how feeding practices affect growth velocity amongst HIV-unexposed children in the critical early months of life and how both these factors are related to later overweight and obesity.

1.4.3 Paper II

It is now widely accepted that malnutrition, through alterations in the immune system function, increases susceptibility to infectious disease and that infectious disease in-turn worsens malnutrition (Scrimshaw and SanGiovanni, 1997, Scrimshaw et al., 1968). Empirical data from fifteen studies that evaluated the association between infant HIV infection and growth in LMICs supports this assertion and demonstrates poor postnatal growth in HIV-positive children (Isanaka et al., 2009). PMTCT interventions increase the chance of HIV-exposed children to remain HIV-uninfected (Filteau, 2009, Mofenson, 2015). As the effect of HIV exposure on the infant is unclear this paper set out to ascertain whether HIV exposure has any effect on the postnatal growth rates of a cohort of children that were followed-up before the availability of ART. Furthermore, of the few studies that have evaluated the effect of HIV infection and exposure on child growth, almost all of them assessed attained growth between HIV-exposed and -unexposed children (Isanaka et al., 2009). This paper went a step further and studied both attained growth and growth velocity so as to better understand rates of growth of HIV-exposed and -unexposed children .

1.4.4 Paper III

Notwithstanding the benefit of ART for preventing MTCT, some studies have reported adverse birth outcomes with in-utero ART exposure (Jao and Abrams, 2014). These observations could potentially have significant public health implications given that many countries around the globe, including South Africa, have now adopted the WHO PMTCT Option B+ strategy of initiating all HIV-positive pregnant women on life-long ART for maintenance of their health and prevention of vertical transmission (National Department of Health, 2014, World Health Organization, 2013a) (Figure 6). The few studies that have assessed these associations in South Africa were hospital based and majority of them had small sample sizes (Aniji et al., 2013, Jao and Abrams, 2014, Malaba et al., 2016, Malaba et al., 2017b, Mehta, 2017, Moodley et al., 2016). This paper therefore sought to evaluate whether in-utero HIV and ART exposure had any adverse effects on infant birth and growth outcomes in a nationally representative sample of HEU children attending primary health care facilities.

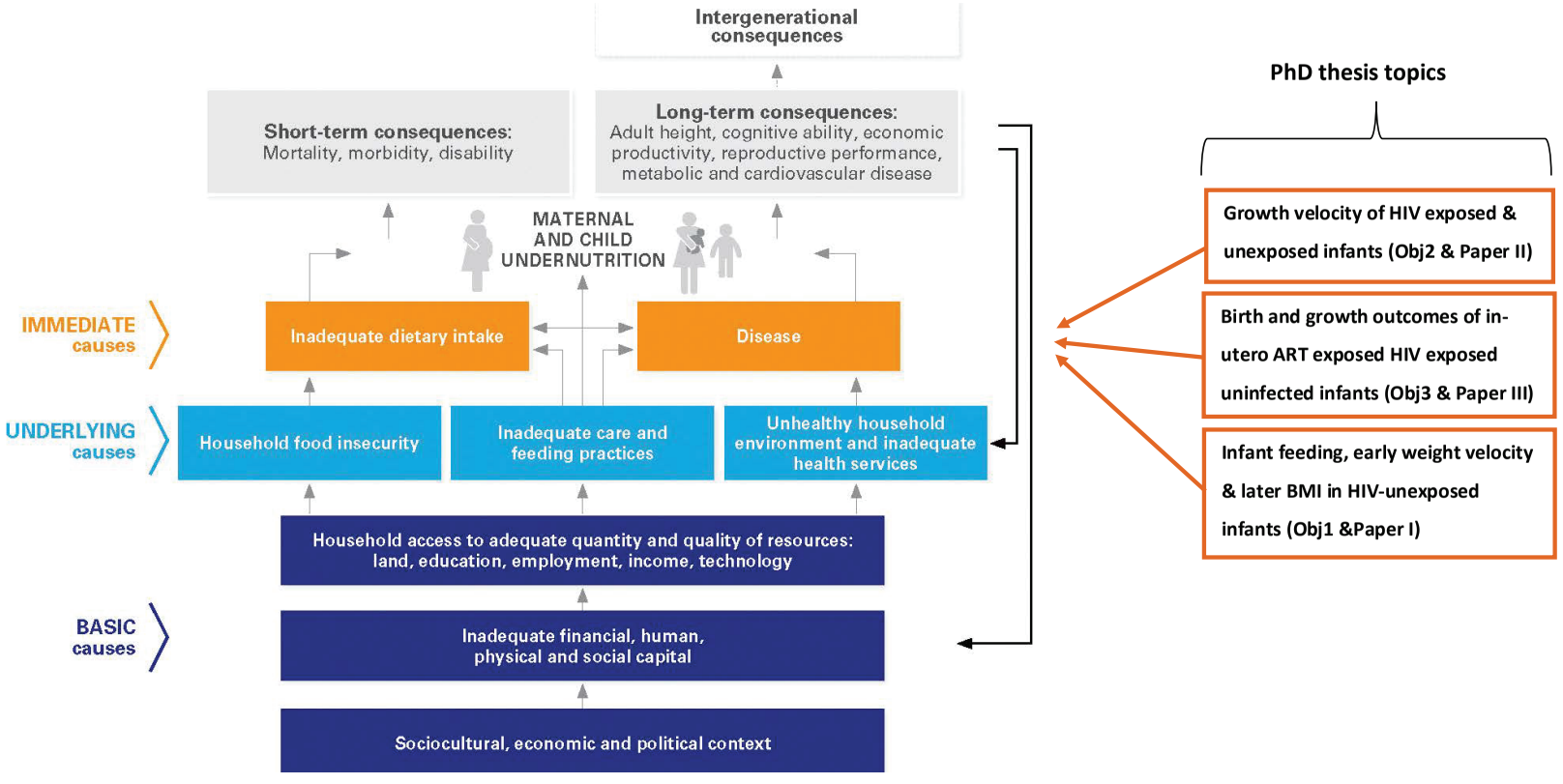

Figure 5: Adapted UNICEF conceptual framework for the causes of malnutrition in relation to the PhD topics. Reproduced from open source documents: (UNICEF, 1990, UNICEF, 2015)

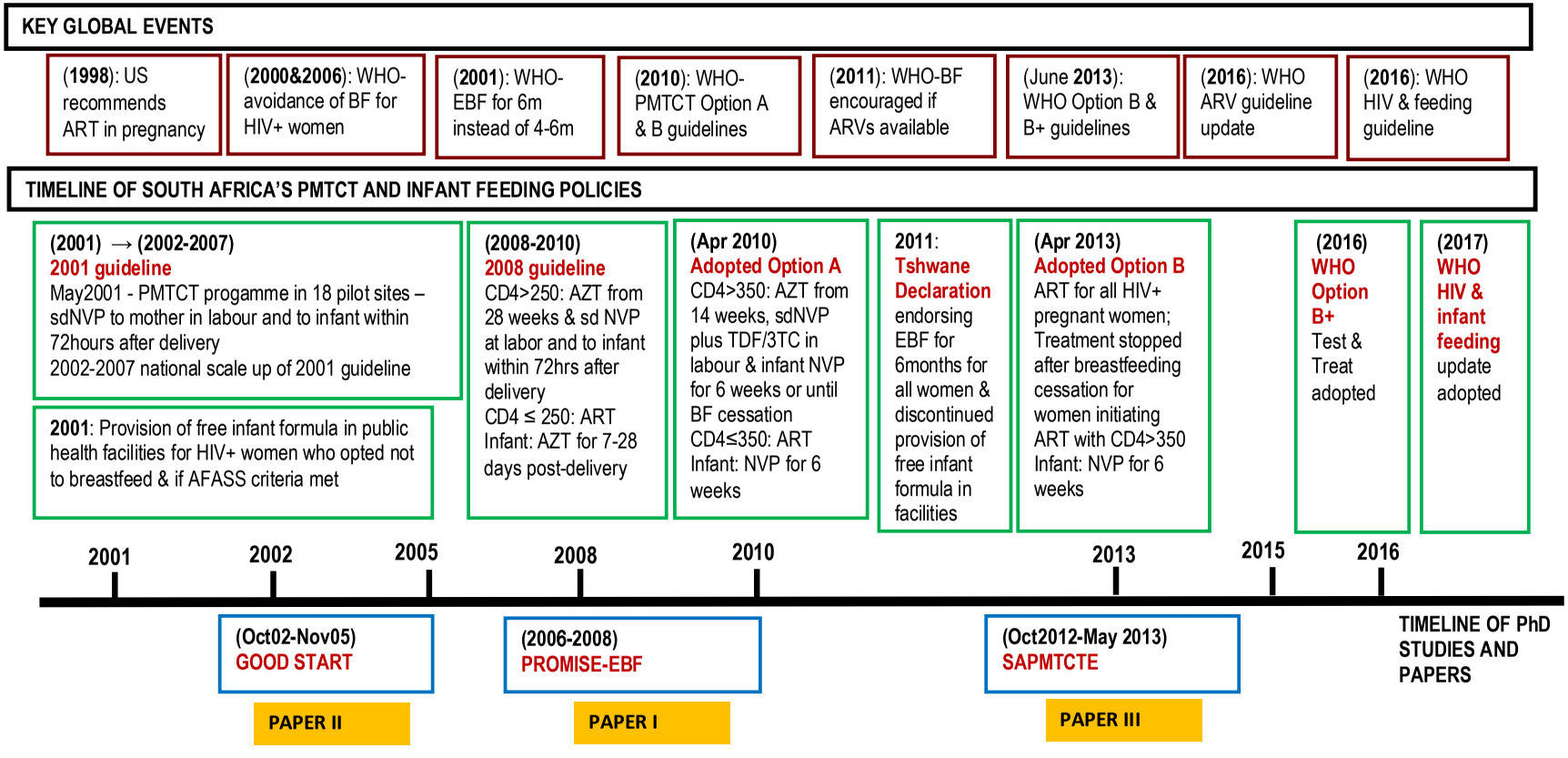

Figure 6: Overview of studies in the context of PMTCT and infant feeding policies in South Africa

Chapter 2: Thesis objectives

2.1 General objective

The general objective of this thesis is to describe child growth and its determinants in South Africa, a country with high malnutrition and HIV prevalences, under different PMTCT policies between 2002 and 2013, using the latest WHO growth standards.

2.2. Specific objectives

- Amongst HIV-unexposed children, to assess (1) the effect of early infant feeding practices on growth velocity in the first 6 months, and (2) the effect of both on BMI-for-age Z-score at 2 years of age using the WHO standards: **Paper I**
- To compare the growth velocity of HIV-exposed and -unexposed children in South Africa using the WHO growth velocity standards: **Paper II**
- To study the effect of in-utero HIV and ART exposure on PTD, LBW and SGA at birth, and UFA at six weeks postpartum in children attending primary health care facilities in South Africa during established implementation of WHO PMTCT Option A policy using the WHO and Intergrowth-21st standards: **Paper III**

Chapter 3: Study participants and methods

This chapter describes the study settings and participants and summarises the methods that were used to collect, manage and analyse the thesis data.

3.1 Study settings

The studies were conducted in South Africa (Figure 7), a country with the lowest fertility rate in sub-Saharan Africa (Moultrie and Timaeus, 2003), estimated at 2.84 in 2001 (Moutrie and Dorringtol, 2004) and declining to 2.57 by 2014 (Statistics South Africa, 2014).

Data for the first and second objectives (papers I and II) were collected from three of the 18 pilot districts selected for the 2001 South African PMTCT programme evaluation. These sites were selected to reflect PMTCT programme effectiveness in settings with varying HIV prevalences and socio-economic conditions. Paarl is a peri-urban area located 60km from Cape Town in the Cape Winelands District of the Western Cape Province. This site was selected because it had relatively better socio-economic conditions than the other areas, an antenatal HIV prevalence of 9.0% at the time of the study and a well-performing PMTCT programme. Most women residing in the area delivered at the Paarl Regional Hospital (Jackson et al., 2007). Rietvlei is a rural area about 90km from Port Shepstone and is located in Umzimkhulu local municipality in the Harry Gwala District (previously Sisonke District) of the Kwa-Zulu Natal Province. At the time of the study Umzimkhulu formed part of Alfred Nzo District in the Eastern Cape Province. In accordance with census 2001 data, this area had some of the most deprived wards in the Eastern Cape at the time of the study (Noble 2010). Rietvlei was selected to evaluate PMTCT programme effectiveness in an under-resourced setting with a high antenatal HIV prevalence (28.0% in 2004)(Barron et al., 2005). Umlazi is a peri-urban township situated approximately 23km from Durban in the eThekwini District of Kwa-Zulu Natal Province. The antenatal HIV prevalence was 47.0% in 2004. This area was selected to assess the PMTCT programme effectiveness in a moderately resourced area with a high antenatal HIV prevalence. Clinics in the area had access to obstetric services at the Prince Mshiyeni Regional Hospital at the time of the study.

Data for the third objective (paper III) were collected in a nationally representative sample of 580 primary health clinics and community health facilities offering immunization services in South Africa. These facilities were part of the South African PMTCT Evaluation (SAPMTCTE) and were selected within each province based on their annual 6 week immunisation coverage and the 2008 national antenatal maternal HIV prevalence.

a) South African map with boundaries for provinces and districts delineated

b) Paarl c) Rietvlei d) Umlazi

Figure 7: Maps showing the study areas

3.2 Data collection

3.2.1 Study designs

The data for this thesis comes from three studies spanning over an eleven year period between 2002 and 2013 (Figure 6 and Table 2).

For the first objective of this thesis (paper I) data were drawn from the PROMISE-EBF study, a cluster randomised trial that recruited women in pregnancy and followed them up at 3, 6, 12 and 24 weeks and 2 years postpartum. Data collection took place between 2006 and January 2008. This study aimed to assess the effect of home-based EBF counselling on EBF rates at 12 weeks.

For the second objective of this thesis (paper II) data were drawn from the Good Start Study, a prospective cohort study that enrolled pregnant women antenatally (between 34 and 36 weeks gestation) or postnatally while still hospitalized. Eight hundred and eighty three consented mother-infant pairs were recruited between October 2002 and November 2004 and followed-up at 3, 24 and 36 weeks postpartum. This study aimed to assess the HIV vertical transmission rate and HIV-free survival by 36 weeks in three purposively selected sites in South Africa.

For the third objective of this thesis (paper III) data were drawn from the nationally representative SAPMCTE cross-sectional survey. Based on their annual 6 week immunisation coverage and the 2008 national antenatal maternal HIV prevalence, 580 primary health clinics and community health centres offering immunisation services were sampled within each province using the probability proportional to size approach. Data collection took place between October 2012 and May 2013. This study aimed to assess early PMTCT impact by measuring MTCT amongst children receiving their six weeks immunisation in selected facilities, regardless of their known HIV exposure or PMTCT status.

3.2.2 Measurements

Standard anthropometric measurement techniques were used to measure weight, using calibrated weight scales, and recumbent length or standing height, using length boards or roller meters and stadiometers respectively, in papers I and II. Routinely collected anthropometric data, obtained using standard clinic protocols and instruments, were analysed for paper III. Routinely collected birthweights and gestational ages, extracted from the participant held road to heath booklets, were used in all three papers. In each study structured questionnaires were used to gather data pertaining to important risk factors for child nutrition, such as the mother's health information including her HIV status and ARV drug use, child morbidity data and infant feeding practices. Infant dietary intake was ascertained using the 24-hour and 7-day dietary recall questions in the questionnaires. Infant blood samples were also collected in the studies to ascertain the infant HIV exposure and infection status (Table 2).

3.3 Data management and analysis

3.3.1 Exposure variables

Only self-reported HIV-negative women and their children were considered in Paper I. The main exposures in this paper were infant feeding practices and growth velocity in the first few months of life. Table 1 shows the infant feeding definitions that were used in this thesis. The child's HIV infection and exposure status, and the additional antiretroviral drug exposure status for children born to HIV-positive mothers, was the main exposure in Papers II and III. In these papers the child's HIV infection status was ascertained by the polymerase chain reaction (PCR) test using dried blood spot specimens obtained from the children by trained data collectors. No HIV testing was performed on the mothers in all the primary studies therefore the HIV exposure status of the children was either determined based on the maternal self-reported HIV status (all three papers) and/or the child HIV antibody test results (paper III). Amongst HIV-infected mothers in Paper III, self-reported ARV use was categorised into three groups: 1) ART-use primarily for mother's health (ART-group), as per Option-A guidelines, 2) antenatal ZDV as MTCT prophylaxis (ZDV-group) and 3) no ARV use

antenatally (None-group). Women on ART were further dichotomised into those who initiated ART pre-conception and those who started ART post-conception. These aforementioned groups formed the ARV exposure categories for the HIV-exposed children.

Table 1: Infant feeding definitions

Variable	Definition
Exclusive breastfeeding (EBF)	Infant received breast milk and no other nutritious foods or liquids. Oral rehydration solution (ORS), syrups (vitamins, minerals, medicines) are allowed
Predominant breastfeeding (PBF)	Infant received breast milk and other liquids e.g. water, fruit juice, ORS, syrups (vitamins, minerals, medicines)
Ever breastfed	Infant was breastfed at any point in time
Mixed breastfed (MBF)	Infant received breast milk with nutritive liquids or solids
Exclusive formula fed (EFF)	Infant received commercial infant formula milk only without any breast milk
Mixed formula fed (MFF)	Infant received commercial infant formula milk with other nutritive liquids and solids, without breast milk

Sources: (Goga et al., 2012, World Health Organization, 2008a)

3.3.2 Outcome variables

In this thesis WAZ, WLZ, LAZ, BMI-for-age Z-scores were estimated to assess postnatal attained growth. These indices were interpreted as follows: children with WAZ, WLZ and LAZ below the minus 2 standard deviation cut-off were classified as moderately underweight, wasted and stunted respectively. Children with BMI-for-age Z-scores above the +2 and +3 cut-off were classified as overweight and obese respectively (World Health Organization, 1995a). Postnatal growth s were described using weight velocity (WVZ) and length velocity Z-scores (LVZ) (World Health Organization, 2009b). For perinatal outcomes universally recognised cut-off points were used to define LBW as birthweight below 2.5kg, PTD as birth before 37 completed weeks of gestation and SGA as birth weight for gestational age Z-score below -1.28, which is equivalent to the 10^{th} percentile (World Health Organization, 1977)

3.3.3 Anthropometric data cleaning

The data cleaning framework depicted in Figure 8 was used to manage the data in this thesis (Van den Broeck et al., 2005). Anthropometric measurements were screened and flagged for verification if they met set criteria. The following criteria were used in Paper I: 1) WAZ<-6 or >5, WLZ<-5 or >5, LAZ<-6 or >6, WLZ>3 and LAZ<-3; 2) extreme changes in LAZ and WLZ (greater than 2.5 or 3) between consecutive visits; 3) BMI-for-age Z-score ≥6. Paper II used these criteria: 1) decrease in length of > 2 cm between consecutive visits; 2) WAZ<-6 or >6, WLZ<-6 or >6, LAZ<-6 or >3, WLZ>3 and LAZ<-3; 3) changes in LAZ >2.5 between consecutive visits and 4) Z-score changes >4 or <-4 between 24 and 36 week visits. Paper III used the following criteria: 1) birthweight-for-gestational age Z-score <-6 or >6; WAZ<-6 or >5. All the flagged observations were assessed and if no reasonable justification could be determined, set to missing.

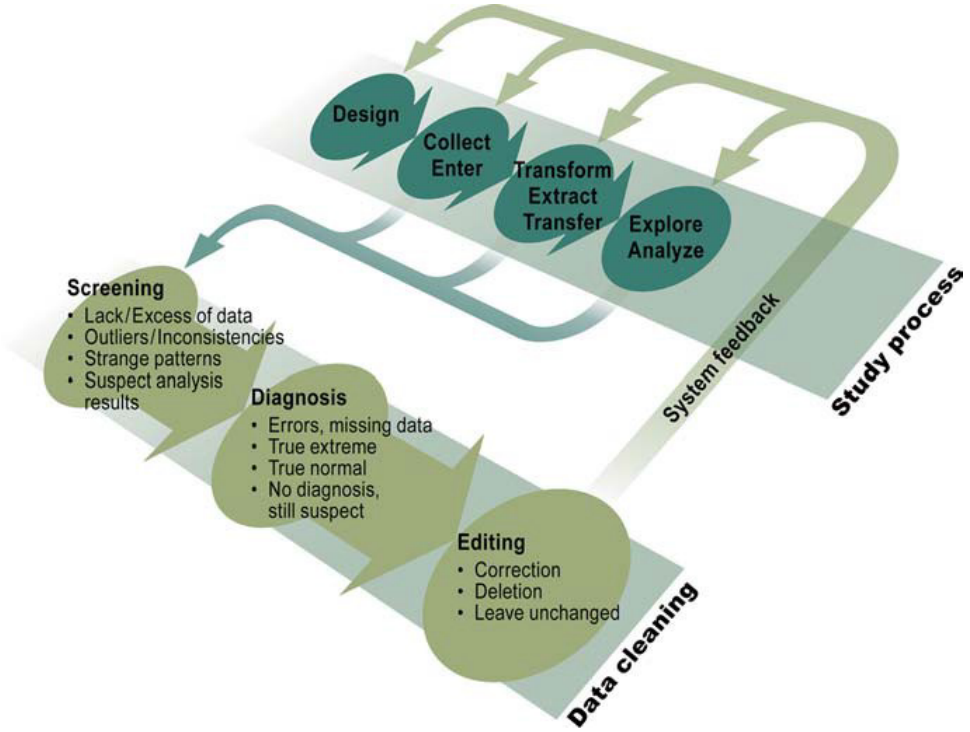

Figure 8: Data cleaning framework. Reproduced from an open access document. Source (Van den Broeck et al., 2005).

3.3.4 Covariates

As aptly stated by Venanzio (1990) “it is not possible to describe the epidemiology of malnutrition with a unidirectional model of causation because its ecology is formed by a complex system in which every variable is affected by a multitude of other variables, giving rise to simultaneous interacting causal models. Poverty, political upheaval, ignorance, taboos and feeding practices…,sanitation, water supply, family size, mother's time for child rearing...low wages, unemployment, epidemics and many other factors interact in a multidirectional way, forming a web of causation whose final outcome is malnutrition.” In this thesis some of the before-mentioned factors (depicted in Figure 9) were selected *a priori* based on their epidemiological or clinical importance and included in the multivariable regression models. As causal relationships were not explored in this thesis, this conceptual framework is descriptive in nature and does not make definitive assumptions about causal pathways, the temporality of certain relationships and possible statistical interactions

3.3.5 Data analysis

Different analytical approaches, ranging from cross-sectional to longitudinal data analysis methods, were used to assess growth in each paper and are summarised in Table 2. Data analyses were performed using STATA (StataCorp), IBM SPSS Statistics and R Software.

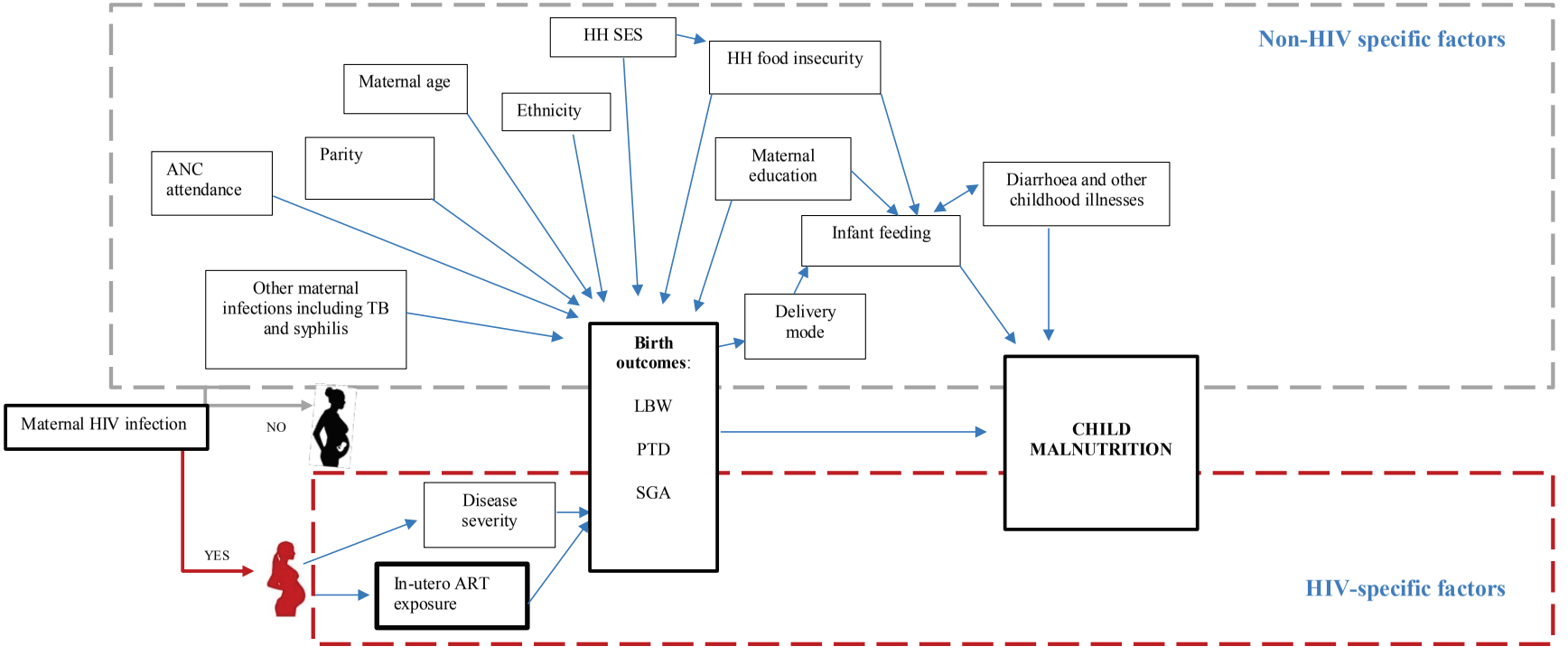

Figure 9: Simplified conceptual framework of factors associated with birth outcomes and child malnutrition Note: The associations depicted in the figure are not necessarily uni-directional and possible statistical interactions are not displayed. Except for ART, which was only considered Paper III, all variables were considered in the PhD papers. Abbreviations: ANC; antenatal clinic, ART; antiretroviral treatment, HH; house hold, LBW; low birth weight, PTD; preterm delivery, SGA; small-for-gestational age.

Table 2: Overview of study methods

Paper	Title	Participants	Methods	Data analyses	Data analysis outcome/s
I	Effects of early feeding on growth velocity and overweight/obesity in a cohort of HIV unexposed South African infants and children	HIV-unexposed infants and their mothers	Study design: Cluster randomised trial of women followed-up at 3, 6, 12, 24 weeks and 2 years postpartum Study sites: Paarl, Rietvlei and Umlazi Interviews: Questionnaires (that included dietary recall questions) were used to conduct face-to-face interviews with the mothers/caregivers Anthropometry: Birthweight extracted from the road to health booklet. Child weight and length measured at 3, 24, 36 weeks and 22 months postpartum using Masskot electronic pan scales, roller meters (Oxford, UK) or Shorr Height-Length Measuring Board (Maryland, USA) & custom-made stadiometer respectively HIV exposure assessment: Self-reported maternal HIV status HIV infection assessment: Infant polymerase chain reaction (PCR) testing at 3 and 24 weeks postpartum	Simultaneous quantile regression 2006 WHO BMI-for-age standard 2009 WHO growth velocity standards	Conditional distribution of BMI-for-age Z-score Mean WVZ and LVZ

II	HIV infection, viral load, low birth weight, and Nevirapine are independent influences on growth velocity in HIV-Exposed South African infants	HIV-exposed infected children, HIV exposed uninfected children and HIV-unexposed children and their mothers	Study design: Community-based prospective cohort study of women enrolled during pregnancy and followed-up at 3, 24 and 36 weeks postpartum Study sites: Paarl, Rietvlei and Umlazi Interviews: Questionnaires (that included dietary recall questions) were used to conduct face-to-face interviews with the mothers/caregivers Anthropometry: Birthweight extracted from the road to health booklet. Infant weight and length measured at 3, 24 and 36 weeks postpartum using Masskot (SOS Series) electronic pan scales and using roller meters (TALC) respectively HIV exposure assessment: Record review HIV infection assessment: Repeat PCR testing at 3, 24 and 36 weeks postpartum	Mixed-effect regression 2009 WHO growth velocity standards	Mean WVZ and LVZ

III	In-utero ART exposure and birth and early growth outcomes amongst HIV exposed uninfected infants attending immunization services: Results from national PMTCT surveillance, South Africa	HIV exposed uninfected and HIV unexposed uninfected children and their mothers	Study design: Nationally representative facility-based cross-sectional survey to measure PMTCT impact at six weeks postpartum Study sites: Primary healthcare and community healthcare facilities in 9 provinces of South Africa. Interviews: Questionnaires (that included dietary recall questions) were used to conduct face-to-face interviews with the mothers/caregivers Anthropometry: Birthweight and 6 weeks weight were extracted from the road to health booklet HIV exposure assessment: HIV antibody testing on infant dried blood spot collected at 6 weeks postpartum HIV infection assessment: PCR testing at 6 weeks postpartum	Multiple correspondence analysis Survey data analysis Logistic regression Intergrowth-21st standards for assessing new born size for term and pre-term born children 2006 WHO weight-for-age standard	PTD LBW SGA UFA

3.4 Ethics

Details pertaining to the ethics approval documents, questionnaires and consent forms for the three primary studies are in the appendix.

3.4.1 PROMISE-EBF Study (Paper I)

Ethical approval for this study was obtained from the University of the Western Cape and the South African Medical Research Council. Informed consent was obtained from participants.

3.4.2 Good Start Study (Paper II)

Ethical approval was obtained from the University of the Western Cape and the University of Kwa-Zulu Natal ethical review boards. All sites used the same standard informed consent and information forms which were developed in English and translated into study languages namely; Xhosa, Zulu and Afrikaans. One consent form was signed for the interviews and another form was signed for HIV related testing of mother and infant. Signed informed consent was obtained from the participants at the time of enrolment into the study. Verbal consent was obtained at each visit or data collection point thereafter, including prior to every blood draw or other specimen collection.

3.4.3 SAPMTCTE Survey (Paper III)

Ethical approval was obtained from the South African Medical Research Council Ethics Committee and United States Centers for Disease Control and Prevention (CDC) Director of Science. Informed consent was also obtained from all mothers/caregivers for participation in the study.

Chapter 4: Summary of Results

This chapter presents a summary of the main findings from the three PhD papers.

4.1 Infant feeding, early weight velocity and later BMI in HIV-unexposed children (Paper I)

Six hundred and forty-one HIV-unexposed children that were followed-up from birth until a median age of 22 months (IQR: 17-26 months), hereafter referred to as the 2 years visit time, were analysed. Although most of the children were initiated on breastmilk early, the proportion of breastfed children decreased in the first 12 weeks of life while the frequency of formula feeding increased. The early introduction of solids such as cereals was also evident in the study. At 2 years, 30% of the children were overweight while 8.7% were obese. In this paper we showed that children who were not breastfed at 12 weeks had higher period-2 (between 12 and 24 weeks) mean WVZ, higher BMI-for-age Z-scores at 2 years and were more likely to be overweight or obese. We also observed a positive association between the period-2 mean WVZ and BMI-for-age Z-score at 2 years.

4.2 Growth velocity of HIV-exposed and -unexposed children (Paper II)

Of the 883 children that were recruited, 783 children (including 65 HIV-positive children, 502 HEU children and 216 HUU children) were analysed. The mean WAZ of HIV-infected children was lower at 3, 24 and 36 weeks than that in the other two groups. Similar results were observed for mean WVZ in period-1 (3-24 weeks postpartum). Although no differences in mean LAZ and WLZ were observed at 3 weeks, HIV-positive children had lower mean LAZ and WLZ at 24 and 36 weeks. These children also had the lowest mean LVZ in period-1. No significant differences in the mean WAZ, LAZ, WLZ, WVZ and LVZ were observed between HEU and HUU children. Mean WVZ varied by feeding mode, with MFF children demonstrating a higher mean WVZ than children that were EBF.

4.3 Birth and growth outcomes of in-utero ART exposed HEU children (Paper III)

Of the 9119 children that were in the final survey sample, 8778 (consisting of 2599 HEU and 6179 HUU children) were included in the analysis. The prevalence (95% confidence intervals) of PTD, LBW, SGA and underweight-for-age (UFA) at six weeks postpartum were 12.5% (11.4%; 13.7%), 10.7% (10.0%; 11.5%), 14.9% (13.8%; 16.1%) and 9.2% (8.6%; 10.0%) respectively in the total sample. As illustrated in Figure 10 below, the prevalence of PTD was similar between HUU (12.3% (11.1%; 13.7%)) and HEU (12.9% (11.12%; 14.7%)) children (P=0.59) in the unadjusted analysis, whereas the latter group had a higher prevalence of LBW (13.0% vs 9.8%, P<0.01), SGA (16.9% vs 14.0%, P=0.03) and UFA (8.4% vs 11.0%, P<0.01). In the multivariable logistic models HEU children demonstrated a higher odds of PTD, LBW, SGA and UFA than HUU children. Within the HEU sub-group, children in the None-group (1.9 (1.1; 3.1)) or those whose mothers initiated ART pre-conception (1.7 (1.1; 2.5)) had almost twice the odds of PTD than children whose mothers initiated ART post-conception. In this study a reduced odds of UFA was observed if the infant was breastfed versus not breastfed while an increased odds of UFA was detected if an infant had experienced diarrheal episodes.

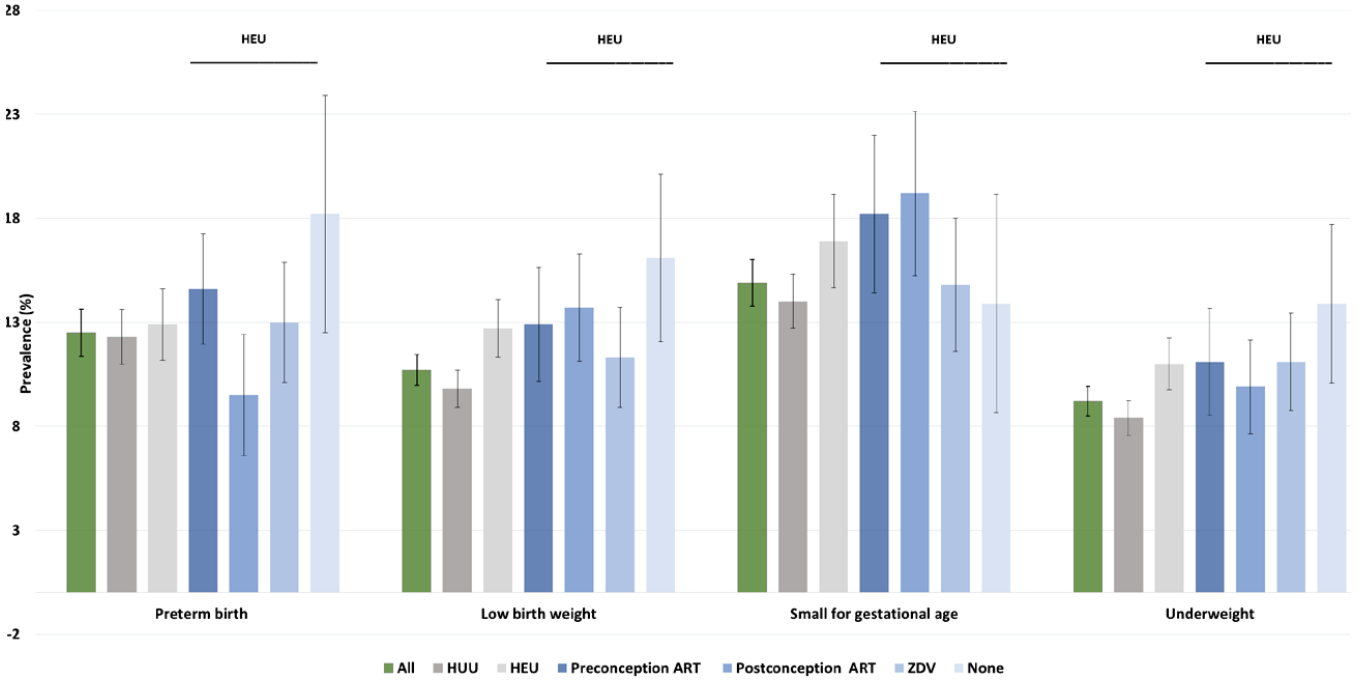

Figure 10: Prevalence of preterm delivery, low birth weight, small-for-gestational age and underweight-for-age at 6week postpartum in HIV exposed uninfected (HEU) and HIV unexposed uninfected (HUU) children. Note: ZDV= zidovudine; None= no ARV drugs received by mother.

Chapter 5: Discussion

5.1 Discussion of main findings and implications

The overall objective of this thesis was to describe child growth and its determinants in South Africa, a country with high malnutrition and HIV prevalences, under different PMTCT policies between 2002 and 2013, using the latest WHO growth standards. To achieve this objective data from two prospective follow-up studies and one nationally representative cross-sectional survey, spanning over an eleven year period between 2002 and 2013, were analysed and the WHO attained growth and growth velocity standards were applied to the data. For the cross-sectional study Intergrowth-21st standards for the foetal growth of both term and preterm born children were also applied. This thesis therefore makes several empirical and methodological contributions to the field of nutritional epidemiology.

5.1.1 Contribution of the thesis and future areas of research

The HIV-infected child

The results presented in chapter 4 contributed to the existing literature by demonstrating that HIV-infected children are not only more underweight, wasted and stunted compared to HEU and HUU children, but also have lower growth rates in the first few of months of life. The majority of the previously published studies only assessed attained growth between HIV-exposed and -unexposed children (Isanaka et al., 2009). Paper II went a step further and assessed both attained growth and growth velocity so as to better understand rates of growth in HIV-exposed and -unexposed children. The study demonstrated that maternal viral load, low birth weight and Nevirapine use were independent predictors of growth velocity in HIV-

exposed children and that HIV-positive children had increased infectious morbidity compared to HUU children.

The HIV exposed uninfected child

Over the past decade significant progress has been made in reducing vertical HIV transmission through the scale-up of PMTCT interventions such as maternal ART use during pregnancy. As a result HEU children now constitute the majority of the children affected by the HIV pandemic (Filteau, 2009, Mofenson, 2015). Very few follow-up studies have examined the growth of HEU children compared to a healthy group of HUU children (the controls) in LMICs. Of the few that have, some did not adequately characterise the maternal and child HIV status, thereby making it difficult to compare growth by HIV exposure and infection status (Isanaka et al., 2009). Paper II, using data from a well characterised group of HIV-exposed and -unexposed children followed-up before the generalised use of ART for PMTCT, demonstrated that HEU children had similar attained growth and growth velocities compared to HUU children in the absence of maternal ART. These findings confirm those reported in other longitudinal studies that were conducted before the wide scale use of ART (Isanaka et al., 2009). However some studies have reported differences in the growth of HIV-exposed compared to HIV-unexposed children (Masaka M., 2007, Rosala-Hallas et al., 2017). The generalised use of ART by pregnant and lactating women in recent years makes it more difficult to measure the true effect of HIV exposure on child growth. Unlike in paper II, and in line with more recent evidence (Evans et al., 2016, Jao and Abrams, 2014, Powis et al., 2016, Sudfeld et al., 2016) data from Paper III, drawn from research conducted when South Africa was scaling up its ART programme, demonstrated that HEU children had poorer birth and early postnatal growth outcomes compared to HUU children. In Paper III, as not all HIV-infected women were on ART, for various reasons including ART eligibility criteria, we could compare outcomes by HIV and ART exposure status. In line with findings from Turner et al. (2013), the results in Paper III demonstrated that HEU children born to women with unmanaged HIV infection had an increased odds of being born preterm than children born to women on ART. These results provide reassuring evidence for the current WHO and South

African PMTCT guidelines that recommend that HIV-infected pregnant women should be placed on ART for maintenance of maternal health status and prevention of vertical transmission (National Department of Health, 2014, World Health Organization, 2013a). Similar to recent data from other observational studies, the results in Paper III also demonstrated that children whose mothers initiated ART before conception had an increased odds of preterm delivery than children whose mothers started ART after conception (Uthman et al., 2017) , but no increased odds for other outcomes. Although the observed associations could be due to selection bias (Stringer et al., 2017), this data suggests that the 1) timing of ART initiation maybe an important predictor of child health outcomes, and 2) need for pregnancy registers or similar pharmacovigilance surveillance systems to monitor adverse outcomes in order to inform future policy. More in-depth research is also required to understand the mechanisms through which in-utero HIV and ART exposure may affect child health outcomes. As a way of unpacking possible mechanisms, Evans et al. (2016) summarised current knowledge in a conceptual framework depicting the complex network of factors that may lead to the growth failure, morbidity and mortality of HEU children through pathways that operate through immunodeficiency and chronic inflammation. Taken together these results suggest that both the exposure to a disease and the infection are important predictors of child malnutrition. This medical dilemma where the need to initiate HIV-positive pregnant women on ART early to improve maternal health and prevent MTCT is juxtaposed against the need to prevent potential drug-related adverse infant outcomes remains to be resolved. Additional in-depth research is needed.

Infant feeding

In line with findings from other studies (Sibiza et al., 2015), results presented in Paper I indicate that poor breastfeeding practices still persist in South Africa despite the known benefits of breastfeeding. Studies have found that breastfeeding can be protective against infectious morbidity such as diarrhoea and respiratory infections, child hospital admissions and mortality (Victora et al., 2016). Findings from Paper I demonstrated that breastfeeding

conferred a protective effect not only against underweight, but was also protective against rapid weight gain (weight velocity) in the first few months of life and, similar to findings in other studies (Singhal and Lanigan, 2007, Victora et al., 2016), later overweight or obesity. This is an important finding as South Africa has persistent chronic undernutrition in children and an increasing burden of overnutrition in the child and adult population (National Department of Health et al., 2017, Said-Mohamed et al., 2015). In addition the prevalence of obesity in South Africa ranks first in Sub-Saharan Africa (Ng et al., 2014). The latest South African Demographic and Health Survey results indicate that 68% of women and 31% of men are overweight or obese (National Department of Health et al., 2017). Breastfeeding, which has been identified as one of the key nutrition-specific interventions for addressing the immediate determinants of foetal and child nutrition, could help curb the rise of childhood overweight or obesity and the subsequent epidemic in the adult population (Ruel et al., 2013). Some studies have found that breastfeeding is not only beneficial for the infant but may also protect the nursing mother against type 2 diabetes; its effect on maternal post-delivery weight change is however inconclusive (Victora et al., 2016). Although the prevalence of EBF in children aged 0-6 months has increased from 8% to 32%, more efforts are required to promote, protect and support breastfeeding in South Africa as it is still below the WHA target of increasing the 0-6 months EBF rates to at least 50% by 2025 (World Health Organization, 2014a).

5.2 Methodological considerations

5.2.1 Study design

This thesis aimed to describe child growth and its determinants in South Africa, a country with high malnutrition and HIV prevalences, under different PMTCT policies between 2002 and 2013, using the latest WHO growth standards. Therefore the study designs used in this thesis were not only descriptive, giving the occurrence of a disease in a population, but also analytical enabling the researcher to examine how particular exposures relate to the development of certain disease outcomes. The following study designs were therefore used:

Cluster randomised trial: Paper I

Although the primary research study for Paper I had a cluster randomised trial design, which is experimental in nature, for the purposes of this thesis the data were treated as data from a cohort study given that the trial intervention (infant feeding counselling (Tylleskar et al., 2011)) was not the exposure of interest in this thesis. The study was therefore treated as a prospective cohort study of HIV-negative women and their children followed-up from 3 weeks until 2 years postpartum. Unlike randomised controlled trials, which try to achieve counterfactuality between the comparison groups by 1) minimising the variation in the outcome that is due to extraneous factors (referred to as confounders) by randomly allocating the treatment or exposure, 2) measuring any residual variation that exists so it can be taken into account in the analyses and 3) blinding the participants and investigators, cohort studies are subject to selection bias which can affect the exchangeability of the comparison groups (Rothman et al., 2008a). To minimise this bias potential confounders were adjusted for in the regression models.

Prospective analytical cohort study: Paper II

The research study for Paper II had a prospective analytical cohort study design which is observational in nature, and entailed following-up mother-infant pairs from pregnancy until 36 week postpartum. This study design enabled us to 1) establish that the exposure preceded the outcome and therefore to examine the association between infant HIV exposure and infection and postnatal growth rates and 2) explore associations between the outcome and more than one exposure. However this cohort study design limited the extent to which we could infer that observed associations were causal in nature because of potential selection bias and confounding. As with most cohort studies, this study had participants that were lost to follow (LTFU) (Myer and Karim, 2007). A comparison of characteristics between participants that were observed until the end of the study versus those that were LTFU failed

to identify significant differences between the groups. Therefore we do not believe that LTFU biased the study results. Furthermore to minimise the possible effect of selection bias potential confounders were adjusted for in the regression models.

Analytical cross-sectional survey: Paper III

Paper III utilised a cross-sectional survey design. Unlike in the cohort studies, which had a follow-up period, in this cross-sectional study the exposures and outcomes were assessed at one point in time. Although this feature makes it difficult for most investigators conducting cross-sectional studies to ascertain the temporal relationships between the exposures and outcomes, the theoretical basis of the main relationships we considered in this study makes us believe that the likelihood of reverse causality was low. However information bias may have affected our results as some of our key variables were not objectively measured. For example, maternal ART use during pregnancy was obtained by recall and the gestational age data that were analysed were estimated by public healthcare facility staff, likely using the last menstrual period (LMP) method which tends to overestimate the prevalence of preterm delivery compared to ultra-sound based preterm estimates (Malaba et al., 2017a). The major strength of this study design is that it is more affordable, compared to study designs such as cohort studies, so it enabled us to examine our research question in a large sample.

5.2.2 Sample sizes

Given that it is impractical to study research questions by collecting data from an entire population, a representative sample of the population of interest is often studied and the study findings generalised at the population level. The research questions addressed in this thesis were not the principal questions in the studies undertaken, therefore the sample sizes and statistical power estimations for the secondary outcomes were not determined *a priori*. Samples sizes that were used in this thesis were therefore the result of the sample size estimations done for the primary research questions.

At the time of the analysis, some participant data were incomplete because 1) they were determined to be implausible during the data cleaning process and therefore coded as missing, or 2) participant non-response during the interviews, or 3) participants were lost to follow-up (LTFU) during the studies. Missing data can introduce bias, type I and II error in an analysis (Little et al., 2016). In Paper I some participants were not followed up until the 2 year visit time. However we do not believe that this LTFU biased the results because the baseline characteristics of the LTFU participants were very similar to those of participants that were observed at 2 years. In addition all three PhD papers used maximum likelihood estimation which models parameters and standard errors using all available data and assumes that any missing data are missing at random (Little et al., 2016). In Paper III survey weights were added to all analyses in order to maintain the sampling structure of the survey structure. Additional weighting for missing gestational age data (which were missing for 29% of the participants) was applied to the PTD and SGA analyses.

5.2.3 Internal validity

Confounding, selection and information bias can compromise the internal validity of a study (Greenland et al., 2008). In this thesis potential confounders were identified *a priori* based on literature and were primarily controlled for using multivariable regression modelling. Only potential confounders that were measured were controlled for therefore residual confounding may have remained after the adjustments in the multivariable regression models. Selection bias is also possible in this thesis as, for ethical and logistical reasons, each study excluded participants in accordance with certain exclusion criteria. For example, sick children needing emergency care, children that died before the 6 week clinic visit and children attending small remote health facilities were excluded from the study that contributed to Paper III. The exclusion of some the most vulnerable children in this study may explain why our rate of LBW was lower than the national estimate which is based on all lives births in South Africa.

To minimise information bias all data collectors in the research studies undertaken for this thesis received standardized training on anthropometric techniques. For papers I and II the anthropometry data collection was also validated periodically in order to improve validity and reduce inter-observer and intra-observer bias. The use of routinely collected anthropometric

data in paper III precluded the ability of the researchers to minimise these biases in paper III. Recall bias is also a type of information bias. The results presented in this thesis are subject to recall bias as certain data were ascertained through recall. For example in all 3 research studies participants were asked questions about what they fed their children in the previous 24 hours and seven days using dietary recall questionnaires. The validity of the dietary information acquired using this method may be compromised because participants may 1) alter their dietary patterns if the study is focused on their diets, a phenomenon called the Hawthorne effect, 2) participants may not recall all the foods they have consumed (Walsh and Joubert, 2007). In addition short-term dietary recall data, such as feeding practices reported for the previous 24 hours or seven days, does not accurately capture long-term feeding patterns (Bland et al., 2003). Nevertheless the use of this dietary assessment method in this thesis has the following advantages: 1) unlike the dietary records method which relies on participants to accurately record their diets, the dietary recall questionnaires were administered by well-trained data collectors so illiterate participants could also be included in the studies, 2) group-level dietary data collected using this method are fairly valid (Thompson and Byers, 1994). The misclassification of participants may have also occurred in this thesis. In Papers I and II no maternal HIV and child HIV antibody tests were done during the course of the studies therefore some of the HIV-negative women may have seroconverted leading to the misclassification of children as HIV-unexposed. In Paper III HIV-positive mothers were further categorized into ARV groups based on self-reported ARV drug use which is subject to recall bias. Therefore some participants may have been misclassified in the ARV groups. All the studies that contributed to this thesis had some degree of missing data including biologically implausible anthropometric measurements that were set to missing during data cleaning. In Paper I mixed effect regression was therefore used to analyze the incomplete longitudinal data as it uses all available longitudinal data without discarding data for children with missing data. These models also adjust for the correlation that exists between repeated measurements taken from the same child or group of children (Fitzmaurice et al., 2011).

5.2.4 External validity

External validity is concerned with generalisability of the study findings to the larger population or populations in other settings (Rothman et al., 2008b) and is closely linked to the inclusion and exclusion criteria that were used to select participants for the study. Therefore the generalisability of the study findings in this thesis is limited to populations that have similar characteristics (such as social, demographic, economic and epidemiological patterns of HIV and malnutrition in the setting) to those of the participants that were studied.

Chapter 6: Conclusions and recommendations

This thesis highlights the importance of not only addressing the double burden of malnutrition in South Africa, but also the burden of colliding disease epidemic of communicable and non-communicable diseases. The strong association observed between infant HIV infection and poor growth highlights the importance of addressing the unfinished agenda of combating the HIV epidemic by striving towards the global UNAIDS goals of ensuring that 90% of all people living with HIV know their status, 90% of all those who are diagnosed with HIV are sustained on ART and 90% of those on ART are virally suppressed in order to prevent vertical transmission and improve both maternal and infant health. There is also an urgent need to tackle persistent undernutrition in both HIV-exposed and -unexposed South African children while curbing the concomitant rise of overnutrition, possibly thorough facility and community based support programmes that encourage longer durations of breastfeeding and avoidance of early introduction of complementary foods. As more HIV-positive women are placed on ART vertical transmission declines and more children are born HEU. While early initiation of HIV-positive women on is important for preventing MTCT and maintenance of maternal health, data does signal that pre-conception initiation of ART may have an adverse effect on PTD. As access to ART increases routine surveillance system should be set up to monitor adverse outcomes. The emergence of a large population of HEU for whom there are limited data also warrants the urgent need for the close follow-up, through surveillance systems and in-depth cohort studies, of this sub-population of children in order to assess not only short term outcomes but also longer-term outcomes of HIV and ART exposure as the intrauterine programming may lead to adult disease.

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Appendix

PhD paper I

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Effects of early feeding on growth velocity and overweight/obesity in a cohort of HIV unexposed South African infants and children

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Abstract

Background: South Africa has the highest prevalence of overweight/obesity in Sub-Saharan Africa. Assessing the effect of modifiable factors such as early infant feeding on growth velocity and overweight/obesity is therefore important. This paper aimed to assess the effect of infant feeding in the transitional period (12 weeks) on 12–24 week growth velocity amongst HIV unexposed children using WHO growth velocity standards and on the age and sex adjusted body mass index (BMI) Z-score distribution at 2 years.

Methods: Data were from 3 sites in South Africa participating in the PROMISE-EBF trial. We calculated growth velocity Z-scores using the WHO growth standards and assessed feeding practices using 24-hour and 7-day recall data. We used quantile regression to study the associations between 12 week infant feeding and 12–24 week weight velocity (WVZ) with BMI-for-age Z-score at 2 years. We included the internal sample quantiles (70th and 90th centiles) that approximated the reference cut-offs of +2 (corresponding to overweight) and +3 (corresponding to obesity) of the 2 year BMI-for-age Z-scores.

Results: At the 2-year visit, 641 children were analysed (median age 22 months, IQR: 17–26 months). Thirty percent were overweight while 8.7% were obese. Children not breastfed at 12 weeks had higher 12–24 week mean WVZ and were more overweight and obese at 2 years. In the quantile regression, children not breastfed at 12 weeks had a 0.37 (95% CI 0.07, 0.66) increment in BMI-for-age Z-score at the 50th sample quantile compared to breast-fed children. This difference in BMI-for-age Z-score increased to 0.46 (95% CI 0.18, 0.74) at the 70th quantile and 0.68 (95% CI 0.41, 0.94) at the 90th quantile . The 12–24 week WVZ had a uniform independent effect across the same quantiles.

Conclusions: This study demonstrates that the first 6 months of life is a critical period in the development of childhood overweight and obesity. Interventions targeted at modifiable factors such as early infant feeding practices may reduce the risks of rapid weight gain and subsequent childhood overweight/obesity.

Introduction

The first 1000 days from conception to 2 years are a critical period in the growth and development of infants [1]. Insults or stimuli in the intrauterine environment, including maternal body composition and diet, can "programme" the expression of genes and lead to permanent physiological or morphological changes in the fetus [2-4]. Programming can extend into the postnatal period where infant feeding and infant growth patterns can further predispose infants to later cardiovascular disease, overweight and obesity and other chronic disease; a phenomenon called "developmental origins of health and disease"[2,5]. Evidence from twin studies suggests that rapid weight gain between birth and 3 months is primarily influenced by modifiable environmental influences in term babies [6-13], such as nutrition [14], whereas from 5 months onwards genetic factors play a larger role [7,9,15].

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The World Health Organisation (WHO) recommends exclusive breastfeeding (EBF) until 6 months of age and timely introduction of appropriate complementary foods with continued breastfeeding up to 2 years or beyond [16]. EBF protects infants from rapid weight gain in the postnatal period, a risk factor for insulin resistance and later overweight and obesity which are both prevalent in South Africa [17,18]. Breastfeeding practices are still poor in South Africa as only 8% of infants 0–5 months are EBF [19]. We have shown that a third of infants who initiated breastfeeding in our sample were introduced to other foods, particularly formula, from as early as 3 days [20]. By 12 weeks postpartum 20% and 40% of HIV negative and positive women respectively had stopped all breastfeeding and transitioned to other liquids and solids Data from observational studies showed that children introduced to complementary foods earlier than 3 or 4 months, compared with later introduction, were more likely to be overweight or obese [21-24]. A randomised controlled trial also showed that high protein intake in infant formula was associated with rapid weight gain in the first 2 years of life [25]. Here we aimed to assess the effect of infant feeding in the transitional period (12 weeks) on 12–24 week growth velocity amongst HIV unexposed children using WHO growth velocity standards [26], which can adequately describe the growth of South African children [27], and on the age and sex adjusted body mass index (BMI) Z-score distribution at 2 years. We used data from the PROMISE-EBF (ClicinalTrials.gov.no: NCT00397150) trial; a multi-country cluster randomized community trial primarily assessing the effect of home based EBF counselling on EBF rates at 12 weeks [28].

Methods

Study design and participants

The present paper includes data from the three South African sites of the PROMISE-EBF behavioural-intervention trial that sought to improve EBF rates through peer counselling, conducted between 2006 and January 2008: Paarl (mixed peri-urban/rural area), Rietvlei (rural area) and Umlazi (peri-urban formal township). Trial methods have been described in detail elsewhere [28,29]. Briefly, pregnant women in their last trimester of pregnancy were screened for inclusion into the study. A total of 964 HIV negative and 184 HIV positive women and their singleton children were enrolled at the 3 week postnatal visit and followed up at 6, 12 and 24 weeks . Six hundred and fifty four HIV unexposed children (67.8% of original cohort) followed up again between March and September 2008 at a median age of 22 months (IQR: 9–34 months), which we refer to as the 2 year visit, were considered for this analysis due to the described negative effect of HIV infection on growth [27]. We compared baseline characteristics of participants that were followed-up at 2 years with those that were not (see Additional file 1) and observed no systematic differences, besides the proportion of male children, between the groups. This suggests that the sample that was followed-up is generally representative of the children in the whole cohort. A further 13 children were excluded because of extreme and implausible anthropometric values leading to a final sample of 641 children.

Data collection

Standardised questionnaires were used to collect interview data during pregnancy and postnatally at 3, 6, 12 and 24 weeks, and at the primary endpoint of 2 years of age. Maternal variables included: age, parity and education which were captured during recruitment; delivery mode and reported HIV status collected at the 3 week visit. The questionnaires also addressed infant feeding practices through 24-hour and 7-day recall of a list of 23 foods commonly consumed in the study sites. No food diaries were used. Data on child birth weight were extracted from perinatal records.

Field staff measured child weight and recumbent length/height during the 3, 6, 12, and 24 weeks visits and at 2 years. Children were weighed to the nearest 0.1 kg on Masskot (SOS Series) electronic pan scales, which were calibrated weekly using a 2 kg weight, wearing minimum clothing and no shoes. Depending on the study site, recumbent length measurements were obtained to the nearest 0.1 cm using TALC roller meters (Oxford, UK) or Shorr Height-Length Measuring Board (Maryland, USA) while height was measured using a validated ustom-made stadiometer. All field workers were trained on anthropometric techniques. In order to improve validity and reduce inter and intra-observer bias, the anthropometry data collection was validated periodically. Child age was calculated using the date of birth from the Road to Health card and the date of the interview.

Data were double-entered into a Microsoft Access database and analysed using Stata SE 12 [30] and IBM SPSS Statistics 21 [31].

Anthropometric scoring

The primary outcome measure was BMI-for-age Z-scores at 2 years; secondary outcomes were weight velocity Z-scores (WVZ) and length velocity Z-scores (LVZ). We calculated BMI-for-age Z-scores at the 12 week and 2 year visits, standardised for sex and actual age at the respective visit, using the WHO growth standards [32]. We considered children as "overweight" and "obese" if their BMI-for-age Z-scores were above +2 and +3 respectively as recommended by the World Health Organisation [33]. A macro based on the WHO-2009

growth velocity standards was used to compute the WVZ and LVZ. Velocities were calculated for a first period, namely from 3 or 6 to 12 weeks post-delivery, and for a second period, namely 12 to 24 weeks post-delivery. In cases where the 3 or 6 week weight was missing we used the birth weight for the calculation of velocity in the first period. The age intervals and child ages observed in the study did not always correspond exactly with those of the velocity standards. Thus the velocity Z-scores were calculated, as recommended by WHO, by identifying the best-fitting age interval for each child period observed and linearly extrapolating the observed increment in the child period to the duration of the best-fitting target interval [26].

Data cleaning

Anthropometric measurement values and Z-scores were flagged for verification if any of the following criteria were met: a) decrease in length of more than 2 cm between two consecutive visits; b) WAZ <-6 or >5, WLZ <-5 or >5, LAZ <-6 or >6, WLZ >3 and LAZ <-3; c) extreme changes in LAZ between visits defined as LAZ at 3 weeks < 2 and LAZ at 24 weeks > 2.5, or LAZ at 24 weeks < 2 and LAZ at 36 weeks > 2.5; d) changes > 4 or <-4 Z-scores between 24 and 36 weeks and BMI-for-age Z-score ≥6. All the flagged anthropometric observations were assessed and values treated as missing if no plausible explanation was determined.

Feeding pattern

We used a combination of 24-hour and 7-day infant feeding recall data at each follow-up visit to generate time specific food consumption indicator variables (for breast milk, water, sugar water, formula, cereals, fruits/vegetables, traditional herbs, prescribed and non-prescribed medicines) with 3 categories: yes, no and missing. For example if the caregiver said that she gave the child breast milk in the previous 24-hours or 7-days then we coded that child as having received breast milk. If the caregiver said "no" to both questions on breast milk, the child was then considered as one that did not receive breast milk. The response was coded as "missing" for breast milk if data were missing for both questions. Cross-tabulation of the 12 week breast milk and formula indicators revealed that all children had consumed at least one of the two foods. Based on exploratory analysis we combined two of the three combinations of these feeding indicators and this resulted in a binary 'ever breastfed" variable with the following categories: yes (received breast milk with other solids and liquids which may include formula) and no (received formula and other liquids and solids except breast milk). The 12 week breastfeeding cessation variable was defined as no breastfeeding at the 12 week interview (based on 24-hour and 7-day recall) and no breastfeeding reported for the subsequent final 24-week interview. Only children who initiated breastfeeding by the 3 week visit were considered in this definition.

Statistical analysis

Unlike the ordinary least squares (OLS) regression which only considers the conditional mean function, we used quantile regression which is a statistical technique that provides a more detailed analysis of the relationship between the dependent variable and its independent variables because it provides conditional regression coefficients for each quantile, [34,35]. We used univariate and multivariate simultaneous quantile regression to test whether 12 week infant feeding and 12–24 week growth velocity (adjusting for other variables) had increased effects over the upper tails of the conditional distribution of BMI-for-age Z-scores at 2 years. For this analysis we included the internal sample quantiles (70th and 90th centiles) that approximate the reference cut-offs of +2 and +3 Z-scores for BMI-for-age around 2 years. We also performed OLS regression modelling. The following variables were adjusted for in the multivariate models because of their epidemiologic or clinical importance: birth weight, maternal age, parity, maternal education, study arm and site. Although the child's age and sex were taken into account in the BMI-for-age Z-score estimations, based on previous literature [36] we included an interaction term between the infant feeding and sex variables in initial regression models to test whether sex modifies the relationship between feeding and BMI-for-age Z-score. This interaction term was excluded from the final models as no effect measure modification was detected. Maternal age and parity were excluded from the final multivariate model because they were not significantly associated with 2-year BMI in the univariate analysis. The Breusch-Pagan / Cook-Weisberg test was used to check for heteroskedasticity and trends across the quantile regression percentiles were also tested. Continuous data are presented as mean ± SD or median (IQR) while categorical variables are presented as frequencies. We used the Student *t*-test to compare means and the Pearson chi-square test to examine associations in the cross-tabulations. Statistical tests were two-sided and performed at the 5% significance level. Kernel density functions were used to estimate the 2 year BMI-for-age Z-score distribution stratified by the 12 week overweight and breastfeeding while the two-sample Kolmogorov-Smirnov test was used to test for equality of the distribution functions.

Ethical approval

The PROMISE-EBF trial was approved by the Regional Committees for Medical and Health Research Ethics

(REK VEST) in Norway (issue number 05/8197), University of the Western Cape (research registration number 0607/8) and the South African Medical Research Council (protocol ID: ECO7-001). Informed consent was obtained from all participants.

Results

Study population

Six hundred and forty one HIV unexposed children with valid weight and length data (median age 22 months, IQR: 17–26 months) were analysed at the 2-year visit (Figure 1). Table 1 summarises the characteristics of these children and their mothers. Rietvlei had slightly fewer male children compared to the other sites and more children with birth weight >4 kg. About half the children were firstborn and Paarl had the highest frequency of vaginal births.

Infant feeding

The proportion of children consuming any breast milk decreased from 89.3% (95% confidence interval (CI), 86.5, 91.7%) at 3 weeks to 79.4% (95% CI 75.8, 82.6%) at 12 weeks (Table 2). In contrast there was an increase in the proportion of children consuming formula from 48.5% (95% CI 44.5, 52.5%) to 62.9% (95% CI 58.8, 66.9%) and cereal from 28.6% (95% CI 25.1, 32.3%) to 63.4% (95%CI 59.4, 67.4%) between the 3 and 12 weeks. Cereals were consumed by 79.5% (95% CI 71.0, 86.4%) of children who were not breastfed at 12 weeks and 59.6% (95% CI 54.9, 64.1%) of those who were breast-fed (P < 0.001). Formula was consumed by all non-breastfed children at 12 weeks and 53.7% (95% CI 49.0, 58.4%) of the breastfed children (P < 0.001).

Weight and length velocity

The overall mean WVZ varied between the feeding groups in both postnatal periods while no differences were observed for mean LVZ (Table 3). In period-1, children who received no breast milk at 3 weeks had a higher mean WVZ compared to children who received any breast milk (P < 0.01). The same association was observed between 12 week feeding and the period-2 mean WVZ. Comparable results were obtained when mean weight and length values were compared in place of velocity Z-scores (Additional file 2). Period-2 mean WVZ also differed by breastfeeding cessation with children who stopped all breastfeeding by 12 weeks (n = 116) having a higher (P = 0.02) mean WVZ than children who continued breastfeeding (n = 47), data not shown.

Breastfed and non-breastfed children

Thirty percent of all children were overweight at 2 years while 8.7% were obese. The estimated 2 year BMI-for-age Z-score density distribution for our sample is depicted in Figure 2 and is shifted to the right of the referent standard expected normal distribution. The unadjusted effect of breastfeeding status at 12 weeks (P = 0.08) on 2 year BMI-for-age Z-score is depicted in Figure 3. The mean and upper tail of the BMI-for-age Z-score density distribution for children who were not breastfed at 12 weeks are shifted to the right of the distribution for breastfed children. The adjusted linear regression showed similar results with the mean BMI-for-age Z-score at 2 years for children

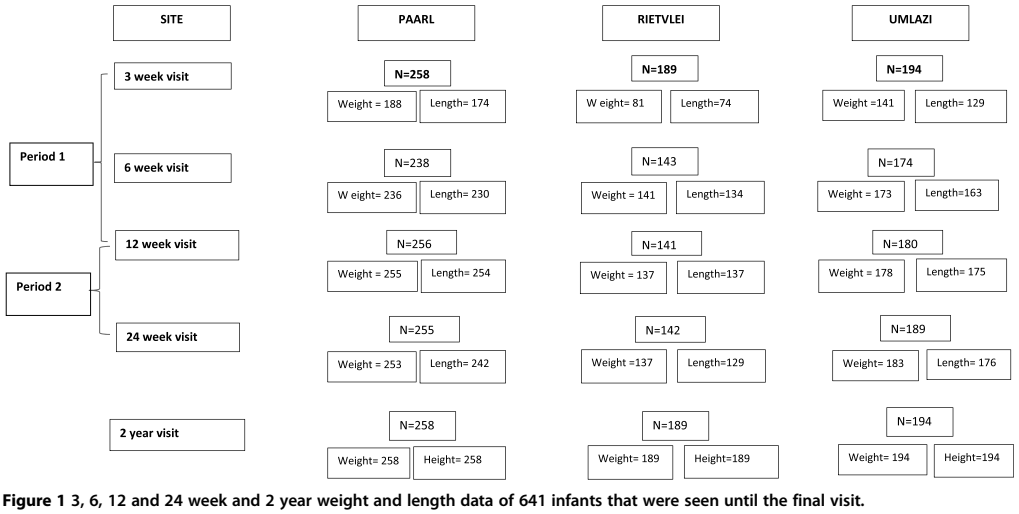

Figure 1 3, 6, 12 and 24 week and 2 year weight and length data of 641 infants that were seen until the final visit.

Table 1 Participant characteristics by site

Variables	Paarl (N = 258)		Rietvlei (N = 189)		Umlazi (N = 194)	
	n	%	n	%	n	%
Infant gender, (% male)	142	55.0	92	48.7	113	58.3
Birth weight, (%)						
Low birth weight (<2.5Kg)	21	8.1	6	3.2	11	5.7
Normal birth weight (2.5- < 4Kg)	219	84.9	134	70.9	172	88.7
Macrosomia (≥4Kg)	11	4.3	16	8.5	7	3.6
Missing	7	2.7	33	17.5	4	2.1
Maternal age (y), %						
15–25	160	62.0	122	64.6	134	69.1
26–44	98	37.9	67	35.5	60	30.9
Parity, %						
Primipara	126	48.8	78	41.3	114	41.2
Multipara	132	51.2	111	58.7	80	41.2
Maternal education (grade), %						
0–7	28	10.9	37	19.6	9	4.5
8–10	117	45.4	108	57.1	53	27.3
11–12	109	42.3	44	23.3	114	588
>12	4	1.6	0	0	18	9.3
Delivery mode, %						
Vaginal	217	84.1	148	78.3	116	59.8
C-section	37	14.3	26	13.8	74	38.1
Missing	4	1.6	15	7.9	4	2.1

who were not breastfed at 12 weeks 0.32 (95% CI 0.04, 0.61) higher than for breastfed children (Table 4).

Table 4 and Figure 4 present the adjusted OLS and multivariate quantile regression models (detailed table with the adjusted OLS and multivariate quantile regression models for all the covariates is in Additional file 3). The adjusted quantile regression model showed an overall significant linear trend between breastfeeding and BMI-for-age Z-score at 2 years (P = 0.01). Breastfeeding had an increasingly protective effect on the 2 year BMI-for-age Z-score conditional distribution from the 60th quantile (Table 4 and Figure 4), and this effect was most pronounced at the 90th quantile which corresponds with the definition for obesity in our sample. Children who were not breastfed at 12 weeks had a 0.37 (95%CI 0.07, 0.66) increment in BMI-for-age Z-score at the 50th quantile compared to breast-fed children. This difference in BMI-for-age Z-score increased to 0.46 (95% CI 0.18, 0.74) at the 70th quantile and was 0.49 (95% CI 0.16, 0.83) and 0.68 (95% CI 0.41, 0.94) at the 80th and 90th quantiles respectively. No significant point estimates were observed for infant feeding between the 10th and 40th quantiles. The 12 week BMI-for-age Z-score and period-2 WVZ regression coefficients showed uniform positive effects across the quantiles of 2 year BMI-for-age Z-score. The birth weight regression estimate tended to be positively associated with quantiles of the 2 year BMI-for-age Z-score and increased towards the upper tails of the distribution (Additional file 3). The assumption of homoscedasticity was not violated in the model (P = 0.52).

Discussion

Maternal and child malnutrition, which include both under and over nutrition, are a growing concern in low and middle income countries [37]. South Africa has the highest prevalence of obesity in Sub-Saharan Africa with 42% of women, aged ≥20 years, obese and a quarter of girls younger than 20 years overweight/obese [38]. This high prevalence of obesity among women of child bearing age raises concerns as pre-pregnancy overweight/obesity and excessive gestational weight gain are risk factors for gestational diabetes, pregnancy induced hypertension [39] and adverse birth outcomes such as macrosomia. Furthermore, evidence shows that infants of obese mothers have twice the odds of being obese at 2 years compared to infants of non-obese mothers [40]. Childhood obesity is in turn a risk factor for adult obesity, diabetes and non-communicable disease [41]. To prevent this cycle of obesity, both the determinants and

Table 2 Food consumption at 3, 12 and 24 weeks based on 24-hour and 7-day recall

	3 week feeding (N = 629)		12 week feeding (N = 629)		24 week feeding (N = 629)	
	n	%	n	%	n	%
Breast milk						
Yes	527	89.3*	458	79.4*	395	67.4*
Water						
Yes	269	42.8	264	45.8*	358	61.1*
Sugar water						
Yes	189	30.05	286	49.6*	154	26.3*
Traditional herbs						
Yes	424	67.4	167	28.9*	155	26.5*
Commercial infant formula						
Yes	305	48.5	363	62.9*	366	62.5*
Cereals						
Yes	180	28.6	366	63.4*	527	89.9*
Fruits/vegetables						
Yes	66	10.5	168	29.1*	400	68.3*
Prescribed medicines						
Yes	260	41.4*	231	89.5*	289	100.0*
Non-prescribed medicines						
Yes	444	70.6	355/577	61.5*	258	44.0*

*Percentage calculations were based on the total number of available data for each food item. The denominator was 629 unless otherwise indicated by the asterisk.

the critical period for overweight and obesity development must be identified.

Previous studies have identified the first 3 months of life as a critical period when infant growth is mainly nutrition dependent [14]. In this study we investigated the association between the effect of early infant feeding practices on growth velocity in the first 6 months, and the effect of 12 week infant feeding and 12–24 week growth velocity on BMI-for-age Z-score at 2 years of age amongst HIV unexposed children.

The proportion of overweight and obese children in our sample was high considering that recent findings from the first South African National Health and Nutrition Examination Survey (SANHANES-1) indicate a prevalence of overweight (BMI: 25–29.9) and obesity (BMI >30) of 17.5%(95% CI 11.4,23.6%) and 4.4%(95% CI 2.2,6.5%) respectively among boys aged 2–5 years; 18.9%(95% CI 14.1,23.4%) and 4.9%(95% CI 2.9,7.0%) among girls [42].

Consistent with results from Chirwa *et.al.* [43], our study found higher weight velocity in the first few

Table 3 Period-1 (the 3/6 week–12 week) and period-2 (12–24 weeks) mean weight velocity (WVZ) and length velocity (LVZ) by infant feeding[1]

3 week feeding					
Period 1: 3/6–12 weeks	Never breastfed	Breastfed[3]			
	n	Mean ± SD	n	mean ± SD	P-value[2]
WVZP1 (N = 522)	60	1.58 ± 1.72	462	0.99 ± 1.60	<0.01
LVZP1 (N = 402)	46	1.69 ± 2.62	356	1.37 ± 2.44	0.41
12 week feeding					
Period 2: 12–24 weeks	Never breastfed	Breastfed[3]	P-value[2]		
WVZP2 (N = 494)	98	1.07 ± 1.75	394	0.64 ± 1.57	0.02
LVZP2 (N = 477)	93	0.82 ± 2.62	382	0.85 ± 2.52	0.93

[1]Values are mean ± SD of velocity Z-scores based on WHO standard. LVZ, length velocity Z-score; P1, Period-1; P2, Period-2; WVZ, weight velocity Z-score.
[2]Student test P values for group comparisons a 5% significance level.
[3]Children received breast milk in addition to other solids and liquids.

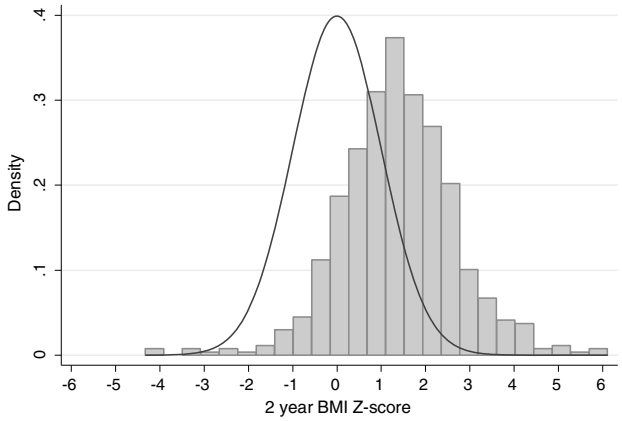

Figure 2 Univariate density distribution of the sample BMI-for-age Z-scores at 2 years (blue distribution) and a normal reference distribution (red distribution). The sample distribution is shifted to the right of the reference distribution.

months of life to be strongly associated with higher BMI-for-age Z-score at 2 years. We also demonstrated that children who were not breastfed at 12 weeks had higher mean WVZ between 12 and 24 weeks, higher BMI-for-age Z-scores at 2 years and were more likely to be overweight/obese. This suggests that the protective effect of breastfeeding against childhood obesity risk is partly based on its mediating effect on infant weight velocity. The higher BMI-for-age Z-scores in non-breastfed children could partly be attributed to their earlier introduction to nutrient dense cereals and formula which may have led to the rapid weight gain observed in the first 6 months of life; consistent with previous systematic reviews [44-46].

Commercial infant formulae, such as the NAN Pelargon® that was commonly used in South Africa at the time of the study, also have higher protein content (14 g/day) [47] compared to breast milk (9 g/day) [48] around 3 months. A high protein intake in excess of metabolic requirements stimulates the secretion of insulin and insulin growth factor 1 (IGF-1) axis and subsequently increases weight gain in infancy [25,49] through heightened cell proliferation and adipogenic activity (adipocyte differentiation) [50]. Insulin and IGF-1 levels are therefore generally higher in

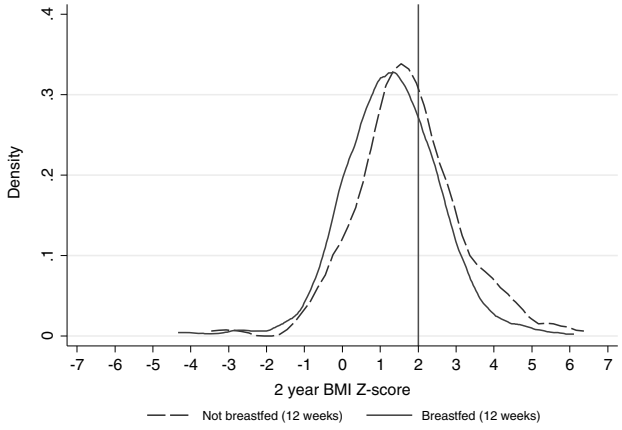

Figure 3 2 year BMI-for-age Z-score estimated density distributions with regards to 12 week breastfeeding status. The vertical red reference line indicates the +2 BMI-for-age Z-score threshold which corresponds with overweight status.

Table 4 Multivariate quantile regression and ordinary least squares (OLS) coefficients for 2 year BMI-for-age Z-score[1]

	OLS mean	10th percentile	20th percentile	30th percentile	40th percentile	50th percentile	60th percentile	70th percentile	80th percentile	90th percentile
Not breastfed at 12 weeks = Yes [Ref: No]	0.32 (0.04, 0.61)	0.04 (−0.36, 0.44)	0.08 (−0.35,0.52)	0.31 (−0.10,0.72)	0.29 (−0.02,0.60)	0.37 (0.07,0.66)	0.34 (0.05,0.63)	0.46 (0.18, 0.74)	0.49 (0.16, 0.83)	0.68 (0.41, 0.94)
R-squared/pseudo R-squared	0.2182	0.1204	0.1238	0.1299	0.1251	0.1165	0.1147	0.1200	0.1216	0.1640

[1]Values are ordinary least square (OLS) and quantile regression beta-coefficients (adjusted for birth weight, weight velocity between 12–24 weeks, 12 week BMI-for-age Z-score, maternal education, study site and intervention arm) with respective 95% confidence intervals in brackets. 466 observations were assessed in the models. With the exception of study arm, only variables that had significant association with BMI-for-age Z-score in the bivariate analysis were included in the final model. R-squared/pseudo R-squared.

formula fed infants compared to breastfed infants [51]. Based on this "early protein hypothesis"[50] Koletzko and colleagues [25] conducted a randomized clinical trial that assessed whether growth differed between breastfed children and those fed a low protein (1.77 g/100 Kcal) formula before 6 months. Their results showed no significant difference in mean BMI and obesity risk between these two groups from 3 months of age until 6 years [25]. Nonetheless, exclusive breastfeeding continues to be strongly recommended as it has many other advantages, such as protection against gastrointestinal infections [52], which are particularly important in resource limited settings where formula feeding may not be acceptable, feasible, affordable, sustainable and safe. Breast milk also has a lower average caloric density (kcal/100 mL) [53] compared to infant formula, and the energy per kg of bodyweight in breastfed infants is 10–18% lower than for formula fed infants aged between 3 and 12 months [50]. Furthermore evidence shows that breastfed infants self-regulate the quantity and frequency of milk intake better than formula-fed infants, who tend to empty their bottles [54]. Formula-fed infants are also twice as likely to complete their meals later in infancy, even when satiated, compared to breastfed infants [55]. While cereals were given more frequently to formula-fed infants in this study, early introduction of solids was also highly practiced in breastfed infants. This early introduction of cereals could have displaced some of the breastfeeding and may therefore partially explain the higher BMI-Z-scores observed in the sample breastfed infants compared to the international reference. Previous observational studies showed that children introduced to complementary foods earlier than 3 or 4 months, compared with later introduction, were more likely to be overweight or obese [21,24,56]. Collectively, these results indicate that any breastfeeding in the first 3 months of life is protective against rapid weight gain in infancy and therefore plausibly protects against subsequent later overweight/obesity [18,36,57,58]. This further highlights the importance of early infant feeding as a key intervention to prevent childhood overweight/obesity. It is therefore of serious concern that amongst this group of HIV unexposed children, approximately half had been introduced to commercial infant formula milk by the age of three weeks. The decade of provision of free commercial infant formula milk at clinics through the Prevention of Mother to Child Transmission of HIV (PMTCT) services, together with the absence of a clear message to mothers regarding infant feeding has likely contributed to this situation [59]. Enormous efforts, detailed in the revised 2013 Infant and Young Child Feeding Policy which unambiguously supports breastfeeding as the optimal feeding mode for infants, are required to improve the EBF prevalence in South Africa [60]. Changing practices however takes time and will require collective action from all stakeholders, including communities and

Figure 4 Quantile regression and ordinary least squares (OLS) coefficients. The figure presents the never breastfed coefficients from the final model for the 10^{th},20^{th},30^{th},40^{th}, 50^{th}, 60^{th},70^{th}, 80^{th} and 90^{th} 2 year BMI-for-age Z-score quantiles. The respective values are connected by the maroon solid line with accompanying estimated 95% confidence interval intervals shaded in grey. The OLS value is depicted by the horizontal blue line with 95% confidence limits depicted by the black dotted lines.

healthcare workers. Interventions to support breastfeeding in the workplace are also critical [61].

Our study has several strengths. To our knowledge this is the first study to assess the effect of early infant feeding on childhood BMI in African children [18,36,57,58].We are also not aware of any previous studies that have examined the effect of early weight velocity on later BMI using the WHO growth standards in this population group. We used simultaneous quantile regression, a more detailed technique than OLS regression, to examine the effect of weight velocity and infant feeding across the BMI-for-age Z-score distribution. As with Beyerlain and colleagues [62], this statistical approach enabled us to observe the differential effect of breastfeeding, which was not detected by OLS regression, on the upper tail of the conditional BMI-for-age Z-score distribution at 2 years.

Limitations of the present study were the absence of data on maternal nutritional status and smoking during pregnancy, which are known determinants of infant weight velocity and childhood overweight/obesity [63,64]. In this study we used maternal HIV status reported at recruitment; this information is subject to bias. We only included those women that reported being HIV negative in this paper; some women may have seroconverted during the follow-up period and would therefore be misclassified. The small group of HIV positive women and their children was excluded from this analysis because 1) infant HIV test results were not obtainable for one of the study sites and 2) relevant data to adjust for in this group (e.g. viral load, CD4 count and detailed information on antiretroviral (ARV) drug use) were not available. Birth weights were used to estimate the period-1 velocity for only 15 children (2.34% of the analysed sample) that did not have 3 week weight data. We therefore do not think that this small proportion of the sample skewed the results. Our feeding exposure variable was based on short term reporting of feeding practices, and not on a longitudinal assessment of feeding, at the assumed time of 12 weeks. Recall bias together with lack of precise information on the timing of the consumption of each food item and the exact quantities consumed may have therefore introduced variability in the data that is evidenced by wide confidence intervals observed in infant feeding quantile regression estimates. Furthermore, data were missing for some of the food variables because participants did not respond when asked about the consumption of that particular food item. There was also overlap between the confidence intervals of the OLS and quantile regression models and this may largely be due to the relatively modest sample size we used for this analyses approach (n = 466) compared to other studies [36,62,65].

Conclusion

This study demonstrates that infant feeding practices in the first 12 weeks of life can predict the development of childhood overweight and obesity. Early life therefore presents a crucial window of opportunity for interventions to address modifiable factors such as infant feeding practices in order to reduce the risks of rapid weight gain in infancy and subsequent childhood overweight/obesity.

Additional files

Additional file 1: Participant characteristics stratified by 2-year follow-up participation.

Additional file 2: Mean weight, length/height and BMI-for-age Z-score by infant feeding[1]. [2]Student test P values for group comparisons a 5% significance level.

Additional file 3: Multivariate quantile regression and ordinary least squares (OLS) coefficients for 2 year BMI-for-age Z-score.

Abbreviations

BMI: Body mass index; EBF: Exclusive breastfeeding; LAZ: Length-for-age Z-score; LVZ: Length velocity Z-score; PMTCT: Prevention of mother to child transmission of HIV; WVZ: Weight velocity Z-score; WAZ: Weight-for-age Z-score; WLZ: Weight-for-length Z-score.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TD WZ, DJJ, and CL designed research; WZ, TD, VR and DJJ conducted research; VR, CL and JVDB conducted data analysis; VR, JVDB, MC, TD, DJ, LTF, AEG, IMSE, and WZ wrote the paper. All authors (except MC and JVDB who are deceased) read and approved the final manuscript.

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Additional file 1: Participant characteristics stratified by 2-year follow-up participation

Variables	**With 2-year follow-up (N=746)**		**Without 2-year follow-up (N=402)**	
Infant	**n**	**%**	**n**	**%**
Male gender, %	392	52.55	172	42.79
Birth weight, %				
Low birth weight (<2.5Kg)	47	6.30	15	3.73
Normal birth weight (2.5-<4Kg)	610	81.77	326	81.09
Macrosomia (≥4Kg)	39	5.23	18	4.48
Missing	50	6.70	43	10.70
Maternal				
Age (y), %				
15-25	473	63.40	248	61.69
26-44	273	36.60	154	38.31
Missing	0		0	
Parity, %				
Primipara	391	52.41	214	53.23
Multipara	355	47.59	188	46.77
Missing	0		0	

Education (grade), %				
0-7	85	11.39	39	9.70
8-10	313	41.96	149	37.06
11-12	320	42.90	190	47.26
>12	28	3.75	24	5.97
Missing	0		0	
Delivery mode				
Vaginal	545	73.06	278	69.15
C-section	172	23.06	92	22.89
Missing	29	3.89	32	7.96

Additional file 2. Mean weight, length/height and BMI-for-age Z-score by infant feeding[1]

3 week feeding					
	Never breastfed		Breastfed[3]		
	n	mean±SD	n	mean±SD	P-value[2]
Weight (Kg) 3 weeks	43	4.01 ±0.75	347	4.00 ±0.62	0.91
6 weeks	55	4.84±0.82	483	4.93±0.79	0.43
Length (cm) 3 weeks	41	51.69±2.34	336	51.86±2.50	0.67
6 weeks	55	54.69±2.76	472	55.12±2.93	0.31
12 week feeding					
	Never breastfed		Breastfed[3]		P-value[2]
Weight (Kg) 12 weeks	117	6.32±0.89	451	6.21±.92	0.25
24 weeks	101	8.04±1.12	403	7.89±1.11	0.22
2 years	119	12.57±2.1	458	11.62±1.84	<0.001

Length/height (cm) 12 weeks	116	60.29±3.05	450	60.03±3.05	0.41
24 weeks	100	66.41±3.10	400	66.49±3.49	0.83
2 years	119	82.27±4.78	458	80.44±4.96	<0.001
BMI-for-age Z-score 2years	119	1.72±1.36	458	1.31±1.29	<0.01

[1]Values are mean±SD of weight, length/height and BMI-for-age Z-score.
[2]Student test P values for group comparisons a 5% significance level.
[3]Children received breast milk in addition to other solids and liquids.

Additional file 3: Multivariate quantile regression and ordinary least squares (OLS) coefficients for 2 year BMI-for-age Z-score [1]

	OLS mean	**10th percentile**	**20th percentile**	**30th percentile**	**40th percentile**	**50th percentile**	**60th percentile**	**70th percentile**	**80th percentile**	**90th percentile**
Constant	0.26 (-0.54; 1.06)	-1.03 (-2.15;0.09)	-0.49 (-1.60;0.62)	-0.13 (-1.12;0.87)	0.06 (-1.05;1.17)	0.08 (-1.09;1.26)	0.42 (-0.63;1.44)	0.86 (-0.26;1.97)	1.37 (-0.01;2.74)	1.21 (-0.29;2.70)
Not breastfed at 12 weeks										
Yes [Ref=No]	0.32 (0.04;0.61)	0.04 (-0.47; 0.55)	0.08 (-0.32;0.48)	0.31 (-0.07;0.68)	0.29 (-0.02;0.59)	0.37 (0.09;0.64)	0.34 (0.13;0.29)	0.46 (0.27;0.65)	0.49 (0.24;0.75)	0.68 (0.31;1.03)
Birth weight	0.30 (0.06;0.53)	0.21 (-0.13;0.54)	0.19 (-0.07;0.45)	0.20 (-0.03;0.42)	0.24 (-0.3;0.51)	0.32 (0.07;0.58)	0.29 (0.10;0.49)	0.27 (0.03;0.51)	0.26 (-0.10;0.62)	0.59 (0.23;0.95)
WVZP2	0.19 (0.12;0.25)	0.16 (0.06;0.26)	0.21 (0.13;0.29)	0.19 (0.12;0.26)	0.19 (0.11;0.27)	0.20 (0.12;0.28)	0.20 (0.12;0.29)	0.19 (0.10;0.28)	0.15 (0.05;0.25)	0.18 (0.08;0.28)
12 week BMI-for-age Z-score	0.35 (0.26;0.45)	0.43 (0.27;0.59)	0.40 (0.29;0.52)	0.34 (0.23;0.44)	0.38 (0.29;0.47)	0.33 (0.23;0.43)	0.34 (0.26;0.42)	0.32 (0.24;0.40)	0.30 (0.21;0.39)	0.37 (0.21;0.53)
Education (grade)										
8-10	-0.11 (-0.49;0.26)	-0.03 (-0.51;0.45)	0.29 (-0.27;0.83)	0.18 (-0.29;0.64)	0.04 (-0.38;0.46)	-0.06 (-0.46;0.34)	-0.04 (-0.50;0.43)	-0.15 (-0.63;0.32)	-0.18 (-0.91;0.55)	-0.34 (-1.08;0.39)
11-12	-0.15 (-0.54;0.24)	0.12 (-0.31;0.55)	0.09 (-0.38;0.55)	0.05 (-0.32;0.42)	-0.05 (-0.42;0.31)	-0.12 (-0.50;0.27)	-0.09 (-0.56;0.39)	-0.26 (-0.75;0.24)	-0.19 (-0.94;0.54)	-0.53 (-0.36;0.29)

>12 [Ref= 0-7]	0.30 (-0.42;1.02)	0.78 (0.06; 1.50)	0.56 (-0.23;1.36)	0.46 (-0.26;1.17)	0.30 (-0.34;0.94)	0.28 (-0.37;0.94)	0.06 (-0.71;0.82)	-0.19 (-0.88;0.50)	-0.48 (-2.12;1.17)	1.01 (-0.94;2.96)
Site										
Rietvlei	-0.38 (-0.71;-0.06)	-0.35 (-0.76;0.06)	-0.30 (-0.59;-0.02)	-0.43 (-0.77;-010)	-0.25 (-0.62;0.13)	-0.19 (-0.58;0.21)	-0.16 (-0.56;0.23)	-0.23 (-0.61;0.15)	-0.34 (-0.75;0.08)	-0.72 (-1.10;0.35)
Umlazi [Ref= Paarl]	-0.07 (-0.33;0.18)	-0.05 (-0.46;0.36)	-0.03 (-0.27;0.21)	-0.08 (-0.27;0.13)	-0.04 (0.28;0.21)	0.07 (-0.16;0.30)	0.04 (-0.14;0.21)	-0.04 (-0.30;0.22)	-0.18 (-0.45;0.09)	-0.24 (-0.63;0.16)
Arm										
Intervention [Ref= control]	0.10 (-0.13;0.32)	0.23 (-0.10;0.56)	0.01 (-0.22;0.24)	0.04 (-0.16;0.23)	-0.03 (-1.16;0.10)	0.01 (-0.17;0.19)	0.06 (-0.10;0.23)	0.19 (-0.03;0.41)	0.12 (-0.12;0.35)	-0.17 (-0.41;0.06)
R^2 / Pseudo R^2	0.2182	0.1204	0.1238	0.1299	0.1251	0.1165	0.1147	0.1200	0.1216	0.1640

[1]Values are ordinary least square (OLS) and quantile regression beta-coefficients with respective p-values in brackets. 466 observations were assessed in the models. With the exception of study arm, only variables that had significant association with BMIZ-score in the bivariate analysis were included in the model. WVZP2, weight velocity Z-score in period-2 (12-24 weeks

PhD paper I Erratum

The data cleaning section of Paper I had a few errors that needed to be corrected. The data cleaning section will be replaced with the following text which has been submitted to the International Journal of Breastfeeding:

"Anthropometric measurement values and Z-scores were flagged for verification if any of the following criteria were met: WAZ<-6 or >5, WLZ<-5 or >5, LAZ<-6 or >6, WLZ>3 and LAZ<-3; 2) extreme changes in LAZ and WLZ (greater than 2.5 or 3) between consecutive visits; 3) BMI-for-age Z-score ≥6. All the flagged anthropometric observations were assessed and values treated as missing if no plausible explanation was determined."

Paper III (accepted manuscript)

III

Title: In-utero ART exposure and birth and early growth outcomes amongst HIV exposed uninfected infants attending immunization services: Results from national PMTCT surveillance, South Africa

Running title: Birth and growth outcomes of HEU infants

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ABSTRACT

Background

Despite the recognised benefit of antiretroviral therapy (ART) for preventing and treating HIV; some studies have reported adverse birth outcomes with in-utero ART exposure. We evaluated the effect of infant in-utero HIV and ART exposure on preterm delivery (PTD), low birth weight (LBW), small-for-gestational age (SGA) and underweight-for-age (UFA) at six-weeks.

Methods

We surveyed 6179 HIV-unexposed-uninfected (HUU) and 2599 HIV-exposed-uninfected (HEU) infants. HEU infants were stratified into three groups: ART; Zidovudine alone; no antiretrovirals (None). The ART group was further stratified to explore pre- or post-conception exposure. Multivariable logistic regression evaluated effects of HIV and ARV exposure on the outcomes.

Results: We found higher odds of PTD, LBW, SGA and UFA in HEU than HUU infants. HEU in the None group (adjusted odds ratio [AOR], 1.9; 95% CI, 1.2, 3.0) or those whose mothers initiated ART pre-conceptually (AOR, 1.7; 1.1, 2.5) had almost twice the odds of PTD than infants whose mothers started ART post-conceptually, but no increased odds for other outcomes.

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Conclusions: ART resulted in better birth outcomes, however, there was an association between pre-conception ART and PTD. As ART access increases, pregnancy registers or similar surveillance should be in place to monitor outcomes to inform future policy.

Key words: HIV; antiretroviral therapy; birth outcome; South Africa

INTRODUCTION

With the success of the prevention of mother to child HIV transmission (PMTCT) programme, HIV-exposed-uninfected (HEU) children now constitute the majority of the children affected by the HIV pandemic [1]. In January 2015 South Africa (SA), which has a maternal HIV prevalence of 29%, adopted the 2013 World Health Organization (WHO) recommendation of lifelong antiretroviral treatment (ART) for all HIV-positive pregnant women, referred to as WHO-PMTCT Option-B+, as a PMTCT strategy and to maintain maternal health [2, 3]. Although the benefits of ART outweighs the potential adverse effects, this population of HEU children requires monitoring to better understand the short and long term health effects of HIV and ART exposure [4]. The shift to Option-B+, and the possible future use of pre-exposure prophylaxis (PreP) as a primary prevention method in HIV-uninfected adolescents and young women [5, 6], will increase the number of children exposed to ART during critical intrauterine, intrapartum and postpartum periods. It is well established that HIV infection in children is associated with poor birth [7] and postnatal [8-10] outcomes. However the effect of exposure to maternal HIV infection and ART use on the birth outcomes of HEU infants in resource-limited settings, where HEU children have a higher mortality risk than HUU children [11], and where endemic under-nutrition and co-infections can compound any potential adverse effect of HIV or ART exposure, is less clear. Using data from a nationally representative facility-based PMTCT survey we studied the effect of infant in-utero HIV and ART exposure on preterm delivery (PTD), low birth weight (LBW) and small-for-gestational age (SGA) at birth, and underweight-for-age (UFA) at six weeks postpartum.

METHODS

Study design

The 2012-13 South African PMTCT Evaluation (SAPMTCTE) was a nationally representative facility-based cross-sectional survey, conducted between October 2012 and May 2013, to measure vertical HIV transmission at 4-8 weeks postpartum. During this study, ART use was criteria-led (WHO PMTCT 'Option-A'), changing to 'universal test and treat' for all HIV-positive pregnant women throughout breastfeeding ('Option-B') in April 2013. Under Option-A, antiretroviral drug (ARV) naïve HIV-infected pregnant women were placed on ART (recommended tenofovir disoproxil fumarate (TDF) + Lamivudine (3TC/FTC) + Nevirapine (NVP)) if CD4 cell count $\leq$350 cells/mm^{3}and Zidovudine (ZDV) from 14 weeks gestation, (with infant NVP for six weeks or until one week post-breastfeeding) if CD4 >350 cells/mm^3 [12].

The study methods have been described elsewhere ([13] and supplementary materials). In brief, 580 primary health facilities offering immunisation services were sampled using a probability proportional to size approach. Consenting mother-infant pairs, attending immunisation services, were consecutively or systematically enrolled, regardless of maternal HIV status, in each facility. Sick infants needing emergency care and those aged <4 weeks or >8 weeks were excluded.

Exposure measures

Maternal HIV infection and ART use were the primary exposures. Trained nurse data collectors drew infant dried blood spot (iDBS) specimens during the study visits. All iDBS received HIV antibody (serological) testing and, antibody-positive samples or samples from self-reporting

HIV-positive mothers, were tested for both HIV-1 proviral DNA and HIV-1 RNA (see supplementary methods). Data collectors used electronic questionnaires to gather data on the mother's self-reported ARV drug use and timing of initiation. As no maternal blood specimens were collected, a mother was defined as HIV-negative if the infant's antibody result was negative, and HIV-infected if two infant HIV antibody test results were positive. An infant was defined as HEU if (i) the HIV antibody result was positive and PCR result was negative, or (ii) the antibody result was positive and PCR equivocal or rejected (1% of sample), or HUU if both results were negative. Amongst HIV-infected mothers, self-reported ARV use was categorised into three groups: namely (i) ART-use primarily for mother's health (ART-group), as per Option-A guidelines, (ii) antenatal ZDV as MTCT prophylaxis (ZDV-group) and (iii) no ARV use antenatally (None-group). Given that ART, particularly TDF which has been associated with poor birth outcomes, has low bioavailability in breastmilk [14], infants whose mother's only started ART postnatally were excluded from this analysis. In an effort to (i) make the periods of exposure to ARV drugs comparable between women on ZDV vesrsus ART and (ii) compare outcomes by duration of ART exposure, women on ART were further dichotomised by ART duration and those who initiated ART post-conception treated as the reference.

Outcomes

The outcomes of interest were PTD, LBW, SGA at birth, and UFA at 6 weeks postpartum. Birth weight and length, 6-week weight and length, and gestational age were extracted from the infants' routine road to health booklets at 4-8 weeks (median 6 weeks) postpartum. The anthropometric measurements were conducted by routine health facility staff using facility procedures and equipment. Length data were excluded in this analysis due to measurement errors and missing data. Health facility staff routinely estimated infant gestational age at delivery using the last menstrual period (LMP). PTD was defined as birth before 37 completed

weeks gestation, LBW as birthweight <2.5Kg, and SGA as birthweight-for-gestational-age Z-score below -1.28 (equivalent to <10th percentile)[15, 16]. We estimated birthweight-for-gestational-age Z-scores using recently published international Intergrowth-21st standards for assessing new born size for term and pre-term born infants [17] and LMS growth [18]. We estimated weight-for-age Z-scores (WAZ) in infants aged 4-8 weeks using the WHO growth standards [19] and considered infants as UFA if their WAZ was below -2 [20].

Data cleaning

Anthropometric measurements and Z-scores were flagged for verification if any of the following criteria were met: birthweight-for-gestational Z-score <-6 or >6; WAZ less than -6 or >5. Except for gestational age, which had 1% observations set to missing after verification (including 3 gestational ages outside of the range for the Intergrowth standards (20-23 weeks)), the remaining measurements and Z-scores had <1% observations omitted.

Covariates

Covariates in the models were selected a priori based on literature [21] and a conceptual framework (supplementary Figure 1). Participants were defined as food insecure if they ever ran out of food in the previous year. Multiple correspondence analysis (MCA) was used to to construct the socio-economic status (SES) index (see supplementary materials). Infant feeding practices were established through 24-hour and 7-day day recall infant feeding questions and infants were categorised into two groups: 1) 'breastfed' if they received any breastmilk and 2) 'none-breastfed' if they received no breastmilk. As SA has racial inequalities, race was included in the models as a potential social determinant of the study outcomes [22]. Based on the reported race, study infants were classified as: 1) "black", 2) "coloured", a multiracial group

or 3) "other" comprised of very small samples of infants defined as "white", "indian" and "other".

Statistical analysis

Analyses were survey based and additional weighting for missing gestational age data was applied to the PTD and SGA analyses (see supplementary materials). Categorical variables were compared using the Pearson chi-squared test while linear regression was used to test equality of means. The Wald test was used for multiple hypothesis testing. In the modelling we first generated four multivariable logistic regression models to assess the effect of in-utero HIV exposure on the outcomes in the total sample of HUU and HEU infants. We then restricted the analysis to HEU infants and compared the outcomes by ART exposure status and duration using four additional models. To avoid bias introduced by adjustment of potential mediators in the presence of unmeasured common causes, the "LBW paradox" [23], variables such as birthweight were not included in the UFA models. In each full model we also included interaction terms to test whether infant HIV exposure modifies the effect of other covariates on birth outcomes. Statistical analyses were performed at a 5% significance level using STATA-14 (Stata Corp., College Station, Texas, USA, 2007), R Software-3.1.2 and IBM-SPSS Statistics-22 (SPSS Inc, Chicago, IL, USA).

Ethical considerations

Ethical approval was obtained from the South African Medical Research Council and the Office of Associate Director of Science at the United States Centers for Disease Control and Prevention . All participants provided informed consent.

RESULTS

There were 9119 live born infants in the SAPMCTE study of which 8976 were of a singleton birth (Figure 1). The final sample analysed included 6179 HUU and 2599 HEU infants after some exclusions. Notably, women who initiated ART pre-conception were older, had a higher parity, and higher frequencies of TB and syphilis than women in the other ARV groups (Table 1).

Prevalence of PTD, LBW, SGA and 6 weeks childhood underweight

The prevalences (95% confidence intervals [CI]) of PTD, LBW and SGA were 12.5% (11.4%; 13.7%), 10.7% (10.0%; 11.5%), and 14.9% (13.8%; 16.1%) respectively in the total sample (Table 2). The prevalence of PTD was similar between HUU (12.3% (11.1%; 13.7%)) and HEU (12.9% (11.12%; 14.7%)) infants (P=0.59) in the unadjusted analysis, whereas the latter group had a higher prevalence of LBW (13.0% vs 9.8%, P<0.01) and SGA (16.9% vs 14.0%, P=0.03). UFA at 6 weeks postpartum was observed in 9.2% (8.6%; 10.0%) of the total sample. A higher proportion of infants were underweight in HEU (11.0%) versus HUU (8.4%) infants (P<0.01).

Factors related to PTD, LBW and SGA

Multivariable analyses demonstrated a higher odds of PTD if the infant was HEU versus HUU (adjusted odds ration (aOR), 97% CI: 1.2 (1.0; 1.5)), was of coloured versus black race (1.7 (1.3; 2.3)), was born to a mother wth less than a secondary education (1.4 (1.1; 1.7) and resided in a poorer household (1.7(1.2; 2.5)). There was a reduced odds of PTD if the infant was born to a mother aged 30-35 versus <20 years (0.7 (0.5; 0.9)) and had more than 5 antenatal care (ANC) visits versus ≤5 (0.7 (0.6; 0.9)) (Table 3). Within the HEU sub-group, infants in the None-group (1.9 (1.1; 3.1)) or those whose mothers initiated ART pre-conception (1.7 (1.1;

2.5)) had almost twice the odds of PTD than infants whose mothers initiated ART post-conception.

A higher odds of LBW was observed if an infant was HEU versus HUU (1.6 (1.3; 1.9)), born to a mother with TB during pregnancy (1.6 (1.1; 2.5)) and if the infant was of coloured versus black race (2.0 (1.5; 2.6)) was born to a mother wth less than a secondary education (1.3 (1.0; 1.6) and resided in a poorer household (1.5(1.1; 2.0)); a reduced odds of LBW if the infant was born to an older mother versus a mother aged <20 years (0.6 (0.5; 0.8)), a mother who had more than 5 versus ≤5 ANC visits (0.8 (0.6; 0.9)) or if the infant was male versus female (0.8 (0.7; 0.9)) (Table 4).

A higher odds of SGA was observed if an infant was HEU versus HUU (1.3 (1.1; 1.6)) and born of coloured versus black race (1.6 (1.3; 2.0)). Factors protective against SGA were older maternal age (aged 26-29) versus aged <20 years (0.6 (0.4; 0.8)) and attendance of 5 versus ≤5 ANC visits (0.8 (0.7; 1.0)) (Table 5).

Factors related to UFA at six weeks postpartum

A greater odds of UFA at 6 weeks postpartum was observed if an infant was HEU versus HUU (1.5 (1.2; 1.8)) , of coloured versus black race (2.2 (1.6; 2.8)), was born by C-section versus vaginal delivery (1.4 (1.1; 1.7)) , had experienced diarrheal episodes (1.9 (1.3; 2.8)), was born to mother who had TB during pregnancy (1.8 (1.2; 2.8)) or had less than a secondary education (1.4 (1.1; 1.8) and resided in a poorer household (1.5(1.1; 2.0)). A reduced odds of underweight was observed if the infant was breastfed versus not breastfed (0.8 (0.6; 1.0) and if the infant was born to a mother who attended more than 5 ANC visits versus ≤5 (0.8 (0.6; 1.0)) (Table 6). None of the models showed evidence of effect measure modification by HIV exposure status (data not shown).

DISCUSSION

In a nationally representative survey we observed a greater odds of PTD, LBW, SGA and underweight among HEU than HUU infants.

We noted that HEU infants whose mothers did not receive any ARV drugs carried higher odds of PTD than ART exposed infants but no increased odds for LBW, SGA and underweight. In addition, among ART exposed pregnancies, PTD was more common among infants whose mothers initiated ART pre-conception than postconception. We also identified several independent risk factors for poor birth and growth outcomes, including coloured race, which was associated with all study outcomes; birth by C-section, was associated with UFA; exposure to maternal TB during pregnancy was associated with LBW and UFA and diarrhea was a risk factor for UFA; a lower maternal education and household SES, which were associated with PTD, LBW and UFA. Factors that were protective against all outcomes included older maternal age and more frequent ANC attendance. Additonal protective factors included male gender for LBW and UFA and breastfeeding for UFA.

Our finding that HEU infants have more adverse birth outcomes than HUU infants has been reported in other African studies ([24] supplementaty Table 2). Within SA, data from a hospital-based cohort study in the Western Cape showed a higher prevalence of PTD and LBW, but not SGA, in HIV-exposed versus HUU infants [25]. Surveillance pregnancy registry data in KwaZulu-Natal also showed a higher prevalence of LBW in HIV-exposed than HUU infants[26]. Another cohort study in the same region reported a higher prevalence of SGA but not PTD in HIV-exposed infants [7]. The mechanisms through which maternal HIV infection results in specific adverse birth outcomes in HEU infants are still unclear. Current evidence suggests that HIV exposure elicits chronic immune activation and systemic inflammation in HEU infants [27, 28], particularly in infants born to women with higher viral loads [29], which

has been associated with PTD [30, 31] and LBW [31]. These proposed mechanisms may partly explain why, similar to other studies [32, 33], our study infants born to women with untreated HIV infection had higher rates of PTD compared to infants born to women who initiated ART during pregnancy. These results support the current "test and treat" strategy of initiating all newly diagnosed HIV-positive pregnant women on ART.

However, exposure to ART during pregnancy, a critical period of foetal growth and development, may carry some risks. Evidence on the effect of in-utero ART exposure on adverse outcomes is mixed ([34] and supplementary Table 2), and there is still uncertainty whether observed adverse effects are specific to particular drugs or combinations thereof. Moreover as more countries adopt the Option-B+ strategy, many more women will be on ART drugs at conception, highlighting the importance of setting up pregnancy registers or similar surveillance systems to monitor whether earlier ART initiation adversely affects birth outcomes in order to inform future policy. Such monitoring fits into the broader child health goals of reducing child mortality while optimising good health and well-being [35]. Consistent with findings from a recent review [36], our data shows that PTD is higher amongst women who initiated ART pre-conception than amongst mothers initiating ART post-conception. These findings are in contrast to the null associations reported in single-site hospital based retropective [37] and prospective[25] cohort studies conducted in SA and could be biased by the unmeasured maternal disease severity or could be due to selection bias[38]. In line with these studies, however, LBW and SGA did not differ by timing of ART initiation in our sample.

The success of PMTCT programmes make it possible for most HIV-exposed infants to remain uninfected [1]. Therefore, while the proportion of under-five deaths attributed to HIV infection has decreased in SA, the proportion of under-5 deaths due to neonatal conditions, which contribute the most to under-five mortality, has increased. Given that PTD complications constitute the bulk of these neonatal conditions, it is important that risk factors of PTD are

addressed through better care of both mothers and infants [39, 40]. Our findings that older maternal age and more frequent ANC visits were protective against all adverse study outcomes, and that infants born to women with lower maternal education and SES had a greater risk for PTD, LBW and UFA, highlight the importance of health system strengthening and the need for further investment in multi-sectoral "nutrition-sensitive" interventions [41] that address the multifactorial etiology of these outcomes. The protective effect of breastfeeding on childhood underweight further emphasises the importance of supporting early initiation of breastfeeding for all infants.

Our study describes birth outcomes in infants that survived their first month of life, therefore, our overall LBW rate is lower than the national estimate of 14.8%, which is based on data from all live births [42]. Background national estimates of PTD and SGA are not readily available. Our PTD rate was higher than the 8.0% modelled by Blencowe, et al. [43] based on data from two hospital-based studies, while our SGA rate is lower than 23% and 21.8% reported by Lee, et al. [44] among preterm and term born infants respectively.

This study has some limitations. First, we were limited by the lack of key clinical data, such as maternal obstetric history, substance use, accurate CD4 cell count, HIV viral load and whether or not the PTD was induced or spontaneous. While data collectors used a chart to assist HIV-infected mothers recall their self-reported ARV-drug use, which is subject to recall bias and may cause misclassification of participants, the lack of detailed information on the specific drug and dose used precluded the extent to which study outcomes could be assessed by ART regimen. However, data does show there is no significant difference in PTD between infants exposed to TDF-containing ART and other combinations [45] although a greater risk of PTD has been reported with protein-inhibitor compared to nucleoside-reverse-transcriptase-based ART [46, 47]. Furthermore, some women initiated on ART pre-cenception may have been on different ART regimens, probably second-line treatment due to drug resistance or poor

adhenrance, than women started post-concepton. Although self-reported CD4 cell count data were also available, we did not include them in the final analysis as the exact timing of the CD4 count testing was unknown, many mothers did not know their results and those who had the information only reported one result. The lack of CD4 count and viral load data precluded the extent to which the effect of maternal immune suppression and viremia respectively, could be assessed. We also could not stratify the ARV analyses by CD4 categories in order to minimize bias by indication. Secondly, as this was an observational study we could not establish causal relationships. Nevertheless in an effort to minimize bias we included, based on a conceptual framework, factors known to influence our outcomes in the multivariable analyses. Thirdly, we obtained gestational age from the infant's health card, which contained LMP-based gestational age data. Whilst this method remains the most commonly used method in SA, it has several limitations including poor recall of the date of LMP and a tendancy to over estimate PTD [48]. However, evidence suggests that LMP is a fairly reliable measure of gestational age in resource limted settings [49]. We also used routinely collected infant weight data which are subject to measurement error. Fourthly, in accordance with the 2010 WHO PMTCT guidelines, HIV-positive pregnant women that were not eligible for ART were started on ZDV from 14 weeks gestation to ensure sufficient drug exposure time by late pregnancy [50] as this period carries the highest risk of MTCT [51]. Eligible HIV-positive pregnant women were however started on ART immediately in order to improve maternal health and reduce MTCT. We were therefore concerned about potential lead time bias when comparing the outcomes of the ZDV versus ART HEU infants. Although an analysis of our data revealed that the time of exposure to the drugs was similar between the ARV groups we restricted the ZDV versus ART comparisons to women who initiated ART post-conception to minimize this bias. Lastly, selection bias is possible as sick infants needing emergency care, infants that died before the 6-week clinic visit and infants attending small remote facilities were excluded. As

these infants represent particularly vulnerable groups our estimates of LBW, SGA, PTD and UFA could be underestimated.

Notwithstanding these, our study has some strengths. Firstly, this is the largest nationally representitve study of birth outcomes and growth to date among HEU and HUU infants in SA, although it has inherent cross-sectional study limitations related to temporality. Secondly, the availability of laboratory HIV-1 ELISA and PCR results enabled us to exclude HIV-infected infants from the analyses, although NVP exposure may cause false-negative results[52]. Thirdly, we collected data, although not exhaustive, on maternal, infant and health system characteristics which enabled us to explore the independent effect of these factors and adjust for them as potential confounders. Fourthly, we estimated birthweight-for-gestational age Z-scores using the recent Intergrowth standard for term and pretern born infants. Lastly, we collected data before the wide-scale implementation of Option-B+ in SA, which enabled us to compare outcomes of in-utero ZDV exposed versus ART-exposed infants.

In conclusion, ART resulted in better birth outcomes, however, there was an association between pre-conception ART and PTD. As ART access increases, pregnancy registers or similar routine surveillance should be in place to monitor outcomes to inform future policy.

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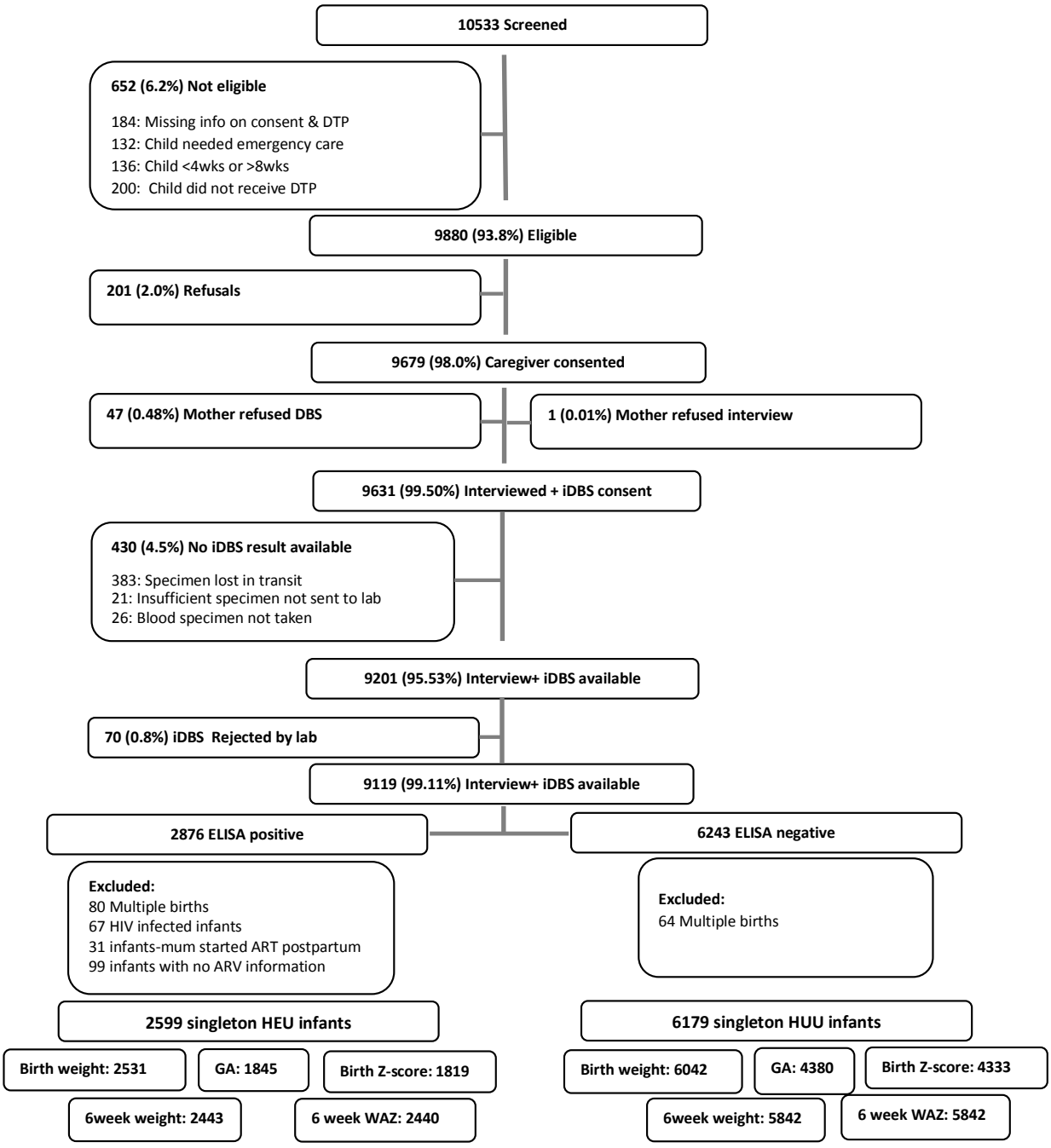


Figure 1: SAPMTCTE study profile

Note: gestational age; GA

Table 1: Weighted analysis of participant characteristics by HIV and antiretroviral exposure status

Characteristics	HUU (N=6179)	HEU (N=2599)				Total (N=8778)
		Pre-conception ART (N=616)	Post-conception ART (N=780)	ZDV (N=873)	None (N=330)	
Maternal						
Age at enrolment (y)						
<20	18.3 (17.1; 19.6)	1.8 (1.0; 3.2)	3.1 (2.0; 4.9)	7.0 (5.0; 9.6)	5.2 (3.2; 8.4)	14.0 (13.1; 15.0)
20-25	39.6 (38.2; 41.1)	10.0 (7.8; 12.8)	28.5 (25.2; 32.0)	36.2 (32.6; 40.0)	34.9 (29.0; 41.3)	35.9 (34.7; 37.1)
26-29	17.9 (16.8; 19.0)	23.3 (19.7; 27.2)	27.2 (24.1; 30.5)	26.1 (23.1; 29.4)	24.5 (19.7; 30.1)	20.3 (19.3; 21.3)
30-35	14.0 (12.9; 15.1)	35.2 (31.1; 39.4)	29.8 (26.5; 33.3)	19.1 (16.5; 22.0)	20.7 (16.2; 26.0)	17.5 (16.5; 18.5)
>35	10.0 (9.1; 10.9)	29.8 (26.5; 33.3)	14.6 (12.0; 17.7)	11.6 (9.5; 14.0)	14.7 (10.8; 19.7)	12.2 (11.4; 13.0)

Missing	0.3 (0.2; 0.5)	0	0	0	0	0.2 (1.0; 3.0)
Parity						
1	43.0 (41.7: 44.4)	15.4 (12.5; 18.8)	24.8 (21.7; 28.2)	30.6 (26.7; 34.7)	24.1 (19.2; 29.7)	37.3 (36.1; 38.4)
2-3	44.4 (43.0; 45.7)	63.6 (59.5; 67.6)	65.0 (61.2; 68.6)	58.8 (54.7; 62.8)	63.7 (57.6; 69.3)	50.0 (48.8; 51.1)
4+	8.54 (7.8; 9.3)	21.0 (17.6; 24.9)	10.2 (8.1; 12.9)	10.64 (8.7; 13.0)	12.3 (8.9; 16.7)	10.0 (9.3; 10.7)
Missing	4.1 (3.5; 4.7)	0	0	0	0	2.8 (2.4; 3.3)
Education (grade)						
≤grade 7	11.0 (10.0; 12.2)	16.9 (14.8; 19.2)	15.1 (12.6; 17.9_	21.8 (17.3; 27.1)	22.1 (15.0; 31.3)	12.9 (11.9; 14.0)
>grade 7	88.7 (87.5; 89.7)	83.1 (80.8; 85.2)	85.0 (82.1; 87.4)	78.2 (72.9; 82.7)	74.5 (64.9; 82.3)	82.9 (81.1; 84.6)
Missing	0.3 (0.2; 0.5)	0	0	0	0	0.2 (0.1; 0.4)
Delivery mode						
Vaginal	76.8 (75.4; 78.1)	71.3 (66.9; 75.3)	73.2 (69.3; 76.8)	76.8 (73.5; 79.9)	78.0 (71.9; 83.1)	76.1 (74.7; 77.4)

C-section	23.1 (21.8; 24.4)	28.7 (24.7; 33.1)	26.5 (22.9; 30.4)	23.2 (20.1; 26.5)	21.9 (16.8; 28.0)	23.8 (22.5; 25.1)
Missing	0.2 (0.1; 0.3)	0	0.3 (0.1; 1.1)	0	0.1 (0.0; 0.8)	1.5 (0.8; 2.8)
ANC visits						
1-5	44.6 (42.2; 47.2)	41.9 (40.0; 47.0)	38.0 (32.8; 43.5)	39.2 (35.2; 43.3)	48.4 (42.3; 54.6)	43.4 (41.0; 45.8)
5+	23.4 (21.1; 25.8)	31.7 (26.6; 37.3)	28.7 (24.4; 33.5)	26.91(23.1; 31.1)	21.7 (16.8; 27.6)	24.8 (22.5; 27.2)
Missing	32.0 (29.2; 34.9)	26.4 (21.8; 31.5)	33.3 (27.6; 39.4)	33.9 (28.7; 39.6)	29.9 (24.0; 36.5)	31.8 (29.0; 43.8)
Syphilis during pregnancy						
Positive	1.1 (0.8; 1.5)	8.1 (4.8; 13.1)	7.6 (4.6; 12.2)	4.6 (2.9; 7.1)	2.6 (1.3; 5.1)	2.7 (2.0; 3.6)
Negative	68.3 (65.5; 71.0)	64.9 (59.0; 70.4)	66.8 (61.3; 71.8)	70.4 (66.4; 74.2)	58.1 (51.4; 64.5)	67.8 (65.1; 70.3)
Missing	30.6 (28.0; 33.4)	27.1 (22.2; 32.6)	25.7 (21.4; 30.4)	25.0 (21.4; 28.9)	39.24 (32.89; 46.0)	29.6 (27.1; 32.2)
Tuberculosis during pregnancy						

Yes	1.3 (1.0; 1.6)	9.5 (7.1; 12.5)	5.1 (3.4; 7.5)	2.2 (1.2; 4.1)	1.89 (0.75; 4.73)	2.4 (2.0; 2.8)
No	94.5 (93.7; 95.2)	90.0 (87.0; 92.4)	94.4 (91.7; 96.2)	97.6 (95.7; 98.7)	97.88 (95.07; 99.10)	94.6 (93.9; 95.2)
Missing	4.2 (3.7; 4.9)	0.5 (0.1; 1.6)	0.5 (0.2; 1.4)	0.1 (0.0; 0.7)	0.23 (0.03; 1.61)	3.0 (2.6; 3.5)
No food	14.0 (12.4; 15.7)			18.6 (15.4; 22.2)	14.9 (10.4; 20.8)	
Household socio- economic quintile						
Poorest	15.2 (13.5; 17.0)	16.5 (13.4; 20.2)	15.1 (12.4; 18.2)	16.3 (13.5; 19.6)	21.6 (17.0; 27.0)	15.6 (14.0; 17.4)
Second	16.9 (15.4; 18.6)	17.6 (14.3; 21.5)	21.0 (17.8; 24.7)	20.9 (17.8; 24.3)	18.0 (13.9; 23.0)	17.8 (16.3; 19.4)
Third	17.8 (16.4; 19.3)	20.5 (17.2; 24.2)	21.3 (17.9; 25.1)	23.0 (19.4; 27.0)	24.0 (19.4; 29.3)	19.1 (17.8; 20.5)
Fourth	35.8 (33.5; 38.1)	35.2 (30.9; 39.7)	34.1 (29.6; 35.2)	30.6 (26.3; 35.2)	28.1 (22.9; 33.9)	34.7 (32.6; 37.0)
Least poor	14.3 (12.8; 16.0)	10.2 (7.5; 13.8)	8.6 (6.5; 11.2)	9.3 (7.0; 12.2)	8.4 (4.6; 14.7)	12.7 (11.4; 14.2)
Missing	0	0	0	0	0	0

Infant						
Male	51.4 (50.0; 52.7)	50.5 (46.2; 54.8)	48.5 (44.2; 52.8)	54.8 (51.2; 58.4)	51.8 (46.1; 57.4)	51.4 (50.3; 52.6)
Race						
Black	85.7 (83.0; 88.1)	97.3 (94.9; 98.5)	98.2 (97.1; 99.0)	96.2 (94.1; 97.5)	97.5 (94.5; 98.9)	89.3 (87.2; 91.1)
Coloured	12.6 (10.4; 15.2)	2.7 (1.5; 5.1)	1.8 (1.0; 3.0)	3.3 (2.0; 5.3)	2.0 (0.9; 4.7)	9.5 (7.8; 11.5)
Other	1.7 (1.1; 2.6)	0	0	0.6 (0.2; 1.9)	0.5 (0.1; 3.5)	1.3 (0.8; 1.9)
Breast feeding						
Yes	88.0 (86.9; 89.1)	58.2 (52.5; 63.6)	62.8 (59.1; 66.4)	65.7 (62.2; 69.0)	73.0 (66.7; 78.6)	80.6 (79.3; 81.8)
No	12.0 (10.9; 13.1)	41.8 (36.4; 47.5)	37.2 (33.6; 40.9)	34.3 (31.0; 37.8)	27.0 (21.4; 33.3)	19.4 (18.2; 20.7)

Data are percentages with 95% confidence intervals. Definitions: ART= antiretroviral treatment, HEU= HIV exposed uninfected infants, HUU= HIV unexposed infants, LBW= low birthweight, None= infant was not exposed to in-utero antiretroviral drugs, PTD= preterm delivery, SGA= small-for-gestational age, UFA= underweight-for-age, ZDV= Zidovudine prophylaxis

Table 2: Adverse birth and growth outcomes by HIV and ARV exposure status

Group		PTD n [%(95%CI)]	LBW n [%(95%CI)]	SGA n [%(95%CI)]	UFA n [%(95%CI)]
HUU	N=6179	568 [12.3 (11.1; 13.7)]	624 [9.8 (8.9; 10.7)]	590 [14.0 (12.8; 15.4)]	531 [8.44 (7.7; 9.29)
HEU	Pre-conception ART n=616	67 [14.6 (11.6; 18.3)]	84 [12.9 (10.4; 15.9)	79 [18.2 (14.7; 22.3)]	74 [11.1 (8.8; 13.9)]
	Post-conception ART n=780	61 [9.5 (7.1; 12.5)]	111 [13.7 (11.3; 16.5)]	106 [19.2 (15.5; 23.5)]	82 [9.9 (7.9; 12.4)]
	ZDV n=873	84 [13.0 (10.4; 16.2)]	93 [11.3 (9.1; 13.9)]	90 [14.8 (11.9; 18.3)]	91 [11.1 (8.9; 13.7)]
	None n=330	39 [18.2 (13.2; 24.6)]	51 [16.1 (12.5; 20.5)]	30 [13.9 (9.4; 20.0)	43 [13.9 (10.5; 18.2)]

	Total N=2599	251 [12.9 (11.2; 14.7)]	339 [13.0 (11.6; 14.4)]	305 [16.9 (14.7; 19.2)]	290 [11.0 (9.9; 12.4)]
Grand Total	N=8778	819 [12.5 (11.4; 13.7)]	963 [10.7 (10.0; 11.5)]	895 [14.9 (13.8; 16.1)]	821 [9.2 (8.6; 10.0)]
Note: 205 (1.7%) participants had missing data for LBW, 2553 (26.1%) for PTD and 2626 (26.8%) for SGA. Definitions: ART; antiretroviral treatment, low birthweight; LBW, None; infant was not exposed to in-utero antiretroviral drugs, preterm delivery; PTD, HEU; HIV exposed uninfected infants, HUU; HIV unexposed infants, small-for-gestational age; SGA, underweight-for-age; UFA, ZDV; Zidovudine prophylaxis					

Table 3: Weighted multivariable logistic regression models for factors related to preterm delivery (PTD) in A) HIV exposed uninfected and HIV unexposed uninfected infants and B) HIV exposed uninfected infants[a]

Variable	A) HEU and HUU[b] combined		B) HEU only [c]	
	AOR (95%CI)	p-value [d]	AOR (95%CI)	p-value
HIV exposure status				
HEU	1.2 (1.0; 1.5)	0.04	----	----
HUU	Ref			
ARV	----			0.04*
Pre-conception ART			1.7 (1.1; 2.5)	0.02
ZDV			1.4 (0.9; 2.0)	0.11
None			1.9 (1.1; 3.1)	0.01
Post-conception ART			Ref	
Syphilis serology				
Positive	0.7 (0.4; 1.2)	0.22	0.7 (0.3; 1.3)	0.22

Negative	Ref		Ref	
Tuberculosis				
Yes	1.1 (0.7; 1.9)	0.65	1.1 (0.5; 2.3)	0.83
No	Ref		Ref	
Maternal age		0.08*		0.08*
20-25	0.9 (0.7; 1.1)	0.36	1.5 (0.7; 3.2)	0.31
26-29	0.9 (0.7; 1.3)	0.63	1.5 (0.7; 3.5)	0.35
30-35	0.7 (0.5; 0.9)	0.02	1.0 (0.5; 2.3)	0.94
>35	1.0 (0.6; 1.4)	0.79	2.0 (0.9; 4.6)	0.11
<20	Ref		Ref	
Parity		0.20*		0.08*
2-3	0.8 (0.7; 1.0)	0.06	0.7 (0.4; 0.9)	0.02
4+	0.9 (0.6; 1.2)	0.40	0.7 (0.4; 1.2)	0.14
1	Ref		Ref	
Maternal education				

≤ grade 7	1.4 (1.1; 1.7)	<0.01	1.1 (0.8; 1.6)	0.62
> grade 7	Ref		Ref	
ANC visits				
+5	0.7 (0.6; 0.9)	<0.01	0.7 (0.5; 1.0)	0.08
1-5	Ref		Ref	
Household socio economic quintile		<0.01*		0.02*
Poorest	1.7 (1.2; 2.5)	<0.01	3.0 (1.3; 6.8)	<0.01
Second	1.2 (0.9; 1.7)	0.26	1.7 (0.7; 3.8)	0.24
Third	1.3 (1.0; 1.8)	1.00	2.6 (1.2; 5.9)	0.02
Fourth	1.1 (0.8; 1.5)	0.68	2.1 (0.9; 4.4)	0.07
Least poor	Ref		Ref	
Household food insecurity				
Yes	0.8 (0.6; 1.0)	0.07	1.0 (0.6; 1.5)	0.82

No	Ref		Ref	
Infant race		<0.01*		0.41*
Coloured	1.7 (1.3; 2.3)	<0.01	1.4 (0.7; 2.7)	
Other	1.3 (0.5; 3.4)	0.56	----	
Black	Ref		Ref	
Infant gender				
Male	0.9 (0.8; 1.0)	0.12	1.1 (0.8; 1.5)	0.55
Female	Ref		Ref	

[a]The values in the models are adjusted odds ratio, AOR (95%CI). HEU, HIV exposed uninfected; HUU, HIV unexposed; Ref; Reference category.

[b] Model included 6214 HIV exposed and unexposed infants.

[c] Model included 1839 HIV exposed uninfected infants.

[d] Except for the asterisk below, the p-values in this table are t test p-values. The 5% significance level was used in all analyses

[*] This p-value is derived from the joint hypothesis testing adjusted Wald test

Table 4: Weighted multivariable logistic regression models for factors related to low birth weight (LBW) in A) HIV exposed uninfected and HIV unexposed uninfected infants and B) HIV exposed uninfected infants[a]

Variable	A) HEU and HUU[b]		B) HEU[c]	
	AOR (95%CI)	p-value[d]	AOR (95%CI)	p-value
HIV exposure status				
HEU	1.6 (1.3; 1.9)	<0.01	----	
HUU	Ref			
ARV	----			0.27*
Pre-conception ART	----		0.9 (0.6; 1.3)	0.54
ZDV			0.8 (0.6; 1.1)	0.14
None			1.1 (0.8; 1.6)	0.47
Post-conception ART			Ref	
Syphilis serology				
Positive	0.8 (0.5; 1.3)	0.29	0.6 (0.3; 1.2)	0.15
Negative	Ref		Ref	
Tuberculosis				
Yes	1.6 (1.0; 2.5)	0.03	1.3 (0.7; 2.6)	0.46
No	Ref		Ref	
Maternal age		<0.01*		0.02*
20-25	0.8 (0.6; 1.0)	0.02	1.0 (0.5; 1.9)	0.95
26-29	0.6 (0.5; 0.8)	<0.01	0.8 (0.4; 1.6)	0.59

30-35	0.6 (0.4; 0.8)	<0.01	0.7 (0.4; 1.5)	0.38
>35	0.9 (0.6; 1.2)	0.49	1.5(0.7; 3.0)	0.28
<20	Ref		Ref	
Parity		0.45*		0.40*
2-3	1.0 (0.8; 1.2)	0.87	0.9 (0.6; 1.2)	0.46
4+	0.8 (0.6; 1.1)	0.23	0.7 (0.4; 1.2)	0.18
1	Ref		Ref	
Maternal education				
≤grade 7	1.3 (1.0; 1.6)	0.03	1.0 (0.7; 1.4)	0.98
>grade 7	Ref		Ref	
ANC visits				
+5	0.8 (0.6; 0.9)	0.01	0.7 (0.5; 1.0)	0.07
1-5	Ref		Ref	
Household socio economic quintile		0.03*		0.43*
Poorest	1.1 (0.8; 1.5)	0.66	1.2 (0.6; 2.1)	0.66
Second	1.2 (0.9; 1.7)	0.20	1.1 (0.6; 2.1)	0.78
Third	1.5 (1.1; 2.0)	0.01	1.5 (0.8; 2.8)	0.22
Fourth	1.3 (0.9; 1.7)	0.16	1.4 (0.7;2.6)	0.34
Least poor	Ref		Ref	

Household food insecurity				
Yes	1.1 (0.9; 1.4)	0.22	1.2 (0.9; 1.7)	0.27
No	Ref		Ref	
Infant race		<0.01*		
Coloured	2.0 (1.5; 2.6)	<0.01	3.4 (1.8; 6.6)	<0.01
Other	1.2 (0.5; 2.8)	0.67	----	
Black	Ref		Ref	
Infant gender				
Male	0.8 (0.7; 0.9)	<0.01	0.8 (0.6; 1.0)	0.03
Female	Ref			

[a]The values in the models are adjusted odds ratio (95%CI). Definitions: AOR, Adjusted odd ratio; HEU, HIV exposed uninfected; HUU, HIV unexposed; Ref; Reference category.

[b] Model included 8476 HIV exposed and unexposed infants

[c] Model included 2510 HEU infants.

[d] Except for the asterisk below, the p-values in this table are t test p-values. The 5% significance level was used in all analyses

[*] This p-value is derived from the joint hypothesis testing adjusted Wald test

Table 5: Weighted multivariable logistic regression models for factors related to small-for-gestational age (SGA) in A) HIV exposed uninfected and HIV unexposed uninfected infants and B) HIV exposed uninfected infants[a]

Variable	A) HEU and HUU[b]		B) HEU[c]	
	AOR (95%CI)	p-value[d]	AOR (95%CI)	p-value
HIV exposure status				
HEU	1.3 (1.1; 1.6)	<0.01	----	
HUU	Ref			
ARV	----			0.14*
Pre-conception ART			0.9 (0.6; 1.3)	0.52
ZDV			0.7 (0.5; 1.0)	0.05
None			0.7 (0.4; 1.1)	0.08
Post-conception ART			Ref	
Syphilis serology				
Positive	1.3 (0.8; 2.3)	0.30	1.6 (0.9; 2.8)	0.15
Negative	Ref		Ref	
Tuberculosis				
Yes	1.3 (0.8; 2.1)	0.31	1.1 (0.6; 2.2)	0.76
No	Ref		Ref	
Maternal age		<0.01*		0.03*
20-25	0.8 (0.6; 1.0)	0.10	0.8 (0.4; 1.5)	0.45
26-29	0.6 (0.4; 0.8)	<0.01	0.6 (0.3; 1.2)	0.17

30-35	0.7 (0.5; 1.0)	0.05	0.6 (0.3; 1.2)	0.15
>35	1.0 (0.7; 1.4)	0.91	1.2 (0.6; 2.5)	0.66
<20	Ref		Ref	
Parity		0.67*		0.65*
2-3	0.9 (0.8; 1.1)	0.42	0.9 (0.7; 1.3)	0.64
4+	0.9 (0.6; 1.2)	0.46	0.8 (0.5; 1.3)	0.36
1	Ref		Ref	
Maternal education				
≤grade 7	1.1 (0.9; 1.4)	0.37	1.4 (1.0; 2.0)	0.04
>grade 7	Ref		Ref	
ANC visits				
+5	0.8 (0.7; 1.0)	0.02	0.9 (0.7; 1.3)	0.69
1-5	Ref		Ref	
Household socio economic quintile		0.35*		0.65*
Poorest	1.3 (1.0; 1.8)	0.09	1.3 (0.7; 2.3)	0.41
Second	1.4 (1.0; 1.9)	0.06	1.2 (0.6; 2.3)	0.60
Third	1.4(1.0; 1.9)	0.07	1.3 (0.6; 2.4)	0.52
Fourth	1.3 (0.9; 1.8)	0.14	1.0 (0.5; 1.9)	0.97

Least poor	Ref		Ref	
Household food insecurity				
Yes	1.0 (0.8; 1.3)	0.87	0.8 (0.6; 1.2)	0.27
No	Ref		Ref	
Infant race		<0.01*		
Coloured	1.6 (1.3; 2.0)	<0.01	2.1 (1.0; 4.4)	0.04
Other	1.5 (0.3; 7.4)	0.62	----	
Black	Ref		Ref	
Infant gender				
Male	1.0 (0.9; 1.2)	0.67	0.9 (0.7; 1.2)	0.60
Female	Ref		Ref	

[a] The values in the models are adjusted odds ratio (95%CI). Definitions: AOR: Adjusted odd ratio; HEU, HIV exposed uninfected; HUU, HIV unexposed, Ref; Reference category. Only variables that had a significant association with low birth weight in the bivariate analysis were included the final multivariable models.

[b] Model included 6142 HIV exposed and unexposed infants.

[c] Model included 1813 HIV exposed uninfected infants.

[d] Except for the asterisk below, the p-values in this table are t test p-values. The 5% significance level was used in all analyses

[*] This p-value is derived from the joint hypothesis testing adjusted Wald test

Table 6: Weighted multivariable logistic regression models for factors related to underweight-for-age (UFA) in A) HIV exposed uninfected and HIV unexposed uninfected infants and B) HIV exposed uninfected infants[a]

Variable	HEU and HUU[b]		HEU[c]	
	AOR (95%CI)	p-value[d]	AOR (95%CI)	p-value
HIV exposure status			----	
HEU	1.5 (1.2; 1.8)	<0.01		
HUU	Ref			
ARV				0.43*
Pre-conception ART	----		1.1 (0.7; 1.6)	0.78
ZDV			1.1 (0.8; 1.6)	0.64
None			1.4 (0.9; 2.2)	0.12
Post-conception ART			Ref	
Syphilis serology				
Positive	0.7 (0.4; 1.1)	0.12	0.5 (0.3; 1.0)	0.05
Negative	Ref		Ref	
Tuberculosis				
Yes	1.8 (1.2; 2.8)	<0.01	1.8 (1.0; 3.2)	0.04
No	Ref		Ref	
Maternal age		0.04*		0.15*

20-25	0.8 (0.6; 1.0)	0.06	0.8 (0.4; 1.4)	0.34
26-29	0.7 (0.5; 0.9)	0.01	0.7 (0.4; 1.3)	0.30
30-35	0.7 (0.5;0.9)	0.02	0.7 (0.4; 1.3)	0.21
>35	0.9 (0.6; 1.3)	0.48	1.1 (0.6; 2.2)	0.72
<20	Ref		Ref	
Parity		0.33*		0.62*
2-3	0.9 (0.7; 1.1)	0.15	0.9 (0.6; 1.3)	0.52
4+	0.8 (0.6; 1.2)	0.27	0.8 (0.4; 1.4)	0.33
1	Ref		Ref	
Maternal education				
≤7	1.4 (1.1; 1.8)	<0.01	1.20 (0.8; 1.7)	0.32
>7	Ref		Ref	
ANC visits				
+5	0.8 (0.6; 1.0)	0.02	0.9 (0.6; 1.2)	0.31
1-5	Ref		Ref	
Household socio economic quintile		0.01*		0.59*
Poorest	1.5 (1.1; 2.0)	0.02	1.4 (0.8; 2.7)	0.25
Second	1.2 (0.9; 1.7)	0.23	1.2 (0.6; 2.4)	0.58

Third	1.6 (1.2; 2.1)	<0.01	1.6 (0.8; 2.9)	0.16
Fourth	1.2 (0.9; 1.6)	0.32	1.3 (0.7; 2.5)	0.38
Least poor	Ref		Ref	
Household food insecurity				
Yes	1.2 (0.9; 1.5)	0.14	1.1 (0.8; 1.6)	0.63
No	Ref		Ref	
Delivery method				
C-section	1.4 (1.1; 1.7)	<0.01	1.4 (1.0; 1.9)	0.03
Vaginal	Ref		Ref	
Infant race		<0.01*		
Coloured	2.2 (1.6; 2.8)	<0.01	2.6 (1.4; 5.0)	<0.01
Other	1.3 (0.5; 3.5)	0.67		----
Black	Ref		Ref	Ref
Infant gender				
Male	1.2 (1.0; 1.4)	0.04	1.4 (1.0; 1.8)	0.04
Female	Ref		Ref	Ref
Feeding				
Breastfed	0.8 (0.6; 1.0)	0.05	1.1 (0.8; 1.5)	0.56
None breastfed	Ref		Ref	
Diarrhoea				

Yes	1.9 (1.3; 2.8)	<0.01	1.8 (0.8; 4.0)	0.18
No	Ref		Ref	

[a] The values in the models are adjusted odds ratio (95%CI). Definitions: AOR, Adjusted odd ratio; HEU, HIV exposed uninfected; HUU, HIV unexposed; Ref, Reference category. Only variables that had a significant association with low birth weight in the bivariate analysis were included the final multivariable models.

[b] Model included 8202 HIV exposed and unexposed infants.

[c] Model included 2414 HIV exposed uninfected infants.

[d] Except for the asterisk below, the p-values in this table are t test p-values. The 5% significance level was used in all analyses

[*] This p-value is derived from the joint hypothesis testing adjusted Wald test

Supplementary material

Sampling

580 primary health clinics (PHCs) and community health centres (CHCs) offering immunisations services were stratified into 3 strata, within each province, based on their annual 6 week immunisation coverage: small (<130 annual immunisations); medium (130-300 annual immunisations) and large (>300 annual immunisations) facilities. Small facilities were excluded from the final sample due to logistical constraints. Using the 2008 national antenatal maternal HIV prevalence (29.0%) as a cut-off point, facilities in the large immunisation stratum were further stratified to below or above the national average for antenatal HIV prevalence generating a total of 23 strata. A stratified two-stage sampling approach then followed. In the first stage, the primary sampling units (health facilities) were randomly selected proportional to the size of each stratum. In the second stage, a fixed number of infants, comprising of the median number of infants expected within a stratum in a sampling window of 3-4 weeks, was consecutively or randomly selected in each facility. Based on a design effect of 2 and relative precisions for MTCT risk of 30-50% across provinces, a target sample size of 12200 was estimated to generate valid provincial and national estimates for vertical HIV transmission at 6 weeks postpartum [1].

Laboratory testing

Infant dried blood spot (iDBS) specimens were collected by trained nurse data collectors and sent to the National Institute for Communicable Disease (NICD), National Health Laboratory Services (NHLS) for HIV antibody serology testing and, for antibody positive samples, testing for both HIV-1 proviral DNA and HIV-1 RNA using an enzyme immunoassay (EIA) [Genscreen HIV1/2 Ab EIA Version2, Bio-rad Laboratories, France]. Antibody positive specimens collected at the 6week study visit, which underwent a second confirmatory test

[Vironostika HIV Uni-form II plus O, bioMérieux Clinical Diagnostics, Marcy-L'Etoile, France], were tested for both HIV-1 proviral DNA and HIV-1 RNA using a qualitative Polymerase Chain Reaction (PCR) COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) Qualitative assay version 1.0, Roche Diagnostics, Branchburg, NJ] to determine infection status. Ten percent of antibody negative tests were re-tested to confirm negativity.

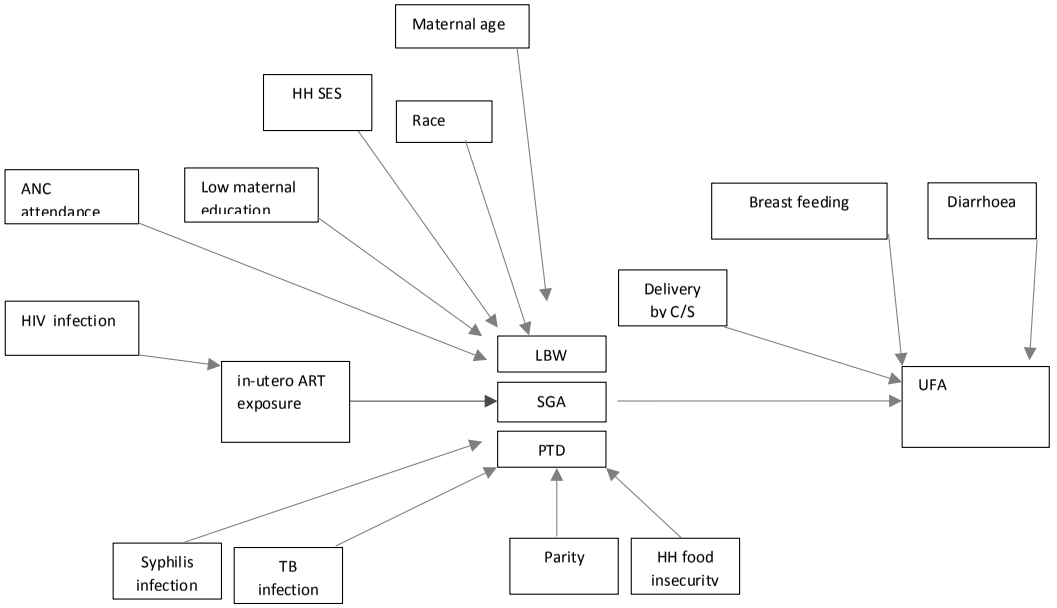
Construction of socio economic status variable

Given that our asset variables were discrete we used multiple correspondence analysis (MCA) rather than the principal components analysis (PCA) method [2], which is better suited for normally distributed continuous variables, to construct our socio-economic index [3] using binary variables on household asset ownership (television, refrigerator, stove, phone and car), facilities (access to piped water, electricity and flush toilet) and housing characteristics (brick or non-brick structure).These variables were checked for internal consistency (Cronbach's alpha=0.73). Using index scores from the first dimension, which explained 84.5% of the variation, we classified households into quintiles: quintile one representing the poorest households and five the least poor. The reliability of the final index was assessed by checking the distribution of certain variables (access to a refrigerator, stove, television, telephone, car, flush toilet and electricity, housing type and mother's employment status) across the quintiles [4].

Handling of missing data

Twenty nine percent of the study participants did not have gestational age information largely because the data were not recorded in their RtHB. As a complete case analysis may introduce bias, we used the inverse probability weighting method to account for the missing gestational age data in the preterm delivery (PTD) and small for gestational age (SGA) analyses. First we generated a predictive logistic regression model for the probability that an individual is missing

the binary outcome variable, preterm delivery. The following “fully observed”[5] factors were considered in the model: maternal education and HIV status, child gender and race, household socioeconomic status and an indicator variable for presence of a road-to-health booklet. The goodness-of-fit of the model was then assessed using the Hosmer Lemeshow test [6]. The final weight for the PTD and SGA analyses was estimated as a product of the survey weight and the inverse probability that an individual has missing data for PTD. Only the survey weight was applied to the low birth weight (LBW) and underweight-for-age (UFA) analyses.



Supplementary figure 1: Simplified conceptual framework of factors associated with birth outcomes and childhood underweight. Note: The associations depicted in the figure are not necessarily uni-directional and possible statistical interactions are not displayed. Abbreviations: ANC; antenatal clinic, ART; antiretroviral treatment, HH; house hold, LBW; low birth weight, PTD; preterm delivery, SGA; small-for-gestational age, Underweight-for-age; UFA

Supplementary table 1: Prevalence estimates

Outcome	Numerator	Denominator
PTD	Number of infants with gestational age<37 weeks	Number of infants with gestational age data
LBW	Number of infants with birth weight <2.5Kg	Number of infants with birth weight data
SGA	Number of infants with small for gestational age Z-score <-1.28	Number of infants with small for gestational age Z-score data
UFA	Number of infants with weight-for-age Z-score <-2	Number of infants with weight-for-age Z-score data
Note: Weights were applied to all prevalence estimates. Definitions: low birthweight; LBW, preterm delivery; PTD, small-for-gestational age; SGA, underweight-for-age; UFA,		

Supplementary table 2: Studies comparing birth outcomes in HIV exposed and unexposed African infants

Study; design	Setting and data collection period	Adverse birth outcome	PTD %	LBW %	SGA %	Measure of association
Aniji 2013;[7] Hospital based retrospective cohort study	South Africa (Johannesburg); Oct2008- Mar2009	Pre-conception ART	21	21	----	
		Post-conception ART	24	25	----	
Chen 2012[8]; Hospital-based record reviews	Botswana; May2009-April2011	Overall	19.6	----	13.5	
			23.7		18.4	
			17.2		11.5	
Ekouevi 2008[9]; Birth cohort	Côte d'Ivoire (Abidjan); Mar 2001-Jul2003	ART	----	HIV exposed	----	
		ZDV	----	HIV unexposed	----	

Ekouevi 2011[10]; Facility based retrospective cohort	Côte d'Ivoire (Abidjan); 2003-2009	EFV based ART	9.5	17.2	----	
		NVP based ART	12.7	24.2	----	
Fowler 2016[11]; Randomised controlled trial	India, Malawi, South Africa,Tanzania, Uganda, Zambia & Zimbabwe; period 1(April2011-Sep2012); period 2(Oct2012-Oct2014)	ZDV-based ART: Period1	20.5	23.0		
		TDF-based ART		16.9	----	
		ZDV	13.1	12.0	----	
		ZDV-based ART: Period2	19.7	20.4	----	

		TDF-based ART	18.5	16.9	----	
		ZDV	13.5	8.9	----	
Kesho Bora Study Group 2011[12]; randomised controlled trial in antenatal clinics	Burkina Faso, Kenya, South Africa. Jun2005-Aug2008	ART	13.0	11.0	----	
		ZDV & NVP	11.0	7.0	----	
Koss 2014[13]; Hospital based open-label single-site RCT	Uganda (Tororo); 2009-2013	LPV/r based ART	16.2	----	----	
		EFV based ART	14.7	----	----	
Liu 2014[14]; antiretroviral drug safety study in hospitals and	South Africa and Zambia; Oct2010-Apr2011	ART: South Africa	18.4	15.8	----	

primary health care facilities						
		ART: Zambia	29.7	18.1	----	
Li 2016[15]; Clinic-based prospective observational study	Tanzania (Dar es Salaam); Nov2004-Sep2011	No ARVs	39	17	17	
		All ARV exposed	29	18	21	
		ZDV	27	15	9	
		Pre-conception ART	38	26	16	
		Post-conception ART	26	21	15	
Malaba 2016[16]; Hospital based prospective cohort study	South Africa (Gugulethu); Apr2013-Aug2015	HIV exposed	22.0	14.0	11.0	

		HUU	13.0	9.0	9.0	
		pre-conception ART	24.0	15.0	11.0	
		post-conception ART	25.0	14.0	12.0	
Marazzi 2011[17]; retrospective cohort study	Malawi and Mozambique; Jul2005-Dec2009	Overall	19.1	11.5	----	
		No ART	70	----	----	
		ART	8.5	----	----	
Mehta 2017[18]; Pregnancy registry surveillance study	South Africa (Kwa-zulu Natal); Oct2013-Oct2014	Overall	----	12.4	----	
		HIV exposed	----	14.5	----	
		HIV unexposed	----	11.0	----	

Moodley 2016[19]; Hospital based retrospective record review	South Africa (Kwa-Zulu Natal); Jul-Dec2011 & Jan-Jul2014	Overall: 2011	22.0	12.0	7.2	
		Overall: 2014	19.5	12.8	8.2	
		No ARVs	32.4	23.0	10.1	
		ZDV/NVP	20.1	10.0	7.5	
		NVP based ART	24.5	15.3	9.2	
		EFV based ART	21.1	13.5	8.0	
Ndirandu 2012[20]; Non-randomised antenatal clinic-based intervention cohort	South Africa (Kwa-Zulu Natal) 2001-2004	Overall	21.4	----	16.6	
		HUU	21.8	----	15.1	
		HEU	20.9	----	18.1	

Nlend 2014[21]; hospital based cross-sectional study	Cameroon (Yaounde) 2008-2013	Overall	9.7	11.6	----	
		Pre-conception ART	8.1	11.7	----	
		During pregnancy	10.1	11.6	----	
Powis 2011[22]; randomised controlled trial	Botswana; enrolment between Jul2006-May2008	LPV-based ART	21.4	----	----	
		ABC-based ART	11.8	----	----	
Rempis 2017; cross-section study[23]	Uganda (Fort Portal); Feb-Dec2013	HUU	28.2	----	11.3	
		Pre-conception ART	28.9	----	8.3	

		Post-conception ART	27.6	----	13.8	
Sofeu 2015[24]; hospital based cohort study	Cameroon; 2007-2010	HUU	----	4.6	3.5	
		HEU	----	7.5	6.3	
Taha 2012[25]; Six prospective cohort studies	Malawi (Blantyre); 1989-1994; 2000-2007	HIV exposed	7.6	12.9		
Turner 2013[26]; prospective cohort study	Malawi (Blantyre); 2000-2004					
		HIV exposed	16.0	21.0	----	
		Peripheral viral load increase & low birth weight				PR=1.4

		Placental viral load increase & low birth weight				PR=1.2
		Placental viral load increase & preterm delivery				PR=1.3
Young 2012[27]; prospective clinical trial	Uganda (Tororo); 2009-2013	HEU	17.7	19.6	15.1	
Zash 2016[28]; hospital-based record review	Botswana (Gaborone & Francistown); May2009-April2011; April2013-April2014	Overall	21	----	18	
		Initiated EFV-based	18.2	----	11.9	

		ART in pregnancy				
		Initiated other ART in pregnancy	20.7	----	21.1	
		Initiated ZDV in pregnancy	16.4	----	20.9	
		EFV-based ART at conception	28.0	----	----	
		Other ART at conception	31	----	----	

Abbreviations: ABC**;** Abacavir**,** ARV; antiretroviral, ART: antiretroviral treatment, EFV; Efavirenz, HEU; HIV exposed uninfected, HUU; HIV unexposed, LPV/r; Lopinavir/Ritonavir, low birthweight; LBW, preterm delivery; PTD, small-for-gestational age; SGA, NVP; Nevirapine, ZDV; Zidovudine, underweight-for-age; UFA

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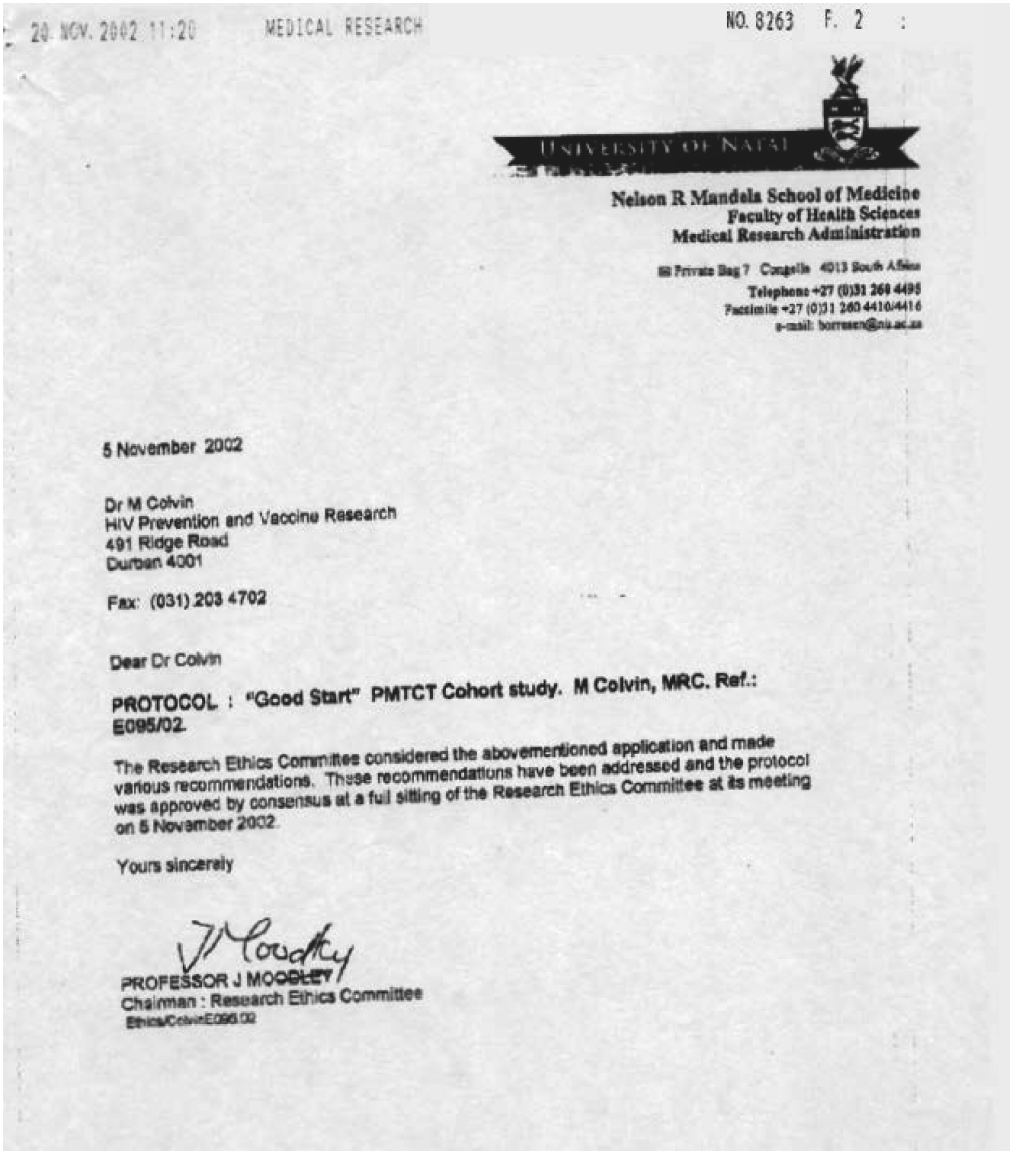
Paper II: Ethics document



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5 November 2002

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HIV Prevention and Vaccine Research
491 Ridge Road
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Fax: (031) 203 4702

Dear Dr Colvin

PROTOCOL : "Good Start" PMTCT Cohort study. M Colvin, MRC. Ref.: E095/02.

The Research Ethics Committee considered the abovementioned application and made various recommendations. These recommendations have been addressed and the protocol was approved by consensus at a full sitting of the Research Ethics Committee at its meeting on 5 November 2002.

Yours sincerely

PROFESSOR J MOODLEY
Chairman : Research Ethics Committee
Ethics/ColvinE095.02

Paper I: Ethics document

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL

ETHICS COMMITTEE

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http:// www.sahealthinfo.org/ethics/ethics.htm

20 March 2007

Dr M Chopra
Director: Health Systems Research Unit
MRC Cape Town

Dear Dr Chopra

Protocol ID: EC07-001
Protocol title: Good Start community-based intervention study
Meeting date: 26 February 2007

Thank you for your application to the Ethics Committee. Your application was discussed at the February 2007 meeting. I am pleased to inform you that ethics approval was granted for the study.

Wishing you well with your research.

Yours sincerely

PROF. D DU TOIT
CHAIRPERSON: MRC ETHICS COMMITTEE

Paper III: Ethics document

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL
SOUTH AFRICA

ETHICS COMMITTEE

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25 September 2012

Dr A Goga
Health Systems Research Unit
MRC Pretoria

Dear Dr Goga

Protocol ID: EC09-002
Protocol title: Survey/evaluation of the effectiveness of the national prevention of mother-to-child transmission (PMTCT) programme on infant HIV until 18 months postpartum in South Africa
Meeting date: 21 September 2012

Thank you for your application for an amendment, dated 3 and 14 September 2012. The Committee granted ethics approval for the amendment, Protocol Version 3 (August 2012).

Wishing you well with your research.

Yours sincerely

PROF. D DU TOIT
CHAIRPERSON: MRC ETHICS COMMITTEE

Questionnaires and consent forms

The study questionnaires and consent forms can be obtained from the following contacts:

PROMISE-EBF Study (Paper I): Professor Tanya Doherty (tanya.doherty@mrc.ac.za);

Good Start Study (Paper II): Professor Tanya Doherty (tanya.doherty@mrc.ac.za);

SAPMTCTE Study (Paper III): Professor Ameena Goga (ameena.goga@mrc.ac.za);

Errata for
Growth of HIV-exposed and HIV-unexposed children in South Africa

Anthropometric nutritional status and growth rates

Vundli Ramokolo

Thesis for the degree philosophiae doctor (PhD)

at the University of Bergen

(signature of candidate)

(signature of faculty)

14/11/17

Errata

Errata list

Errata	Was	Now reads
Page 9 first para	HIV positive. .	HIV positive.
Page 9 second para	BMI-for-age age Z-score	BMI-for-age Z-score
Page 10	For Paper II, the majority of the HIV-positive and -negative women in Paper II were also	For Paper II, the majority of the HIV-positive and -negative women were also
Page 22 para 3	development	developed
Page 27	As they a long-term	As they are a long term-term
Page 31 para 1	Figure 5	Figure 6
Page 40	US adopts Option B	US recommends ART in pregnancy
Page 40	2005-2008	2006-2008
Page 166	Paper I: Ethics document	Paper II: Ethics document
Page 167	97%	95%
Page 167	Paper II: Ethics document	Paper I: Ethics document